2. HEALTH EFFECTS

2.1 INTRODUCTION

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

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

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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

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

LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of *n*-hexane are indicated in Table 2-1 and Figure 2-1.

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

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

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

1.2.1 Inhalation Exposure

2.2.1.1 Death

No studies were located describing death in humans after inhalation exposure to *n*-hexane. This includes cases of occupational exposure where severe neurological effects occurred (see Section 2.2.1.4).

No studies were located describing death in test animals after acute-duration inhalation exposure to pure *n*-hexane. An LC_{50} (lethal concentration, 50% kill) of 73,680 ppm was reported in male Long-Evans rats exposed for 4 hours to a C6 aliphatic hydrocarbon fraction containing only *n*-hexane and its isomers (Hine and Zuidema 1970). All deaths occurred during the 4-hour exposures with the exception of 1 rat exposed at 81,800 ppm, which had convulsions during and after exposure and died during the sixth day. Rats that survived were uncoordinated, prostrate, or comatose during exposure but recovered within a few hours after removal from the chamber. Concentrations resulting in death in test animals are far above the reported explosive limit for *n*-hexane vapor (approximately 11,000 ppm) (see Chapter 3).

In other acute-duration inhalation studies on *n*-hexane in experimental animals, no deaths were reported in pregnant Fischer 344 rats exposed to 1,000 ppm *n*-hexane for 6 hours a day for 5 or 9 days (Bus et al. 1979) or male Sprague-Dawley rats exposed to 5,000 ppm for 24 hours and observed for a further 14 days (De Martino et al.1987). Exposures at 5,000 ppm in pregnant rats for 20 hours a day for 14 days, or in male mice for 20 hours a day for 5 consecutive days, also resulted in no deaths (Mast et al.1987).

Deaths have been reported after intermediate-duration inhalation exposures to relatively high concentrations of *n*-hexane. These deaths appear to be related to a failure to gain weight, compounded by development of severe neuropathy which makes eating and drinking difficult. In male Wistar rats exposed to 3,040 ppm *n*-hexane 12 hours a day for 16 weeks, 2 of 7 rats died shortly before the end of the study (after 109 and 111 days of exposure). Both animals showed unsteady gait and foot-drop before death (Takeuchi et al. 1980). No deaths occurred in rats exposed at similar concentrations to the structurally related chemicals *n*-pentane or *n*-heptane for the same duration. Deaths were also reported in young male Fischer 344 rats (80 days old) after an 11-week exposure to 1,000 ppm *n*-hexane for 24 hours a day, 6 days a week (Howd et al.1983). Two rats died during the 11th week of exposure; 2 died during week 12 (first week of recovery); and 1 died during week 14 (third week of recovery). The deaths appeared to be related to weight loss (treated rats weighed only 46% as much as age-matched controls). Advanced neuropathy and

subsequent muscle weakness made eating and drinking difficult. No deaths occurred in male weanling rats (20 days old at initiation) exposed similarly (Howd et al.1983). Four of 8 male Fischer 344 rats died within 6 weeks after an 11-week exposure to 1,500 ppm *n*-hexane for 24 hours a day, 5 days a week (Rebert and Sorenson 1983). Signs preceding death were not stated. Deaths also occurred in 2 of 12 male New Zealand rabbits exposed to 3,000 ppm *n*-hexane for 8 hours a day, 5 days a week for 24 weeks (Lungarella et al.1984). The time to death was not reported. Before death, the rabbits had signs of breathing difficulties (gasping, lung rales, mouth breathing). In a study where male Sprague-Dawley rats were exposed to 5,000 ppm *n*-hexane for 16 hours a day for up to 6 weeks, the authors stated that after 4 weeks, some animals had to be removed from treatment to prevent death after severe paralysis developed (De Martino et al.1987).

Other intermediate-duration inhalation exposures to *n*-hexane in experimental animals report no deaths as the result of treatment (Abou-Donia et al.1985, 1991; Altenkirch et al.1982; Bio/Dynamics 1978; Cavender et al.1984; Dunnick et al.1989; Huang et al.1989; IRDC 1981; NTP 1991). Generally, the daily exposure in these studies was 6-8 hours. The highest *n*-hexane concentration used in these studies was 10,000 ppm 6 hours a day, 5 days a week for 13 weeks in $B6C3F_1$ mice (Dunnick et al.1989; NTP 1991) and Fischer 344 rats (Cavender et al.1984). The longest duration exposure was 8 hours a day for 40 weeks at 700 ppm *n*-hexane in male Wistar rats (Altenkirch et al.1982).

Reliable LOAEL values for death in rats and rabbits for intermediate-duration exposure are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.2 Systemic Effects

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1. No chronic-duration inhalation studies for exposure to *n*-hexane in experimental animals were located.

Respiratory Effects. Hexane was one of 16 industrial solvents (hydrocarbons, alcohols, ketones, esters, and ethyl ether) tested for irritation potential on an average of 10 volunteers of mixed sexes for 3-5 minutes in an inhalation chamber (Nelson et al.1943). The purity and the isomer composition of the hexane was not specified. Hexane was the only one of the 16 solvents which caused no irritation to the eyes, nose, or throat at the highest concentration tested (500 ppm). No odor was reported.

Table 2-1. Levels of Significant Exposure to n-Hexane - Inhalation

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
ACUTE EXPOSURE							
Systemic							
1	Rat (Hybrid)	10 d 6 hr/d Gd 6-15	Bd Wt	409 F			Litton Bionetics 1979
2	Rat (Sprague-Dawley)	14 d 20 hr/d Gd 6-19	Bd Wt	1000 F		5000 F (31% reduced weight gain in dams)	Mast et al. 1987
3	Rat (Fischer- 344)	2 wk 5 d/wk 24 hr/d	Bd Wt		1500 M (approx. 11% decreased body weight at 2 weeks)		Rebert and Sorenson 1983
			Metab	1500 M			
4	Mouse (Swiss)	12 d 20 hr/d	Bd Wt	5,000			Mast et al. 1988
5	Mouse (B6C3F1)	5 d 20 hr/d	Bd Wt	5000 M			Mast et al. 1989a
6	Mouse (Swiss CD-1)	5 d 20 hr/d	Bd Wt	5000 M			Mast et al. 1989b
7	Rabbit (New Zealand)	14 d 5 d/wk 8 hr/d	Resp		3000M (nasal discharge, gasping, lung rales, mouth breathing)		Lungarella et al. 1984
Neurological							
8	Rat (Sprague-Dawley)	1 wk 6 d/wk 16 hr/d			5000M (11% decrease in MCV)		De Martino et al. 1987

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

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
Reproductive							
9	Rat (Sprague-Dawley)	24 hr				5000 M (spermatid and spermatocyte degeneration, exfoliation)	*De Martino et al.* 1987
10	Rat (Sprague-Dawley)	2-8 d 16 hr/d				5000 M (spermatid and spermatocyte degeneration, exfoliation)	De Martino et al. 1987
11	Mouse (B6C3F1)	5 d 20 hr/d		5000 M			Mast et al. 1989a
12	Mouse (B6C3F1)	5 d 20 hr/d		5000 M			Mast et al. 1989b
Developmental							
13	Rat (Fischer-344)	5 d 6 hr/d Gd 8-12		1000			Bus et al. 1979
14	Rat (Fischer-344)	5 d 6 hr/d Gd 12-16		1000			Bus et al. 1979
15	Rat (Fischer-344)	9 d 6 hr/d Gd 8-16			1000 (temporary decrease in pup growth rate)		Bus et al. 1979
16	Rat (Hybrid)	10 d 6 hr/d Gd 6-15		409			Litton Bionetics 1979
17	Rat (Sprague-Dawley)	14 d 20 hr/d Gd 6-19		200	1000 (7% decrease in fetal weight in males only)		Mast et al. 1987

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

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
18	Mouse (Swiss)	12 d GD 6-17 20 hr/d				5000 (decreased live fetuses per litter, female fetus weight, gravid uterine weight)	Mast et al. 1988
INTERMEDIATE EXPOSURE							
Death							
19	Rat (Fischer- 344)	11 wk 6 d/wk 24 hr/d				1000 M (5/10 died in young adult group)	Howd et al. 1983
20	Rat (Fischer- 344)	11 wk 5 d/wk 24 hr/d				1500 M (4/8 died)	Rebert and Sorenson 1983
21	Rat (Wistar)	16 wk 7 d/wk 12 hr/d				3040 M (2/7 died)	Takeuchi et al. 1980
22	Rabbit (New Zealand)	24 wk 5 d/wk 8 hr/d				3000 M (2/12 died)	Lungarella et al. 1984
Systemic							
23	Rat (Sprague-Dawley)	8-26 wk 5 d/wk 6 hr/d	Hemato	129			Bio/Dynamics 1978
			Bd Wt	129			
			Metab	26 M 129 F	129M (higher fasting glucose)		

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

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
24	Rat (Sprague-Dawley)	8-26 wk 7 d/wk 21 hr/d	Hemato	126			Bio/Dynamics 1978
			Hepatic	27 F 126 M	126F (decreased blood urea nitrogen)		
			Bd Wt	126			
25	Rat (Fischer- 344)	13 wk 5 d/wk 6 hr/d	Resp	10000			Cavender et al. 1984
			Cardio	10000			
			Gastro	10000			
			Hemato	10000			
			Renal	6500 M 10000 F	10000M (decreased urine pH)		
			Endocr	10000			
			Dermal	10000			
			Ocular	10000			
			Bd Wt	6500 M 10000 F	10000M (11% decrease)		
			Metab	10000			
26	Rat (Fischer- 344)	11 wk 6 d/wk 24 hr/d	Bd Wt			1000 M (54 & 33% decreased body weight at end of exposure in young adults and weanlings, respectively)	Howd et al. 1983
27	Rat (Wistar)	16 wk 7 d/wk 12 hr/d	Bd Wt	500 M	1200M (13% decrease)		Huang et al. 1989

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

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
28	Rat (Sprague-Dawley)	6 mo 7 d/wk 22 hr/d	Resp	500 M			IRDC 1981
			Cardio	500 M			
			Gastro	500 M			
			Musc/skel		500 M (mild atrophy in gastrocnemius muscle in 3/10)		
			Hepatic	500 M			
			Renal		500 M (increases in kidney weight and incidence and severity of chronic nephritis)		
			Endocr	500 M			
			Dermal	500 M			
			Ocular	500 M			
			Bd Wt			500 M (30% decrease)	
29	Rat (Sprague-Dawley)	28-61 d 7 d/wk 18-21 hr/d	Musc/skel			986 M (severe hindlimb atrophy)	Nylen et al. 1989
30	Rat (Fischer- 344)	11 wk 5 d/wk 24 hr/d	Bd Wt		500 M (10% decrease at 8 weeks)	1000 M (20% decrease at 8 weeks)	Rebert and Sorenson 1983
			Metab	1500 M			
31	Rat (Wistar)	16 wk 7 d/wk 12 hr/d	Musc/skel			3040 M (atrophy, denervation, irregular fibers, disordered myofilaments)	Takeuchi et al. 1980
			Bd Wt			3040 M (33% decrease)	

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

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
32	Mouse (B6C3F1)	13 wk 5 d/wk 22 hr/d	Resp		1000 (mild multifocal regeneration, metaplasia in olfactory epithelium)		Dunnick et al. 1989; NTP 1991
			Cardio	1000			
			Gastro	1000			
			Hemato	1000			
			Hepatic	1000			
			Renal	1000			
			Endocr	1000			
			Dermal	1000			
			Bd Wt		1000M (10% decrease)		
			Metab	1000			
33	Mouse (B6C3F1)	13 wk 5 d/wk 6 hr/d	Resp	500	1000 (multifocal regeneration and metaplasia in olfactory epithelium)	10000 (multifocal erosion, regeneration, inflammation, metaplasia in olfactory and respiratory epithelium, sneezing)	Dunnick et al. 1989; NTP 1991
			Cardio	10000			
			Gastro	10000			
			Hemato	4000	10000M (increase in segmented neutrophils)		
			Hepatic	10000			
			Renal	10000			
			Endocr	10000			
			Dermal	10000			
			Bd Wt	4000 M	10000M (17% decrease)		
			Metab	10000			

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

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
34	Rabbit (New Zealand)	24 wk 5 d/wk 8 hr/d	Resp			3000 M (centrilobular emphysema, fibrosis, goblet cell metaplasia. epithelial desquamation, respiratory tract irritation)	Lungarella et al. 1984
			Hemato	3000 M			
			Bd Wt	3000 M			
35	Chicken (Leghorn)	90 d 24 hr/d	Bd Wt		1000F (12% decrease)		Abou-Donia et al. 1985
36	Chicken (Leghorn)	30 d 24 hr/d	Bd Wt			1008 F (21% decrease)	Abou-Donia et al. 1991
Immunological/Lymphoreticular							
37	Rat (Sprague-Dawley)	6 mo 7 d/wk 22 hr/d		500 M			IRDC 1981
38	Mouse (B6C3F1)	13 wk 5 d/wk 6h/d		10000			Dunnick et al. 1989; NTP 1991
39	Mouse (B6C3F1)	13 wk 5 d/wk 22 hr/d		1000			Dunnick et al. 1989; NTP 1991
Neurological							
40	Rat (Wistar)	9 wk 7 d/wk 22 hr/d				500 M (narcosis, paralysis, axonal degeneration in peripheral nerve, axonal swelling in cervical spinal cord)	Altenkirch et al. 1982

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

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
41	Rat (Wistar)	40 wk 7 d/wk 8 hr/d		700 M			Altenkirch et al. 1982
42	Rat (Fischer- 344)	13 wk 5 d/wk 6 hr/d		3000 M 10000 F	6500M (axonal swelling in sciatic nerve in 1/5)		Cavender et al. 1984
43	Rat (Sprague-Dawley)	1-6 wk 6 d/wk 16 hr/d				5000 M (20-35% decrease in MCV, peripheral neuropathy, paralysis)	De Martino et al. 1987
44	Rat (Sprague-Dawley)	7 wk 5 d/wk 9 hr/d		5000 M			Frontali et al. 1981
45	Rat (Sprague-Dawley)	14 wk 5 d/wk 9 hr/d		1500 M		5000 M (tibial nerve axonal degeneration)	Frontali et al. 1981
46	Rat (Sprague-Dawley)	30 wk 5-6 d/wk 9-10 hr/d		500 M		2500 M (tibial nerve axonal degeneration)	Frontali et al. 1981
47	Rat (Fischer- 344)	11 wk 6 d/wk 24 hr/d				1000 M (hindlimb paralysis)	*Howd et al. 1983*
48	Rat (Wistar)	16 wk 7 d/wk 12 hr/d		500 M		1200 M (decreased grip strength and MCV, axonal swelling and demyelination)	Huang et al. 1989
49	Rat (Sprague-Dawley)	6 mo 7 d/wk 22 hr/d				500 M (gait disturbance, peripheral nerve atrophy)	IRDC 1981

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

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
50	Rat (Fischer- 344)	11 wk 5 d/wk 24 hr/d				500 M (decreased fore- & hindlimb grip strength; increased latency of evoked potentials in CNS)	Rebert and Sorenson 1983
51	Rat (Sprague-Dawley)	42-162 d 7 d/wk 24 hr/d				400-600 (central and peripheral neuropathy, foot-drop, waddling gait, limb weakness, swollen axons, axonal degeneration)	Schaumburg and Spencer 1976
52	Rat (Wistar)	16 wks 7 d/wk 12 hr/d				3040 M (gait disturbances, axonal swelling, neurofilament accumulation, denervated neuromuscular junctions)	Takeuchi et al. 1980
53	Mouse (B6C3F1)	13 wk 5 d/wk 6 hr/d		4000	10000 (decreased locomotor activity, paranodal swellings in tibial nerve)		Dunnick et al. 1989; NTP 1991
54	Mouse (B6C3F1)	13 wk 5 d/wk 22 hr/d			1000 (paranodal swellings in tibial nerve)		Dunnick et al. 1989; NTP 1991
55	Rabbit (New Zealand)	24 wk 5 d/wk 8 hr/d		3000 M			Lungarella et al. 1984
56	Chicken (Leghorn)	90 d 24 hr/d				1000 F (axonal degeneration in ventral columns of thoracic spinal cord in 1/5)	Abou-Donia et al. 1985
57	Chicken (Leghorn)	30 d 24 hr/d		1008 F			Abou-Donia et al. 1991

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

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
	Reproductive						
58	Rat (Fischer- 344)	13 wk 5 d/wk 6 hr/d		10000			Cavender et al. 1984
59	Rat (Sprague-Dawley)	1-6 wk 6 d/wk 16 hr/d				5000 M (testicular aplasia)	De Martino et al. 1987
60	Rat (Fischer- 344)	11 wk 6 d/wk 24 hr/d			1000 M (decreased testes weight)		Howd et al. 1983
61	Rat (Sprague-Dawley)	6 mo 7 d/wk 22 hr/d		500 M			IRDC 1981
62	Rat (Sprague-Dawley)	28-61 d 7 d/wk 18-21 hr/d				1000 M (testicular atrophy)	Nylen *et al. 1989*
63	Mouse (B6C3F1)	13 wk 5 d/wk 6 hr/d		10000			Dunnick et al. 1989; NTP 1991
64	Mouse (B6C3F1)	13 wk 5 d/wk 22 hr/d		1000			Dunnick et al. 1989; NTP 1991
65	Mouse (CD-1)	8 wks 5 d/wk 6 hr/d		396 M			Litton Bionetics 1980

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

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (ppm)	LOAEL Less serious (ppm)	LOAEL Serious (ppm)	Reference
Cancer							
66	Rabbit (New Zealand)	24 wk 5 d/wk 8 hr/d				3000 M (CEL: papillary tumors in bronchiolar epithelium)	Lungarella et al. 1984
CHRONIC EXPOSURE							
Neurological							
67	Human	9.1 yr			69 (decreased MCV and motor action potential amplitude)		Mutti et al. 1982a
68	Human	4.5 yr			195 F (decreased MCV)		Mutti et al. 1982b
69	Human	6.2 yr			58[b] M (decreased MCV)		Sanagi et al. 1980
70	Human	5 yr				190 (peripheral neuropathy, gait disturbance, decreased MCV)	Wang et al. 1986
Cancer							
71	Mouse (B6C3F1)	2 yr 5 d/wk 6 hr/d				9018[c] F (CEL: increased incidence of hepatocellular adenomas and carcinomas)	Biodynamics 1995b Mixed Hexanes

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

[b]Used to derive a chronic-duration inhalation minimal risk level (MRL) of 0.6 ppm, based on a less serious LOAEL of 58 ppm for decreased motor nerve conduction velocity in humans. Concentration divided by an uncertainty factor of 100 (10 for use of a LOAEL and 10 for human variability).

[c]Exposure was to commercial hexane containing 51.5% *n*-hexane and the remainder other hexane isomers.

approx. = approximately; Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; CNS = central nervous system; d = day(s); Endocr = endocrine; F = female; Gastro = gastrointestinal; Gd = gestational day; Hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; Metab = metabolic; MCV = motor nerve conduction velocity; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); yr = year(s)

Figure 2-1. Levels of Significant Exposure to *n*-Hexane - Inhalation

Acute (≤14 days)

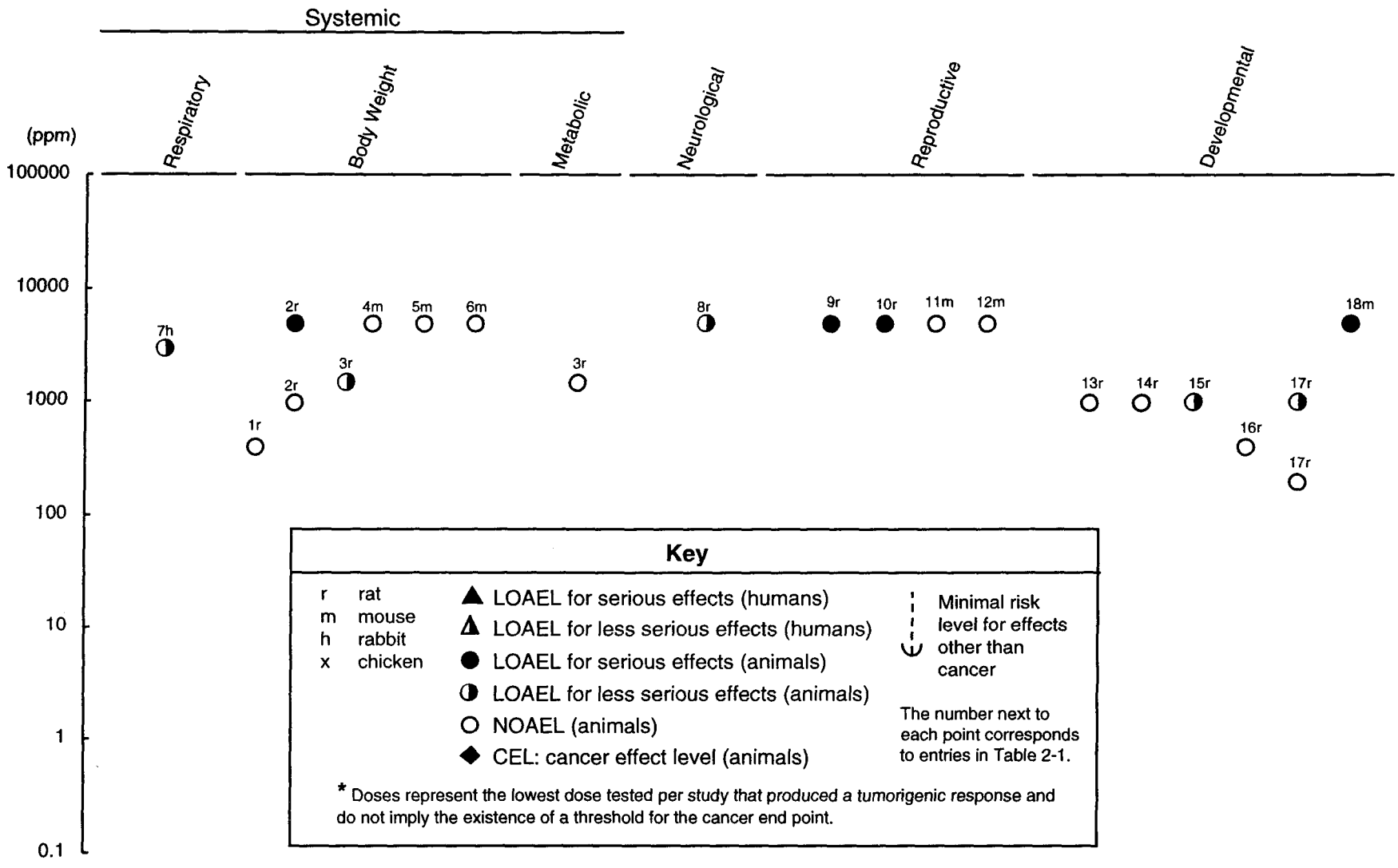


Figure 2-1. Levels of Significant Exposure to *n*-Hexane - Inhalation (cont.)

Intermediate (15-364 days)

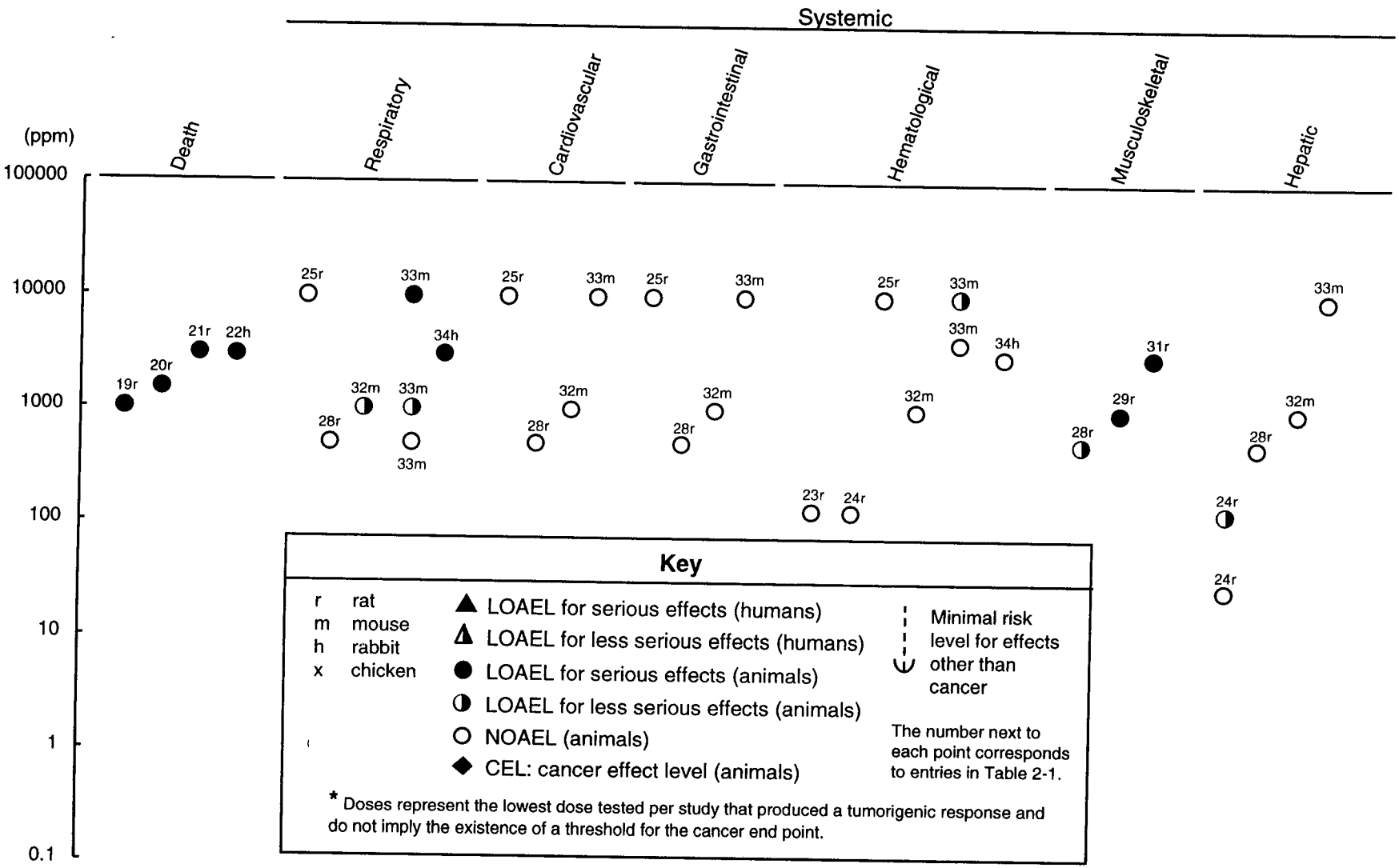


Figure 2-1. Levels of Significant Exposure to *n*-Hexane - Inhalation (cont.)

Intermediate (15-364 days)

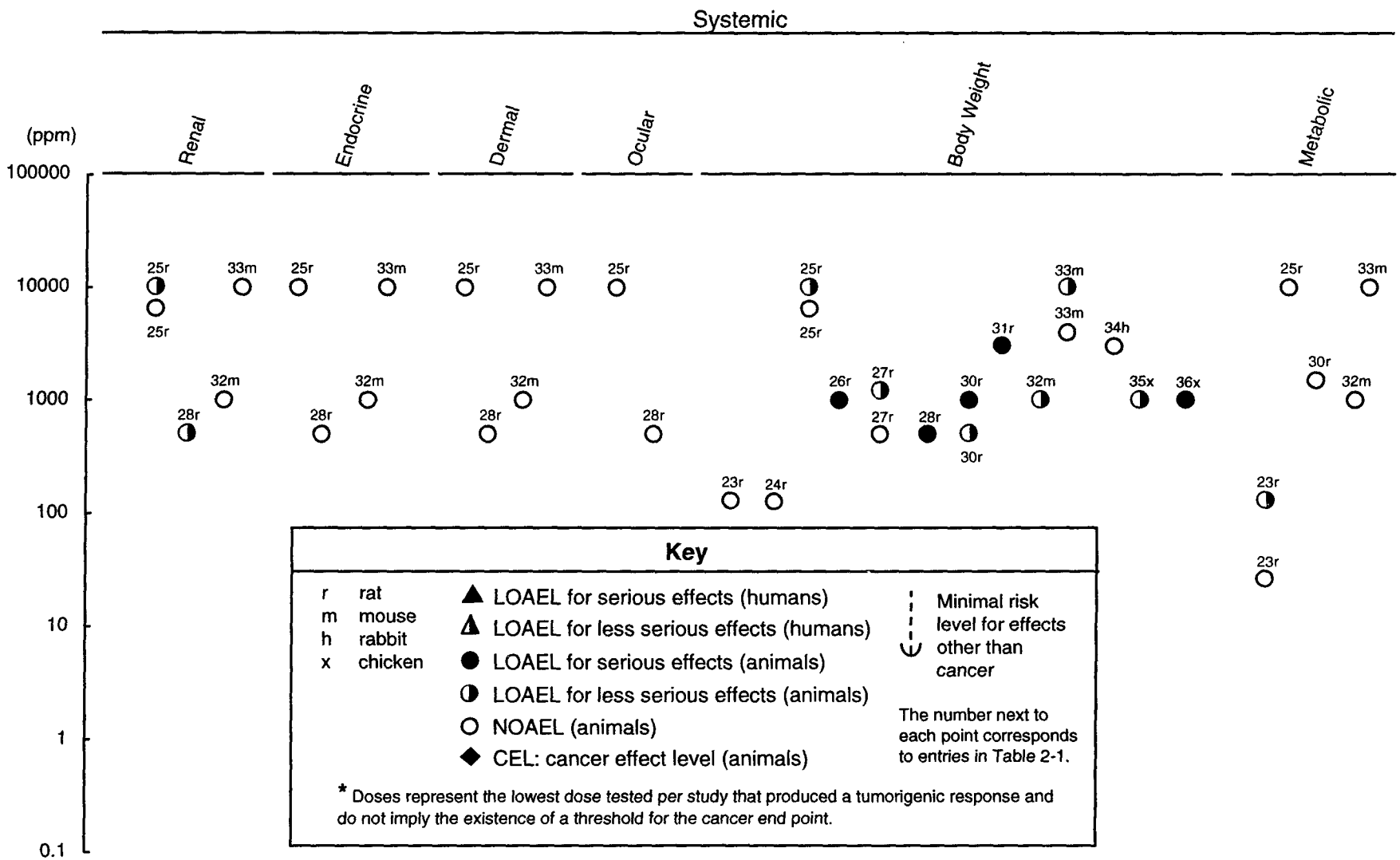


Figure 2-1. Levels of Significant Exposure to *n*-Hexane - Inhalation (cont.)

Intermediate (15-364 days)

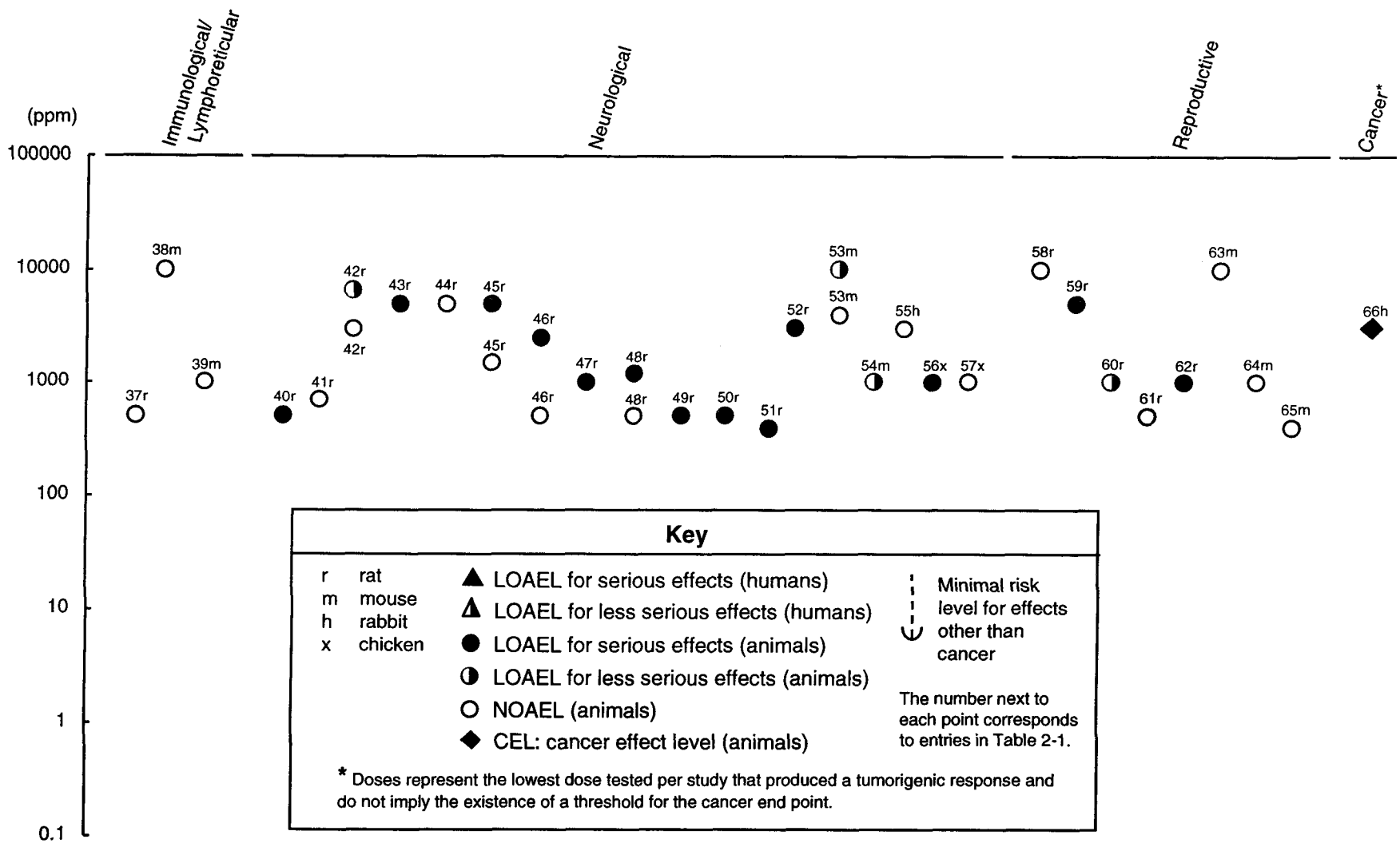
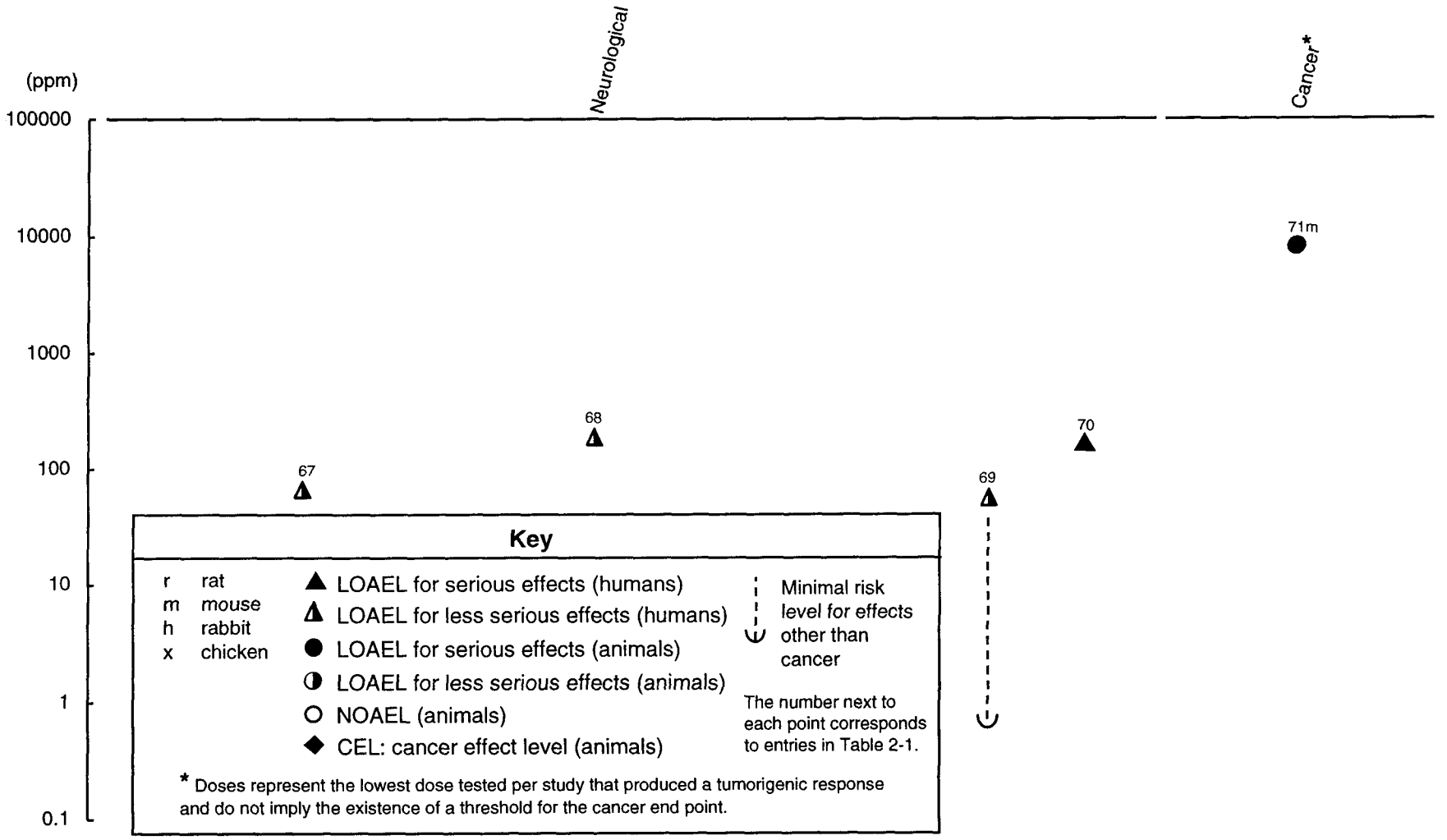


Figure 2-1. Levels of Significant Exposure to *n*-Hexane - Inhalation (cont.)

Chronic (≥365 days)



2. HEALTH EFFECTS

No information on respiratory effects after acute-duration inhalation exposure to *n*-hexane was located except a statement that male New Zealand rabbits exposed to 3,000 ppm 8 hours a day, 5 days a week for 24 weeks showed signs of respiratory tract irritation (nasal discharge) and breathing difficulties (gasping, lung rales, mouth breathing) throughout the study (Lungarella et al.1984).

More serious respiratory effects were seen upon histological examination of the respiratory tract at the end of this study. Gross lung changes observed in the rabbits included collapsed dark red areas, hyperemia, and the accumulation of mucous material. The trachea and the major bronchi showed areas of epithelial desquamation, relative atrophy, flattening of the mucosa, and foci of goblet cell metaplasia. The distal regions of terminal bronchioles and proximal portions of the alveolar ducts contained air space enlargements (consistent with centrilobular emphysema), scattered foci of pulmonary fibrosis, and papillary tumors of the bronchiolar epithelial cells. None of these alterations were found in the control rabbits. Two of 12 rabbits died during the study, possibly due to respiratory failure. To examine the reversibility of these effects, a group of 5 rabbits that had been kept for 120 days after exposure ceased were examined. No microscopic pathologic changes were visible in the mucosa of the trachea and the major bronchi except for small, scattered foci of goblet cell metaplasia. However, irregular foci of cellular proliferation and papillary tumors in terminal bronchiolar and alveolar ducts were observed.

Milder respiratory tract lesions resulting from inhalation *n*-hexane exposure were observed in 13-week exposure studies in male and female B6C3F_1 mice (Dunnick et al.1989; NTP 1991). In an intermittentexposure study (6 hours a day, 5 days a week), sneezing (a sign of respiratory tract irritation) was seen in both sexes at 10,000 ppm, beginning at week 4 and continuing until the end of the study. Multifocal regeneration and metaplasia in the olfactory epithelium were observed at 1,000 ppm. At 10,000 ppm, multifocal erosion, regeneration, inflammation, and metaplasia in both the olfactory and respiratory epithelium were observed. In a companion study where mice were exposed to 1,000 ppm *n*-hexane (22 hours a day, 5 days a week), mild multifocal regeneration and metaplasia in the olfactory epithelium were observed (Dunnick et al.1989; NTP 1991). No respiratory effects were observed in this study in mice exposed to 500 ppm *n*-hexane (6 hours a day, 5 days a week).

In other studies where histopathological examination of the respiratory tract was performed after inhalation of *n*-hexane, no lesions were noted in male Sprague-Dawley rats exposed to up to 500 ppm *n*-hexane for 22 hours a day, 7 days a week for 6 months (IRDC 1981) or in Fischer 344 rats of both sexes exposed to up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks (Cavender et al.1984).

It should be noted that respiratory effects observed in these animal studies occurred at concentrations that are substantially higher than needed to cause neurotoxicity in rats (see Section 2.2.1.4). Thus, respiratory effects do not appear to be a sensitive indicator of *n*-hexane toxicity.

Cardiovascular Effects. Histopathological examination of the heart and aorta revealed no treatmentrelated lesions in male Sprague-Dawley rats exposed to up to 500 ppm *n*-hexane for 22 hours a day, 7 days a week for 6 months (IRDC 1981) or in Fischer 344 rats of both sexes exposed to up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks (Cavender et al.1984). Similar results were noted in $B6C3F_1$ mice exposed to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks or 1,000 ppm for 22 hours a day, 5 days a week for 13 weeks (Dunnick et al.1989; NTP 1991). No other studies regarding cardiovascular effects after inhalation exposure to *n*-hexane in experimental animals were located.

Gastrointestinal Effects. Histopathological examination of gastrointestinal tissues (salivary glands, esophagus, stomach, small intestine [duodenum, jejunum, ileum], large intestine [cecum, colon, rectum], and pancreas) revealed no treatment-related lesions in male Sprague-Dawley rats exposed to up to 500 ppm *n*-hexane for 22 hours a day, 7 days a week for 6 months (IRDC 1981) or in Fischer 344 rats of both sexes exposed to up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks (Cavender et al.1984). Similar results were noted in $B6C3F_1$ mice exposed to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks or 1,000 ppm for 22 hours a day, 5 days a week for 13 weeks (Dunnick et al.1989; NTP 1991). No other studies regarding gastrointestinal effects after inhalation exposure to *n*-hexane in experimental animals were located.

Hematological Effects. In a study where rats (12/sex/dose) were exposed to *n*-hexane for up to 6 months for either 6 hours a day, 5 days a week (0,6,26, or 129 ppm) or 21 hours a day, 7 days a week (0, 5, 27, or 126 ppm), some hematological parameters differed statistically from controls at 3 or 6 months (Bio/Dynamics 1978). Hematological analysis was performed in 4 animals in each group of 12. Among rats exposed for 6 hours a day, 5 days a week, mean hemoglobin and hematocrit values were within normal limits for all groups of males at 3 and 6 months. At 6 months, significantly increased leukocyte counts were observed in males at 6 and 129 ppm *n*-hexane, but not at 26 ppm. In females, hematocrit was reduced at 3 months at 6 and 26 ppm, but was elevated at 129 ppm. Erythrocyte counts were also lower at 6 and 26 ppm. Clotting time at 6 ppm was elevated at 3 months. No difference from control was seen in any female group at 6 months. None of the parameters measured in males or females was outside normal biological limits. Among rats exposed for 21 hours a day, 7 days a week, the only differences from control in hematological parameters seen in any group at 3 or 6 months were significantly lower erythrocyte counts

at 3 months at 5 ppm and a significantly depressed leukocyte count at 5 ppm at 6 months. None of the parameters measured in males or females was outside normal biological limits. Furthermore, when the 21 hours a day, 7 days a week experiment is compared to the 6 hours a day, 5 days a week experiment and the lack of a dose-response is considered, it is clear that there were no significant hematological effects in this study.

Hematological parameters were within normal limits in Fischer 344 rats (15/sex/dose) exposed to up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks (Cavender et al.1984). Similarly, hematological parameters were within normal limits in $B6C3F_1$ mice (18/sex/dose) exposed to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks except for an increase in segmented neutrophils in males exposed to 10,000 ppm. The authors ascribed this to chronic active inflammation in the nasal mucosa of some of the male mice (Dunnick et al.1989; NTP 1991). In mice in this study exposed to 1,000 ppm *n*-hexane for 22 hours a day, 5 days a week for 13 weeks, all hematological parameters were within normal limits. No significant changes were observed in hematological parameters in male New Zealand rabbits (n=12) exposed to 3,000 ppm *n*-hexane 8 hours a day, 5 days a week for 24 weeks (Lungarella et al.1984). No other studies examining hematological effects after inhalation exposure to *n*-hexane were located.

Musculoskeletal Effects. Muscle wasting and atrophy have been reported in humans occupationally exposed to *n*-hexane (Yamamura 1969). These effects occurred in individuals with severe neurotoxicity.

Muscle atrophy is a common finding after intermediate-duration inhalation exposure to *n*-hexane in experimental animals. This atrophy is secondary to *n*-hexane-induced neurotoxicity which results in muscle denervation (see Section 2.2.1.4). Hindlimb atrophy characterized as *"*severe*"* was reported in 10 of 11 male Sprague-Dawley rats exposed to 986 ppm *n*-hexane for 28 or 61 days (Nylen et al.1989). Mild atrophy of the gastrocnemius muscle was observed in 3 of 10 male Sprague-Dawley rats exposed to 500 ppm *n*-hexane for 22 hours a day for 7 days a week for 6 months (IRDC 1981). Degenerative changes in the muscle were not observed. Electron microscopy of the gastrocnemius and soleus muscles in male Wistar rats exposed to 3,040 ppm *n*-hexane for 12 hours a day, 7 days a week for 16 weeks, revealed denervation, irregular fibers, disordered myofilaments, zig-zagging of the Z-band, and numerous invaginations of the plasma membrane (Takeuchi et al.1980).

Hepatic Effects. Decreased blood urea nitrogen, indicating an effect on protein catabolism, was noted in female, but not male, Sprague-Dawley rats (12/sex/dose, 4 analyzed per group) exposed to 126 ppm *n*-hexane for 21 hours a day, 7 days a week for 26 weeks (Bio/Dynamics 1978). Histopathological

examination of the liver after intermediate-duration inhalation exposure revealed no treatment-related lesions in male Sprague-Dawley rats (n=20) exposed to 500 ppm *n*-hexane daily for 6 months, 22 hours a day (IRDC 1981); or in Fischer 344 rats of both sexes (15/sex/dose, 10 examined per group) exposed to up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks (Cavender et al.1984). Similar results were observed in $B6C3F_1$ mice (18/sex/dose, 8-10 examined per group) exposed to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks or 1,000 ppm for 22 hours a day, 5 days a week for 13 weeks (Dunnick et al.1989; NTP 1991).

Renal Effects. An increased incidence and severity of chronic nephropathy (a common age-related condition in male rats) was noted in male Sprague-Dawley rats (n=20, 10-11 examined) exposed to 500 ppm *n*-hexane daily for 6 months, 22 hours a day (IRDC 1981). Increased kidney weight was also observed. The authors stated that it was unclear whether the increased incidence and severity was due to exacerbation of the process seen in the control group or if the *n*-hexane exposure caused additional tubular injury. No lesions were reported in the urinary bladder. The authors did not investigate what role the unique male rat protein $\alpha_{2\mu}$-globulin might be playing in these renal effects. Other substances that apparently bind to this carrier protein include a number of hydrophobic xenobiotics such as petroleumderived hydrocarbons, including decalin and the gasoline constituent trimethylpentane. These substances cause an $\alpha_{2\mu}$-globulin nephropathy syndrome in male rats (EPA 1991). A decrease of urine pH in male rats exposed to 10,000 ppm but no histopathological lesions in the kidney were reported in Fischer 344 rats of both sexes (15/sex/dose, 10 examined per group) exposed to up to 10,000 ppm *n*-hexane for 6 hours a day, 5 days a week for 13 weeks (Cavender et al.1984). Histopathological examination of the kidney and urinary bladder showed no treatment-related lesions in $B6C3F_1$ mice (1 g/sex/dose, 8-10 examined per group) exposed to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks or 1,000 ppm for 22 hours a day, 5 days a week for 13 weeks (Dunnick et al.1989; NTP 1991).

Endocrine Effects. Histopathological examination of endocrine tissues (thyroid, parathyroid, adrenals, pituitary, pancreas) after intermediate-duration inhalation exposure revealed no treatment-related lesions in male Sprague-Dawley rats exposed to 500 ppm *n*-hexane daily for 6 months, 22 hours a day (IRDC 1981) or in Fischer 344 rats of both sexes exposed to up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks (Cavender et al.1984). Similar results were seen in $B6C3F_1$ mice exposed to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks or at 1,000 ppm for 22 hours a day, 5 days a week for 13 weeks (Dunnick et al.1989; NTP 1991). Tissues examined were the thyroid, parathyroid, adrenals, pituitary, and pancreas.

Dermal Effects. Coldness, reddishness, or roughness of the skin in the distal extremities was observed in 59.2% of 93 workers with peripheral neuropathy after occupational inhalation exposure to *n*-hexane (Yamamura 1969).

Histopathological examination of the skin after intermediate-duration inhalation exposure revealed no treatment-related lesions in male Sprague-Dawley rats exposed to 500 ppm *n*-hexane daily 22 hours a day for 6 months (IRDC 1981), or in Fischer 344 rats of both sexes exposed to up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks (Cavender et al.1984). Similar results were seen in $B6C3F_1$, mice exposed to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks or 1,000 ppm for 22 hours a day, 5 days a week for 13 weeks (Dunnick et al.1989; NTP 1991).

Ocular Effects. Histopathological examination of the eye and optic nerve after intermediate-duration inhalation exposure revealed no treatment-related lesions in male Sprague-Dawley rats exposed to 500 ppm *n*-hexane 22 hours a day for 6 months (IRDC 1981); or in Fischer 344 rats of both sexes exposed to up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks (Cavender et al.1984). Effects caused by direct contact of *n*-hexane vapor with the eye are discussed in Section 2.2.3 (Dermal Exposure).

Body Weight Effects. The body weights of male Fischer 344 rats exposed to 1,500 ppm *n*-hexane 24 hours a day, 5 days a week were 11% below those of control rats within 2 weeks (Rebert and Sorenson 1983). In a developmental study where pregnant Sprague-Dawley rats were exposed to *n*-hexane concentrations of 0, 93, and 409 ppm over gestation days 6-15, no effects on body weight of the dams were observed (Litton Bionetics 1979); however, reduced body weight gain was seen at 5,000 ppm (but not at 200 or 1,000 ppm) in pregnant rats exposed 20 hours a day during gestation days 6-19 (Mast et al.1987).

Effects on body weight are common during intermediate-duration exposure of rats to *n*-hexane and tend to occur prior to the development of neurotoxicity (see Section 2.2.1.4). In male Wistar rats exposed to 0, 500, 1,200, or 3,000 ppm *n*-hexane daily for 12 hours a day, body weight was lower in the treated groups from 4 weeks of exposure (Huang et al.1989). Significantly decreased grip strength was noted at 13 weeks and, at study termination (16 weeks), body weights in the 1,200 and 3,000 ppm groups were 13% less than control. In another study with male Wistar rats exposed daily to 3,040 ppm *n*-hexane, reduction in body weight compared to control was significant at 4 weeks, and final weight was 33% less than control at 16 weeks (Takeuchi et al.1980). In this study, reductions in nerve conduction velocity were observed at 4 weeks, and clinical signs of neurotoxicity occurred at 10 weeks. Similarly, Sprague-Dawley rats exposed to 500 ppm for 22 hours a day showed significant reduction in body weight compared to controls at 7 weeks and clinical signs of neurotoxicity at 16 weeks (IRDC 1981). At study termination

after 6 months, treated animals weighed 30% less than controls. Severe body weight effects were observed in male Fischer 344 rats exposed for 24 hours a day, 6 days a week for 1 I weeks at 1,000 pph (I?owd et al.1983). At the end of this study, the body weight of young adults (80 days old) was 54% below that of controls, and that of weanlings (20 days old) was 33% below control. The young adults in this study failed to gain any weight over the 11-week exposure. Body weight effects were concentration-related in male Fischer 344 rats exposed for 24 hours a day, 5 days a week (Rebert and Sorenson 1983). Body weight was 10% below control at 500 ppm and 20% below control at 1,000 ppm after 8 weeks.

Intermittent exposure has only mild effects on body weight even at high concentrations of *n*-hexane. In Fischer 344 rats exposed to up to 10,000 ppm for 6 hours a day, 5 days a week, the only effect seen after 13 weeks was a 11% decrease in body weight compared to controls in males, with no effect seen in females (Cavender et al.1984). No effect on body weight was observed in rats exposed 21 hours a day to 126 ppm or intermittently (6 hours a day, 5 days a week) to 129 ppm *n*-hexane for 26 weeks (Bio/Dynamics 1978).

Less-severe body weight effects were observed in species that are less susceptible to *n*-hexane-induced neurotoxicity (see Section 2.2.1.4). In male B6C3F_1 mice exposed to 1,000 ppm *n*-hexane for 22 hours a day, 5 days a week for 13 weeks, a 10% depression in the final body weight relative to control was observed (Dunnick et al.1989; NTP 1991). No change in weight was found in females. In male B6C3F_1 mice exposed to 10,000 ppm *n*-hexane for 6 hours a day, 5 days a week for 13 weeks, a 17% depression in the final body weight relative to control was observed. NOAELs were 4,000 ppm in males and 10,000 ppm in females (Dunnick et al.1989; NTP 1991). No effect on body weight was observed in male New Zealand rabbits exposed to 3,000 ppm *n*-hexane for 8 hours a day, 5 days a week for 24 weeks (Lungarella et al.1984). Variable weight loss was observed in Leghorn chickens exposed to 1,000 ppm *n*-hexane continuously for 30 days (21%) and 90 days (12%) (Abou-Donia et al.1991). Weight loss was greatly exacerbated in the 90-day study (up to 35%) when chickens were exposed to both 1,000 ppm *n*-hexane and 1,000 ppm methyl isobutyl ketone.

Metabolic Effects. In studies where metabolic parameters (blood pH, electrolytes, glucose) were measured, no effects were seen after inhalation exposure to *n*-hexane in Fischer 344 rats at up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks (Cavender et al.1984) or in B6C3F_1 mice similarly exposed (Dunnick et al.1989; NTP 1991). Significantly higher mean-fasting glucose was observed in male Sprague-Dawley rats (n=4) exposed for 6 hours a day, 5 days a week at 6 and 129 ppm, but not at 26 ppm (Rio/Dynamics 1978). Female fasting glucose levels were unaffected by exposure in this study. No effect on this parameter was seen in a parallel experiment at similar concentrations for 21 hours a day. For this reason, and because of the small group size (n=4), this finding is of doubtful toxicological

significance. Body temperature in rats was unaffected by continuous exposure to up to 1,500 ppm *n*-hexane after 2 and 11 weeks (Rebert and Sorenson 1983).

2.2.1.3 Immunological and Lymphoreticular Effects

One report of immunological effects in humans after exposure to *n*-hexane was located (Karakaya et al. 1996) describing a reduction in immunoglobulin levels in a group of 35 male workers compared to a control group of 23 (matched by age, other characteristics of the groups not reported). The reductions correlated with 2,5-hexanedione in urine but not with workplace *n*-hexane concentrations (23-215 ppm). The reductions also remained well within the normal ranges for immunoglobulins in blood, so the toxicological significance of these findings can not be assessed without confirmatory studies (Jackson et al. 1997). Cell counts (lymphocytes, neutrophils, monocytes, eosinophils) were unaffected by *n*-hexane exposure. No reports of dermal sensitization after exposure to *n*-hexane in humans were located.

Information on imrnunological/lymphoreticular effects in test animals is limited to histopathological examination of tissues after intermediate-duration inhalation exposure to *n*-hexane. No treatment-related lesions were observed in the cervical, bronchial, or mesenteric lymph nodes, thymus, bone marrow (sternum), or spleen of male Sprague-Dawley rats exposed to 500 ppm *n*-hexane 22 hours a day for 6 months (IRDC 1981) or in mandibular and mesenteric lymph nodes, thymus, bone marrow, or spleen of Fischer 344 rats of both sexes exposed to up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks (Cavender et al.1984). Similar results were observed in mesenteric lymph nodes, thymus, bone marrow (sternum), and spleen in $B6C3F_1$ mice exposed to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks or 1,000 ppm for 22 hours a day, 5 days a week for 13 weeks (Dunnick et al.1989; NTP 1991).

The highest NOAEL values for immunological/lymphoreticular effects in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.4 Neurological Effects

The neurotoxicity of *n*-hexane was first observed in the shoe industries of Japan and Italy in the 1960s and early 1970s. A number of epidemiological studies were initiated in response to outbreaks of apparent peripheral neuropathy in shoe workers. While the clinical course of the disease was well described, elucidation of a dose-duration response relationship has been difficult. In most cases, concentrations of *n*-hexane

in the workplace air were not measured until after disease developed. Also, in almost all cases, workers were concurrently exposed to other chemicals which may have affected their response to *n*-hexane.

One of the first large epidemiological investigations carried out was a case series of 93 cases of peripheral neuropathy in workers exposed to *n*-hexane from glues and solvents used in sandal manufacture (Yamamura 1969). After several cases of advanced quadriplegia were noted in the Fukaya district, Mie prefecture, Japan, an epidemiological investigation was carried out. Medical examination was performed on 296 of 1,662 workers checked by questionnaire. Of this group, 93 cases of peripheral neuropathy (both sensory and motor) were diagnosed. Urinalysis, hematology and serum chemistry, electromyography, and nerve conduction tests were done on 42-44 of the 93 total cases. The group was composed of 21 males and 72 females with an average age of 40.6 years. On the basis of symptoms, the subjects were divided into 3 groups: Group I, sensory neuropathy only (53 cases); Group II, sensorimotor neuropathy (32 cases); and Group III, sensorimotor neuropathy with muscle atrophy (8 cases). The grade of disorder was dependent on working conditions (work hours, work load, vapor concentration in room). The authors stated that *n*-hexane concentrations in the patients' work areas (primarily the home) ranged from 500 to 2,500 ppm. Duration of exposure was not specifically mentioned although the case of 1 female who had been exposed for 8 months was described in detail. The most common initial symptom was numbness in the distal portions of the extremities (88%); the second most common was muscle weakness (14%). Major clinical findings were numbness (100%); muscle weakness (43%); hypoactive reflexes (38.7%); and coldness, reddishness, or roughness of the skin (59.2%). Pyramidal tract signs (indicating central nervous system effects) were not observed in any patient. All the cases in Group III showed a continuing increase in the severity of their conditions for 1-4 months after exposure ceased. A follow-up of 36 patients showed complete or near-complete recovery in 3-18 months, including 6 of 8 in Group II. Residual atrophy and muscle weakness were still present in 2 individuals from Group III. No fatalities occurred. Electromyography revealed the appearance of fibrillation voltages (indicating denervation) and positive sharp waves in 15.3 and 19.9% of examined muscles in groups II and III, respectively. In the median and ulnar nerves, reduction of motor nerve conduction velocity below 45 meters per second (msec) was observed in 22 and 16 cases, respectively. In the tibia1 nerve, motor nerve conduction velocity was reduced below 40 m/sec in 31 cases. In the peroneal nerve, the motor nerve conduction velocity was below 40 rn!sec in 21 cases, and no response was obtained in 5 cases. The authors stated that reduction in motor nerve conduction velocity was greatest in Group III, followed by Group II and then Group I. Muscle biopsies of the anterior tibia1 muscle in 3 cases revealed atrophy of muscle fibers but no significant variation among groups. Myofibrils and striations were preserved and degenerative changes were minimal. Biopsies of peripheral nerve in 6 cases revealed *"*striking*"* demyelination and infiltration of leukocytes in

perivascular areas. Axonal degeneration was also present, although the authors stated that demyelination was the more obvious feature.

A case series of workers in a furniture factory in the Bronx, New York, illustrates the typical clinical presentation of *n*-hexane neurotoxicity (Herskowitz et al.1971). This report describes the cases of 3 women who worked as cabinet finishers wiping glue off furniture with rags soaked in a solvent which contained *n*-hexane. An open drum of this solvent was used in a small, poorly ventilated room. Air measurements of *n*-hexane averaged 650 ppm, although peaks of up to 1,300 ppm also occurred. The number of measurements was not reported. Neurological signs of both motor and sensory impairment were noted in all 3 women with an onset 2-4 months after beginning employment. Initial symptoms and clinical findings were similar in all three women. In the first case (a 23-year-old woman), initial symptoms were a burning sensation in the face, numbness of the distal extremities, and an insidious, progressive distal symmetrical weakness in all extremities. Frequent headaches and abdominal cramps were also reported. After being admitted to the hospital (6 months after beginning work), muscle testing revealed a moderate distal symmetrical weakness and a bilateral foot-drop gait. There was a moderate decrease of pin and touch perception and mild impairment of vibration and position sense in the lower extremities. Tendon reflexes were slightly hyperreflexic (1+) and symmetrical throughout, except for absent Achilles tendon reflexes. No Babinski sign (indicating central nervous system toxicity) was present. A complete blood count, blood urea nitrogen, fasting blood sugar, electrolytes, liver-function tests, transaminases and enzymes, and thyroxine levels were within normal limits. Serum lead screening was also negative. An electromyogram revealed fibrillation potentials in the small muscles of the hands and feet. Nerve conduction velocities were 45 m/s in the left ulnar nerve (normal range in the general population, 49-75), 26 m/s in the right median nerve (normal range, 50-75), and 23 m/s in the left peroneal nerve (normal range, 40-60). Sural nerve biopsy was unremarkable, although electron microscopic examination showed a few myelinated axons containing dense bodies and exceptionally numerous mitochondria. Muscle biopsy showed scattered groups of small angulated fibers and many fibers with clear central zones, consistent with denervation. Electron microscopy of nerve branches within the muscle showed an increased number of neurofilaments with abnormal membranous structures and clumping and degeneration of mitochondria with dense bodies. Increased numbers of mitochondria, glycogen granules, and degenerated mitochondria were noted in the motor endplates.

In a study where exposure appeared to be limited to only *n*-hexane and acetone, 2 age-matched groups consisting of 14 control workers and 14 exposed workers employed in a factory producing tungsten carbide alloys were compared (Sanagi et al.1980). The groups were matched with respect to age, stature, weight, alcohol consumption, and smoking habits. Exposure was estimated with 22 personal samples taken from

the breathing zones over a period of 2 years (this number of samples is fewer than optimal for measuring air levels). Eight-hour time-weighted average exposure to solvent vapors consisted of *n*-hexane at 58±41 ppm and acetone at 39±30 ppm; no other solvent vapors were detected. The exposure duration ranged from 1 to 12 years with an average of 6.2 years. Both groups completed questionnaires and underwent clinical neurological examinations with reference to cranial nerves, motor and sensory systems, reflexes, coordination, and gait. Neurophysiological studies performed included electromyography on muscles of the forearm and leg. Nerve stimulation studies were performed with a surface electrode (motor nerve conduction velocity, residual latency). Conduction velocities and distal latencies in the control group were similar to those reported in other studies (Goodgold and Eberstein 1983; Johnson et al.1983). In the questionnaire, only the prevalence of headaches, dysesthesia of limbs, and muscle weakness was higher in the exposed group compared to the control; complaints of hearing deficits which were thought to be related to noise from ball mills were also greater in the exposed group. Cranial nerve examinations and motor and sensory nerve examinations did not reveal any statistically significant abnormal neurological signs; however, paresthesia of the extremities was observed in 3 exposed workers and 1 worker in the control group. Differences ($p < 0.05$) in the jump test (muscle strength) and the tuning fork test (vibration sensation) were noted. A general trend of diminished muscle strength reflexes was found in the biceps and knees of exposed workers; however, the difference was not statistically significant. Significant differences in the nerve conduction velocities of the right median, ulnar, and posterior tibia1 nerves were not found. However, a statistically significant decrease was detected in the posterior tibia1 nerve. An increased residual latency (time from onset of stimulus to recording) of motor conduction and decreased maximal motor nerve conduction velocities were reported in the exposed workers. Residual latency was 2.21±0.34 m&c in controls versus 2.55±0.48 rn/sec in exposed subjects; maximal motor nerve conduction velocity was 48.3±2.1 m/sec in controls versus 46.6±2.3 rn/sec in exposed subjects. Normal values for the posterior tibia1 nerve have been reported as 2.1-5.6 m/sec for distal latency and 44.8-51.2 m/sec for conduction velocity (Goodgold and Eberstein 1983). The subjects in this study were age matched because these parameters vary with increasing age (conduction velocity decreases and distal latency increases).

It is not entirely clear whether the acetone co-exposure in the Sanagi et al. (1980) study contributed to the observed effects. Indirect evidence from an occupational study (Cardona et al.1996) showed that workplace acetone concentrations had a statistical correlation with the ratio of urinary *n*-hexane metabolites to *n*-hexane air concentration, although it did not correlate with measured urinary metabolites. No animal studies are available describing the effects of inhalation co-exposure to acetone and *n*-hexane, although there are several studies which report interactions between acetone and the neurotoxic metabolite of *n*-hexane, 2,5-hexanedione, by the oral route (See Section 2.4, Mechanisms of Action). Oral administration of acetone has been reported to potentiate the neurotoxicity caused by oral exposure to

2,5-hexanedione in rats (Ladefoged et al.1989, 1994). Oral exposure to acetone alone in rats at 650 mg/kg/day resulted in a statistically significant decrease in motor nerve conduction velocity after 6 weeks; co-exposure to acetone and 2,5-hexanedione resulted in greater effects than those seen with 2,5-hexanedione alone (Ladefoged et al.1989). It is possible that acetone may potentiate *n*-hexane neurotoxicity by decreasing body clearance of 2,5-hexanedione (Ladefoged and Perbellini 1986). Simultaneous subcutaneous injection of acetone and 2,5-hexanedione increased the peak concentration of 2,5-hexanedione in rat sciatic nerve compared to injection of 2,5-hexandione alone (Zhao et al.1998). Acetone also influences the action of many chemicals by its induction of the cytochrome P-450 isozyme CYP2E1 (Patten et al.1986). *n*-Hexane is metabolized by P-450 isozymes (see Section 2.4); induction by acetone may result in an increased production of the neurotoxic metabolite 2,5-hexanedione.

In a cross-sectional study of press-proofing workers in Taipei resulting from the diagnosis of 2 workers with peripheral neuropathy, a total of 59 workers from 16 press-proofing factories were examined (Wang et al.1986). Criteria for inclusion in the study were not specified. Exposure to *n*-hexane-containing solvents occurred during a cleaning process. The mean age of the workers was 25.8 years; the employment duration ranged from 2 months to 25 years, with a mean of 5.8 years. The workers were interviewed to obtain demographic information and occupational and medical histories. Neurological examinations of the workers (57 male and 2 female) were conducted. These examinations included the evaluation of motor reflexes, muscle strength, and sensory function. In addition, nerve conduction velocity studies were conducted in 54 of the 59 workers. The authors defined polyneuropathy as the presence of objective signs (the inability to walk-o*n*-heels and/or walk-o*n*-toes) plus at least one abnormally slow conduction velocity in both the upper and the lower extremities or two abnormally slow nerve conduction velocities in the lower extremities. Age-matched controls were not used in this study; an *"*abnormally low conduction velocity*"* was defined as less than 45 m/sec in the upper extremities and less than 40 m/sec in the lower extremities. Of the 59 workers examined, 15 (25%) were diagnosed with neuropathy. Among the individuals with neuropathy, the duration of employment ranged from 7 months to 5 years. There were 3 factories where workers had polyneuropathy; 8 of these workers were from a factory which had since been closed, so no air measurements were possible. Two of these workers were from a factory where the measured *n*-hexane concentration was 22 ppm, but this is likely to be an underestimate since the door was open and ventilation fans were running, while the usual condition was a closed-off work area. Six of the workers with polyneuropathy worked in a factory where the measured *n*-hexane concentration was 190 ppm. In all cases, air samples were collected for a single random 1-hour period only, thus these estimates should be viewed with caution. In addition, most of these workers were exposed for more than 8 hours a day as a result of overtime; 12 of 13 who regularly slept in the factory had polyneuropathy compared to only 3 of 46 who slept elsewhere. No significant correlation between neuropathy and length of employment or age

was found. Chemical analysis showed that *n*-hexane, ranging in content from 10 to 65%, was present in solvents in all 16 factories. Other chemicals known to cause neurotoxicity (acrylamide, tri-*ortho*-cresyl phosphate, methyl *n*-butyl ketone, mercury, carbon disulfide, manganese) were not present in significant amounts. All cases of neuropathy occurred in factories using solvents with ≥50% *n*-hexane. The sural nerve biopsies of three diagnosed individuals showed axonal degeneration and secondary changes in the myelin sheath, which are compatible with toxic neuropathy. Decreased motor nerve conduction velocities were observed in some workers exposed to less than 100 ppm *n*-hexane.

An outbreak of peripheral neuropathy in an offset printing factory in Hong Kong provides information on possible sensitive indicators of *n*-hexane neurotoxicity (Chang et al.1993). In this incident, 20 of 56 employees developed peripheral neuropathy after exposure to solvents containing *n*-hexane. Mean air measurements of *n*-hexane (taken after the outbreak began) were 63 ppm (range, 30-110 ppm) in the plant and 132 ppm (range, 80-210) for personal air samples from the machine operators. The work period was 12 hours a day, 6 days a week. Clinical signs were similar to those seen in other cases of *n*-hexane neurotoxicity; of the 36 remaining asymptomatic workers, 26 had abnormalities on nerve conduction tests (conduction velocity, distal latency, potential amplitude) and were considered to have a subclinical peripheral neuropathy. In the 10 *"*healthy*"* workers, a significantly reduced amplitude of sensory action potentials was observed compared to a control group of 20 unexposed individuals. Other parameters measured (motor action potential amplitude, motor and sensory nerve conduction velocities) were similar between the groups.

A decrease in the amplitude of the sensory nerve action potential has also been observed in a group of 20 asymptomatic workers exposed to *n*-hexane (Pastore et al.1994). The subjects of this study were selected on the basis of urinary levels of the *n*-hexane metabolite 2,5-hexanedione (See Sections 2.3 and 2.7) exceeding 5 mg/L and compared to a group of unexposed laboratory workers. Mean years worked was 8.13 (range, 1.5-23 years). Sensory and motor nerve conduction velocities and distal latencies were normal in all nerves tested. However, significant decreases were found in sensory nerve action potential amplitude in the median, sural, and ulnar nerves. Neither the level of 2,5-hexanedione in urine nor age correlated with the changes in amplitude; however, there was a significant correlation between years worked and amplitude.

Differential effects on large myelinated fibers (fast-conducting) and small myelinated fibers (slowconducting)have also been observed after exposure to *n*-hexane (Yokoyama et al.1990). Three workers, 23-27 years old, developed peripheral neuropathy after being exposed to *n*-hexane in the workplace for approximately 6 months. A single measurement of air *n*-hexane was 195 ppm; individual near-face

sampling on each worker was not done. Neurological exams were performed on the workers, and the distribution of conduction velocities in the sensory sural nerve was measured. This technique simulates individual fiber action potentials from the compound action potential from the nerve, giving a distribution of velocities. This distribution shifted to lower velocities when compared to a group of control subjects (11 males, aged 23-40; mean age, 32). Sural nerve biopsy in one patient 10 weeks after cessation of exposure showed a decrease in large myelinated fibers and a mild decrease in small myelinated fibers, confirming the conduction velocity distribution results. Teased fiber preparations showed paranodal swellings, retracted myelin, and focal demyelination.

In another cross-sectional study, possible central nervous system effects of inhalation exposure to *n*-hexane were examined in workers exposed to *n*-hexane-containing glues or *n*-hexane-containing solvents used in vegetable oil extraction (Seppalainen et al.1979). Concentrations of *n*-hexane in workers' breathing zones were not stated. Visual evoked potentials (VEPs) and averaged extraocular electroretinograms (ERGS) were recorded from 15 workers occupationally exposed to *n*-hexane for 5-21 years and from 10 healthy controls. (In this type of study, a visual stimulus is presented and the resulting electrical activity in the brain is measured with scalp electrodes.) Of the subjects, 11 were male and 4 female, the mean age was 46, and the mean exposure to *n*-hexane was 12 years (range, 5-21 years). The mean age of the control subjects was 34.9 years. Each peak of the VEP recording was designated by standard symbols and peak amplitude measured. The amplitude of the VEP components was significantly smaller among the exposed subjects compared with controls with the exception of N0, which tended to be larger. In addition, the latencies of P1 and N1 were longer among the exposed workers, while that of P2 was slightly shorter. The peak-to-peak amplitude of the ERGS was also diminished among the exposed subjects. This study is limited by the small number of subjects. Also the control group was on average 11 years younger than the exposed group.

Several studies have demonstrated sub-clinical alteration in neurological function after inhalation exposure to *n*-hexane. In a cross-sectional study using age-matched controls, workers in a shoe factory exposed to *n*-hexane were compared to a control group, which had not been exposed, from the same factory (Mutti et al.1982a, 1982c). The control group consisted of 12 males and 40 females with a mean age of 29.6 years and employment time of 10.2 years. The exposed group was composed of 24 males and 71 females with a mean age of 30.9 years and employment time of 9.1 years. The exposed group was divided into a mildand high-exposure group on the basis of time-weighted average breathing-zone air samples (a total of 108 samples were taken over a 2-year period). The participants were surveyed for neurological symptoms

and motor nerve action potential amplitude, and duration; and motor nerve conduction velocities were measured in the median, ulnar, and peroneal nerves. Mean breathing-zone *n*-hexane air concentrations were 69 ppm in the mild-exposure group and 134 ppm in the high-exposure group. Cyclohexane, methyl ethyl ketone, and ethyl acetate were also detected. Methyl ethyl ketone (which appears to potentiate *n*-hexane neurotoxicity in both humans and test animals [Altenkirch et al.1977, 19821) concentrations were 22 ppm in the mild-exposure group and 76 ppm in the high-exposure group. Symptoms more frequent during the workday in the exposed than the control group were sleepiness and dizziness. Chronic symptoms more frequent in the exposed group were weakness, paraesthesia, and hypoesthesia. Motor action potential amplitude in all three examined nerves was significantly decreased compared to controls in both exposed groups. Motor nerve conduction velocity was significantly decreased in median and peroneal nerves, but not in the ulnar nerve. In the median nerve, motor nerve conduction velocity was significantly decreased in the high-exposure group compared to the mild-exposure group.

A group of 15 women from a shoe factory (mean age 26.6 years, mean exposure time 4.5 years) was compared to a control group of 15 healthy age-matched women from other shoe factories who had not been exposed to neurotoxic chemicals (Mutti et al.1982b). Measurements included motor conduction velocity of the median, ulnar, and peroneal nerves, and distal sensory conduction velocity of the median and ulnar nerves. In addition, somatosensory-evoked potentials in the brain were measured by two monopolar needle electrodes inserted into the scalp. Potentials were evoked by stimulation of the median nerve at the wrist. The mean time-weighted average *n*-hexane air concentration was 195 ppm for 36 samples taken over a 3-year period in the factory; methyl ethyl ketone concentration was 60 ppm. The authors stated that these concentrations had been substantially reduced 3 months earlier when industrial hygiene had improved and *n*-hexane had fallen to *"*trace*"* amounts at the time the conduction measurements were taken. All nerve conduction velocities (motor and sensory) were significantly slowed in exposed workers compared to controls. Sensory nerve action potential peak latency (time from onset of sensory stimulus to peak response at point of measurement) was significantly higher in the median and ulnar nerves of the exposed workers. Ten workers had one or more nerve conduction velocities or sensory peak latencies more than two standard deviations from the mean. Nerve conduction velocities were age-dependent in the control group, but not in the exposed group. The somatosensory-evoked potential recording could be broken down into 10 peaks; significantly greater latency was observed for the first 2 peaks in the exposed group compared to the controls. There was a negative linear relationship between distal sensory conduction velocity and latency of the earliest evoked potential (P15).

In a follow-up study, a group of 90 shoe manufacturing workers (27 men and 63 women) diagnosed in the past with *n*-hexane polyneuropathy were studied again at least 1 year after cessation of *n*-hexane exposure (Valentino et al.1996). Subjects were referred by the Italian government to confirm disability status and thus may not be representative of all those originally diagnosed. Urinary 2,5-hexanedione levels were analyzed in the urine in more than half of the workers to confirm that *n*-hexane exposure had ceased. Subjects were classified on the basis of the duration of time since the diagnosis. Group A was made up of 63 subjects who were studied after a period shorter than 10 years (mean, 4.5 years), with a mean duration of exposure of 8.9 years. Group B was made up of 27 subjects who were studied after a period longer than 10 years (mean 12.9 years), with a mean duration of exposure of 9.2 years. At the time of the follow-up clinical and neurophysiological evaluation, the mean age of subjects in group A was 44.4 years (SD 14.1) and the mean age of subjects in Group B was 52.9 years (SD 5.2). A control group of 18 men and 20 women with a mean age of 38.8 years (SD 4.9) was used (Group C). Groups A and B were not significantly different with respect to symptoms related to polyneuropathy. Paresthesia and weakness in legs or arms were reported by 22% and 28% of Group A subjects, respectively, and by 28% and 35% of Group B subjects, respectively. The percentage of subjects with abnormal leg deep tendon reflexes (knee, ankle), leg cutaneous sensitivity or vibration sensation, and arm vibration sensation was statistically higher in subjects who had ceased *n*-hexane exposure for less than 10 years than in the other subjects. No differences were found between Group A and Group B for arm deep tendon reflexes and arm cutaneous sensation, or in electrophysiological parameters. Motor nerve conduction velocities and distal latencies had improved from those observed at the time of diagnosis and were similar to the control group. However, sensory nerve conduction velocities and distal latencies, while improved from those at diagnosis, were still statistically different from controls.

In studies with test animals, signs of narcosis (prostration, coma) and incoordination have been reported in male Long-Evans rats exposed for 4 hours to a C6 aliphatic hydrocarbon fraction containing only *n*-hexane and its isomers (Hine and Zuidema 1970). One rat exposed at 81,800 ppm had convulsions during and after exposure and eventually died during the sixth day. Rats that survived recovered within a few hours after removal from the chamber. Motor nerve conduction velocity was significantly decreased as early as 1 week into treatment (11% less than control litter mates) in male Sprague-Dawley rats exposed to 5,000 ppm *n*-hexane for 16 hours a day, 6 days a week for 6 weeks (De Martino et al.1987). Mean reductions ranging from 20 to 34% were seen from the second to the fourth week. Between four and six weeks of treatment, clinical signs of neurotoxicity became evident in most animals. No other studies

describing neurological effects in experimental animals after acute-duration inhalation exposure to *n*-hexane were located.

Signs of neurological toxicity similar to those seen in humans after inhalation exposure to *n*-hexane have been observed in many intermediate-duration studies with rats. In Sprague-Dawley rats (sex not specified) exposed continuously to 400-600 ppm *n*-hexane for up to 162 days, animals developed an unsteady, waddling gait after 45-69 days of exposure (Schaumburg and Spencer 1976). Further exposure resulted in a progressive, symmetrical, distal hindlimb weakness with foot-drop. Severely affected animals also developed distal weakness of the upper extremities. Pathological changes, including giant axonal swellings and fiber degeneration, were detected in the peripheral and central nervous systems of the 4 animals exposed for 49 days. The changes were most striking in tibia1 nerves supplying calf muscles and in selected areas of the cerebellum, medulla, and spinal cord. In contrast to the usual picture associated with dying-back disease, the distal regions of proximal nerve fibers supplying calf muscles degenerated before equivalent regions of longer fibers supplying the hindfeet. Electron microscopic examination showed the swollen regions contained densely packed masses of 10 nm neurofilaments. Groups of mitochondria and neurotubules were displaced to the periphery of the axon or segregated into bundles.

The sequence of events in *n*-hexane-induced neuropathy has been described in rats (Spencer and Schaumburg 1977a). The process appears to begin by increases in the number of 10 nm axonal neurofilaments and accumulation in swellings on the proximal sides of the nodes of Ranvier in distal regions of large myelinated fibers. As exposure continues, there is a retrograde spread of axonal swellings up the nerve and smaller myelinated and unmyelinated fibers became involved. The nerve terminal is unaffected until late in the process. The enlarged axons displace the paranodal myelin sheaths leaving denuded swellings in areas near the nodes of Ranvier. This process occurs before functional impairment is evident, and can be reversed on cessation of exposure as swelling diminishes and proliferation of Schwann cells occurs at these sites with subsequent remyelination of the axons. If exposure to *n*-hexane continues, axonal restoration and remyelination do not take place at some swellings, and the length of the nerve fiber between the swelling and the terminal undergoes breakdown, very similar to that seen when fibers are transected. Axon sprouting is often seen at the intact portion of a degenerated fiber even while intoxication continues. When intoxication ends, this regenerative process can reestablish motor and sensory function.

The nerve fibers most vulnerable to *n*-hexane exposure in rats were the branches of the tibia1 nerve serving the calf muscles, followed in order by the plantar nerve branches supplying the flexor digitorum brevis

muscle, and then sensory plantar nerve branches innervating the digits. As intoxication continued, axonal degeneration ascended the plantar and tibia1 nerves (Spencer and Schaumburg 1977b). Examination of control animals indicated that the most sensitive fibers were also the largest. Effects on the central nervous system have also been observed in rats exposed to *n*-hexane or its neurotoxic metabolite, 2,5-hexanedione. Axonal swelling and degeneration were observed in the anterior vermis, spinocerebellar tract in the medulla oblongata, and gracile tracts of the spinal cord (Spencer and Schaumburg 1977b).

The neurotoxicity of *"*pure*"* *n*-hexane (99%) has been compared to *"*mixed hexanes*"* (a mixture containing the *n*-hexane isomers 2-methylpentane, 3-methylpentane, cyclohexane, methyl cyclopentane, and 2,3-dimethyl butane with approximately 1% *n*-hexane) (IRDC 1981). The mixture was intended to be more representative of products used commercially. In this experiment, groups of Sprague-Dawley rats were exposed to *n*-hexane alone (500 ppm), mixed hexanes (494 ppm) or *n*-hexane plus mixed hexanes (992 ppm) daily for 6 months, 22 hours a day. No deaths occurred as a result of treatment. Body weight declines were observed in both groups exposed to *n*-hexane, but not in the mixed-hexanes group, and first became significant at 7 weeks in the *n*-hexane-alone group. Gait disturbance developed in both *n*-hexane-treated groups, the earliest incidence was at week 16 in the *n*-hexane-alone group. Exposure to *n*-hexane either alone or in combination with mixed hexanes for 6 months produced neuronal atrophy with secondary skeletal muscle atrophy. In the *n*-hexane-alone group, the incidence of *"*trace/mild*"* peripheral nerve atrophy was 14 of 16 as opposed to 0 of 8 in controls. In the group treated with both *n*-hexane and mixed hexanes, the incidence of *"*trace/mild*"* peripheral nerve atrophy was 8 of 17. Axonal degeneration was not observed in either group. No clinical or histopathological signs of neurotoxicity were noted in the group exposed to mixed hexanes alone. No histopathological lesions were observed in any group in the brain, spinal cord, or neuroganglia (lumbar, sacral, dorsal).

In a study comparing continuous to intermittent exposure, male Wistar rats exposed to 500 or 700 ppm *n*-hexane 22 hours a day for 9 weeks showed signs of narcosis (Altenkirch et al.1982). All exposed animals developed limb weakness beginning in the hindlimbs, leading to paralysis, and eventually to quadriplegia. Complete hindlimb paralysis was exhibited in the ninth week by all animals exposed to 500 ppm hexane. Animals exposed to 700 ppm *n*-hexane exhibited hindlimb paralysis in the fourth week of exposure. Light-microscopic examination of peripheral nerves revealed characteristic patterns of scattered multifocal giant axonal swellings localized primarily in the branches of the tibia1 nerve supplying the calf muscles and also in other portions of the ischiatic nerve. Breakdown of axons and myelin degradation were also visible distal to axonal swellings. Axonal swellings were also observed in the gracile tract

of the spinal cord at cervical levels. In experiments where animals were co-exposed to *n*-hexane and methyl ethyl ketone, the onset of clinical and pathological changes occurred earlier than in animals exposed to *n*-hexane alone. In contrast to these results, daily exposure at 700 ppm for up to 40 weeks, but for only 8 hours a day, did not result in clinical signs of neurotoxicity (Altenkirch et al.1982).

In an extensive toxicological study of *n*-hexane inhalation exposure in the rat (Cavender et al.1984), no significant differences from controls were noted in neurological function for either sex in Sprague-Dawley rats exposed up to 10,000 ppm *n*-hexane for 6 hours a day, 5 days a week for 13 weeks. Assessments included posture, gait, tone and symmetry of the facial muscles, and an examination of reflexes (pupillary, palpebral, extensor thrust, and cross-extensor thrust). Brain weights were significantly lower in the 10,000 and 3,000 ppm group males compared to controls, but not in the 6,500 ppm group males. Isolated, greatly enlarged axons were noted in the medulla of one male in the 10,000 ppm group; no other histopathologic lesions were present in the brain that could be attributed to *n*-hexane exposure. Neuropathological studies on peripheral nerves revealed signs of axonopathy in the tibia1 nerve in 4 of 5 males from the 10,000 ppm and 1 of 5 males from the 6,500 ppm group. The lesions were at an early stage of development and consisted of paranodal axonal swelling in teased fibers from the nerve. The most extreme examples of these swellings were noted in the smaller branches of the sciatic nerve. No evidence of segmental demyelination or axonal degeneration was observed. No consistent changes were present in any female rats or in males from the 3,000 ppm group.

The dose-duration relationship for *n*-hexane neurotoxicity was examined in male Sprague-Dawley rats in another intermittent exposure to *n*-hexane at higher concentrations (0, 500, 1,500, 2,500, or 5,000 ppm) for 9-10 hours a day, 5 days a week for 7, 14, or 30 weeks. No clinical signs of neurotoxicity (gait disturbance, hindlimb weakness) were noted in any of the treated groups (Frontali et al.1981). Histopathological examination showed no effect on tibia1 nerve branches at 500 and 1,500 ppm for any duration up to 30 weeks. Pathological alterations (giant axonal degeneration) were seen in rats exposed to 2,500 ppm for 30 weeks or to 5,000 ppm for 14 weeks. Exposure at 5,000 ppm for 7 weeks was without effect.

The effect of *n*-hexane exposure on nerve conduction velocity was investigated in male Wistar rats exposed to *n*-hexane at 0, 500, 1,200, or 3,000 ppm 12 hours a day for 16 weeks (Huang et al.1989). From week 12, a marked decrease in grip strength and *"*slowness of action*"* were observed in the 3,000 ppm and 1,200 ppm exposed rats. However, by the end of exposure, no rat displayed definite quadriplegia or hindlimb paralysis. After week 8, motor nerve conduction velocity in the 3,000 ppm and 1,200 ppm treated

rats was significantly reduced. At week 16, inspection of graphical data indicated a control velocity of approximately 32 m/sec, 27 m/sec in the 1,200 ppm group (14% decrease) and 23 m/sec in the 3,000 ppm group (28% decrease). No significant decrease was seen at 500 ppm. Paranodal swellings and demyelination as well as remyelination of the peripheral nerve in the 3,000 ppm treated rats were observed. Similar changes were observed in the 1,200 ppm exposed rats but to a lesser degree. No abnormalities were found in the 500 ppm and the control rats.

In a study which compared the toxicity of several straight-chain alkanes, groups of male Wistar rats were exposed to target concentrations of 3,000 ppm *n*-pentane, *n*-hexane, or *n*-heptane 12 hours a day for 16 weeks (Takeuchi et al.1980). An unsteady and waddling gait was observed in 1 of 7 rats in the *n*-hexane group (actual concentration 3,040 ppm) after 10 weeks of exposure. After 12 weeks, 4 of 7 had an unsteady, waddling gait and 2 had foot-drop. The 2 rats with foot-drop died 1 and 3 days before the end of the 16-week-exposure period. The 5 surviving rats all showed unsteady waddling gait at the end of 16 weeks, and 2 had foot-drop. Motor nerve and mixed nerve conduction velocities were significantly decreased in the *n*-hexane group by 4 weeks and became progressively slower during the study. Distal latencies (time from onset of stimulus to recording of response at the distal nerve end) were increased. Histological examination showed that rats in the *n*-hexane group had paranodal swelling in myelinated nerves and accumulations of neurofilaments in the axoplasm. Many denervated neuromuscular junctions were also observed. None of these signs were seen in the *n*-pentane or *n*-heptane groups.

The effect of age on the rate of development and severity of effects of *n*-hexane exposure was studied in weanling (21 days old) and young adult (80 days old) male Fischer 344 rats exposed to 0 or 1,000 ppm for 24 hours a day, 6 days a week for 11 weeks (Howd et al.1983). Forelimb and hindlimb grip strength, and amplitude and conduction time of the compound action potential in the ventral caudal nerve of the tail were monitored weekly. Brainstem auditory-evoked responses were recorded beginning after 4 weeks of exposure. In general, effects had an earlier onset and were more severe in young adults than in weanlings. Mild signs (slight ataxia) were seen in the weanlings at 8 weeks (2 of 10), all weanlings had these signs by the end of exposure (11 weeks). More serious signs (difficulty walking, flaccid hindlimbs) were not observed in the weanlings. Weanlings recovered completely over the next 4 weeks. In contrast, slight ataxia was observed in young adults by 7 weeks (2 of 10), and all young adults were affected by 8 weeks. Difficulty in walking was observed in 3 of 10 young adults at 8 weeks; by the end of exposure at 11 weeks, all young adults had flaccid paralysis of the hindlimbs or had died (2 of 10). Only slight recovery took place over the next 4 weeks. Within 2 weeks of exposure, decreases in grip strength were apparent in rats

of both ages. Hindlimb grip strength was more severely decreased than forelimb grip strength by exposure to *n*-hexane, and the young adults were more severely affected than weanlings with respect to their agematched controls during exposure and recovery. In both weanling and young adult exposed rats, motor nerve action potential latency (time from onset of stimulus to the motor nerve to recording of the action potential distally) began to increase at 7-8 weeks while the amplitude decreased. By the end of the exposure period, action potential amplitude had decreased so much that action potentials could not be detected in many of the exposed rats during the recovery period. There were no differences between weanling and young adult rats in their brainstem auditory-evoked responses when measured after 4 weeks of exposure. The latency of the first component of the brainstem auditory-evoked responses increased in *n*-hexane-exposed groups compared to controls. This effect was significant from week 7 through week 9 in weanlings and from week 7 through the last week of exposure in young adults. Complete recovery was seen in both age groups. The conduction time in the central auditory tracts between the first and fifth components of the brainstem auditory-evoked response was significantly prolonged in the *n*-hexane exposed rats (both ages) from the fourth week. This effect persisted throughout the exposure, after which there was some recovery. The most sensitive of the measures in revealing the onset of the developing neuropathy was hindlimb grip strength. The authors suggested that the relative resistance of the weanling rats to *n*-hexane neuropathy may be due to shorter, smaller-diameter axons, or to a greater rate of growth and repair in their peripheral nerves compared to those of adults.

Evoked responses in brain and peripheral nerve during and after *n*-hexane exposure were investigated in male Fischer 344 rats exposed to 0, 500, 1,000 or 1,500 ppm for 24 hours per day, 5 days per week, for 11 weeks and observed for a 6-week recovery period (Rebert and Sorenson 1983). Fore- and hindlimb grip strengths were significantly decreased at all concentrations by 4 weeks; recovery was concentration-dependent, being most rapid at 500 ppm and slowest at 1,500 ppm. Ventral caudal nerve action potential latency was unaffected at 500 ppm, but increased significantly in the 1,500 ppm group at 3 weeks. Latencies continued to increase during the recovery period in the 1,000 and 1,500 ppm groups. Somato-sensory-evoked responses (recorded in the brain) were also unaffected in the 500 ppm group, but both latency and amplitude were affected in the 1,000 and 1,500 groups. Little recovery occurred in the affected groups. In contrast, effects on the brainstem auditory-evoked response and cortical auditory-evoked response did recover after exposure. The authors suggested that the differences in recovery may be accounted for by the greater prevalence of longer, larger-diameter fibers in the somatosensory system.

Mice appear to be less susceptible to *n*-hexane neurotoxicity than rats. In $B6C3F_1$ mice exposed to 0, 500, 1,000,4,000, or 10,000 ppm *n*-hexane for 6 hours a day, 5 days a week for 13 weeks, the only neurobehavioral finding observed in exposed animals was a decrease in locomotor activity in female mice at 10,000 ppm (Dunnick et al.1989; NTP 1991). Paranodal swellings were detected in 6 of 8 mice (3 of 4 females and 3 of 4 males) from the 10,000 ppm group. Incidences in the affected mice ranged from 1 of 59 to 10 of 88 axons examined. More serious signs (segmental demyelination, distal axonal degeneration, and axonal swelling in the spinal cord) were not observed in mice from any treatment groups. No treatment-related lesions were observed in the brain in any group. In mice exposed to 1,000 ppm *n*-hexane for 22 hours a day, 5 days a week for 13 weeks, neurobehavioral test results were similar between control and exposed groups. Paranodal swellings were detected in 6 of 8 mice (3 of 4 females and 3 of 4 males) from the exposed group, but not in the control group. Incidences in the affected mice ranged from 1 of 59 to 6 of 60 axons examined. No other lesions were observed in the peripheral or central nervous systems.

New Zealand rabbits exposed to 3,000 ppm *n*-hexane for 8 hours a day, 5 days a week for 24 weeks, showed no signs of peripheral neurotoxicity (hindlimb weakness, foot dragging) (Lungarella et al.1984).

The effect of co-exposure to *n*-hexane and methyl isobutyl ketone was investigated in chickens (Abou-Donia et al.1985). (While non-mammalian species are not commonly used in toxicology studies, the chicken has proven to be a valuable model for a human neurotoxicity caused by organophosphates which is clinically similar to that caused by *n*-hexane [Abou-Donia and Lapadula 19901). During a continuous 90-day exposure, chickens exposed to 1,000 ppm *n*-hexane alone developed mild ataxia (1 on a scale of 5) while chickens exposed to 1,000 ppm methyl isobutyl ketone alone showed signs of leg weakness. Co-exposure to *n*-hexane and methyl isobutyl ketone caused severe neurologic deficits progressing to paralysis. Time to onset and severity correlated with methyl isobutyl ketone concentration. The spinal cord of one chicken exposed to 1,000 ppm *n*-hexane showed equivocal histologic changes in the lumbar region. Another hen exhibited unequivocal degeneration of the axons and myelin in the ventral columns of the thoracic spinal cord. No changes were seen in peripheral nerves. No histologic changes were seen in chickens exposed to 1,000 ppm methyl isobutyl ketone alone. Lesions in the nervous tissues of chickens exposed to mixtures of *n*-hexane and methyl isobutyl ketone were dependent on methyl isobutyl ketone concentration, period of exposure, and duration of intoxication. In another study examining the effect of *n*-hexane alone and in combination with other chemicals, a 30-day, 24-hour-a-day exposure to 1,008 ppm had no effect (Abou-Donia et al.1991). However, concurrent exposure to *n*-hexane, methyl isobutyl ketone, and the organophosphate O-ethyl O-nitrophenyl phenylphosphonothioate (EPN) greatly increased

the neurotoxicity observed compared to chickens treated simultaneously with methyl isobutyl ketone and EPN.

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

2.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to *n*-hexane. Maternal toxicity (reduced weight gain in dams) was noted in pregnant rats exposed for 20 hours a day to 5,000 ppm *n*-hexane over gestation days 6-19 (Mast et al.1987). No effects were seen at 200 or 1,000 ppm in this study. No evidence of maternal toxicity was seen in pregnant rats exposed to 93 or 409 ppm *n*-hexane for 6 hours a day during gestation days 6-15 (Litton Bionetics 1979). There were no differences in body weight, and all animals were normal in appearance throughout the study.

Reproductive tissue lesions were observed in male Wistar rats exposed to 5,000 ppm *n*-hexane for various periods up to 6 weeks (De Martino et al.1987). The earliest lesions were observed (3 of 6 animals for testis, 4 of 6 for epididymis) after a single 24-hour treatment and involved focal degeneration of primary spermatocytes from the leptotene to the middle pachytene stages and cytoplasmic swelling of spermatids at late stages of maturation in the testis; at the same time, numerous, exfoliated, injured germ cells reached the epididymis. After the 24-hour treatment was suspended, damage to the seminiferous epithelium increased for the first 7 days, while the epididymis also showed focal infiltration by inflammatory cells. Recovery to normal occurred over days 14-30. Lesions were generally more severe in groups treated 16 hours a day for 2, 4, 6, or 8 consecutive days compared to the group treated continuously for 24 hours. After 8 days, massive exfoliation of apparently normal and degenerated spermatids and spermatocytes at various stages of differentiation was observed. Numerous spermatocytes at meiotic metaphase had undergone degeneration characterized by basophilic cytoplasm. Sertoli cells showed retraction of apical cytoplasm and vacuolization. The lumen of the epididymis contained degenerated spermatids and spermatocytes; amorphous coagulated material often lined the apical cytoplasm of the epithelium. Thickening and sclerosis of the arteriolar media were observed in the interstitium. Recovery was not followed in these groups of animals. Reproductive lesions were generally more severe as the duration of treatment increased. Treatment for 2-4 weeks resulted in nuclear vacuolated and/or multinucleated round spermatids and

spermatocytes, massive exfoliation and degeneration of spermatids and prophase spermatocytes, drastic increase of necrotic spermatocytes at metaphase, and a reduction in number of spermatogonia. Sertoli cells showed nuclear swelling and vacuolization. Numerous degenerated germ cells were found in the epididymal tubule. Five to six weeks of treatment induced a gradual reduction in diameter and collapse of the seminiferous tubules and, in some cases, development of tubules containing only Sertoli cells and rare spermatogonia (aplasia). Numerous lipid droplets were visible in the cytoplasm of Sertoli cells. Testicular damage continued to progress during the follow-up period after treatment ended. Most animals reached aplasia. Numerous inflammatory cells were visible in the interstitium and inside the epithelium of the caput epididymis.

In contrast, acute-duration exposure to up to 5,000 ppm *n*-hexane for 20 hours a day for 5 consecutive days caused no changes in mouse sperm morphology when sperm were examined 5 weeks later (Mast et al. 1989a). Similar exposure also had no effect on the fertility of male mice over the following 8 weeks (Mast et al.1989b).

Intermediate-duration inhalation exposure to *n*-hexane has also caused reproductive effects in animals. Male reproductive tissues were examined in Sprague-Dawley rats after 28 or 61 days of daily exposure to 1,000 ppm *n*-hexane for 18-21 hours a day (Nylen et al.1989). Following *n*-hexane exposure, 4 of 6 rats had bilateral testicular damage and a reduced body weight 2 weeks after exposure, and 3 of 6 rats had bilateral testicular damage and reduced body weight 10 months post-exposure. The extent of body weight loss was not reported. Testes of affected rats were markedly reduced in size and weight. The muscles of the hind limbs in all rats with testicular damage were severely atrophic. Atrophic changes of seminiferous tubules throughout the testes were found 2 weeks, 10, 12, and 14 months after cessation of exposure. The testicular tissue of the macroscopically affected *n*-hexane-treated rats was severely disturbed, with total absence of a nerve growth factor-immunoreactive cell population. Total loss of the germ cell line was found in a fraction of animals up to 14 months postexposure, indicating permanent testicular damage. No impairment of androgen synthesis or androgen dependent accessory organs was observed. In a study where the responses to *n*-hexane exposure of weanling (21 days old) and young adult Fischer 344 rats (80 days old) were compared, both absolute and relative testes weights were significantly lower in *n*-hexane-exposed rats compared to controls (Howd et al.1983). In this study, exposure was 24 hours a day for 11 weeks to up to 1,500 ppm *n*-hexane. No differences were noted between the two age groups.

In contrast, no reproductive effects were observed in male Sprague-Dawley rats exposed to 500 ppm *n*-hexane 22 hours a day for 6 months (IRDC 1981). No treatment-related lesions were noted in any of the reproductive tissues examined (seminal vesicles, prostate, testis, epididymis). Similar results were reported in both sexes of weanling Fischer 344 rats exposed to up to 10,000 ppm *n*-hexane 6 hours a day, 5 days a week for 13 weeks (Cavender et al.1984). No treatment-related histopathologic lesions were present in any of the following reproductive tissues: ovaries, uterus, oviducts, vagina, cervix, seminal vesicles, prostate, testis, or epididymis.

Male mice do not appear to be sensitive to *n*-hexane-induced reproductive effects after intermediateduration exposure to *n*-hexane. The fertility of male CD-1 mice was unaffected by exposure to 99 or 396 ppm *n*-hexane for 6 hours a day, 5 days a week for 8 weeks (Litton Bionetics 1980). Fertility indices of females were similar between those mated to control and treated rats for 2 weeks following exposure.

Histopathological examination of $B6C3F_1$ mice exposed to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks or 1,000 ppm for 22 hours a day, 5 days a week for 13 weeks (Dunnick et al.1989; NTP 1991) revealed no treatment-related lesions in any of the reproductive tissues examined (seminal vesicles, prostate, testis, epididymis, ovary, uterus).

No chronic-duration exposure inhalation studies in animals were located for *n*-hexane.
The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.6 Developmental Effects

In a developmental study using 30 pregnant Sprague-Dawley rats exposed for 20 hours a day to 0, 200, 1,000, or 5,000 ppm over gestation days 6-19, *n*-hexane had no effect on the number of implantations, the mean percentage of live pups per litter, the mean percentage of resorptions per litter, or on the fetal sex ratio compared to controls (Mast et al.1987). There were no maternal deaths and no clinical signs of toxicity were noted. No significant differences were observed in intrauterine death rate, or in fetal body weight, or in the incidence of fetal malformations. A statistically significant reduction in fetal body weight relative to controls was observed for males at the 1,000 and 5,000 ppm exposure levels (7 and 15%), respectively, but there was maternal toxicity (reduced weight gain) in dams at 5,000 ppm. In pregnant

female Wistar rats exposed to 500 ppm *n*-hexane for 23 hours a day throughout gestation (21 days), reduced body weight of offspring was reported ranging from 22% at postnatal day 9 to 13% at postnatal day 25 (Stoltenburg-Didinger et al.1990). Delayed histogenesis of the cerebellar cortex in the offspring of exposed dams was also reported during the first 30 postnatal days. The number of offspring examined in this study was not reported and statistical analysis of body weights was not performed.

In a study on the effect of *n*-hexane exposure at various times during gestation, no significant adverse developmental effects were found (Bus et al.1979). Pregnant Fischer 344 rats (7 control and 7 treated) were exposed to 1,000 ppm *n*-hexane for 6 hours per day during gestation days 8-12, 12-16, or 8-16. No significant alterations in fetal resorptions, body weights, visible anomalies, or the incidence of soft tissue and skeletal anomalies were noted in any of the treatment groups. A temporary decrease in pup weight gain was seen in the offspring from dams exposed during gestation days 8-16. A low incidence of pyelectasis (enlarged renal pelvis) was noted in each of the three treatment groups; however, this was only observed when the litters contained fewer than three fetuses. A low, nonsignificant incidence of misaligned fourth sternebrae was noted in each of the treatment groups. The number of fetuses examined per group ranged from 18 to 36.

Similar results were observed in pregnant rats exposed to 93 or 409 ppm for 6 hours a day during gestation days 6-15 where larger groups of fetuses per treatment group (150-188) were examined (Litton Bionetics 1979). There were no compound related deaths and all dams were normal in appearance throughout the study. Enlarged salivary glands were noted at necropsy in one control and two animals from each treated group but were judged not to be treatment-related. Mean body weight and food consumption were not affected by treatment. Live litters, implantation sites, resorptions, mean litter size, and average fetal weight were not affected by treatment. No soft-tissue abnormalities were observed. There was no statistically significant difference in skeletal abnormalities between control and treated groups.

Concentration-related developmental effects were observed in groups of 35 pregnant Swiss mice exposed to *n*-hexane for 20 hours a day during gestation days 6-17 at 0, 200, 100, and 5,000 ppm (Mast et al.1988). Maternal body weight was significantly reduced (6%) at 5,000 ppm, but this was accompanied by a decrease in mean gravid uterine weight. There was no effect on body weight in a group of 10 non-pregnant mice co-exposed to *n*-hexane at 5,000 ppm in this experiment. The mean ratio of uterine weight to extra-gestational weight gain for all treatment groups was less than for the control groups, this difference was statistically significant for the 5,000 ppm group. The number of live fetuses per litter was significantly

reduced at 5,000 ppm, with a significant concentration dependent trend. The number of resorptions per litter was significantly increased at 200 ppm, but not at higher concentrations. Fetal weights (male and female combined) were slightly, but not significantly, reduced for all treatment groups compared to controls. However, the decrease was significantly correlated to increasing *n*-hexane concentration. Male fetal weights for *n*-hexane exposure groups were not significantly affected compared to controls, but female fetal weights were significantly reduced for the 5,000 ppm group compared to controls. There was no increased incidence of malformations or variations in any group exposed to *n*-hexane.

Exposure to much higher concentrations of *n*-hexane as a component of a commercial hexane mixture (53.45%) did not cause developmental effects in Sprague-Dawley rats (Neeper-Bradley 1989a). Groups of pregnant Sprague-Dawley rats (n=25/group) were exposed to commercial hexane vapor for 6 hours/day on gestational days 6-15. Exposure concentrations were 0, 914, 3,026, and 9,017 ppm. No significant differences between groups were observed for the number of viable implantations per litter, number of nonviable implantations per litter, sex ratio, fetal body weights (total, male and female), incidence of individual or pooled external, visceral or skeletal malformations or total malformations, the incidence of variations by category, or of total variations. Some maternal toxicity occurred during the exposure period as reflected by reduced weight gain, but total weight gain throughout pregnancy was unaffected by exposure. The authors concluded that exposure to commercial hexane vapor by inhalation during organogenesis in Sprague-Dawley rats resulted in maternal toxicity at 3,026 and 9,017 ppm, with no apparent developmental toxicity at any level.

In a parallel experiment in CD-1 mice (Neeper-Bradley 1989b) under the same exposure conditions (30 dams/group), no significant differences between groups were observed for the number of viable implantations per litter, number of nonviable implantations per litter, sex ratio, or fetal body weights (total, male and female). Slight maternal toxicity (color changes in the lungs at necropsy) was observed at 3,026 and 9,017 ppm. A significantly increased incidence of poor ossification occurred at 2 of the 84 sites examined (bilateral bone island at the first lumbar arch and all intermediate phalanges of the hindlimb unossified) in the 9,017 ppm group. There were no significant differences among groups for the incidences of variations by category (external, visceral, or skeletal) or by total variations. The authors concluded that exposure to commercial hexane vapor by inhalation during organogenesis in the CD-1 mouse resulted in slight maternal toxicity at 3,026 and 9,017 ppm and slight developmental toxicity (in the absence of malformations) at 9,017 ppm.

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

2.2.1.7 Genotoxic Effects

Structural abnormalities in sperm were observed in Sprague-Dawley rats after exposure to 5,000 ppm *n*-hexane for 16 hours a day for 2-8 days (De Martino et al.1987). Multinucleated round spermatids and spermatocytes were observed.

In contrast, sperm abnormalities were not observed in $B6C3F_1$ mice exposed to up to 5,000 ppm *n*-hexane for 5 days for 20 hours a day (Mast et al.1989a). Analysis of sperm obtained 5 weeks post-exposure showed no significant effects on morphology compared to the control group, while a significant doserelated reduction in the percentage of normal sperm was seen with the positive control agent, ethyl methanesulfonate.

In a dominant lethal assay in male CD-1 mice, *n*-hexane exposure at 99 or 396 ppm for 6 hours a day, 5 days a week for 8 weeks did not cause dominant lethal mutations (Litton Bionetics 1980). (The dominant lethal assay is designed to determine the ability of a test compound to induce genetic damage in the germ cells of treated male mice that could lead to death or developmental failure of zygotes heterozygous for such a lesion). The average number of implantations per pregnant female was not affected by *n*-hexane exposure, while it was significantly reduced in the positive control. The average resorptions or dead implants were not significantly increased by *n*-hexane exposure but were increased by the positive control. A further comparison between the proportions of females with one or more dead implants also showed no adverse effect from *n*-hexane exposure. Similar results were observed in another dominant lethal mutation study at higher concentrations in which male Swiss mice were exposed to up to 5,000 ppm *n*-hexane for 20 hours a day for 5 days (Mast et al.1989b).

There was no increase in the incidence of micronucleated normochromatic erythrocytes or polychromatic erythrocytes in the peripheral blood of male and female mice exposed to 1,000, 4,000, or 10,000 ppm *n*-hexane, 6 hours a day, 5 days a week for 13 weeks or in mice exposed to 1,000 ppm for 22 hours a day for 13 weeks (NTP 1991). Other genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

Papillary tumors, apparently derived from Clara cells, in the bronchiolar epithelium have been reported in a group of 12 New Zealand rabbits exposed to 3,000 ppm *n*-hexane for 8 hours a day, 5 days a week for 24 weeks (Lungarella et al.1984), but the incidence was not reported.

In a chronic-duration study in $B6C3F_1$ mice (50/sex/group) where exposure to commercial hexane (51.5% *n*-hexane) was for 6 hours/day, 5 days a week for 2 years, a statistically significant treatment-related increase in hepatocellular neoplasms (adenoma and carcinoma) was observed among females exposed at 9,018 ppm (Bio/Dynamics 1995b). Incidences of adenoma were: 4/50, 6/50,4/50, and 10/50 at 0, 900, 3,000, and 9,018 ppm, respectively. Incidences of carcinoma at these exposures were 3/50, 2/50, 5/50, and 6/50 and total neoplasms 7/50, 8/50, 9/50, and 16/50. In males, liver neoplasms were observed but were not treatment-related (total neoplasms 17/49, 16/50, 17/50, 13/50, respectively). There was no treatment-related increase in any other lesions of the liver, including foci of cellular alteration among males and females. In 9,018 ppm group females, liver tumor incidence was similar to control males. A significant treatment-related decrease in severity of cystic endometrial hyperplasia of the uterus was also observed among 9,018 ppm group females. The authors suggested that the decrease in severity of cystic endometrial hyperplasia may indicate a possible treatment-related alteration in the hormonal balance (e.g., a decrease in estrogenic stimulation of the uterus), resulting in the female mice showing the normal incidence of male liver neoplasms. It is unclear what components of the hexane mixture caused the neoplasms.

No increased incidence of neoplasms at any site was observed in Fischer 344 rats of either sex (50/sex/group) similarly exposed to commercial hexane in a parallel experiment (Bio/Dynamics 1995a). The Cancer Effect Level (CEL) for rabbits after intermediate-exposure is recorded in Table 2-1 and plotted in Figure 2-1.

2.2.2 Oral Exposure

2.2.2.1 Death

In an LD_{50} (lethal dose, 50% kill) test with Sprague-Dawley rats (sex not specified), an LD_{50} of 15,840 mg/kg was reported for 14-day-old rats (Kimura et al.1971). Values of 32,340 and 28,710 mg/kg

were reported for young (80-160 g) and older (300-470 g) adults, respectively. An LD_{50} for newborns could not be derived because of dose-volume limitations.

In a gavage study where rats were administered 570, 1,140, or 4,000 mg/kg/day *n*-hexane for 90-120 days, 3 rats died due to gavage error (chemical pneumonitis immediately following dosing), but no other deaths were reported (Krasavage et al.1980).

As part of a developmental study on oral exposure to *n*-hexane, pregnant 60-90-day-old outbred albino mice (CD-1) received *n*-hexane (99%) once daily at doses up to 2,200 mg/kg/day on gestation days 6-15 by cottonseed oil gavage. One of 14 mice died after receiving 10 daily doses of 2,200 mg/kg/day (Marks et al.1980). In a second study where doses were given 3 times a day, 2 of 25 died at 2,830 mg/kg/day, 3 of 34 at 7,920 mg/kg/day, and 5 of 33 at 9,900 mg/kg/day (Marks et al.1980).]

No deaths occurred in chickens receiving 100 mg/kg/day *n*-hexane for 90 consecutive days (Abou-Donia et al.1982).

The LOAEL values and LD_{50} from each reliable study for death in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

No studies were located regarding systemic effects after oral exposure to *n*-hexane in humans. The only studies regarding systemic effects in animals after oral exposure to *n*-hexane reported body weight effects in rats and chickens.

The highest NOAEL values and all LOAEL values from each reliable study for body weight in rats and chickens during acute- and intermediate-duration exposures are recorded in Table 2-2 and plotted in Figure 2-2.

Body Weight Effects. An unspecified but statistically significant decrease in body weight was observed in male Sprague-Dawley rats receiving 5 consecutive daily doses of 10,000 mg/kg/day *n*-hexane (Linder et al.1992). Body weight had returned to normal 13 days after treatment. A body weight decrease of approximately 10% was seen in COBS rats exposed by gavage to 1,140 mg/kg/day *n*-hexane for

Table 2-2. Levels of Significant Exposure to n-Hexane - Oral

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less serious (mg/kg/day)		Serious (mg/kg/day)	
	ACUTE EXPOSURE							
	Death							
1	Rat (Sprague-Dawley)	once (G)					15840 (LD_{50})	Kimura et al. 1971
2	Mouse (CD-1)	10 d Gd 6-15 1 x/d (GO)					2200 F (1/14 died)	*Marks et al. 1980*
3	Mouse (CD-1)	10 d Gd 6-15 3 x/d (GO)					2830 F (2/25 died)	*Marks et al. 1980*
	Systemic							
4	Chicken (Leghorn)	once or twice Day 0, 21 1 x/d (G)	Bd Wt	2000 F				Abou-Donia et al. 1982
	Neurological							
5	Chicken (Leghorn)	once or twice Day 0, 21 1 x/d		1000 F	2000 F	(mild leg weakness)		Abou-Donia et al. 1982
	Reproductive							
6	Rat (Sprague-Dawley)	1 d 2x (G)			20000M	(transient decreased sperm head count per gram testis)		*Linder et al. 1992*

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

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	Developmental						
7	Mouse (CD-1)	10 d Gd 6-15 1 x/d (GO)		2200			Marks et al. 1980
8	Mouse (CD-1)	10 d Gd 6-15 3 x/d (GO)		2830	7920 (reduced fetal weight)		Marks et al. 1980
	INTERMEDIATE EXPOSURE						
	Systemic						
9	Rat (COBS)	90-120 d 5 d/wk 1 x/d (G)	Bd Wt	570 M	1140M (10% decrease)		Krasavage et al. 1980
10	Rat (Wistar)	8 wk 7 d/wk 1 x/d (GO)	Bd Wt	1251 M			Ono et al. 1981
11	Chicken (Leghorn)	90 d 1 x/d (G)	Bd Wt		100 F (19% decrease)		Abou-Donia et al. 1982
	Neurological						
12	Rat (COBS)	90-120 d 5 d/wk 1 x/d (G)		1140 M		4000 M (severe hindlimb paralysis, axonal swelling, myelin retraction)	Krasavage et al. 1980

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

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
13	Rat (Wistar)	8 wk 7 d/wk 1 x/d (GO)			1251 M (decreased motor and mixed nerve conduction velocity)		Ono et al. 1981
14	Chicken (Leghorn)	90 d 1 x/d (G)			100 F (leg weakness)		Abou-Donia et al. 1982
	Reproductive						
15	Rat (COBS)	90-120 d 5 d/wk 1 x/d (G)		1140 M		4000 M (atrophy of testicular germinal epithelium)	Krasavage et al. 1980

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

Bd Wt = body weight; d = day(s); F = female; Gd = gestational day; (G) = gavage; (GO) = gavage in oil; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; wk = week(s); x = times

Figure 2-2. Levels of Significant Exposure to *n*-Hexane - Oral

Acute (≤14 days)

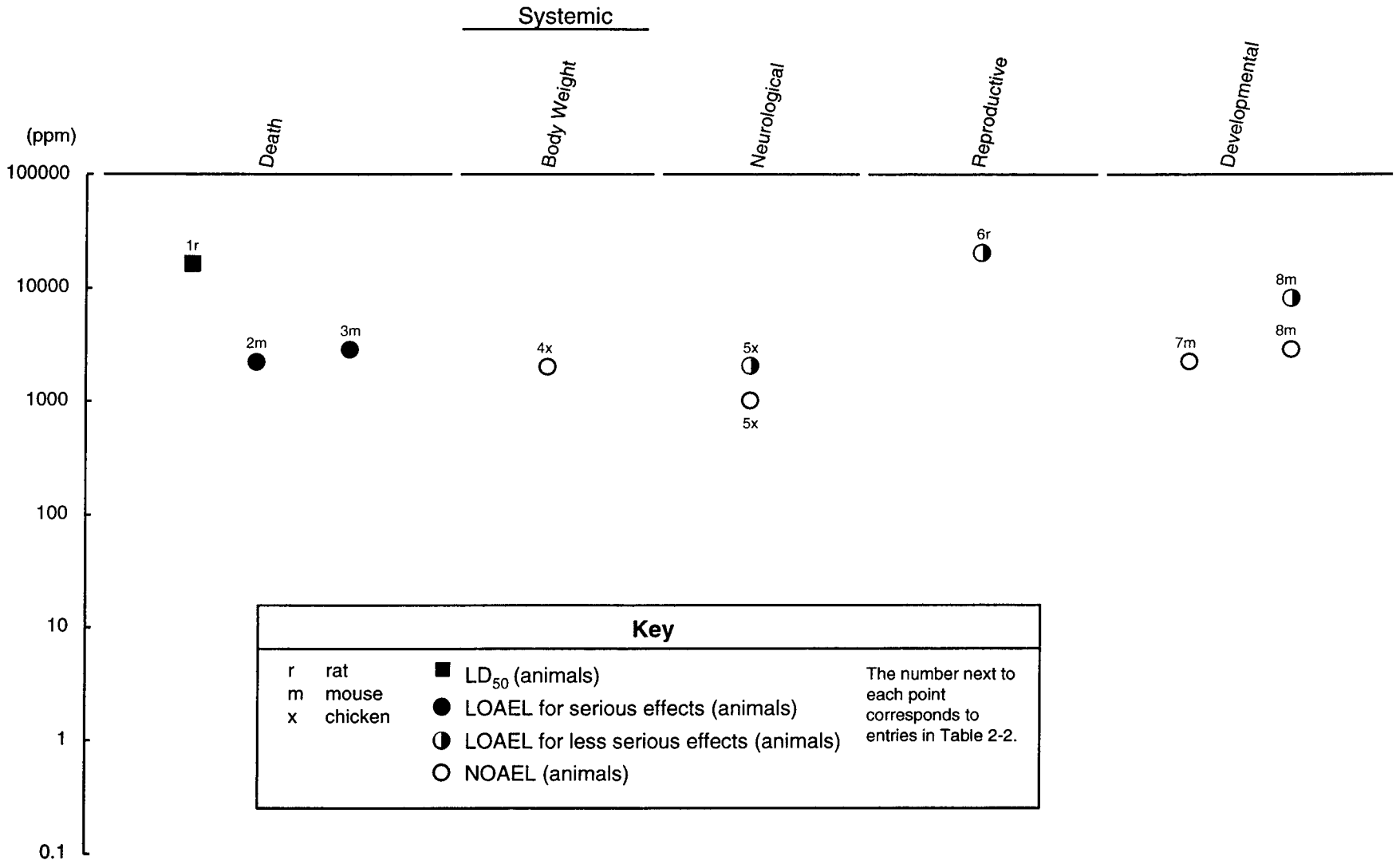
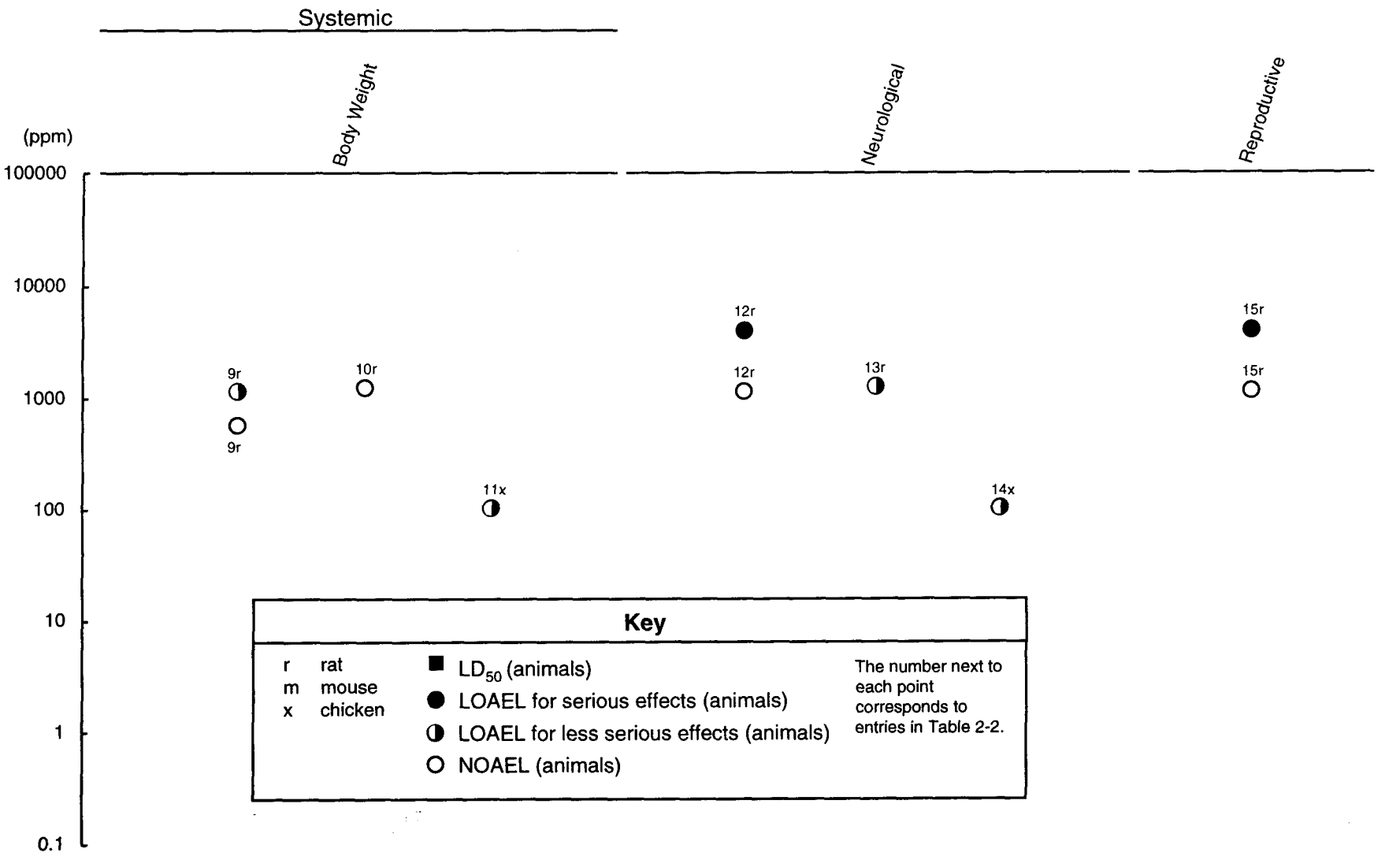


Figure 2-2. Levels of Significant Exposure to *n*-Hexane - Oral (cont.)

Intermediate (15-364 days)



90 days (Krasavage et al.1980). Decreased body weight was observed at 570 mg/kg/day in this study (approximately 14%), along with a decrease in food consumption. Daily doses of 1,251 mg/kg/day of *n*-hexane had no effect on body weight in male Wistar rats over an 8-week period (Ono et al.1981). Two doses of up to 2,000 mg/kg/day given to chickens 21 days apart had no effect on body weight (Abou-Donia et al.1982). However, 100 mg/kg/day for 90 days caused a 19% decrease in body weight in this species.

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological/lymphoreticular effects after oral exposure to *n*-hexane in humans or animals.

2.2.2.4 Neurological Effects

Decreases in motor nerve conduction velocities were noted in rats after oral exposure to *n*-hexane (Ono et al.1981). In Wistar rats receiving an average daily dose of 1,251 mg/kg/day, the motor nerve conduction velocity in the *n*-hexane-treated group was significantly less than control after 4 and 8 weeks of administration. After 8 weeks of treatment with similar doses of the *n*-hexane isomer methylcyclopentane, the motor nerve conduction velocity was also decreased. No changes were observed with 2-methylpentane or 3-methylpentane. The distal mixed nerve conduction velocity in the *n*-hexane group was less than control after 4 weeks of administration; however, a significant difference with the other solvents was not observed. The proximal mixed nerve conduction velocity in the *n*-hexane group was less than control after 6 weeks of administration and after 8 weeks with 2-methylpentane and methylcyclopentane. No changes in behavior or clinical signs of peripheral neurotoxicity were noted. The authors speculated that the conduction velocity decreases observed with 2-methylpentane and methylcyclopentane may be the result of metabolism to the neurotoxic metabolite of *n*-hexane, 2,5-hexanedione (see Section 2.4).

In a study comparing oral administration of *n*-hexane with its metabolites, groups of 5 male COBS rats were exposed by gavage 5 days a week for 90-120 days with *n*-hexane, 2-hexanol, 2-hexanone, 2,5-hexanedione, 2,5-hexanediol, or 5-hydroxy-2-hexanone (Krasavage et al.1980). Practical grade hexane (40% *n*-hexane, 24% each 3-methylpentane and dimethylbutane, 9% cyclopentane, 2.5% cyclohexane, and 1.2% 2-methylpentane) was also tested. Test compounds were given as equimolar doses of 6.6 mmollkg. *n*-Hexane was also given at 13.2 mmol/kg and 46.2 mmol/kg (up to 120 days); doses were 570, 1,140, or 4,000 mg/kg/day *n*-hexane. Practical grade hexane was also tested at 4,000 mg/kg/day.

Clinical signs of neurotoxicity (severe hindlimb weakness or paralysis) did not occur over the 90-day dosing period at 570 or 1,140 mg/kg/day *n*-hexane. However, at 4,000 mg/kg/day, these signs were present after 101 days in 3 of 4 rats. Clinical signs of neurotoxicity were seen with all the other chemicals tested except practical grade hexane. Time to onset was as little as 16.8 days with 2,5-hexanedione. No histologic evidence of tibia1 nerve alterations (multifocal axonal swellings, adaxonal myelin infolding, paranodal myelin retraction) was observed with *n*-hexane at 570 or 1,140 mg/kg/day, but was observed at 4,000 mg/kg/day. One rat of 5 treated with 4,000 mg/kg/day practical grade hexane showed histological lesions; none showed clinical signs of neurotoxicity.

Leghorn chickens (12 months old, 1.7 kg) were given oral doses of *n*-hexane and observed for clinical signs of neurotoxicity (Abou-Donia et al.1982). (While non-mammalian species are not commonly used in toxicology studies, the chicken has proven to be a valuable model for a human neurotoxicity caused by organophosphates which is clinically similar to that caused by *n*-hexane [Abou-Donia and Lapadula 19901.) One chicken was given a single dose of 1,000 mg/kg, and 3 chickens were given 2,000 mg/kg, one dose at day 0 of the experiment and another at day 21. The 2,000 mg/kg dose caused mild leg weakness followed by full recovery after 2-4 days; no effect was seen at 1,000 mg/kg. Oral administration of the *n*-hexane metabolites 2,5-hexanedione, 2-hexanone, or 2,5-hexanediol caused ataxia leading to paralysis in this study. In a 90-day oral exposure at 100 mg/kg/day, leg weakness was observed, but no serious signs of neurotoxicity (Abou-Donia et al.1982).

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

2.2.2.5 Reproductive Effects

n-Hexane was 1 of 14 compounds used to evaluate multiple end points of spermatotoxicity in shortduration tests (Linder et al.1992). Three days after treatment ended, body weight and prostate weight were significantly decreased (amount unspecified) in rats receiving 10,000 mg/kg/day *n*-hexane for 5 consecutive days. No changes were noted 13 days post-treatment, or in rats receiving 20,000 mg/kg on a single day. No changes in male reproductive tissue histology were noted in 20,000 mg/kg *n*-hexane-treated rats, nor were there any changes in sperm motility or morphology after either 1-day exposure or 5-consecutive-day exposure. A significant decrease was noted in total sperm head counts per gram of testis 2 days after 1-day treatment (20,000 mg/kg), but not at 14 days. Total sperm head count, and specific counts in the caput and cauda region of the epididymis were unchanged after either 1-day exposure at 20,000 mg/kg (2 and 14 days after exposure) or 5-consecutive-day exposure at 10,000 mg/kg/day (3 and 13 days after the last exposure).

Changes in all these parameters were noted in rats receiving known reproductive toxicants such as benomyl and boric acid. In the group receiving a single 2,000 mg/kg dose of the *n*-hexane metabolite 2,5-hexanedione, no histopathological changes were detected 2 days after treatment; but at 14 days, testicular debris was observed in the proximal caput, sloughed epididymal cells were observed in the cauda lumen as was retention in the lumen of Step 19 (mature) spermatids in Stage IX-XII tubules. In a study where rats received 0, 570, 1,140, or 4,000 mg/kg/day *n*-hexane for 90-120 days (Krasavage et al.1980), varying stages of atrophy of testicular germinal epithelium were noted at 4,000 mg/kg/day but not at the lower doses.

In a developmental study in mice orally exposed to *n*-hexane, signs of maternal toxicity (1 of 14 died, reduced body weight gain) were observed at 2,200 mg/kg/day (Marks et al.1980). Maternal toxicity was also observed at higher doses: 2 of 25 dams at 2,830 mg/kg/day, 3 of 34 at 7,920 mg/kg/day, and 5 of 33 at 9,900 mg/kg/day died.

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

2.2.2.6 Developmental Effects

In a developmental study, pregnant 60-90-day-old outbred albino mice (CD-1) received n-hexane (99%) once daily by cottonseed oil gavage at doses up to 2,200 mg/kg/day on gestation days 6-15 (Marks et. Al.1980). No statistically significant differences were observed between treated and control litter for total number of implants, numbr of resorptions, fetal deaths, sex ratio, number of stunted fetuses, live fetuses per dam, or fetal weight. No differences in the incidence in the incidence of malformed fetuses (visceral or skeletal) were observed . Signs of maternal toxicity (1 of 14 died reduced body weight gain) were observed. Signs of maternal toxicity (1 of 14 died, reduced body weight gain) were observed at 2,200 mg/kg/day. In a second study at higher does, pregnant mice received n-hexane (99%) 3 times daily by cottonseed oil gavage at doses up to 9,900 mg/kg/day on gestation days 6-15 (Marks et al.1980). The higher hexane doses were toxic: 2 of 25 dams at 2,830 mg/kg/day, 3 of 34 at 7,920 mg/kg/day, and 5 of 33 at 9,900 mg/kg/day died. At the 7,920 and 9,900 mg/kg/day doses, the average fetal weight was significantly reduced, but the incidence of malformations in treated- and vehicle-control groups did not differ significantly. *n*-Hexane was not teratogenic even at doses toxic to the dam.

The highest NOAEL values and the LOAEL value from the two reliable studies for developmental effects in mice for an acute duration are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects after oral exposure to *n*-hexane in human or animals.

Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

No studies were located regarding cancer effects after oral exposure to *n*-hexane in humans or animals.

2.2.3 Dermal Exposure

2.2.3.1 Death

Topical application of a single 2 mL dose of undiluted *n*-hexane had no effect on survival or body weight in exposed guinea pigs observed for 35 days after exposure (Wahlberg and Boman 1979). Deaths and/or effects on body weight were seen with similar doses of other common industrial solvents tested in this study (carbon tetrachloride, dimethylformamide, ethylene glycol monobutylether, 1,1,1-trichloroethane, and trichlorethylene).

2.2.3.2 Systemic Effects.

The only reports regarding systemic effects in humans after dermal exposure to *n*-hexane are two studies describing dermal effects and ocular effects in volunteers. Ocular effects in rabbits and body weight effects in guinea pigs have also been reported.

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

Dermal Effects. *n*-Hexane was 1 of 11 solvents tested for dermal toxicity in a male volunteer (Wahlberg 1984). Analytical grade *n*-hexane (1.5 mL; test area, 3.1 cm^2) within a glass ring was applied to the volar forearm of the volunteer and left on the skin for 5 minutes. Blood flow values (expressed as a relative, dimensionless value) after dermal application of 1.5 mL of neat *n*-hexane appeared to increase

Table 2-3. Levels of Significant Exposure to n-Hexane - Dermal

Species (Strain)	Exposure/ Duration/ Frequency	System	NOAEL	LOAEL			Reference
				Less serious		Serious	
ACUTE EXPOSURE							
Systemic							
Human	once	Dermal	0.1 mL M	1.5 mL M	(increased blood flow, slight and transient erythema, stinging and/or burning sensation)		Wahlberg 1984
Gn Pig (NS)	once	Bd Wt	2.0 mL				Wahlberg and Boman 1979
Rabbit (New Zealand)	1 wk 5 d/wk 8 hr/d	Ocular		3000 M ppm	(lacrimation, hyperemia of conjunctiva)		Lungarella et al. 1984
INTERMEDIATE EXPOSURE							
Systemic							
Rabbit (New Zealand)	24 wk 5 d/wk 8 hr/d	Ocular		3000 M ppm	(lacrimation, hyperemia of conjunctiva)		Lungarella et al. 1984

Bd Wt = body weight; d = day(s); Gn Pig = guinea pig; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; NS = not specified; wk = week(s)

about 6-fold at 15 minutes post-application. Blood flow had returned to control approximately 60 minutes after application. Unlike the other solvents tested (DMSO, trichlorethylene, toluene, 1,1,2-trichloroethane), blood flow increased for about 10 minutes after the end of the application. With the other solvents, blood flow either fell or remained unchanged after application. A slight transient erythema was observed after 10-20 minutes exposure to *n*-hexane and a stinging and/or burning sensation reported by the volunteer. Application of 0.1mL neat *n*-hexane did not cause clinical signs or affect blood flow.

Ocular Effects. Hexane was one of 16 industrial solvents (hydrocarbons, alcohols, ketones, esters, and ethyl ether) tested for irritation potential on an average of 10 volunteers of mixed sexes for 3-5 minutes in an inhalation chamber (Nelson et al.1943). The purity and the isomer composition of the hexane were not specified. Hexane was the only one of the 16 solvents which caused no irritation to the eyes, nose, or throat at the highest concentration tested (500 ppm).

Clinical signs of ocular irritation (lacrimation, hyperemia of the conjunctiva) were observed throughout a 24-week study in rabbits exposed to 3,000 ppm *n*-hexane (Lungarella et al.1984). These effects were the result of direct contact of *n*-hexane vapor with the eye.

Body Weight Effects. Topical application of a single 2 mL dose of undiluted *n*-hexane had no effect on body weight in exposed guinea pigs followed for 35 days after exposure (Wahlberg and Boman 1979). Effects on body weight were seen with similar doses of other common industrial solvents tested in this study (carbon tetrachloride, dimethylformamide, ethylene glycol monobutylether, 1,1,1-trichloroethane, and trichlorethylene).

No studies were located regarding the following health effects in humans or animals after dermal *exposure* to *n*-hexane:

2.2.3.3 Immunological and Lymphoreticular Effects

2.2.3.4 Neurological Effects

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Other genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

No studies were located regarding cancer effects after dermal exposure to *n*-hexane in humans or animals.

2.3 TOXICOKINETICS

Exposure to *n*-hexane takes place predominantly by the inhalation route. This is due to rapid volatilization of liquid *n*-hexane (vapor pressure of 150 mm Hg at 25 °C). *n*-Hexane also has a very low solubility in water (9.5 mg/L at 25 °C), and significant oral exposure through food or drinking water has not been reported. Little toxicokinetic information exists for oral or dermal exposure to *n*-hexane in humans or animals. Inhaled *n*-hexane is readily absorbed in the lungs. In humans, the lung clearance (amount present which is absorbed systemically) of *n*-hexane is on the order of 20-30%. Absorption takes place by passive diffusion through epithelial cell membranes. Absorption by the oral and dermal route has not been well characterized. Inhaled *n*-hexane distributes throughout the body; based on blood-tissue partition coefficients, preferential distribution would be in the order: body fat>>liver, brain, muscle>kidney, heart, lung>blood. *n*-Hexane is metabolized by mixed function oxidases in the liver to a number of metabolites, including the neurotoxicant 2,5-hexanedione. Approximately 10-20% of absorbed *n*-hexane is excreted unchanged in exhaled air, and 2,5-hexanedione is the major metabolite recovered in urine. *n*-Hexane metabolites in the urine and *n*-hexane in exhaled air do not account for total intake, suggesting that some of the metabolites of *n*-hexane enter intermediary metabolism.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

The absorption of inhaled *n*-hexane has been investigated in six healthy male volunteers (Veulemans et al. 1982). Three different trials were performed on each volunteer: 4-hour exposure at 102 ppm *n*-hexane; 4-hour exposure at 204 ppm, and exposure during exercise on a stationary bicycle ergometer at 102 ppm. Each trial was done at least two weeks apart. Lung clearance (from alveolar air to blood) and retention were calculated from *n*-hexane concentrations in inhaled and expired air. After exposure, *n*-hexane in exhaled air was measured for up to 4 hours to determine respiratory elimination. Retention of *n*-hexane (calculated from lung clearance and respiratory minute volume) was approximately 20-25% of the *n*-hexane in the inhaled air. This resulted in calculated absorption rates of 0.84 mg/min at 102 ppm and

1.59 mg/min at 204 ppm. Retention was about the same at both concentrations, indicating that metabolism had not been saturated. Physical exercise at 102 ppm caused a significant increase in lung clearance and at peak loads (60 watts) was more than twice the value at rest, resulting in an increase in absorption rate. Pulmonary excretion of *n*-hexane after exposure ended appeared to be biphasic, with a fast drop in the first 30 minutes and a slower drop for the remainder of the 4-hour observation period. Blood concentrations of *n*-hexane reached steady-state within 100 minutes and were stable until the end of exposure. After exposure, there was a rapid fall to about 50% of the level at the end of exposure in the first 10 minutes, followed by a slower exponential time course with a half-life of 1.5-2 hours.

In a workplace study, lung uptake and excretion of *n*-hexane were studied in 10 workers (sex not specified, 18-30 years old) in a shoe factory (Mutti et al.1984). Simultaneous samples of inhaled and alveolar air (last 100 mL of the tidal volume) were collected 6 times during an 8-hour workday. Breathing-zone air was collected with personal samplers. Median time-weighted average *n*-hexane concentrations were 243 mg/m^3 (69 ppm). 2-Methylpentane, 3-methylpentane, cyclohexane, and *n*-heptane were also present in the air. Alveolar excretion was monitored during a 6-hour post-exposure period. Uptake was calculated from lung ventilation, the retention coefficient (1-[Calv/Cinh]), and environmental concentrations. The total amount of exhaled *n*-hexane was calculated by integration of the decay curve for the concentration of exhaled *n*-hexane. About 25% of inhaled *n*-hexane was retained in the alveoli. Absorption into the blood in relation to total respiratory uptake was about 17%, taking into account the retention coefficient and alveolar ventilation.

2.3.1.2 Oral Exposure

No studies were located that specifically addressed absorption of *n*-hexane after oral exposure in humans or animals. Absorption of *n*-hexane by the oral route in humans can be inferred from the appearance of *n*-hexane in exhaled air and 2,5-hexanedione in urine of volunteers receiving 0.24 or 0.81 mg/kg via a gastric feeding tube (Baelum et al.1998). Absorption of toxicologically significant amounts by this route can be inferred since neurological effects occurred in rats receiving *n*-hexane by gavage (Krasavage et al. 1980; Ono et al.1981). Significant serum levels of the *n*-hexane metabolite 2,5-hexanedione were also measured in rats receiving *n*-hexane by gavage (Krasavage et al.1980).

2.3.1.3 Dermal Exposure

The permeability of human skin to *n*-hexane has been determined *in vitro* in flow-through diffusion cells (Loden 1986). Pieces of full-thickness human skin were exposed to [^{3}H]*n*-hexane in human serum, and the appearance of label in the *trans* compartment measured for 0.5 or 12 hours. The skin was then sectioned with a microtome into 0.25 mm slices and the quantity of label in the skin measured. The rate of resorption (uptake of substance by the receptor fluid beneath the skin [i.e., the amount that passes through the skin]) was calculated. The rate of resorption for *n*-hexane through human skin was calculated to be 0.83 (μg * cm^2/hr). The permeability of *n*-hexane through human skin was much lower (approximately 100-fold) than for other chemicals tested in this study. For example, rates of resorption (in μg * cm^2/hr) were 99 for benzene and 118 for ethylene glycol.

No information is available on whether absorption of *n*-hexane by children differs from that of adults. Since absorption by all routes appears to be by passive diffusion, it is probable that absorption in children is similar to that of adults.

2.3.2 Distribution

Partition coefficients of a series of aliphatic hydrocarbons, including *n*-hexane, have been determined in human tissues (Perbellini et al.1985). The following partition coefficients for *n*-hexane (olive oil/air, blood/air, tissue/air) were determined: olive oil, 146; blood, 0.80; liver, 5.2; kidney, 3; brain, 5; fat, 104; muscle, 5; heart, 2.8; and lung, 1. Saline/air partition was not reported separately for *n*-hexane, but was very low for the range reported for the entire group of compounds (0.1-0.4).

Partition coefficients have also been reported for human milk from a group of 8 volunteers (Fisher et al. 1997). The milk/air coefficient was 4.66 and the blood/air coefficient was 2.13. A milk/blood partition coefficient of 2.10 was calculated from this data.

Partition coefficients for *n*-hexane in male Fischer 344 rats have been reported (blood/air, tissue/air): blood, 2.29; liver, 5.2; muscle, 2.9; and fat, 159 (Gargas et al.1989).

No information is available on whether distribution of *n*-hexane in children differs from that of adults. Transfer across the placenta has been demonstrated in rats for *n*-hexane and two resulting metabolites,

2-hexanone and 2,5-hexanedione (Bus et al.1979); no preferential distribution to the fetus was observed for either *n*-hexane or the metabolites. *n*-Hexane has not been measured in breast milk, although a milk/blood partition coefficient of 2.10 (Fisher et al.1997) ,indicates there would be preferential distribution to this compartment. Due to its relatively rapid metabolism, storage of *n*-hexane in body fat does not appear to occur at air concentrations to which humans are exposed; thus, there is unlikely to be mobilization of stored *n*-hexane upon pregnancy or lactation. The toxic metabolite of *n*-hexane, 2,5-hexanedione, can probably be distributed to germ cells as demonstrated by the testicular effects observed in male rats after drinking water exposure to 2,5-hexanedione. High air concentrations of *n*-hexane can also produce these effects in rats, presumably via 2,5-hexanedione (see Section 2.1.1.5).

2.3.2.1 Inhalation Exposure

In a study where blood *n*-hexane concentrations were determined in volunteers during exposure to 102 or 204 ppm for 4 hours, blood *n*-hexane reached steady-state within 100 minutes and was stable until the end of exposure. Concentrations of *n*-hexane in blood at 100 minutes were 0.202 mg/L at 102 ppm and 0.357 mg/L at 204 ppm. After exposure, there was a rapid fall to about 50% of the level at the end of exposure in the first 10 minutes and a slower exponential time course with a half-life of 1.5-2 hours (Veulemans et al.1982).

In Fischer 344 rats exposed to up to 10,000 ppm *n*-hexane for 6 hours, *n*-hexane achieved an apparent steady state in all tissues within 2 hours (Baker and Rickert 1981). Steady-state concentrations were proportional to dose only in blood and liver. In brain, sciatic nerve, kidney, lung, and testes, exposure to 1,000 ppm resulted in a disproportionately greater concentration than exposure at 500 ppm. Peak blood concentrations of *n*-hexane were 1, 2, 8, and 21 μg/mL, and peak sciatic nerve concentrations were 12, 48, 130, and 430 μg/g at 500, 1,000, 3,000, and 10,000 ppm, respectively. In a study that addressed possible accumulation of *n*-hexane in tissues, *n*-hexane was not detected in any tissue besides sciatic nerve after 2 hours post-exposure in either 1 or 5 day exposures to *n*-hexane at 1,000 ppm for 6 hours a day (Bus et al. 1981). Initial concentrations after a single exposure were: sciatic nerve, 46 μg/g; kidney, 5.8 μg/g; liver, 1.2 μg/g; brain, 3 μg/g, and blood, 0.5 μg/mL. Initial concentrations after 5 daily exposures were similar. No significant difference was found between *n*-hexane blood concentrations in mothers (0.45±0.11 μg/mL) and total fetal concentration (0.61±0.14 μg/g wet weight) after exposure during pregnancy (Bus et al. 1979), indicating that transfer across the placenta takes place. Initial concentrations of *n*-hexane in maternal tissues were: liver, 0.85 μg/g; kidney, 6.33 μg/g; and brain, 0.04 μg/g.

2.3.2.2 Oral Exposure

No studies were located regarding distribution of *n*-hexane after oral exposure in humans or animals.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution of *n*-hexane after dermal exposure in humans or animals.

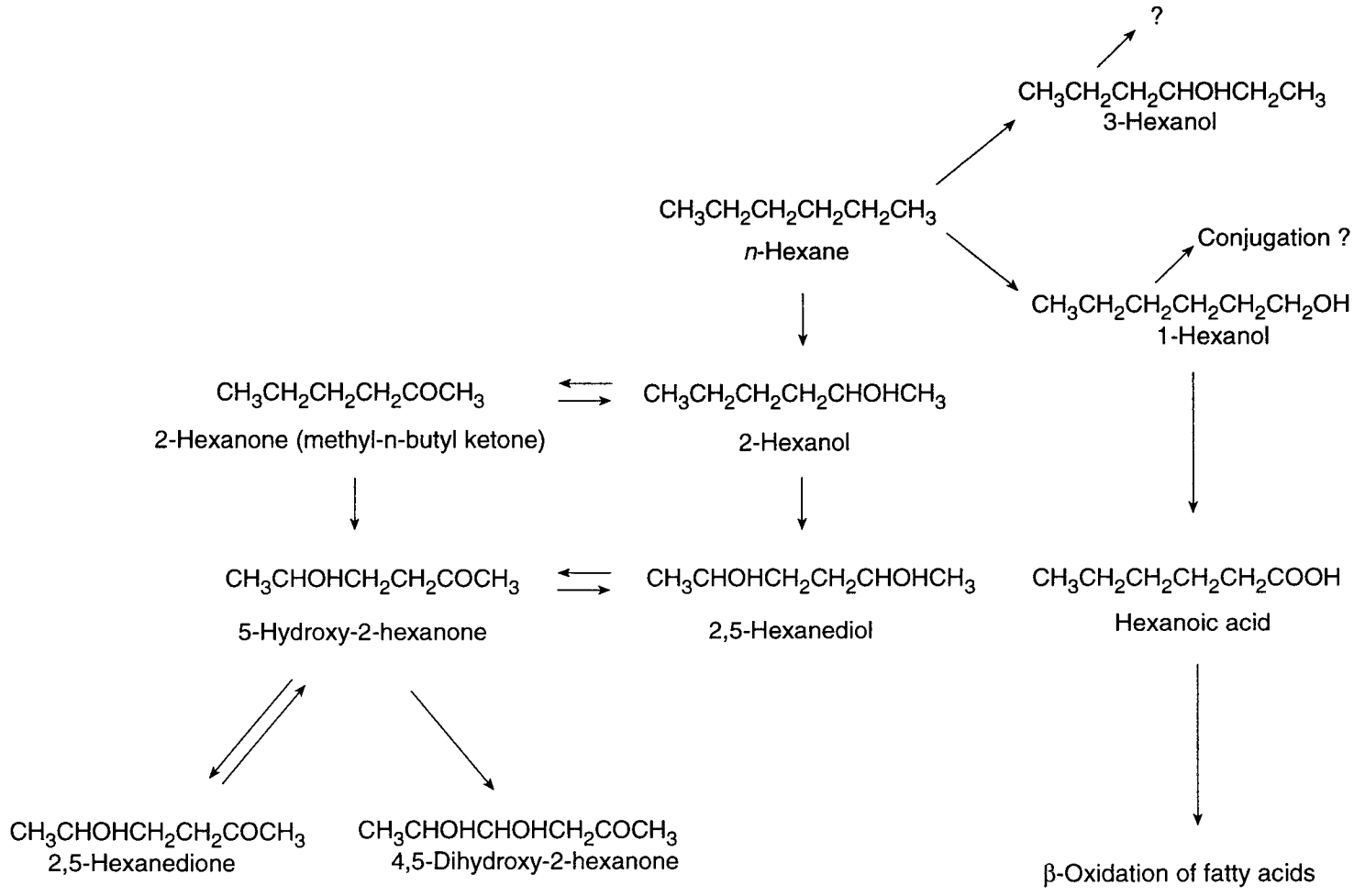
2.3.3 Metabolism

The metabolism of *n*-hexane takes place in the liver. The initial reaction is oxidation by cytochrome P-450 isozymes to hexanols, predominantly 2-hexanol. Further reactions convert 2-hexanol to 2-hexanone, 2,5-hexanediol, 5-hydroxy-2-hexanone, 4,5-dihydroxy-2-hexanone and the neurotoxicant 2,5-hexanedione. Hydroxylation at the 1- and 3- positions can be considered detoxification pathways; hydroxylation at the 2- position is a bioactivation pathway. A diagram of the proposed pathway for mammalian metabolism of *n*-hexane is presented in Figure 2-3.

Approximately 10-20% of *n*-hexane absorbed by inhalation is excreted unchanged in exhaled air; the remainder is metabolized. Metabolism takes place via mixed-function oxidase reactions in the liver. In a study in which metabolites were measured in workers exposed to *n*-hexane (Perbellini et al.1981), mean concentrations of *n*-hexane metabolites in urine were: 2,5-hexanedione, 5.4 mg/L; 2,5-dimethylfuran, 3.7 mg/L; gamma-valerolactone, 3.3 mg/L; and 2-hexanol, 0.19 mg/L. (2,5-Dimethylfuran and gamma-valerolactone are believed to be artifacts of sample preparation and analysis rather than true metabolites of *n*-hexane [Perbellini et al.19811). The first reaction that takes place is hydroxylation of *n*-hexane at the 2 position to form 2-hexanol. Further reactions result in 2,5-hexanedione, presumably through transient intermediates, including 2-hexanone, 2,5-hexanediol, and 5-hydroxy-2-hexanone. Correlations between concentrations of *n*-hexane in air and urinary metabolites were best for total *n*-hexane metabolites (r=0.7858), followed by 2-hexanol (r=0.6851) and 2,5-hexanedione (r=0.6725).

The time-course of the metabolism of inhaled *n*-hexane in a group of 19 volunteers has been estimated by determining serum 2,5-hexanedione during and after a 15.5~minute exposure to 60 ppm *n*-hexane (van Engelen et al.1997). The time to reach the peak concentration varied from 16.2 to 19.8 minutes after the

Figure 2-3. Proposed Scheme for the Metabolism of *n*-Hexane



Source: Modified from NTP 1991

start of exposure (i.e., 1-4 minutes following the cessation of exposure). The rate at which 2,5-hexanedione appeared in the blood ranged from 1.89 to 4.48 μM/hour.

Further studies in humans indicate that a large proportion of the 2,5-hexanedione detected in urine after *n*-hexane exposure is the result of an artifact resulting from treatment with acid to hydrolyze urinary conjugates (Fedtke and Bolt 1987). When urine from a male volunteer exposed to 217 ppm *n*-hexane for 4 hours was hydrolyzed enzymatically with β-glucuronidase, excretion of 4,5-dihydroxy-2-hexanone was approximately 4 times higher than that of 2,5-hexanedione. When the urine was hydrolyzed with acid, 4,5-dihydroxy-2-hexanone was not detected, but the amount of 2,5-hexanedione in the urine increased, indicating conversion of 4,5-dihydroxy-2-hexanone to 2,5-hexanedione by the acid treatment. The fraction of 2,5-hexanedione determined after complete acid hydrolysis minus the 2,5-hexanedione originally present was equal to the 4,5-dihydroxy-2-hexanone. Only *"*minor*"* amounts of 2-hexanol were reported.

2,5-Hexanedione has also been detected after acid hydrolysis of the urine of individuals unexposed to *n*-hexane (Fedtke and Bolt 1986a; Perbellini et al.1993). 2,5-Hexanedione was not detected without acid hydrolysis, indicating that it is formed as a result of conversion of 4,5-dihydroxy-2-hexanone. It is possible that small amounts of *n*-hexane are produced in the body by lipid peroxidation, as has been demonstrated for *n*-pentane (Filser et al.1983). Urinary excretion of 2,5-hexanedione ranged from 0.3 to 1.2 mg in 24 hours for unexposed individuals; workers exposed to approximately 50 ppm *n*-hexane excreted 3-4 mg/24 hours (Perbellini et al.1993).

When male Wistar rats were exposed to *n*-hexane at concentrations up to 3,074 ppm for 8 hours, analysis of urine showed that 2-hexanol was the major metabolite, accounting for about 60-70s of the total metabolites collected over the 48-hour collecting period (Fedtke and Bolt 1987). This is in contrast to humans, in which the major urinary metabolite is 2,5-hexanedione (Perbellini et al.1981). The amounts of metabolites excreted were linearly dependent on the exposure concentration, up to an exposure of about 300 ppm. 2-Hexanol and 2-hexanone were detected in the first sample (obtained during the 8-hour exposure); excretion of 2,5-hexanedione was delayed and was not detected until 8-16 hours after exposure began. The amount of 2,5-hexanedione detected depended on sample treatment; total excreted amounts over 48 hours were approximately 350 μg/kg 2,5-hexanedione without acid treatment and 3,000 μg/kg with total acid hydrolysis, indicating conversion of 4,5-dihydroxy-2-hexanone with acid treatment.

The metabolism of *n*-hexane in rat lung and liver microsomes has been investigated (Toftgard et al.1986). In liver microsomes, the formation of 1-, 2-, and 3-hexanol from *n*-hexane was best described kinetically by a 2-enzyme system, while for lung microsomes, single-enzyme kinetics were indicated for each metabolite. For conversion to 1-hexanol, apparent K, values were 0.4 and 300 μM, and V_{max} values were 0.09 and 1.2 nmol/mg protein/min, respectively. For conversion to 2-hexanol, apparent K_m values were 6 and I, 100 μM, and V_{max} values were 1 and 4.6 nmol/mg protein/mm respectively. Insufficient information was available to estimate the high-affinity activity for 3-hexanol, the low-affinity activity had an apparent K_m of 290 pM and a V_{max} of 0.5 nmol/mg protein/mm. In the lung, K_m values were 9, 50, and 65 μM for 1-, 2-, and 3-hexanol, respectively; V_{max} values were 2.2, 1.3, and 0.2 nmol/mg protein/minute, respectively. Prior induction of P-450 enzymes with phenobarbital markedly increased the rate of formation of 2-hexanol in liver microsomes (1.8 nmol/mg/min control versus 15 nmol/mg/min with phenobarbital) and that of 3-hexanol(0.4 versus 2.8), while the rate of formation of 1-hexanol fell slightly (2 versus 0.7). Antibodies to P-450 isozymes PB-B (CYP2B1-inducible by phenobarbital) and BNF-B (CYP1A1-inducible by fi-naphthoflavone) were used as inhibitors to investigate the specificity of the reactions. In control liver microsomes, anti-PB-B showed no inhibitory effects while anti-BNF-B inhibited the formation of 2- and 3-hexanol by 25 and 40%, respectively, but had no effect on the formation of 1-hexanol. In microsomes from rats induced with phenobarbital, the anti-PB-B antibody reduced the formation of hexanols back to control levels. Purified P-450 isozymes were also tested for their ability to hydroxylate *n*-hexane. The highest activity (nmol metabolite/nmol P-450/min) was found with P-450-PB-B (CYP2B1), followed by P-450-PB-D (CYP2B2) and P-450-BNF-B (CYPl A1). Formation of 2,5-hexanediol from 2-hexanol was catalyzed by a P-450 isozyme different from cytochrome P-450-PB-B (as judged by antibody inhibition) that was present in liver but not in lung microsomes. This process was unaffected by prior induction of cytochrome P-450. Furthermore, alcohol dehydrogenase activity with hexanols or 2,5-hexanediol as the substrate was found exclusively in liver cytosol. These results suggest that inhaled *n*-hexane must be transported to the liver either intact or in the form of 2-hexanol before the neurotoxic metabolite 2,5-hexanedione can be formed. The large increase in hydroxylation of *n*-hexane upon induction (which would favor the production of 2,5-hexanedione via 2-hexanol) is a likely explanation for the potentiating effects of methyl ethyl ketone on *n*-hexane neurotoxicity in humans and rats (Altenkirch et al.1977, 1982) and of methyl isobutyl ketone in chickens (Abou-Donia et al.1985).

The tissue and P-450 isoform specificity of *n*-hexane hydroxylation to hexanols has been investigated in rat tissues and cell lines expressing specific P-450 isoforms (Crosbie et al.1997). The highest activity per mg protein for the production of 2-hexanol (which can be further metabolized to 2,5-hexanedione) was in liver,

followed by lung (about 25% of liver activity), muscle and brain. Activity in muscle and brain was very low compared to the liver. Membrane preparations from cells expressing human CYP2E1 had the same *n*-hexane 2-hydroxylation activity as control cells. In contrast, cells expressing human CYP2B6 had approximately 100 times the 2-hydroxylation activity of the CYP2E1 or control cells. Specific induction of the cytochrome P-450 isozyme CYP2E1 has been reported in male Wistar rats after intraperitoneal injection of *n*-hexane (Nakajima et al.1991). No effects on total liver microsomal protein or total cytochrome P-450 content were observed. trans-1,2-Dichloromethylene (DCE), a specific inhibitor of CYP2E1 in rats, has also been shown to affect the metabolism of *n*-hexane (Mathews et al.1997). Rats exhale a large number of endogenous volatile organic compounds, including *n*-hexane. When CYP2E1 was inhibited by intraperitoneal injection of DCE, levels of exhaled *n*-hexane increased approximately 25-fold within 4 hours and returned to pre-dose levels at approximately 24 hours, closely paralleling the inhibition and resynthesis time-course for CYP2E1. No increase in lipid peroxidation was observed, indicating that the increase in exhaled *n*-hexane was the result of inhibition of metabolism. It is probable that many P-450 isoforms are capable of hydroxylating *n*-hexane (both *in vivo* and under laboratory conditions); it is not possible at this time to specify which forms are definitely involved in *n*-hexane metabolism *in vivo.*

The effect of concentration on the fate of [^{14}C]*n*-hexane after inhalation exposure has been studied in Fischer 344 rats (Bus et al.1982). The disposition of radioactivity was dose-dependent, with 12, 24, 38, and 62% of the acquired body burden excreted as *n*-hexane by the lung with increasing exposure concentration (500, 1,000, 3,000, and 10,000 ppm, respectively). In contrast, 38, 31, 27, and 18% of the body burden of radioactivity was recovered as expired CO_2 and 35,40, 31, and 18% was recovered in the urine with increasing *n*-hexane concentration (expired air and urine were collected for 72 hours after exposure). Radioactivity remaining in the tissues and carcass 72 hours after exposure represented 6.1, 8.8, 7.4, and 5.4% of the body burden for the respective exposures. The dose-dependent elimination of radioactivity was apparently due in part to an inhibition of *n*-hexane metabolism reflected by a decrease in total $^{14}CO_2$ and urinary ^{14}C excretion after 10,000 ppm exposure compared to the 3,000 ppm exposure. Half-lives for excretion were estimated from the data. Urinary half-time for excretion of radioactivity was 12.7 hours at 500 ppm.

In a study in which pregnant rats received a single 6-hour exposure to 1,000 ppm *n*-hexane on gestation day 12 or 20 (Bus et al.1979), *n*-hexane was rapidly and extensively metabolized to methyl-*n*-butyl ketone (2-hexanone) and 2,5-hexanedione. 2-Hexanone and 2,5-hexanedione (the only metabolites measured) were

detected in the maternal liver, kidney, brain, and blood. Fetal concentrations of *n*-hexane and its metabolites (entire fetus) were similar to those in maternal blood at all times after exposure. Results were similar on both gestation days 12 and 20. *n*-Hexane and 2-hexanone were rapidly eliminated from maternal tissues and the fetus, with minimal or nondetectable concentrations reached 8 hours after exposure. In contrast, tissue concentrations of 2,5-hexanedione increased between 0 and 4 hours after exposure and thereafter exhibited a significantly slower elimination rate compared to *n*-hexane and 2-hexanone. 2,5-Hexanedione was not detected in the blood or tissues 24 hours after exposure. The half-life of 2,5-hexanedione in maternal blood was significantly greater than *n*-hexane and 2-hexanone (3.9 hours versus 1.24 and 0.99 hours, respectively).

Concentration time curves for *n*-hexane in a closed exposure system indicated that metabolism in rats was proportional to air concentration up to about 300 ppm (Filser et al.1987). Metabolism was non-linear above 300 ppm and appeared to be saturated at concentrations ≥3,000 ppm.

Little information is available on the metabolism of *n*-hexane after oral exposure, although it appears to be qualitatively similar to that after inhalation exposure. Peak serum concentrations of the *n*-hexane metabolite 2,5-hexanedione of 24, 44, and 53 μg/rnL were observed in rats after a single gavage exposure to 570, 1,140, and 4,000 mg/kg *n*-hexane, respectively (Krasavage et al.1980). Serum 2,5-hexanedione concentrations rose slowly to a peak at 12-16 hours and returned to baseline by 24 hours.

Exposure to other chemicals can influence the metabolism of *n*-hexane. The effect of oral pretreatment with methyl ethyl ketone (MEK) on the metabolism of inhaled *n*-hexane was investigated in male Fischer 344 rats (Robertson et al.1989). Groups of 2-4 rats were given MEK (1.87 mL/kg, approxi-Mately 1,500 mg/kg) by gavage for 4 days prior to a single 6-hour inhalation exposure to *n*-hexane (1,000 ppm). Animals were sacrificed at 0, 1, 2, 4, 6, 8, and 18 hours after exposure ended, and samples of blood, liver, testis, and sciatic nerve were obtained and analyzed for *n*-hexane, MEK, and their metabolites. Significant increases in the levels of the neurotoxic metabolite 2,5-hexanedione and 2,5-dimethylfuran (derived from 2,5-hexanedione) were found in blood and sciatic nerve of rats pretreated with MEK. Levels of 2,5-hexanedione in blood were approximately 10-fold higher than control immediately after *n*-hexane exposure in rats and fell rapidly to approximately 2-fold after 6 hours. In sciatic nerve, increases in 2,5-hexanedione were approximately 6-fold at 2 hours and 3-fold at 4 hours. Similar patterns were found with 2,5-dimethylfuran. 2,5-Hexanedione was not detected in the testis of non-pretreated rats; levels were measurable but very low in pretreated rats (0.3-0.6 μg/g compared to 10-12 μg/g in blood or sciatic

nerve). 2,5-Dimethylfuran was significantly higher in testis (approximately 4-fold) in pretreated animals for the first 6 hours after exposure. 2,5-Hexanedione was not detected in the liver in any group, although 2,5-dimethylfuran was. 2-Hexanol and 2-hexanone were detected in some samples but in small amounts. 5-Hydroxy-2-hexanone amounts were similar to those found with 2,5-hexanedione but were more variable.

In this study, P-450-related enzyme activities (benzphetamine *N*-demethylase, 7-ethoxycoumarin O-deethylase) were also measured in liver homogenates (prepared 24 hours after the last treatment) from rats treated orally with MEK for 1-7 days and compared to the activity obtained with phenobarbital treatment (80 mg/kg intraperitoneally for 3 days) (Robertson et al.1989). Total cytochrome P-450 was also measured. No consistent change was noted in benzphetamine *N*-demethylase activity as the result of MEK treatment, while 7-ethoxycoumarin O-deethylase was over 3 times higher than controls and comparable to phenobarbital induction. Total P-450 levels were increased to approximately 150-200% of controls with MEK and to 570% of control by phenobarbital. The authors concluded that the potentiating effects of MEK on the neurotoxicity of *n*-hexane appear to arise, at least in part, from the activating effects of MEK on selected microsomal enzymes responsible for *n*-hexane activation.

Rats pretreated with xylene or phenobarbital and then exposed to *n*-hexane by inhalation exhibited a markedly increased peak serum concentration of 2,5-hexanedione (Toftgard et al.1983). Peak serum concentrations were approximately 4 μg/mL in control rats, 11 μg/mL in xylene-induced rats, and 13 μg/mL in phenobarbital-induced rats. Peaks were reached in 1-2 hours. The half-life for elimination from serum was approximately one hour for both pretreated and untreated rats. The high serum 2,5-hexanedione concentrations were correlated with an induction of liver microsomal P-450 content (0.56 nmol/mg protein in control rats, 1.03 nmol/mg in xylene-induced rats, and 1.7 nmol/mg protein in phenobarbital-induced rats, respectively).

No information is available as to whether metabolism of *n*-hexane in children differs from that of adults. No studies were located comparing metabolism in young and adult animals. The toxicity of *n*-hexane results from biotransformations yielding the active metabolite, 2,5-hexanedione. The initial step is an oxidation to 2-hexanol catalyzed by a cytochrome P-450 enzyme. Some P-450 enzymes are developmentally regulated (Leeder and Kearns 1997). As the above discussion indicates, it is not completely clear which P-450 enzymes are involved in *n*-hexane metabolism.

2.3.4 Elimination and Excretion

No information is available as to whether excretion of *n*-hexane and its metabolites in children differs from that of adults. No studies were located comparing excretion in young and adult animals.

2.3.4.1 Inhalation Exposure

In a study of workers exposed to *n*-hexane (Mutti et al.1984), the post-exposure alveolar excretion of *n*-hexane was about 10% of the total uptake, and was in 2 phases: a fast phase with a half-life of 11 minutes and a slow phase with a half-life of 99 minutes. Urinary metabolite concentrations were lowest at the beginning of the shift, highest at the end of the shift, and still elevated the next morning. Half-life for urinary excretion of total *n*-hexane metabolites (2,5dimethylfuran, 2-hexanol, 2,5-hexanedione, and gamma-valerolactone were detected) in another group of exposed workers was 13-14 hours (Perbellini et al.1981, 1986). There was a strong correlation (r=0.967) between time-weighted average *n*-hexane air concentration and end of shift 2,5-hexanedione in the urine; end-of-shift samples gave the best estimate of overall exposure. The authors calculated that about 3 mg of 2,5-hexanedione/g creatinine would correspond to about 50 ppm of *n*-hexane in the air (mean daily exposure).

2.3.4.2 Oral Exposure

Excretion of *n*-hexane after oral exposure in humans can be inferred from the appearance of *n*-hexane in exhaled air and 2,5-hexanedione in urine of volunteers receiving 0.24 or 0.81 mg/kg via a gastric feeding tube (Baelum et al.1998). No studies were located regarding excretion of *n*-hexane or *n*-hexane metabolites following oral exposure to *n*-hexane in animals.

2.3.4.3 Dermal Exposure

No studies were located regarding excretion of *n*-hexane or *n*-hexane metabolites following dermal exposure to *n*-hexane.

2.3.5 Physiologically based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

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

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

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

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

model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically-sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 2-4 shows a conceptualized representation of a PBPK model.

A PBPK model for *n*-hexane is discussed below. The overall results and model are discussed in this section in terms of use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

2.351 Summary of PBPK Models.

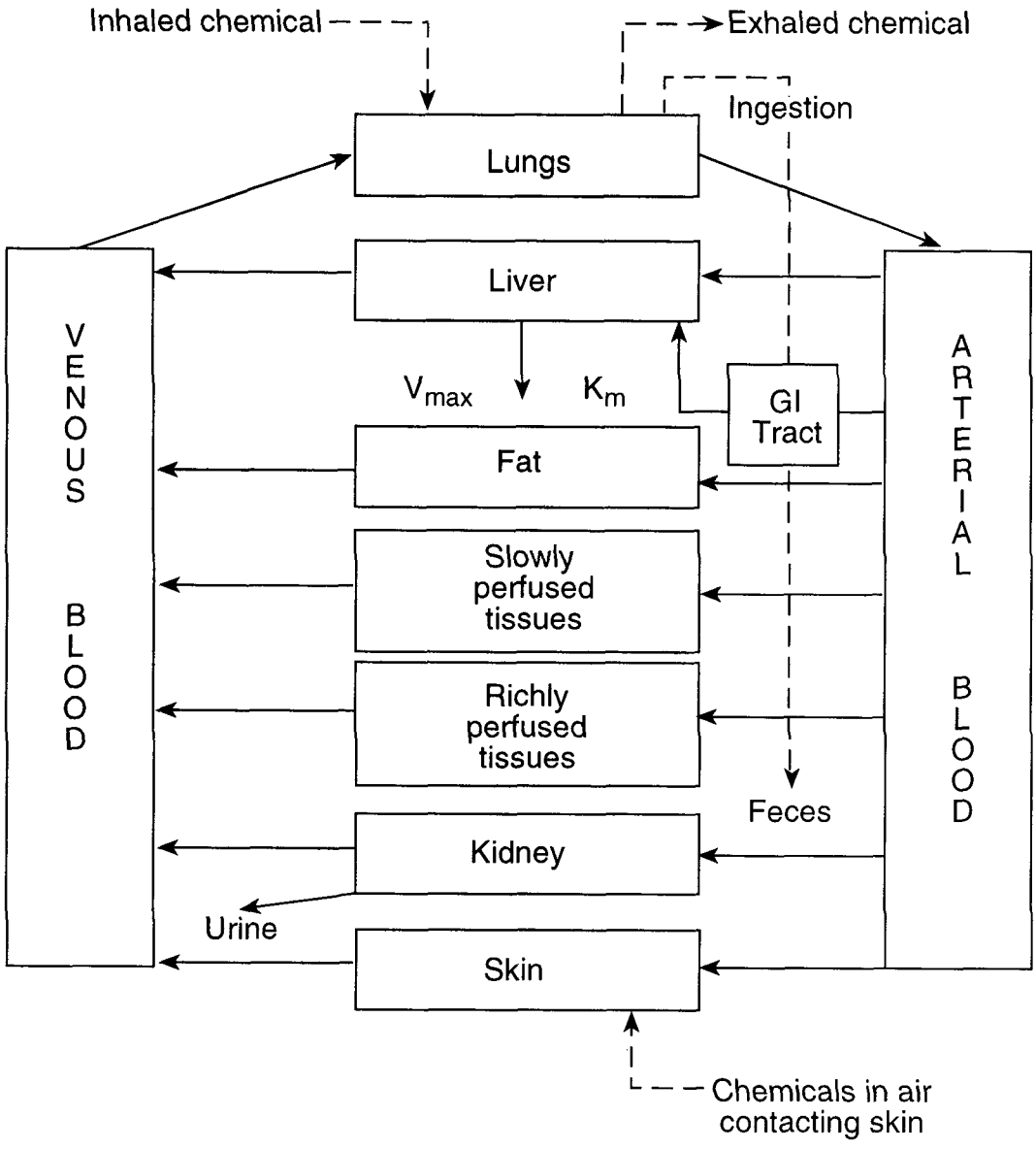
The Perbellini model for *n*-hexane (Perbellini et al.1986, 1990a) is an 8-compartment model which simulates the absorption, distribution, biotransformation, and excretion of *n*-hexane during inhalation exposure. The excretion kinetics of the neurotoxic metabolite of *n*-hexane, 2,5-hexanedione, are also simulated.

A model describing transfer of *n*-hexane via lactation from a mother to a nursing infant is also available (Fisher et al.1997). Human milk/blood partition coefficients for 19 volatile organic chemicals including *n*-hexane were experimentally determined using samples from volunteers. These parameters were used to estimate the amount of *n*-hexane an infant would ingest from milk if the mother was occupationally exposed to *n*-hexane at the Threshold Limit Value (TLV) throughout a workday.

2.3.5.2 n-Hexane PBPK Model Comparison.

The Perbellini PBPK model for *n*-hexane is the only validated model for this chemical identified in the literature. The Fisher model was intended for risk assessment to predict which of 19 volatile organic chemicals may be present in milk at a high enough level after workplace exposure to raise health concerns for a nursing infant.

Figure 2-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

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

2.3.5.3 Discussion of Models.

The Perbellini Model.

Risk assessment. The Perbellini model successfully described alveolar air and venous blood concentrations of *n*-hexane following inhalation exposure in humans. Simulations indicated that exposure to 50 ppm for an 8-hour-workday, 5-day workweek would result in a gradual accumulation of *n*-hexane in body fat which is not completely cleared during the weekend.

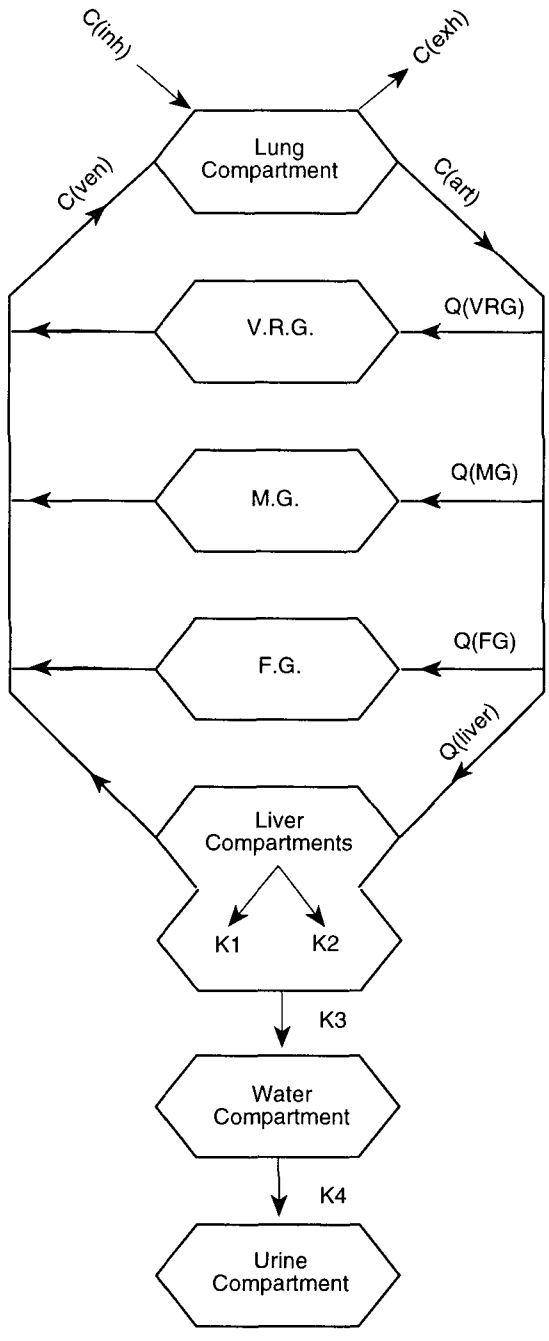
Description of the model. The Perbellini model has eight compartments (See Figure 2-5):

1. A lung compartment where *n*-hexane arrives via inhalation and reaches a concentration that also depends on the *n*-hexane concentration in the alveolar air, in the arterial blood, and in the venous blood;
2. a vessel rich tissue compartment (VRG) including the heart, brain, and kidney;
3. a muscle tissue compartment (MG);
4. a fat tissue compartment (FG);
5. liver tissue compartment #1 describing the input and output of *n*-hexane (catabolism of *n*-hexane);
6. liver tissue compartment #2 describing the synthesis and transfer of 2,5-hexanedione;
7. water compartment concerned with the distribution of 2,5-hexanedione; and
8. urine compartment where 2,5-hexanedione is excreted.

It is assumed that the *n*-hexane concentration instantaneously reaches a balance between alveolar air and arterial blood and the *n*-hexane concentration in venous blood is in constant and dynamic balance with the corresponding tissue concentrations.

Physiological parameters for volumes and blood flow of the compartments are listed in Table 2-4. Physiologic constants (compartment volume, blood flows, etc) were taken from published values. Values for the solubility of *n*-hexane in blood and tissues (partition coefficients) are taken from human tissue (Perbellini et al. 1985). Rate constants (Table 2-4, Figure 2-5) were estimated from animal and human data and are all assumed to be first-order.

Figure 2-5. Perbellini PBPK Model



Souce: Perbellini et al. 1986

C(ven) = concentration of *n*-hexane in venous blood; C(inh) = concentration of *n*-hexane in inhaled air; C(exh) = concentration of *n*-hexane in exhaled air; C(ar) = *n*-hexane in arterial blood; FG = fat tissue group; MG = muscle tissue group; VRG = vessel-rich tissue group

Table 2-4. Parameters Used in the Perbellini PBPK Model for *n*-Hexane

Parameters	Human
	Compartment volumes (L)
Liver	1.7
Lung	1.0
Fat	11.5
Vessel-rich compartment	7.1
Muscle compartment	36.3
	Flows (L/min)
Alveolar ventilation	6
Cardiac output	6.3
	Percentage of cardiac output
Liver	25.0
Fat	4.4
Vessel-rich compartment	55
Muscle compartment	16
	Partition coefficients
Blood/air	0.8
Liver/blood	6.5
Fat/blood	130
Vessel-rich compartment/blood	5
Muscle/blood	6.2
	Metabolic constants (min^{-1})
k_1 (catabolism of *n*-hexane to metabolites)	0.3
k_2 (synthesis of 2,5-hexanedione from *n*-hexane)	0.012
k_3 (transfer of 2,5-hexanedione to body water)	0.009
k_4 (transfer of 2,5-hexanedione from body water to urine)	0.0009

Source: Perbellini et al. 1986, 1990b

Validation of the model. The Perbellini model was validated using a data set for venous blood *n*-hexane values in volunteers exposed for 4 hours (Veulemans et al.1982). The range in the study was 334-368 μg/L during exposure to 204 ppm; the model predicted a value within this range. After 4 hours exposure to 102 ppm, the predicted value for venous blood *n*-hexane concentration was about 10% below that actually observed in humans. The authors also compared their own data from previous studies on the correlation between venous blood *n*-hexane concentrations and workplace concentrations. From the correlation curve, exposure at 102 ppm would predict a venous blood concentration of 176 μg/L; the model predicted 182 μg/L. The urinary excretion rate of 2,5-hexanedione predicted by the model was also compared to a data set from 13 workers followed for 24 hours from the beginning of a workday. The model successfully predicted the rate of 2,5-hexanedione urinary excretion.

Target tissues. Target tissues were not specifically addressed in this model. The target tissue for *n*-hexane is peripheral nerve (via the neurotoxic metabolite 2,5-hexanedione).

Species extrapolation. No species extrapolation was attempted in this model. Results from *in vitro* studies in rat liver homogenates were used to estimate kinetic parameters for the catabolism of *n*-hexane and synthesis of 2,5-hexanedione.

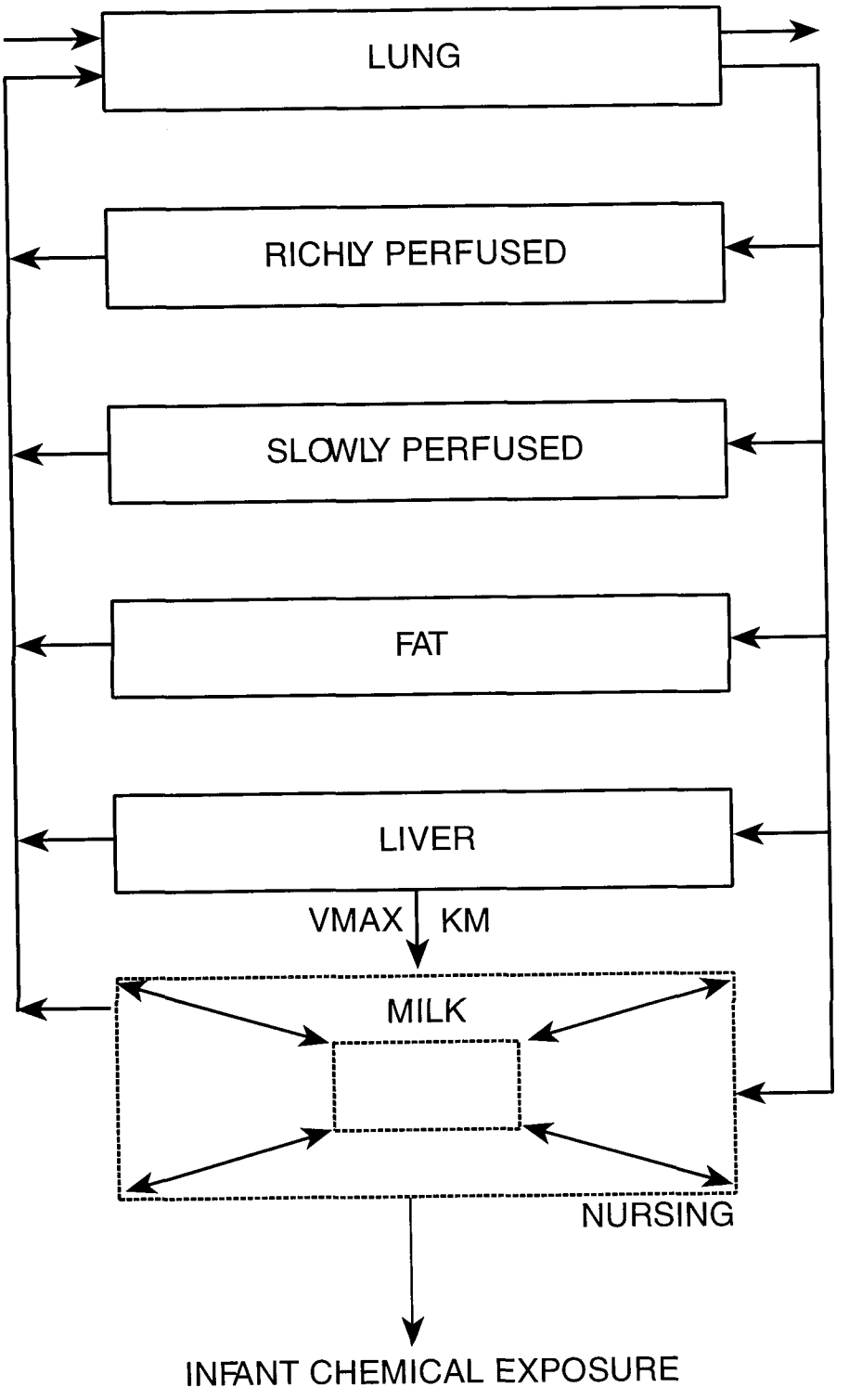
Interroute extrapolation. No interroute extrapolation was attempted in this model.

The Fisher Model.

The Fisher model simulates the transfer of *n*-hexane from a mother to a nursing infant during and after occupational exposure via inhalation (Fisher et al.1997). The model is shown in Figure 2-6, and its parameters are presented in Table 2-5. Blood/air and milk/air partition coefficients were determined with samples from volunteers. Simulations were run over a 24-hour period at the *n*-hexane TLV value of 50 ppm assuming a 9-hour working period containing 2 half-hour and one 1-hour break periods and 8 nursing periods over 24 hours. Total *n*-hexane ingested in milk was compared to the EPA Health Advisory Intake for chronic ingestion of contaminated water by 10 kg children. The model predicted ingestion at the rate of 0.052 mg/day compared to the EPA advisory intake of 4.0 mg/day. Simulated inhalation exposures at the TLV level for some chemicals (e.g., bromochloroethane) resulted in infant exposures via milk that exceeded the EPA advisory intakes for contaminated water.

Risk assessment. The purpose of this study was risk assessment. The transfer to milk of 19 chemicals was simulated to predict those that may result in exposures to infants higher than the EPA

Figure 2-6. Fisher PBPK Model



Source: Fisher et al. 1997

Milk compartment volume changes as a result of nursing.

Table 2-5. Parameters Used in the Fisher PBPK Model for *n*-Hexane

Parameters	Human
	% BW[c] (BW = 60 kg)
Liver	1.5
Richly perfused	10
Slowly perfused	54
Fat	25
Milk	10–125 mL
	Flows (L/min)
Alveolar ventilation	$24 \times BW^{0.74}$
Cardiac output	$15 \times BW^{0.74}$
	Percentage of cardiac output
Liver	29
Fat	410
Richly perfused	35
Slowly perfused	19
Milk	7
	Partition coefficients
Blood/air	2.13
Liver/blood	2.45
Fat/blood	74.74
Richly perfused blood	2.45
Slowly perfused blood	1.36
Milk/air	4.60
Milk/blood	2.10
	Metabolic constants
V_{max}	6.0 mg/kg/hr
K_m	0.3 mg/L
	Milk compartment
Nurse[a]	20/hr
Prod[b]	0.06 L/hr

[a] Nurse is a first-order term to describe the rate of ingestion of breast milk by a nursing infant.
[b] Prod is a zero-order term to describe the rate of breast milk production at 1.3–3 months of lactation.
[c] Body weight
Source: Fisher et al. 1997

Drinking Water Health Advisory Values for ingestion of contaminated water. The predicted amount of *n*-hexane ingested was below this value.

Description of the model. The model contains seven compartments: alveolar space, lung blood, fat, slowly perfused tissues, rapidly perfused tissues, liver, and milk (see Figure 2-6). In this model, standard literature values were used for most parameters while blood/air and milk/air partition coefficients were determined experimentally (see Table 2-5). The milk/blood partition coefficient was derived from the blood/air and milk/air coefficients. Maximum rates of hepatic metabolism (V_{max}) and the K_m value for *n*-hexane were taken from a study in rats. The milk compartment changes in volume in response to nursing, milk letdown from nursing is assumed to be a first-order process and milk production a zero-order process. Minimum and maximum volumes for the milk compartment were 0.010 and 0.125 L, respectively. Simulations were run assuming a *n*-hexane air level of 50 ppm using a chemical exposure and nursing schedule over a 24-hour period (4 maternal exposures to *n*-hexane and 8 nursing periods). The amount of *n*-hexane ingested by the infant was predicted.

Validation of the model. Data sets for levels of *n*-hexane in breast milk after quantified exposures to *n*-hexane are not available so the model was not validated.

Target tissues. Target tissues (peripheral nervous system) were not specifically addressed in this model.

Species extrapolation. No species extrapolation was attempted in this model.

Interroute extrapolation. No interroute extrapolation was attempted in this model.

2.3 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

Absorption. *n*-Hexane is absorbed by passive diffusion in the lungs. Oral and dermal absorption have not been studied, but absorption by these routes is probably by the same process. Alveolar *n*-hexane reaches a steady state with the *n*-hexane in blood; as *n*-hexane is distributed and metabolized in the body more is absorbed from the alveolar air. In studies with humans, there was no evidence of saturation up to

204 ppm (Veulemans et al.1982). During exercise in this study, the alveolar uptake rate decreased, but total intake increased slightly because of the higher ventilation rate.

Distribution. The distribution of *n*-hexane is a function of its high lipid and very low water solubility. Partition coefficients established in human tissues indicate a distribution pattern at equilibrium of body fat>>liver, brain, muscle>kidney>heart>lung>blood (Perbellini et al.1985). *n*-Hexane is transported in blood mainly by partitioning into hydrophobic regions of blood proteins (Lam et al.1990). Transfer to tissues occurs via a similar partitioning process. *n*-Hexane can also leave the blood through the lungs via the pulmonary circulation depending on the alveolar air *n*-hexane concentration.

Storage. Experiments in animals indicate that no significant storage in tissues takes place. *n*-Hexane concentrations fell to minimal or nondetectable in all rat tissues, including body fat, within 8 hours after the end of a 6 hour exposure at 1,000 ppm (Bus et al.1981). A PBPK model predicts some accumulation of *n*-hexane in body fat in humans during a workweek, with some still remaining after the weekend (Perbellini et al.1986, 1990a). Whether this release from body fat after exposure ceases is toxicologically significant is unclear.

Excretion. Some *n*-hexane is exhaled following cessation of exposure. This could amount to approximately 10% of that absorbed (Mutti et al.1984; Veulemans et al.1982). Excretion is rapid and biphasic with half-lives of 0.2 hours and 1.7 hours. Most *n*-hexane is excreted in the urine as metabolites. Radiolabeled $^{14}CO_2$ in exhaled air has been detected after animal exposure to [^{14}C]*n*-hexane (Bus et al. I982), indicating that intermediary metabolism of some metabolites takes place. 2,5-Hexanedione and 4,.5-dihydroxy-2-hexanone are the major urinary metabolites of *n*-hexane in humans. Half-lives of excretion have been estimated to be 13-14 hours (Perbellini et al.1981, 1986).

Effect of Dose and Duration of Exposure on Toxicity. No studies were located where *n*-hexane concentration was measured in workplace air before workers became ill, so no dose-response relationship can be defined for human neurotoxicity as the result of *n*-hexane exposure. Information on duration of exposure leading to toxicity is available from some case series reports. An occupational exposure caused sensory disturbances in the lower extremities after approximately 2 months (Herskowitz et al. I971). A case of peripheral neuropathy after 7 months of exposure was reported among press-proofing workers in Taipei (Wang et al.1986); a serious case resulting in quadriplegia after 8 months of exposure was reported among sandal workers in Japan (Yamamura 1969). Based on case reports, it can be estimated that

workplace exposure to *n*-hexane at or above 500 ppm for several months may result in peripheral neuropathy in some individuals.

The dose-duration relationship to toxicity is clearer in rat studies. Continuous exposure (more than 20 hours a day, 7 days a week) to approximately 500 ppm *n*-hexane results in at least histological signs of peripheral nerve damage in most studies (Altenkirch et al.1982; IRDC 1981; Schaumburg and Spencer 1976) within 7-26 weeks. Intermittent exposure (8 hours a day, 7 days a week) at 700 ppm for 40 weeks produced no effect in rats (Altenkirch et al.1982). In intermittent exposures at higher concentrations, effects were seen at 1,200 ppm after 16 weeks (12 hours a day, 7 days a week) and at 14 weeks at 5,000 ppm (9 hours a day, 5 days a week) (Frontali et al.1981; Huang et al.1989). At the highest concentration/duration exposure reported (5,000 ppm, 16 hours a day, 6 days a week), reduction in motor nerve conduction velocity was observed at 1 week and paralysis was evident by 4 weeks (De Martino et al. 1987).

Route-dependent Toxicity. *n*-Hexane toxicity does not appear to be route-dependent. Peripheral neuropathy can be produced in rats by the oral route (Krasavage et al.1980) at high doses (4,000 mg/kg/day). The clinical and histopathological signs were similar to those seen after inhalation exposure. No reports of neurotoxicity after dermal exposure were located.

2.4.2 Mechanisms of Toxicity

Effects of Metabolism on Toxicity. Neurotoxicity in shoe workers in Japan and Italy was originally linked to glues and solvents containing *n*-hexane (Yamamura 1969). Since *n*-hexane is of low acute toxicity in humans and animals, it was unclear for several years how exposure resulted in toxicity. An outbreak of an almost identical peripheral neuropathy in a plant in Ohio in 1974 (Allen et al.1975) due to the closely related chemical methyl *n*-butyl ketone (2-hexanone) led to the hypothesis that a common metabolite of *n*-hexane and methyl *n*-butyl ketone may be responsible for the observed neurotoxicity. One of these metabolites, 2,5-hexanedione, was found to produce a peripheral neuropathy in rats identical to that produced by the inhalation of *n*-hexane (Schaumburg and Spencer 1976). The time to onset of neurotoxicity with other metabolites depends on the serum levels of 2,5-hexanedione produced, leading to the conclusion that 2,5-hexanedione is the active agent (Krasavage et al.1980). The conversion of *n*-hexane to 2,5-hexanedione takes place in the mixed-function oxidase system of the liver (Toftgard et al. 1983, 1986). There is some evidence in animals that the initial reaction (the conversion of *n*-hexane to

2-hexanol) can take place in the lung (Toftgard et al.1986). It appears unlikely that metabolism in humans would be saturated in the expected range of human exposure. In humans, a good correlation has been found between 2,5-hexanedione levels in the urine and workplace concentrations (Perbellini et al.1981).

Target Organ Toxicity. *n*-Hexane exposure is documented to cause toxicity in peripheral nerves of humans (both sensory and motor). In rats, *n*-hexane exposure causes toxicity in the peripheral and central nervous system and in male reproductive tissues. Effects on respiratory tissue have been observed in mice and rabbits. The toxic agent in nervous system and reproductive tissues is believed to be the *n*-hexane metabolite 25-hexanedione (Graham et al.1995).

The sequence of events in *n*-hexane-induced neuropathy has been described in rats (Spencer and Schaumburg 1977a). The process appears to begin by increases in the number of 10 nm axonal neurofilaments and accumulation in swellings on the proximal sides of the nodes of Ranvier in distal regions of large myelinated fibers. As exposure continues, there is a retrograde spread of axonal swellings up the nerve, and smaller myelinated and unmyelinated fibers become involved. The nerve terminal is unaffected until late in the process. The enlarged axons displace the paranodal myelin sheaths, leaving denuded swellings in areas near the nodes of Ranvier. This process occurs before functional impairment is evident and can be reversed on cessation of exposure as swelling diminishes, and proliferation of Schwann cells occurs at these sites with subsequent remyelination of the axons. If exposure to *n*-hexane continues, axonal restoration and remyelination do not take place at some swellings, and the length of the nerve fiber between the swelling and the terminal undergoes breakdown, very similar to that seen when fibers are transected. Axon sprouting is often seen at the intact portion of a degenerated fiber even while intoxication continues. When intoxication ends, this regenerative process can reestablish motor and sensory function.

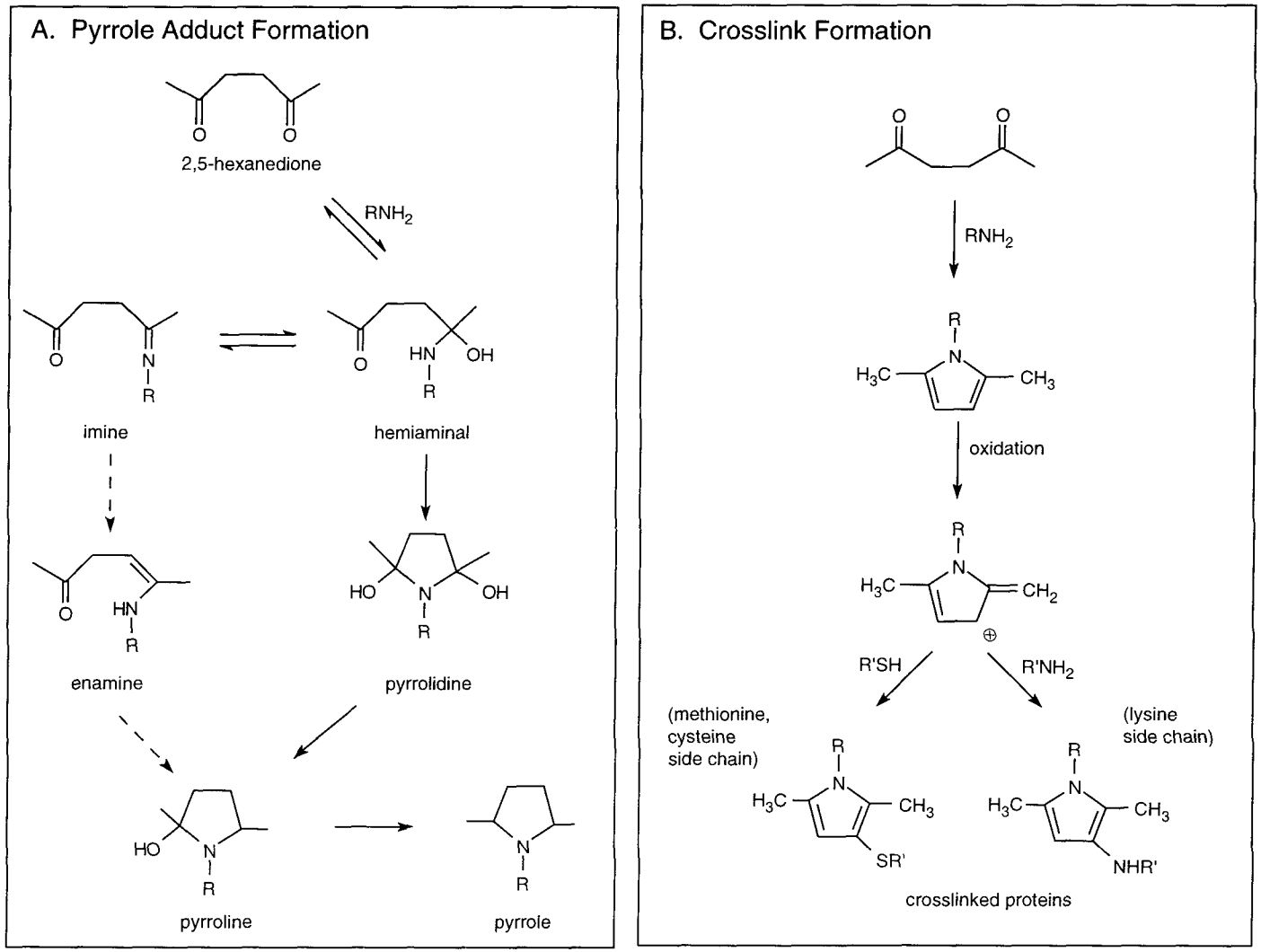
Peripheral neuropathy begins in the hind limbs in the rat model and eventually affects the front limbs. The nerve fibers most vulnerable to *n*-hexane exposure in rats are the branches of the tibia1 nerve serving the calf muscles, followed in order by the plantar nerve branches supplying the flexor digitorum brevis muscle, and then sensory plantar nerve branches innervating the digits. As intoxication continues, axonal degeneration ascends the plantar and tibia1 nerves (Spencer and Schaumburg 1977b). Examination of control animals indicated that the most sensitive fibers were also the largest. Effects on the central nervous system have also been observed in rats exposed to *n*-hexane or its neurotoxic metabolite, 2,5-hexanedione. Axonal swelling and degeneration were observed in the anterior vermis, spinocerebellar tract in the medulla oblongata, and gracile tracts of the spinal cord (Spencer and Schaumburg 1977b).

The chemical structure of 2,5-hexanedione suggested that it could react with lysine side-chain amino groups in proteins to form pyrroles (see Figure 2-7). *In vitro* experiments showed that this was, in fact, the case, and that the modified proteins can undergo secondary reactions to yield oxidized and polymeric products (DeCaprio et al.1982; Graham et al.1982). Oral administration of 2,5-hexanedione produced evidence that this process can take place *in vivo* as demonstrated by the detection of 2,5-dimethylpyrrole adducts in serum and axonal cytoskeletal proteins (DeCaprio and O'Neill 1985). When a series of 2,5-hexanedione analogues were tested for their ability to produce neurotoxicity in rats, it was found that only those with the 2,5 gamma spacing were neurotoxic, and that potency correlated with the rate constant for pyrrole formation (St. Clair et al.1988). The role of oxidation of the pyrrole adduct in the development of neurotoxicity was demonstrated with another 2,5-hexanedione analogue which could form pyrroles but was resistant to oxidation. This analogue (3-acetyl-2,5-hexanedione) caused pyrrolidation of protein in viva, but not neurotoxicity.

The reaction of anti-neurofilament antibodies with high molecular weight aggregates from rat neuronal cytoskeletal proteins provided direct evidence for neurofilament cross-linking after 2,5-hexanedione administration (Lapadula et al.1986). Immunoblotting with antibodies specific for phosphorylated forms of cytoskeletal proteins has demonstrated a reduction of phosphorylation in neurofilament proteins and microtubule-associated-protein 2 (MAP-2) after 2,5-hexanedione treatment (Abou-Donia et al.1988).

Whether neurofilament cross-linking is related to the neurofilament accumulation, axonal swellings, and ultimate axonal degeneration observed in *n*-hexane neurotoxicity or is incidental remains to be elucidated (Graham et al.1995). Since the maintenance of the axon depends on transport of cellular components from the neuronal cell body, the effect of 2,5-hexanedione on axonal transport has been investigated. If 2,5-hexanedione treatment slowed or stopped axonal transport, distal axonal degeneration would be an expected consequence. Measurement of the rate of axonal transport both during and after 2,5-hexanedione intoxication showed accelerated rates of transport that persisted after treatment ended (Pyle et al.1993). Increased rates of axonal transport may reflect a reparative response after neuronal injury (Graham et al. 1995).

Figure 2-7. Reaction of 2,5-Hexanedione with Protein



Source: Adapted from Graham et al. 1995

No information is available as to whether the mechanism of action of *n*-hexane toxicity in children differs from that of adults. Weanling rats (21 days old) were more resistant to the development of *n*-hexane peripheral neuropathy than young adults (80 days old) during an exposure to 1,000 ppm *n*-hexane (Howd et al.1983). The authors suggested that the relative resistance of the weanling rats may have been due to shorter, smaller-diameter axons, or to a greater rate of growth and repair in their peripheral nerves compared to those of adults.

2.4.3 Animal-to-Human Extrapolations

The rat is the major model system for human *n*-hexane neurotoxicity. Inhalation of *n*-hexane in this species produces clinical and histopathological effects similar to those seen in workers exposed to *n*-hexane. However, the toxicokinetics in rats are somewhat different (e.g., less 2,5-hexanedione and more 2-hexanol as a proportion of total urinary metabolites compared to humans [Fedtke and Bolt 1987; Frontali et al. 1981]). Mice do not develop clinical signs of neurotoxicity after exposure to *n*-hexane, although histopathological changes (paranodal axonal swellings) have been observed (Dunnick et al.1989; NTP 1991). A single study in rabbits exposed to high levels of *n*-hexane (3,000 ppm) showed no evidence of neurotoxicity in this species (Lungarella et al.1984).

2.4 RELEVANCE TO PUBLIC HEALTH

Overview. *n*-Hexane is a hydrocarbon produced from crude oil that is a component of many solvents used in industry. *n*-Hexane is also used in certain special glues and adhesives, and is present in gasoline. Because of the high volatility of *n*-hexane, the most likely route of human exposure is inhalation. The risk of health effects in humans depends on the concentration of *n*-hexane in the air and the duration of exposure. Prolonged occupational exposures (months to years) to high concentrations (±500 ppm) have resulted in significant human toxicity. Exposure to very high concentrations (±10,000 ppm, e.g. as the result of a spill) could result in narcosis, although the major hazard in this case would be the risk of explosion and fire. Narcosis has been observed in animals (Hine and Zuidema 1970) but has not been reported in humans.

Human toxicity associated with *n*-hexane was first recognized in the 1960s and early 1970s in Japan and Italy. Workers in the shoe industries in these countries developed a peripheral neuropathy that started with numbness in the feet and hands, followed by weakness in the lower legs and feet. In severe cases, paralysis

developed. Epidemiological investigations revealed that these illnesses were linked with the use of glues and solvents containing high concentrations of *n*-hexane. In all cases, poor ventilation was a major factor in the illness. Removal from the workplace resulted in recovery for the patients over the course of several months to 2 years. There have been hundreds of cases of *n*-hexane neurotoxicity reported from occupational exposure throughout the world, but comparatively few in the United States. This is probably due to different use patterns; in the United States, *n*-hexane is used mainly in closed systems (e.g., for extraction of vegetable oils) while in the shoe industry cases of the 1960s and 1970s, open containers of solvents containing *n*-hexane were present in poorly ventilated workplaces. Issues relevant to children are explicitly discussed in Sections 2.6, Children's Susceptibility, and 5.6, Exposures of Children.

A closely related chemical, 2-hexanone, which is an *n*-hexane metabolite, has also caused peripheral neuropathy in workers (Allen et al.1975). This chemical is the subject of another publication in this series, Toxicol*ogical Profile for 2-Hexanone* (ATSDR 1991).

n-Hexane is metabolized in the body to a number of metabolites. One of these metabolites, 2,5-hexanedione, is believed to be the ultimate toxic agent in *n*-hexane-induced neurotoxicity. These metabolites are excreted from the body in the urine within a few days of exposure. Experiments in animals show that rats are also susceptible to *n*-hexane neurotoxicity. Mild signs of neurotoxicity can be produced by *n*-hexane exposure in mice and chickens, but these do not progress to severe signs like paralysis, as can occur in humans and rats. Several effects occur in animals at very high concentrations, above the expected range of human exposure to *n*-hexane. *n*-Hexane exposure causes damage to male reproductive tissues in rats and signs of respiratory tract and lung damage in mice and rabbits, respectively. Animal studies have generally not shown adverse developmental effects after inhalation and oral exposure to *n*-hexane.

n-Hexane does not appear to be mutagenic in *in vivo* or *in vitro* test systems, nor does it contain *"*structural alerts*"* which have been associated with carcinogenicity (Ashby 1985). No epidemiological studies were located addressing whether there is or is not an association between occupational *n*-hexane exposure and cancer. *n*-Hexane has not been tested for carcinogenicity in animals and has not been categorized as to its potential for carcinogenicity by the International Agency for Research on Cancer (IARC), the National Toxicology Program (NTP), or the EPA. A single report of papillary tumors of the terminal bronchiole epithelium in rabbits after 24 weeks of exposure to 3,000 ppm *n*-hexane was located (Lungarella et al. 1984). In a chronic-duration study in $B6C3F_1$ mice where exposure was to commercial hexane (51.5% *n*-hexane) for 6 hours/day, 5 days a week for 2 years, a statistically significant treatment-related increase in

hepatocellular neoplasms (adenoma and carcinoma) was observed among females exposed at 9,018 ppm (Bio/Dynamics 1995b), but not at 900 or 3,000 ppm. No increases were observed in male mice or in Fischer 344 rats of either sex exposed similarly in a parallel experiment (Bio/Dynamics 1995a).

n-Hexane evaporates very easily so the most likely route of exposure is via inhalation, which is most likely to occur in the workplace. The current U.S. Occupational Safety and Health Administration (OSHA) permissible exposure level (PEL) is 500 ppm for *n*-hexane in workplace air. A new limit of 50 ppm was proposed in 1989, but a U.S. Court of Appeals decision rescinded the 1989 PELs promulgated by OSHA. Only PELs in place prior to 1989 are currently allowed. This decision was based on legal issues and was not specific for *n*-hexane. There is no question that exposure at 500 ppm can cause neurotoxicity in animal models (Spencer et al.1980), and occupational exposure in the range of 500-2,500 ppm resulted in neurotoxicty in 93 of 1,662 workers canvassed in the Japanese shoe industry (Yamamura et al.1969). The American Conference of Governmental Industrial Hygienists (ACGIH) recommends a Threshold Limit Value (TLV) of 50 ppm. Health effects could occur in workplaces if proper industrial hygiene and safety precautions are not followed. Health effects are also possible if consumer products containing *n*-hexane are used without proper ventilation; however, no reports of toxicity after such exposure were located. The exposure of the general population to *n*-hexane is low. Since vegetable oils are extracted with solvents containing *n*-hexane, it is possible that very small amounts are present in these products; these amounts are toxicologically insignificant (see Section 5.4.4). *n*-Hexane has not been detected in drinking water, but it is detectable in the air. The *n*-hexane in the air is at a very low level (ppb) and probably is derived from gasoline. Thus, the risk of adverse health effects in the general population from *n*-hexane exposure appears to be negligible. *n*-Hexane is degraded in the atmosphere in a few days; if present in bodies of water, it evaporates into the atmosphere and is degraded there.

For people living near hazardous waste sites, the potential for adverse health effects would depend on the amount of *n*-hexane to which they were exposed. *n*-Hexane has been detected in at least 60 of the 1,467 hazardous waste sites that have been proposed for inclusion on the EPA National Priority List (NPL) (HazDat 1998). However, the number of sites evaluated for *n*-hexane is not known. The most likely routes of exposure for people living near hazardous waste sites would be by breathing *n*-hexane-contaminated air or skin contact with *n*-hexane-contaminated soil. It is possible, but less likely, to be exposed by drinking contaminated well water. Monitoring of the air, drinking water, and soil levels of *n*-hexane at these sites is necessary to predict the possibility of adverse health effects.

Minimal Risk Levels for *n*-Hexane.

Inhalation MRLs

The database for acute-duration inhalation exposure was insufficient to derive an MRL for this duration. Only one study was identified describing neurological effects for this duration, in which histopathology on nerve tissue was not performed (De Martino et al.1987).

No human studies with adequate documentation of air levels of *n*-hexane were found for intermediateduration inhalation exposure. The animal data were insufficient to derive an MRL for this duration. No NOAELs which were below the LOAELs in the intermediate duration database could be identified for neurological effects, which are the most sensitive.

- An MRL of 0.6 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to *n*-hexane.

The MRL is based on a LOAEL of 58 ppm for reduced motor nerve conduction velocity in occupationally exposed workers (Sanagi et al.1980). This MRL was adjusted by a factor of 10 for use of a LOAEL and a factor of 10 for human variability. Further details are in Appendix A. In this study, where exposure appeared to be limited to only *n*-hexane and acetone, 2 age-matched groups consisting of 14 control workers and 14 exposed workers employed in a factory producing tungsten carbide alloys were compared (Sanagi et al.1980). The groups were matched with respect to age, stature, weight, alcohol consumption, and smoking habits. Exposure was estimated with 22 personal samples taken from the breathing zones over a period of 2 years. This number of samples is fewer than optimal for measuring air levels. Eighthour time-weighted average exposure to solvent vapors consisted of *n*-hexane at 58±41 ppm and acetone at 39±30 ppm; no other solvent vapors were detected. The exposure duration ranged from 1 to 12 years with an average of 6.2 years. Both groups completed questionnaires and underwent clinical neurological examinations with reference to cranial nerves, motor and sensory systems, reflexes, coordination, and gait. Neurophysiological studies performed included electromyography on muscles of the forearm and leg. Nerve stimulation studies were performed with a surface electrode (motor nerve conduction velocity, residual latency). Conduction velocities and distal latencies in the control group were similar to those reported in other studies (Goodgold and Eberstein 1983; Johnson et al.1983). In the questionnaire, the prevalence of headaches, dysesthesia of limbs, and muscle weakness was higher in the exposed group compared to the control; complaints of hearing deficits which were thought to be related to noise from ball

mills were also greater in the exposed group. Cranial nerve examinations and motor and sensory nerve examinations did not reveal any statistically significant abnormal neurological signs; however, paresthesia of the extremities was observed in 3 exposed workers and 1 worker in the control group. Differences ($p<0.05$) in the jump test (muscle strength) and the tuning fork test (vibration sensation) were noted. A general trend of diminished muscle strength reflexes was found in the biceps and knees of exposed workers; however, the difference was not statistically significant. Significant differences in the nerve conduction velocities on the right median, ulnar, and posterior tibia1 nerves were not found. However, a statistically significant decrease was detected in the posterior tibia1 nerve and an increased residual latency (time from onset of stimulus to recording) of motor conduction. Residual latency was 2.21±O.34 m/sec in controls versus 2.55±0.48 m/sec in exposed subjects; maximal motor nerve conduction velocity was 48.3±2.1 m/sec in controls versus 46.6±2.3 m/sec in exposed subjects. Normal values for the posterior tibia1 nerve have been reported as 2.1-5.6 m/sec for distal latency and 44.8-51.2 m/sec for conduction velocity (Goodgold and Eberstein 1983). The subjects in this study were age matched because these parameters vary with increasing age (conduction velocity decreases and distal latency increases). While these changes in the exposed workers remain within the normal range, ATSDR considers these differences in motor nerve conduction velocity and residual latency to be biologically significant.

It is not entirely clear whether the acetone co-exposure in the Sanagi et al. (1980) study contributed to the observed effects. Indirect evidence from an occupational study (Cardona et al.1996) showed that workplace acetone concentrations had a statistical correlation with the ratio of urinary *n*-hexane metabolites to *n*-hexane air concentration, although it did not correlate with measured urinary metabolites. No animal studies are available describing the effects of inhalation co-exposure to acetone and *n*-hexane, although there are several studies which report interactions between acetone and the neurotoxic metabolite of *n*-hexane 2,5-hexanedione (See Section 2.4, Mechanisms of Action). Oral administration of acetone has been reported to potentiate the neurotoxicity caused by oral exposure to the neurotoxic *n*-hexane metabolite 2,5-hexanedione in rats (Ladefoged et al.1989, 1994). Oral exposure to acetone alone in rats at 650 mg/kg/day resulted in a statistically significant decrease in motor nerve conduction velocity after 6 weeks; co-exposure to acetone and 2,5-hexanedione resulted in greater effects than those seen with 2,5-hexanedione alone (Ladefoged et al.1989). It is possible that acetone may potentiate *n*-hexane neurotoxicity by decreasing body clearance of 2,5-hexanedione (Ladefoged and Perbellini 1986). Simultaneous subcutaneous injection of acetone and 2,5-hexanedione increased the peak concentration of 2,5-hexanedione in rat sciatic nerve compared to injection of 2,5-hexandione alone (Zhao et al.1998). Acetone also influences the action of many chemicals by its induction of the cytochrome P-450 isozyme

CYP2E1 (Patten et al.1986). *n*-Hexane is metabolized by P-450 isozymes; induction by acetone may result in an increased production of the neurotoxic metabolite 2,5-hexanedione.

If the neurotoxicity of *n*-hexane was potentiated in this study by co-exposure to acetone, the level of *n*-hexane alone required to produce these effects would be higher than 58 ppm and the MRL level would be higher. Results from simulations with a PBPK model that accurately predicted *n*-hexane blood and 2,5-hexanedione urine levels (Perbellini et al.1986, 1990a) indicate that at concentrations of 50 ppm, the rate-limiting factor in *n*-hexane metabolism is delivery to the liver, not metabolic activity. This suggests that at this concentration (and at the MRL concentration of 0.6 ppm), induction of P-450 enzymes in the liver by acetone or other chemicals would not affect the rate at which 2,5-hexanedione was produced.

Ambient air concentrations of *n*-hexane are in the low-parts-per-billion range. A recent measurement in Chicago (Moschonas and Glavas 1996) was 2 ppb (0.002 ppm).

Oral MRLs.

The database for oral exposure was insufficient to derive MRLs. Only 3 studies were located regarding neurological effects after oral exposure to *n*-hexane, 2 in rats and 1 in chickens (Abou-Donia et al.1982; Krasavage et al.1980; Ono et al.1981). The Krasavage study (1980) in rats resulted in a NOAEL for neurological effects of 1,140 mg/kg/day, and serious effects were seen at 4,000 mg/kg/day (hindlimb paralysis). However, since little is known about the toxicokinetics of *n*-hexane after oral exposure in either humans or test animals, extrapolation of an animal study to predict health effects in humans was not attempted.

Death. No deaths have been reported in humans after exposure to *n*-hexane by any route. An oral LD,, value of 15,840 mg/kg has been reported in 14-day-old Sprague-Dawley rats, indicating low acute toxicity (Kimura et al.1971). LD_{50} values were approximately twice as high in adult animals. Deaths have been reported in animals exposed to relatively high concentrations of *n*-hexane via inhalation for intermediate durations. These deaths appear to be the result of difficulty in eating and drinking as *n*-hexane-related paralysis develops. Continuous exposure (24 hours a day) to 1,000-1,500 ppm *n*-hexane resulted in deaths in male Fischer 344 rats within 11 weeks (Howd et al.1983; Rebert and Sorenson 1983). In contrast to the greater susceptibility of 14-day-old rats than adult rats to oral *n*-hexane exposure, inhalation exposure was more likely to cause lethality in older rats than weanlings (Howd et al.1983; Kimura et al.1971).

Exposure to 3,040 ppm for 16 weeks (12 hours a day) resulted in the death of 2 of 7 rats with signs of neurotoxicity (Takeuchi et al.1980). Exposure to 3,000 ppm for 24 weeks resulted in the death of 2 of 12 rabbits with signs of respiratory effects (Lungarella et al.1984).

Systemic Effects.

Respiratory Effects. Respiratory effects have not been reported in humans after inhalation exposure to *n*-hexane. *n*-Hexane is not irritating to the eyes, nose, or throat at concentrations up to 500 ppm (Nelson et al.1943). Respiratory effects including rales, gasping, and mouth breathing were reported in rabbits throughout a 24-week inhalation exposure to 3,000 ppm *n*-hexane (Lungarella et al.1984). Histopathological examination revealed serious effects in the lung, including centrilobular emphysema and fibrosis. Respiratory effects were also seen in mice exposed via inhalation to up to 10,000 ppm *n*-hexane for 13 weeks (Dunnick et al.1989; NTP 1991). Mild effects were seen in the olfactory epithelium at 1,000 ppm, and in both the olfactory and respiratory tracts at 10,000 ppm. In another study where rats were exposed by inhalation to 500 ppm *n*-hexane for 6 months, no histological changes were seen in respiratory tissues (IRDC 1981).

The effects observed in animals occurred at high concentrations that are well above the probable range of human exposure. It is unlikely that humans in any setting would be exposed to levels high enough to cause respiratory effects.

Cardiovascular Effects. Cardiovascular effects have not been reported in humans after exposure to *n*-hexane. Information from animal studies is limited to histopathological examination of the heart and aorta after intermediate-duration inhalation studies. No treatment-related lesions were seen in $B6C3F_1$ mice exposed to *n*-hexane via inhalation at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks or 1,000 ppm for 22 hours a day, 5 days a week for 13 weeks (Dunnick et al.1989; NTP 1991). Similar results were noted in male Sprague-Dawley rats exposed via inhalation to up to 500 ppm *n*-hexane for 22 hours a day, 7 days a week for 6 months (IRDC 1981).

Subcutaneous administration of *n*-hexane at 143 mg/kg/day for 30 days has been reported to decrease the threshold for ventricular fibrillation in perfused hearts from male Wistar rats (Khedun et al.1996). Myocardial magnesium and potassium levels were reduced in treated rats. When these levels were

corrected by supplementation, the ventricular fibrillation potential was still reduced. Histological alterations (disordered myocardial Z-bands) were also observed in exposed rats.

Gastrointestinal Effects. Gastrointestinal effects have not been reported in humans after exposure to *n*-hexane. Information from animal studies is limited to histopathological examination of gastrointestinal tissues after intermediate-duration inhalation studies. Histopathological examination of gastrointestinal tissues revealed no treatment-related lesions in $B6C3F_1$ mice exposed via inhalation to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks or 1,000 ppm for 22 hours a day, 5 days a week for 13 weeks (Dunnick et al.1989; NTP 1991). Similar results were noted in male Sprague-Dawley rats exposed via inhalation to up to 500 ppm *n*-hexane for 22 hours a day, 7 days a week for 6 months (IRDC 1981).

Hematological Effects. Hematological effects have not been reported in humans after exposure to *n*-hexane. Some minor hematological changes were seen in rats exposed via inhalation to *n*-hexane for 6 months for 6 hours a day, 5 days a week (0, 6, 26, 129 ppm), but were not seen at 21 hours a day, 7 days a week (0,5,27, 126 ppm) and probably have no toxicological significance (Bio/Dynamics 1978). None of the parameters measured in males or females was outside normal biological limits. Hematological parameters were within normal limits in $B6C3F_1$ mice exposed via inhalation to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks, except for an increase in segmented neutrophils in males exposed to 10,000 ppm. The authors ascribed this to chronic active inflammation in the nasal mucosa of some of the male mice (Dunnick et al.1989; NTP 1991). In mice in this study exposed via inhalation to 1,000 ppm *n*-hexane for 22 hours a day, 5 days a week for 13 weeks, all hematological parameters were within normal limits. No significant changes were observed in hematological parameters or serum chemistry in male New Zealand rabbits exposed via inhalation to 3,000 ppm *n*-hexane for 8 hours a day, 5 days a week for 24 weeks (Lungarella et al.1984).

Musculoskeletal Effects. Muscle wasting and atrophy have been reported in humans occupationally exposed to *n*-hexane (Yamamura 1969). These effects occurred in individuals with severe neurotoxicity. Muscle atrophy is a common finding after intermediate-duration inhalation exposure to *n*-hexane in experimental animals. This atrophy is secondary to *n*-hexane-induced neurotoxicity which results in muscle denervation. Hindlimb atrophy characterized as *"*severe*"* was reported in 10 of 11 male Sprague-Dawley rats exposed via inhalation to approximately 1,000 ppm *n*-hexane for 28 or 61 days (Nylen et al. 1989). Mild atrophy of the gastrocnemius muscle was observed in 3 of 10 male Sprague-Dawley rats

exposed via inhalation to 502 ppm *n*-hexane for 22 hours a day, 7 days a week for 6 months (IRDC 1981). Degenerative changes in the muscle were not observed. Electron microscopy of the gastrocnemius and soleus muscles in male Wistar rats exposed via inhalation to 3,000 ppm *n*-hexane for 12 hours a day, 7 days a week for 16 weeks revealed denervation, irregular fibers, disordered myofilaments, zig-zagging of the Z-band, and numerous invaginations of the plasma membrane (Takeuchi et al.1980).

Hepatic Effects. Hepatic effects have not been reported in humans after exposure to *n*-hexane. Histopathological examination of the liver after intermediate-duration inhalation exposure revealed no treatment-related lesions in male Sprague-Dawley rats exposed via inhalation to 500 ppm *n*-hexane daily for 6 months, 22 hours a day (IRDC 1981). Similar results were observed in $B6C3F_1$ mice exposed via inhalation to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks or 1,000 ppm for 22 hours a day, 5 days a week for 13 weeks (Dunnick et al.1989; NTP 1991). Decreased blood urea nitrogen (indicating decreased protein catabolism in the liver) has been reported in female, but not male, rats after 26 weeks of inhalation exposure to 126 ppm *n*-hexane for 21 hours a day, 7 days a week (Bio/Dynamics 1978). However, only 4 animals per group were examined in this study, so the toxicological significance of this finding is doubtful.

Endocrine Effects. Endocrine effects have not been reported after *n*-hexane exposure in humans. Histopathological examination of endocrine tissues (thyroid, parathyroid, adrenals, pituitary, pancreas) after intermediate-duration inhalation exposure revealed no treatment-related lesions in male Sprague-Dawley rats exposed via inhalation to 500 ppm *n*-hexane 22 hours a day for 6 months, (IRDC 1981). Similar results were seen in $B6C3F_1$ mice exposed via inhalation to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks or 1,000 ppm for 22 hours a day, 5 days a week for 13 weeks (Dunnick et al.1989; NTP 1991). Tissues examined were the thyroid, parathyroid, adrenals, pituitary, and pancreas.

Renal Effects. Renal effects have not been observed in humans following exposure to *n*-hexane. Histopathological examination of the kidney and urinary bladder showed no treatment-related lesions in $B6C3F_1$ mice exposed via inhalation to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks or 1,000 ppm for 22 hours a day, 5 days a week for 13 weeks (Dunnick et al. 1989; NTP 1991).

An increased incidence and severity of chronic nephropathy (a common age-related condition in male rats) was noted in male Sprague-Dawley rats exposed via inhalation to 500 ppm *n*-hexane daily for 6 months, 22 hours a day (IRDC 1981). Increased kidney weight was also observed. The authors stated that it was unclear whether the increased incidence and severity was due to exacerbation of the process seen in the control group or if the *n*-hexane exposure caused additional tubular injury. No lesions were reported in the urinary bladder. The authors did not investigate what role the unique male rat protein $\alpha_{2\mu}$-globulin might be playing in these renal effects. Other substances that apparently bind to this carrier protein include a number of hydrophobic xenobiotics such as petroleum-derived hydrocarbons, including decalin and the gasoline constituent trimethylpentane. These substances cause an α_{2U}-globulin nephropathy syndrome in male rats (EPA 1991). A decrease in urine pH, but no histopathological lesions were reported in male rats exposed via inhalation to 10,000 ppm *n*-hexane for 13 weeks (Cavender et al.1984).

Dermal Effects. Dermal effects have been observed in humans following exposure to *n*-hexane. *n*-Hexane was 1 of 11 solvents tested for dermal toxicity in a male volunteer (Wahlberg 1984). A slight transient erythema was observed after 10-20 minutes exposure to 1.5 mL *n*-hexane and a stinging and/or burning sensation reported by the volunteer. Application of 0.1 mL neat *n*-hexane did not cause clinical signs or affect blood flow.

In animal studies, histopathological examination of the skin after intermediate-duration inhalation exposure revealed no treatment-related lesions in male Sprague-Dawley rats exposed via inhalation to 500 ppm *n*-hexane daily for 6 months, 22 hours a day (IRDC 1981). Similar results were seen in $B6C3F_1$ mice exposed via inhalation to *n*-hexane at concentrations up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks or 1,000 ppm for 22 hours a day, 5 days a week for 13 weeks (Dunnick et al.1989; NTP 1991).

Body Weight Effects. Effects on body weight are common during intermediate-duration exposure of rats to *n*-hexane and tend to occur prior to the development of neurotoxicity (see Section 2.2.1.4). In male Wistar rats exposed via inhalation to 0, 500, 1,200, or 3,000 ppm *n*-hexane daily for 12 hours a day, body weight was lower in the treated groups from 4 weeks of exposure (Huang et al.1989). Significantly decreased grip strength was noted at 13 weeks and at study termination (16 weeks) body weights in the 1,200 and 3,000 ppm groups were 13% less than control. In another study with male Wistar rats exposed via inhalation daily to 3,040 ppm *n*-hexane, reduction in body weight compared to control was significant at 4 weeks and was 33% less than control at 16 weeks (Takeuchi et al.1980). In this study, reductions in nerve conduction velocity were observed at 4 weeks and clinical signs of neurotoxicity at 10 weeks.

Similarly, Sprague-Dawley rats exposed via inhalation to 500 ppm showed significant reduction in body weight compared to controls at 7 weeks and clinical signs of neurotoxicity at I6 weeks (IRDC 1981). At study termination after 6 months, treated animals weighed 30% less than controls.

Less severe body weight effects were observed in species that are less susceptible to *n*-hexane-induced neurotoxicity. In male $B6C3F_1$ mice exposed via inhalation to 1,000 ppm *n*-hexane for 22 hours a day, 5 days a week for 13 weeks, a 10% depression in the final body weight relative to control was observed (Dunnick et al.1989; NTP 1991). No change was found in females. In male $B6C3F_1$ mice exposed via inhalation to 10,000 ppm *n*-hexane for 6 hours a day, 5 days a week for 13 weeks, a 17% depression in the final body weight relative to control was observed. NOAELs were 4,000 ppm in males and 10,000 ppm in females (Dunnick et al.1989; NTP 1991). No effect on body weight was observed in male New Zealand rabbits exposed via inhalation to 3,000 ppm *n*-hexane 8 hours a day, 5 days a week for 24 weeks (Lungarella et al.1984). Variable weight loss was observed in Leghorn chickens exposed via inhalation to 1,000 ppm *n*-hexane continuously for 30 days (2 1%) and 90 days (12%) (Abou-Donia et al.1991). Weight loss was greatly exacerbated in the 90-day study (up to 35%) when chickens were co-exposed via inhalation to 1,000 ppm *n*-hexane and 1,000 ppm methyl isobutyl ketone.

Metabolic Effects. Metabolic effects have not been reported in humans after exposure to *n*-hexane. In animal studies where metabolic parameters (blood pH, electrolytes, glucose) were measured, no effects were seen after inhalation exposure to *n*-hexane in rats at up to 10,000 ppm for 6 hours a day, 5 days a week for 13 weeks (Cavender et al.1984) or in $B6C3F_1$ mice similarly exposed (Dunnick et al.1989; NTP 1991). Higher mean-fasting glucose was observed in male Sprague-Dawley rats exposed for 6 hours a day, 5 days a week at 6 and 129 ppm, but not at 26 ppm (Bio/Dynamics 1978). Female fasting glucose levels were unaffected by exposure in this study. No effect on this parameter was seen in a parallel experiment at similar concentrations for 21 hours a day, so this finding is of doubtful toxicological significance, especially since only 4 animals per group were examined in this study. Body temperature was unaffected in rats by continuous exposure to up to 1,500 ppm *n*-hexane at 2 and 11 weeks (Rebert and Sorenson 1983).

Immunological and Lymphoreticular Effects. One report of immunological effects in humans after exposure to *n*-hexane was located (Karakaya et al.1996) describing a reduction in immunoglobulin levels in a group of 35 workers compared to a control group of 23. The reductions correlated with 2,5-hexanedione in urine but not with workplace *n*-hexane concentrations (23-2 15 ppm). The reductions

also remained well within the normal ranges for immunoglobulins in blood, so the toxicological significance of these findings cannot be assessed without confirmatory studies (Jackson et al.1997). Cell counts (lymphocytes, neutrophils, monocytes, eosinophils) were unaffected by *n*-hexane exposure. No reports on dermal sensitization after exposure to *n*-hexane in humans were located in the literature. The animal database is limited to intermediate-duration inhalation studies where tissues were examined histopathologically (e.g, lymph nodes, thymus, bone marrow, spleen), no adverse effects were seen (Cavender et al.1984; Dunnick et al.1989; IRDC 1981; NTP 1991).

Neurological Effects. The major public health concern regarding *n*-hexane exposure is the potential for the development of neurotoxicity. Occupational studies have documented that human exposure to *n*-hexane can result in a peripheral neuropathy that in severe cases can lead to paralysis (Altenkirch et al. 1977; Yamamura 1969; Wang et al.1986). The dose-duration relationship has not been well characterized in humans, but concentrations of 500 ppm and above, and exposure for 2 months or more have been associated with human neurotoxicity. Brief exposure to extremely high concentrations of *n*-hexane may cause signs of narcosis in humans; prostration and coma have been observed in animals exposed to a mixture of hexanes at concentrations of 70,000-80,000 ppm (Hine and Zuidema 1970). At these levels, however, explosion and fire would be the main concern.

ATSDR has developed a chronic-duration inhalation MRL based on neurological effects in humans; the study on which this MRL is based is discussed earlier in this section.

A case series of workers in a furniture factory in the Bronx, New York, illustrates the typical clinical presentation of *n*-hexane neurotoxicity (Herskowitz et al.1971). This report describes the cases of 3 women who worked as cabinet finishers and whose job was to wipe glue off furniture with rags soaked in a solvent which contained *n*-hexane. An open drum of this solvent was used in a small, poorly ventilated room. Air measurements of *n*-hexane averaged 650 ppm, although peaks of up to 1,300 ppm also occurred. Neurological signs of both motor and sensory impairment were noted in all 3 women with an onset 24 months after beginning employment. Initial symptoms and clinical findings were similar in all three women. In the first case (a 23-year-old woman), initial symptoms were a burning sensation in the face, numbness of the distal extremities, and an insidious, progressive distal symmetrical weakness in all extremities. Frequent headaches and abdominal cramps were also reported. After being admitted to the hospital (6 months after beginning work), muscle testing revealed a moderate distal symmetrical weakness and a bilateral foot-drop gait. There was a moderate decrease of pin and touch perception and mild

impairment of vibration and position sense in the lower extremities. Tendon reflexes were slightly hyperreflexic (1+) and symmetrical throughout except for absent Achilles tendon reflexes. No Babinski sign was present. Serum lead screening was negative. An electromyogram revealed fibrillation potentials in the small muscles of the hands and feet. Nerve conduction velocities were 45 m/sec in the left ulnar nerve (normal range in the general population, 49-75), 26 m/sec in the right median nerve (normal range, 50-75), and 23 m/sec in the left peroneal nerve (normal range, 40-60). Sural nerve biopsy was unremarkable, although electron microscopic examination showed a few myelinated axons containing dense bodies and exceptionally numerous mitochondria. Muscle biopsy showed scattered groups of small angulated fibers and many fibers with clear central zones, consistent with denervation. Electron microscopy of nerve branches within the muscle showed an increased number of neurofilaments with abnormal membranous structures and clumping and degeneration of mitochondria with dense bodies. Increased numbers of mitochondria, glycogen granules, and degenerated mitochondria were noted in the motor endplates.

Recovery from *n*-hexane peripheral neuropathy has been examined. In a follow-up study, a group of 90 shoe manufacturing workers (27 men and 63 women) diagnosed in the past with *n*-hexane peripheral neuropathy were studied again at least one year after cessation of *n*-hexane exposure (Valentino et al. 1996). Subjects were referred by the Italian government to confirm disability status and thus may not be representative of all those originally diagnosed. Subjects were classified on the basis of the duration of time since the diagnosis. Motor nerve conduction velocities and distal latencies had improved from those observed at the time of diagnosis and were similar to a control group. However, sensory nerve conduction velocities and distal latencies, while improved from those at diagnosis, were still statistically different from controls.

Peripheral neuropathy has also occurred in humans as the result of solvent abuse of products containing *n*-hexane (Altenkirch et al.1977; Chang et al.1998; Spencer et al.1980). Clinical signs were very similar to those seen after occupational exposure; however, signs of central nervous system toxicity may also be present due to other components in the inhaled mixtures, e.g., toluene (Spencer et al.1980).

Several studies have demonstrated sub-clinical alteration in neurological function in humans after inhalation exposure to *n*-hexane. In a cross-sectional study using age-matched controls, workers in a shoe factory exposed via inhalation to *n*-hexane were compared to a control group, which had not been exposed, from the same factory (Mutti et al.1982a). Mean breathing-zone *n*-hexane air concentrations were 69 ppm

in the mild-exposure group and 134 ppm in the high-exposure group. Methyl ethyl ketone, which has been reported to potentiate *n*-hexane neurotoxicity in humans and animals (Altenkirch et al.1977, 1982), was present at 22 ppm in the mild exposure group and 76 ppm in the high exposure group. Cyclohexane and ethyl acetate were also detected. Symptoms more frequent during the workday in the exposed than the control group were sleepiness and dizziness. Chronic symptoms more frequent in the exposed group were weakness, paraesthesia, and hypoesthesia. Motor action potential amplitude in all three examined nerves was significantly decreased compared to controls in both exposed groups. Motor nerve conduction velocity was significantly decreased in median and peroneal nerves, but not in the ulnar nerve. In the median nerve, motor nerve conduction velocity was significantly decreased in the high-exposure group compared to the mild-exposure group.

A group of 15 women from a shoe factory (mean age 26.6 years, mean exposure time 4.5 years) was compared to a control group of 15 healthy age-matched women from other shoe factories who had not been exposed to neurotoxic chemicals (Mutti et al.1982b). The mean *n*-hexane air concentration was 195 ppm for 36 samples taken over a 3-year period in the factory. Methyl ethyl ketone was present at 60 ppm. All nerve conduction velocities (motor and sensory) were significantly slowed in exposed workers compared to controls. Sensory nerve action potential peak latency was significantly slower in the median and ulnar nerves of the exposed workers. The somatosensory-evoked potential recording could be broken down into 10 peaks; significantly greater latency was observed for the first 2 peaks in the exposed group compared to the controls. There was a negative linear relationship between distal sensory conduction velocity and latency of the earliest evoked potential (P15).

Clinical signs of peripheral neuropathy similar to those seen in human occupational exposures to *n*-hexane can be produced in rats via the inhalation and oral routes, but not in other test species (Altenkirch et al. 1982; De Martino et al.1987; Dunnick et al.1989; Frontali et al.1981; Huang et al.1989; IRDC 1981; Krasavage et al.1980; NTP 1991; Schaumburg and Spencer 1976; Takeuchi et al.1980). Paranodal axonal swelling in mice (Dunnick et al.1989; NTP 1991) and leg weakness in chickens (Abou-Donia et al. 1985) can be produced with inhalation exposure to *n*-hexane, but these conditions do not progress to the severe neurotoxicity observed in humans and rats. The molecular mechanism responsible for the axonal swelling, demyelination, and axonal degeneration seen in human *n*-hexane neurotoxicity is currently unknown. However, animal experiments provide strong evidence that the mechanism involves the formation of protein adducts (pyrrolidation) by the neurotoxic metabolite 2,5-hexanedione (Graham et al. 1995) and possibly crosslinking of neuronal cytoskeletal proteins (e.g., neurofilaments).

Neurological effects have been observed in many intermediate-duration inhalation experiments in rats. In Sprague-Dawley rats exposed continuously to 400-600 ppm *n*-hexane for up to 162 days, animals developed an unsteady, waddling gait after 45-69 days of exposure (Schaumburg and Spencer 1976). Further exposure resulted in a progressive, symmetrical, distal hindlimb weakness with foot-drop. Severely affected animals also developed distal weakness of the upper extremities. Pathological changes including giant axonal swellings and fiber degeneration were detected in the peripheral and central nervous systems of the 4 animals exposed for 49 days. Electron microscopic examination showed the swollen regions contained densely packed masses of 10 nm neurofilaments. Groups of mitochondria and neurotubules were displaced to the periphery of the axon or segregated into bundles.

The sequence of events in *n*-hexane-induced neuropathy has been described in rats (Spencer and Schaumburg 1977a). The process appears to begin by increases in the number of 10 nm axonal neurofilaments and accumulation in swellings on the proximal sides of the nodes of Ranvier in distal regions of large myelinated fibers. As exposure continues, there is a retrograde spread of axonal swellings up the nerve and smaller myelinated and unmyelinated fibers become involved. The nerve terminal is unaffected until late in the process. The enlarged axons displace the paranodal myelin sheaths leaving denuded swellings in areas near the nodes of Ranvier. This process occurs before functional impairment is evident and can be reversed as swelling diminishes, and Schwann cells appear at these sites and remyelinate the axon. As exposure to *n*-hexane continues, axonal restoration and remyelination do not take place at some swellings, and the length of the nerve fiber between the swelling and the terminal undergoes breakdown, very similar to that seen when fibers are transected. Axon sprouting is often seen at the intact portion of a degenerated fiber even while intoxication continues. When intoxication ends, this regenerative process can reestablish motor and sensory function.

The nerve fibers most vulnerable to *n*-hexane exposure in rats were the branches of the tibial nerve serving the calf muscles of the hind limbs, followed in order by the plantar nerve branches supplying the flexor digitorum brevis muscle, and then sensory plantar nerve branches innervating the digits. As intoxication continued, axonal degeneration ascended the plantar and tibia1 nerves (Spencer and Schaumburg 1977b). Examination of control animals indicated that the most sensitive fibers were also the largest.

Effects on the central nervous system have also been observed in rats exposed to either *n*-hexane or its neurotoxic metabolite, 2,5-hexanedione. Axonal swelling and degeneration were observed in the anterior vermis, spinocerebellar tract in the medulla oblongata, and gracile tracts of the spinal cord after inhalation

exposure to *n*-hexane or drinking water exposure to 2,5-hexanedione (Spencer and Schaumburg 1977b). Axonal swellings were also observed in the gracile tract of the spinal cord at cervical levels in rats exposed to 700 ppm *n*-hexane (Altenkirch et al.1982). In both of these cases, severe peripheral neuropathy and axonal demyelination and/or degeneration were present. In other rat studies where central nervous system tissues were examined (brain, spinal cord), no changes were noted in a continuous exposure at 500 ppm for 6 months where gait disturbances developed (IRDC 1981) or in a 13-week exposure at up to 10,000 ppm for 6 hours a day where no clinical signs of neuropathy were observed (Cavender et al.1984). Mild atrophy (IRDC 1981) and axonal swelling (Cavender et al.1984) in peripheral nerve were observed in these studies. One of 15 male, but none of 15 female, rats exposed to 10,000 ppm in the Cavender (1984) study exhibited axonal swelling in the medulla. Evoked responses recorded in the brain (somatosensory, brainstem auditory, cortical auditory) exhibited increased latencies and decreased amplitude in rats exposed to 1,000 or 1,500 ppm *n*-hexane (Rebert and Sorenson 1983). Signs of peripheral neuropathy (reduced hindlimb grip strength) were also present. It appears that while central nervous system effects can be produced by *n*-hexane exposure in rats, the peripheral nervous system is more sensitive. It should also be noted that age-related changes in the central nervous system include axonal swelling and may resemble the early stages of *n*-hexane neurotoxicity (Bio/Dynamics 1978).

Neurological examinations of humans with *n*-hexane-induced peripheral neuropathy have not shown clinical signs of central nervous system toxicity (Herskowitz et al.1971; Yamamura 1969). There have been reports of altered evoked potentials recorded in the brain (increased latency, decreased amplitude) in humans occupationally exposed to *n*-hexane (Mutti et al.1982c; Seppalainen et al.1979). There has been one report of an individual occupationally exposed to *n*-hexane for 38 years who developed Parkinsonism (Pezzoli et al.1995), although the etiology of this case is complicated by the fact that the patient had a sister who was probably affected by Parkinsonism. Further studies, particularly prospective follow-up studies of exposed workers, are necessary before any conclusions can be drawn as to whether exposure to *n*-hexane causes central nervous system effects in humans.

Neurological effects are possible in humans exposed to *n*-hexane. For individuals living near hazardous waste sites, information on the air concentration of *n*-hexane would be necessary to predict the possibility of health effects.

Reproductive Effects. Reproductive effects have not been examined in humans after exposure to *n*-hexane. A dominant-lethal test in mice showed no effect on male fertility (Litton Bionetics 1980). No

effects were seen on reproductive tissues in male rats after intermediate-duration inhalation exposure at 500 ppm (IRDC 1981) or in either sex of mice after intermediate-duration inhalation exposure to up to 10,000 ppm *n*-hexane (Dunnick et al.1989; NTP 1991). However, inhalation exposure in male rats to higher concentrations of *n*-hexane showed effects after acute-duration exposure to 5,000 ppm (spermatid and spermatocyte degeneration and exfoliation) and atrophy of testicular germinal epithelium after intermediate-duration exposure to 1,000 ppm (De Martino et al.1987; Nylen et al.1989). Testicular atrophy in rats was also noted after intermediate-duration oral exposure at 4,000 mg/kg/day (Krasavage et al.1980). Similar to *n*-hexane neurotoxicity after inhalation exposure, effects on the testes in rats can be reproduced by oral administration of the *n*-hexane metabolite 2,5-hexanedione (Chapin et al.1982; Gillies et al.1981). It is currently unknown if similar effects might occur in humans exposed to *n*-hexane. These effects in animal experiments are always accompanied by severe neurotoxicity.

Developmental Effects. Developmental effects have not been examined in humans after exposure to *n*-hexane. Developmental effects were not observed in most acute-duration inhalation animal studies with *n*-hexane except for a temporary decrease in pup weight gain in offspring from pregnant rats exposed via inhalation to 1,000 ppm during gestation days 8-16 (Bus et al.1979), and a decrease in live fetuses per litter and female fetus weight in the offspring of pregnant mice exposed at 5,000 ppm (Mast et al.1988). No effects on offspring were seen in pregnant rats exposed via inhalation to 409 ppm *n*-hexane during gestation days 6-15 (Litton Bionetics 1979). Similar results were seen when pregnant mice were orally exposed to up to 2,830 mg/kg/day *n*-hexane during gestation days 6-1.5 (Marks et al.1980). Reduced fetal weight was seen in this study at 7,920 mg/kg/day; however, maternal toxicity was seen at this dose, so the effect may have been non-specific rather than developmental. Teratogenic effects have not been observed in any animal studies. Based on limited information, developmental effects do not seem likely in humans exposed to *n*-hexane.

Genotoxic Effects. Genotoxic effects have not been examined in humans after *n*-hexane exposure. The database on the genotoxicity potential of *n*-hexane is limited (see Tables 2-6 and 2-7). *n*-Hexane was negative in a dominant lethal test in mice by the inhalation route at up to 396 ppm *n*-hexane (Litton Bionetics 1980). Similar results were observed in another dominant lethal mutation study at higher concentrations in which male Swiss mice were exposed to up to 5,000 ppm *n*-hexane for 20 hours a day for 5 days (Mast et al.1989b). In an inhalation study, morphological alterations in sperm were noted in rats in at 5,000 ppm *n*-hexane (De Martino et al.1987). In contrast, sperm abnormalities were not observed in $B6C3F_1$ mice exposed to up to 5,000 ppm *n*-hexane for 20 hours a day for 5 days (Mast et al.

Table 2-6. *n*-Hexane Genotoxicity *In Vivo*

Species (test system)	End point	Results	Reference
Rat	Sperm morphology	+	De Martino et al. 1987
Mouse (bone marrow)	Sister chromatid exchange	–	NTP 1991
Mouse	Dominant lethal mutation	–	Litton Bionetics 1980
Mouse	Chromosomal exchanges	–	NTP 1991
Mouse	Micronuclei formation	–	NTP 1991
Mouse	Sperm morphology	–	Mast et al. 1989a
Mouse	Dominant lethal mutation	–	Mast et al. 1989b

+ = positive; – = negative

Table 2-7. *n*-Hexane Genotoxicity *In Vitro*

Test system	End point	Results		Reference
		With activation	Without activation	
Non-mammalian cells				
Escherichia coli				
WP2		–	–	McCarroll et al. 1981a
WP2 uvr A		–	–	McCarroll et al. 1981a
CM611		–	–	McCarroll et al. 1981a
WP67		–	–	McCarroll et al. 1981a
WP100		–	–	McCarroll et al. 1981a
WP110		–	–	McCarroll et al. 1981a
p3478		–	–	McCarroll et al. 1981a
Bacillus subtilis				
H17		–	–	McCarroll et al. 1981b
M45		–	–	McCarroll et al. 1981b
Salmonella typhimurium				
TA98	Reverse mutation	–	–	Mortelmans et al. 1986
TA100		–	–	Mortelmans et al. 1986
TA1535		–	–	Mortelmans et al. 1986
TA1537		–	–	Mortelmans et al. 1986
TA92	Reverse mutation	–	–	Ishidate et al. 1984
TA94		–	–	Ishidate et al. 1984
TA98		–	–	Ishidate et al. 1984
TA100		–	–	Ishidate et al. 1984
TA1535		–	–	Ishidate et al. 1984
TA1537		–	–	*Ishidate et al. 1984*
TA98	Reverse mutation	–	–	Houk et al. 1989
TA100		–	–	Houk et al. 1989
TA 98	Reverse mutation	–	–	NTP 1991
TA 100		–	–	NTP 1991
TA 1535		–	–	NTP 1991
TA 1537		–	–	NTP 1991

Table 2-7. *n*-Hexane Genotoxicity *In Vitro* (continued)

Test system	End point	Results		Reference
		With activation	Without activation	
Saccharomyces cerevisiae	Chromosome loss	–	–	Mayer and Goin 1994
Mammalian cells				
Human (lymphocytes)	Unscheduled DNA synthesis	–	–	Perocco et al. 1983
Hamster (CHO)	Chromosomal aberrations	–	–	NTP 1991
Hamster (CHO)	Sister chromatid exchanges	–	–	NTP 1991
Hamster (Chinese CHL)	Polyploidy	ND	+	Ishidate et al. 1984

– = negative; + = positive; ND = not detectable

1989a). Analysis of sperm obtained 5 weeks post-exposure showed no significant effects on morphology compared to the control group. There was no increase in the incidence of micronucleated normochromatic erythrocytes or polychromatic erythrocytes in the peripheral blood of male and female mice exposed via inhalation to 1,000, 4,000, or 10,000 ppm *n*-hexane, 6 hours a day, 5 days a week for 13 weeks or in mice exposed to 1,000 ppm for 22 hours a day for 13 weeks (NTP 1991). In an *in vivo* mouse bone marrow cytogenetics assay, doses of 500, 1,000, or 2,000 mg/kg *n*-hexane dissolved in corn oil and administered by intraperitoneal injection did not increase the incidence of sister chromatid exchanges; chromosomal aberrations were slightly increased, but this increase was not significant (NTP 1991). Results have generally been negative for *n*-hexane in bacterial tester strains such as *Escherichia coli, Bacillus subtilis,* and *Salmonella typhimurium* both with and without metabolic activation (Houk et al.1989; Ishidate et al. 1984; McCarroll et al.1981 a, 1981 b; Mortelmans et al.1986). In studies conducted by the National Toxicology Program (NTP 1991), *n*-hexane was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with a preincubation protocol at doses up to 1,000 μg/plate with or without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 fraction. *n*-Hexane was also negative in an *in vitro* test for induction of chromosome loss in *S. cerevisiae* (Mayer and Goin 1994).

Negative results were also obtained in mammalian cells except for one observation of polyploidy in Chinese hamster CHL cells (Ishidate et al.1984; Perocco et al.1983). Treatment at doses up to 5,000 μg/mL in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 did not induce chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells. Sister chromatid exchanges were induced in CHO cells but only in the presence of S9; no dose-response was apparent (NTP 1991).

The *n*-hexane metabolite 2,5-hexanedione was strongly positive in an *in vitro* test for induction of chromosome loss in *S. cerevisiae* (Mayer and Goin 1994). It was suggested that this effect was due to an effect of 2,5-hexanedione on microtubule function in the yeast cells, resulting in faulty segregation of chromosomes.

Cancer. There is currently little information on the carcinogenic potential of *n*-hexane. No epidemiological studies were located addressing whether there is or is not an association between occupational *n*-hexane exposure and cancer. In a chronic-duration study in $B6C3F_1$ mice (50/sex/group) with exposure to commercial hexane (51.5% *n*-hexane) for 6 hours/day, 5 days a week for 2 years, a statistically significant treatment-related increase in hepatocellular neoplasms (adenoma and carcinoma)

was observed among females exposed at 9,018 ppm (Bio/Dynamics 1995b). Incidences of adenoma were: 4/50, 6/50, 4/50, and 10/50 at 0, 900, 3,000, and 9,018 ppm, respectively. Incidences of carcinoma at these exposures were 3/50, 2/50, 5/50, and 6150, and incidences of total neoplasms were 7150, 8/50, 9/50, and 16/50. In males, liver tumors were observed but were not treatment-related. In the 9,018 ppm group of females, liver tumor incidence was similar to control males. A significant treatment-related decrease in severity of cystic endometrial hyperplasia of the uterus was also observed among females in the 9,018 ppm group. The authors suggested that the decrease in severity of cystic endometrial hyperplasia may indicate a possible treatment-related alteration in the hormonal balance (e.g., a decrease in estrogenic stimulation of the uterus), resulting in the female mice showing the normal incidence of male liver neoplasms. It is not clear what components of the hexane mixture caused the neoplasms. A parallel experiment carried out on rats showed no increase in incidence of neoplasms at any site (Bio/Dynamics 1995a). Papillary tumors have been reported in the bronchiolar epithelium of rabbits after a 24-week exposure to 3,000 ppm *n*-hexane in a study designed to assess respiratory effects (Lungarella et al.1984). *n*-Hexane does not contain any structural alerts associated with carcinogenicity (Ashby 1985) and, as mentioned above, has not been found to be mutagenic. *n*-Hexane has not been categorized as to its carcinogenic potential by the IARC, EPA, or DHHS.

Toxicity of mHexane Metabolites. Since *n*-hexane is metabolized in the body, exposure also occurs to metabolites. The neurotoxicity of *n*-hexane is believed to ultimately result from the effects of one of these metabolites, 2,5-hexanedione, on peripheral nerves (see Section 2.4, Mechanisms of Action). One potential metabolite, 2-hexanone, has also caused neurotoxicity in humans (Allen et al.1975). The other metabolites of *n*-hexane (see Figure 2-3) can also produce neurotoxicity in rats via their subsequent metabolism to 2,5-hexanedione (Krasavage et al.1980). No information was located regarding other mechanisms of toxicity for these metabolites.

2,5-Hexanedione causes a peripheral neuropathy in rats virtually identical to that caused by inhalation of *n*-hexane when administered in drinking water at a concentration of 0.5% (Schaumburg and Spencer 1975; Spencer and Schaumburg 1977a, 1977b). The time to onset of peripheral neuropathy was about 12 weeks. No significant differences in histopathology of peripheral or central nerves were noted between oral exposure to 2,5-hexanedione and inhalation exposure to *n*-hexane.

2,5-hexanedione can also affect testicular tissue in male rats and is, in fact, used as a model for chemically induced sterility (Chapin et al.1982; Krasavage et al.1980). Exposure to drinking water containing 1%

2,5-hexanedione results in severe seminiferous epithelial degeneration and loss of germ cells. In a group of rats receiving a single dose of 2,000 mg/kg 2,5-hexanedione (Linder et al.1992), no histopathological changes were detected 2 days after treatment; however, at 14 days, testicular debris was observed in the proximal caput, sloughed epididymal cells were observed in the cauda lumen as was retention at the lumen and base of Step 19 spermatids in Stages IX-XII.

2.6 CHILDREN'S SUSCEPTIBILITY

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

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 5.6, Exposures of Children.

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

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

Certain characteristics of the developing human may increase exposure or susceptibility while others may decrease susceptibility to the same chemical. For example, the fact that infants breathe more air per kilogram of body weight than adults may be somewhat counterbalanced by their alveoli being less developed, so there is a disproportionately smaller surface area for absorption (NRC 1993).

Cases of *n*-hexane toxicity in humans have occurred as the result of workplace exposure and solvent abuse (Spencer et al.1980). Some of these cases of peripheral neuropathy have occurred in teenagers (particularly with solvent abuse); however, none of the clinical reports indicate differences in physical signs or functional tests between this group and adults (Altenkirch et al.1977; Yamamura et al.1969). While no reports of *n*-hexane toxicity in young children were located, it is probable that similar toxicity would occur if exposure was comparable to that in affected adults. Specific information is not available on whether children are more susceptible than adults to the effects of *n*-hexane.

Animal studies provide limited further information. Only 2 studies were located where the responses to *n*-hexane were compared between young animals and adults. In a study in rats directly comparing the effects of *n*-hexane exposure in weanlings (21 days old) and young adults (80 days old) (Howd et al.1983), peripheral neuropathy occurred in both groups, although onset was more rapid in the young adult group. No deaths were observed over the 11-week exposure period and 3-week recovery period in weanling rats. In young adults, however, 5 of 10 rats died as the result of severe neuropathy. The authors suggested that the relative resistance of the weanling rats may have been due to shorter, smaller-diameter axons, or to a greater rate of growth and repair in their peripheral nerves compared to those of adults. An oral LD_{50} study showed 14-day-old rats were more susceptible to the acute effects of a large dose of *n*-hexane than young

adults (Kimura et al.1970). LD_{50} values for *n*-hexane were 15,840 mg/kg for 14 day olds and 32,340 mg/kg for the young adults. Clinical signs and time to death were not reported.

n-Hexane has not caused teratogenic effects in rodent models (Bus et al.1979; Litton Bionetics 1979; Marks et al.1980; Mast et al.1987; Mast et al.19SS), although some developmental effects have been reported in mice (decreased live fetuses per litter, female fetus weight, gravid uterine weight) exposed during pregnancy to 5,000 ppm (Mast et al.1988). Observation of the offspring after birth to maturity was not performed. There is one report of delayed histogenesis of the cerebellar cortex in the offspring of rats exposed during pregnancy to 500 ppm *n*-hexane during the first 30 postnatal days (Stoltenburg-Didinger et al.1990). The number of offspring examined was not reported, so it is difficult to assess the significance of this report. *n*-Hexane has not been tested in *in vitro* developmental systems. No information is available on whether parental exposure to *n*-hexane can cause transgenerational effects in children. This appears unlikely since *n*-hexane has tested negative for genotoxicity in a number of *in vivo* and *in vitro* tests. One area of potential concern is the finding that very high air concentrations of *n*-hexane (±1,000 ppm) administered for 21-24 hours a day result in signs of testicular damage in rats (De Martino et al.1987; Nylen et al.1989). These signs are also found in rats after large oral doses (Krasavage et al.1980) and the administration of the *n*-hexane metabolite 2,5-hexanedione in drinking water (Chapin et al.1982; Gillies et al.1981). Severe neurotoxicity was evident in all these cases. It is not known whether or not this is a species specific effect, since examination of sperm in a worker population with exposure to *n*-hexane and elevated 2,5 hexanedione urinary levels has not been reported.

No information is available as to whether *n*-hexane or its metabolites cross the placenta in humans. Transfer across the placenta has been demonstrated in rats for *n*-hexane and two resulting metabolites, 2-hexanone and 2,5-hexanedione (Bus et al.1979); no preferential distribution to the fetus was observed for either *n*-hexane or the metabolites. Due to its relatively rapid metabolism, storage of *n*-hexane in body fat does not appear to occur at air concentrations to which humans are exposed; thus, there is unlikely to be mobilization of maternally stored *n*-hexane upon pregnancy or lactation. *n*-Hexane has been detected in samples of human breast milk (Pellizzari et al.1982); however, *n*-hexane was not quantified, nor was any attempt made to assess the subjects' exposure. A human milk/blood partition coefficient of 2.10 (Fisher et al.1997) indicates there would be preferential distribution to this compartment if significant absorption occurred; however no pharmacokinetic experiments have been done to confirm that *n*-hexane or its metabolites are actually transferred to mammalian breast milk. No information is available on *n*-hexane metabolites in breast milk. A PBPK model has been developed that simulates the transfer of *n*-hexane from

a mother to a nursing infant during and after occupational exposure via inhalation (Fisher et al.1997). Blood/air and milk/air partition coefficients were determined with samples from volunteers. Simulations were run over a 24-hour period at the *n*-hexane Threshold Limit Value (TLV) for workplace exposure of 50 ppm, assuming a 9-hour working period containing 2 half-hour and one 1-hour break periods and 8 nursing periods over 24 hours. Total *n*-hexane ingested in milk was compared to the EPA Health Advisory Intake for chronic ingestion of contaminated water by 10-kg children. The model predicted ingestion at the rate of 0.052 mg/day compared to the EPA advisory intake of 4 mg/day.

No information is available on the toxicokinetics of *n*-hexane in children or in young animals compared to adult animals. No information is available as to whether metabolism of *n*-hexane in children differs from that of adults. No studies were located comparing metabolism in young and adult animals. The toxicity of *n*-hexane results from biotransformations yielding the active metabolite 2,5-hexanedione. The initial step is an oxidation to 2-hexanol catalyzed by a cytochrome P-450 enzyme. Some P-450 enzymes are developmentally regulated (Leeder and Kearns 1997); however, it is not completely clear which P-450 enzymes are involved in *n*-hexane metabolism.

No information is available on whether biomarkers for exposure or effect of *n*-hexane validated in adults (exhaled *n*-hexane, 2,5-hexanedion in urine) also are valid for children. Interactions of *n*-hexane with other chemicals have not been reported in children, but have occurred in adults (Altenkirch et al.1977). Since interactions in adults are dependent on toxickinetic parameters, predicting interactions in children requires greater understanding of the metabolism of *n*-hexane in children.

2.7 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of

biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to *n*-hexane are discussed in Section 2.7.1.

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 *n*-hexane are discussed in Section 2.7.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.9, Populations That Are Unusually Susceptible.

2.7.1 Biomarkers Used to Identify or Quantify Exposure to *n*-Hexane

n-Hexane can be measured in exhaled breath during and following exposure (Mutti et al.1984; Raymer and Pellizzari 1996; Veulemans et al.1982). At exposure concentrations of 100-200 ppm, *n*-hexane can probably be detected in exhaled air for about 12-24 hours. While this is the most direct method to identify and quantify exposure to *n*-hexane, these measurements require specialized equipment and are used mainly in research studies.

2. HEALTH EFFECTS

Exposure to *n*-hexane results in the production of metabolites by microsomal oxidative enzymes in the liver. The major metabolite appearing in the urine is the neurotoxic metabolite 2,5-hexanedione. The amount of this metabolite in urine has shown a good correlation with concentrations of *n*-hexane in the workplace air (Mutti et al.1984). Urinary metabolite concentrations were lowest at the beginning of the shift, highest at the end of the shift, and still elevated the next morning. There was a strong correlation (r=0.967) between time-weighted average *n*-hexane air concentration and end-of-shift 2,5-hexanedione in the urine; end-of-shift samples gave the best estimate of overall exposure. In this study, it was found that about 3 mg 2,5-hexanedione/g creatinine would correspond to about 50 ppm of *n*-hexane in the air, the PEL proposed by OSHA in 1989, but struck down in court.

Since *n*-hexane and its metabolites are cleared from the body within a few days, a test for 2,5-hexanedione in the urine is only a biomarker for recent exposure. Another neurotoxic solvent, 2-hexanone (methyl *n*-butyl ketone), also has 2,5-hexanedione as a metabolite, hence exposure to this chemical would have to be ruled out before exposure to *n*-hexane could be confirmed. 2-Hexanone is also a metabolite of *n*-hexane but is present in much smaller quantities in urine after exposure than is 2,5-hexanedione (Fedtke and Bolt 1987).

2,5-Hexanedione is usually measured as *"*total*"* 2,5-hexanedione, a free form accounting for about 10% of the total and 4,5-dihydroxy-2-hexanone, which is converted to 2,5-hexanedione upon acid treatment (acidification of urine samples is routinely performed in order to hydrolyze conjugates that can interfere with analysis). 2,5-Hexanedione has also been detected after acid treatment of urine from individuals not occupationally exposed to *n*-hexane (Fedtke and Bolt 1986a; Perbellini et al.1993). A reference value for 2,5-hexanedione in acid-treated urine in a non-occupationally exposed Italian population (n= 123, 60 males, 63 females) has been determined (Bavazzano et al.1998). This value, defined as the upper unilateral 95% tolerance interval at 95% confidence, was 0.795 mg 2,5-hexanedione/L in males and 0.627 mg/L for females. It is possible that small amounts of *n*-hexane are produced in the body by lipid peroxidation, as has been demonstrated for *n*-pentane (Filser et al.1983). Urinary excretion of 2,5-hexanedione ranged from 0.3 to I .2 mg in 24 hours for unexposed individuals. Workers exposed to approximately 50 ppm *n*-hexane excreted 3-4 mg/24 hours (Perbellini et al.1993).

Pyrrolidation of proteins appears to be a necessary step in *n*-hexane neurotoxicity, and the targets relevant to toxicity are thought to be neuronal axon proteins (Graham et al.1995). However, *n*-hexane metabolites can pyrrolidate a variety of proteins at lysine residues, which upon oxidation can become crosslinked.

Pyrrolidated proteins in rat hair have been measured after intraperitoneal administration of 2,5-hexanedione (Johnson et al.1995). Serial analysis of nose hairs taken during 2,5-hexanedione administration showed a progression with time of the region staining positively for pyrroles. This method may eventually be useful as a biomarker for past exposure to *n*-hexane in humans. A more sensitive and rapid biomarker for 2$hexanedione exposure is the crosslinking of erythrocyte spectrin, where the altered migration of crosslinked spectrin is easily observable in polyacrylamide gels (Anthony et al.1983). Further research is needed to determine whether exposure to *n*-hexane also results in adduct formation and/or crosslinking of spectrin via metabolism to 2,5-hexanedione.

2.7.2 Biomarkers Used to Characterize Effects Caused by *n*-Hexane

There are currently no subtle or sensitive biomarkers of effects associated with exposure to *n*-hexane, although this is an active area of research. Electroneuromyographic testing may prove useful in the detection of nerve conduction abnormalities in their early stages before they are accompanied by clinical manifestations. In a study of 15 women who had been exposed to *n*-hexane in a shoe factory, all nerve conduction velocities (motor and sensory) were significantly slowed in exposed workers compared to controls (Mutti et al.1982b); the effects of the *n*-hexane may have been exacerbated by co-exposure to methyl ethyl ketone. None of these women had clinical signs of peripheral neuropathy. In a study of workers with relatively high urinary 2,5-hexanedione levels (indicating exposure), clinical exams were negative for neuropathy (Pastore et al.1994). Sensory and motor nerve conduction velocities and distal latencies were normal in all nerves tested; however, significant decreases were found in sensory nerve action potential amplitude when compared with an unmatched control group. Neither the level of 2,5-hexanedione in urine nor the age of the workers correlated with the changes in amplitude; however, there was a significant correlation between years worked and decreased amplitude. In contrast, no correlation was found with the length of exposure in another study of asymptomatic workers where 14 of 40 showed abnormalities on electrophysiological testing. Levels of 2,5-hexanedione in the urine correlated with a numerical index for abnormalities (Governa et al.1987).

Pyrrolidation and crosslinking of proteins can be considered biomarkers of either exposure or effect and are discussed in the previous section.

For more information on biomarkers for renal and hepatic effects of chemicals see *ATSDR/CDC Subcommittee Report on Biological indicators* of Organ Damage (1990) and for information on biomarkers for neurological effects see OTA (1990).

2.8 INTERACTIONS WITH OTHER CHEMICALS

Because many other chemicals can affect the enzymes responsible for *n*-hexane metabolism (see Section 2.3.3, Metabolism), the possibility of interactions is a significant concern. The initial step in *n*-hexane metabolism is oxidation to a hexanol by a cytochrome P-450 isozyme; other chemicals can induce these enzymes, possibly increasing the rate of metabolism to the neurotoxic 2,5-hexanedione, or competing with *n*-hexane and its metabolites at enzyme active sites, reducing the rate of metabolism. Interactive effects can be concentration and/or duration dependent.

Altering the *n*-hexane concentration of a paint thinner appears to have been the cause of an outbreak of peripheral neuropathy in Berlin in the 1970s (Altenkirch et al.1977). In a case series of glue-sniffers in Berlin, the neurological symptoms consisted of a symmetrical, progressive, ascending, mainly motor, neuropathy with pronounced muscle atrophy. The height of the disease was reached after 1½ to 2½ months and resulted in quadriplegia in 7 of 17 patients. After 8 months, all patients still had a motor deficit. Nerve biopsy showed paranodal axon swelling, dense masses of neurofilaments, and secondary myelin retraction. The formulation of the thinner had been changed shortly before illness occurred. The *n*-hexane proportion was reduced from 31 to 16%, but methyl ethyl ketone had been added. The authors hypothesized that methyl ethyl ketone had caused a synergistic effect to occur, resulting in *n*-hexane neurotoxicity. In experiments with male Wistar rats, co-exposure to *n*-hexane and methyl ethyl ketone for 9 weeks resulted in an earlier onset of signs of neurotoxicity than with *n*-hexane alone (Altenkirch et al. 1982). Similarly, co-exposure to 2,000 ppm *n*-hexane and 2,000 ppm methyl ethyl ketone over 20 weeks significantly enhanced clinical and electrophysiological signs of neurotoxicity in Wistar rats compared to 2,000 ppm *n*-hexane alone (Ichihara et al.1998). This was accompanied by an approximate doubling in urinary 2,5-hexanedione concentrations.

The potentiation of *n*-hexane neurotoxicity by co-exposure to methyl ethyl ketone may be duration-dependent, as suggested by an experiment in volunteers (Van Engelen et al.1997). Simultaneous exposure to 60 ppm *n*-hexane and either 200 or 300 ppm methyl ethyl ketone for 15.5 minutes had no effect on exhaled *n*-hexane concentrations, and actually lowered 2,5-hexanedione serum concentrations about 3-fold.

Time to peak 2,5-hexanedione concentrations was approximately doubled (18-30 minutes). These results are consistent with methyl ethyl ketone inhibiting the metabolism of *n*-hexane during a single acute exposure. Oral exposure to methyl ethyl ketone prior to inhalation exposure to *n*-hexane significantly increased blood levels of the neurotoxic metabolite, 2,5-hexanedione in Fischer 344 rats (Robertson et al. 1989).

The interaction of *n*-hexane with toluene and trichloroethylene has also been examined in volunteers (Baelum et al.1998). Exposure in these experiments was via a gastric feeding tube at controlled rates equivalent to what the authors stated would be delivered to the liver by inhalation exposure at Danish occupational exposure limits (50 ppm *n*-hexane, 50 ppm toluene, and 30 ppm trichloroethylene). Coexposure to toluene and trichloroethylene slightly increased the area under the curve (AUC) representing concentration versus time for end exhaled *n*-hexane air concentration, but urinary excretion of 2,5-hexanedione was unchanged. The only statistically significant interaction observed with *n*-hexane was an 18% decrease in the urinary excretion of hippuric acid, a toluene metabolite.

Indirect evidence for an effect of co-exposure to acetone on *n*-hexane metabolism in humans has been described (Cardona et al.1996). In this study, the relationship between free and total 2,5-hexanedione (2,5-hexanedione and 4,5-dihydroxy-2-hexanone, See Section 2.7) in urine and workplace air concentrations of *n*-hexane, hexane isomers, acetone, and toluene was analyzed in a group of 87 workers. Median *n*-hexane concentrations were 47 mg/m^3 (range, 4-652 mg/m^3 [13 ppm; range, 1-185 ppm]) and median acetone concentrations (only 70 of the 87 workers were exposed) were 109 mg/m^3 (range, 1-1,826 mg/m^3 [46 ppm; range, 0.4-769 ppm]). A statistically significant correlation was found between air levels of acetone and the ratios of free and total 2,5-hexanedione to air levels of *n*-hexane. Multiple regression analysis indicated that at a given level of *n*-hexane exposure, co-exposure to acetone increases the level of free 2,5-hexanedione in urine while reducing the level of 4,5-dihydroxy-2-hexanone.

Oral administration of acetone has been reported to potentiate the neurotoxicity caused by oral exposure to the *n*-hexane metabolite 2,5-hexanedione in rats (Ladefoged et al.1989, 1994). Oral exposure to acetone alone in rats at 650 mg/kg/day resulted in a statistically significant decrease in motor nerve conduction velocity after 6 weeks; co-exposure to acetone and 2,5-hexanedione resulted in greater effects than those seen with 2,5-hexanedione alone (Ladefoged et al.1989). It is possible that acetone may potentiate *n*-hexane neurotoxicity by decreasing body clearance of 2,5-hexanedione (Ladefoged and Perbellini 1986). Acetone also influences the action of many chemicals by its induction of the cytochrome P-450 isozyme

CYP2E1 (Patten et al.1986). *n*-Hexane is metabolized by P-450 isozymes (see Section 2.4), so induction by acetone may result in an increased production of the neurotoxic metabolite 2,5-hexanedione.

Co-exposure to *n*-hexane and xylene resulted in a loss of auditory sensitivity in male Sprague-Dawley rats (Nylen et al.1994) as measured by the auditory brainstem response. Exposure to *n*-hexane or xylene alone at 1,000 ppm for 61 days for 18 hours a day caused a slight loss of auditory sensitivity when measured 2 days after the end of exposure. Simultaneous exposure to *n*-hexane and xylene (1,000 ppm each) caused a greater and persistent loss of auditory sensitivity which was greater than the sum of effects of exposure to *n*-hexane and xylene separately. These effects were still observed 4 and 10 months after exposure ended. In contrast, combined exposure to *n*-hexane and xylene partially reversed the decreased nerve conduction velocities and action potential amplitudes observed in the group treated with *n*-hexane alone. These effects were persistent from 2 days to 10 months after cessation of exposure.

In a similar experiment with *n*-hexane and toluene (Nylen and Hagman 1994), a reduction in auditory sensitivity compared to controls was observed 2 days after exposure to toluene with *n*-hexane (1,000 ppm each), but not after exposure to *n*-hexane alone. Loss of sensitivity was 5±7 decibels (dB) in the *n*-hexane alone group, 24±11 dB in the toluene alone group, and 31±16 dB in the combined group. The loss in the combined group was significantly higher than in the toluene alone group. The reduction lasted one year after the exposure. Exposure to *n*-hexane alone caused a marked decrease in peripheral nerve conduction velocities. Co-exposure to *n*-hexane and toluene prevented these effects.

In a study where both peripheral and central nervous system effects were measured in rats co-exposed to *n*-hexane and toluene (Pryor and Rebert 1992), toluene exposure at 1,400 ppm for 14 hours a day for 9 weeks prevented the peripheral neurotoxicity (decreased grip strength and nerve conduction velocities) caused by exposure to 4,000 ppm *n*-hexane alone. There was no reciprocal action of *n*-hexane on the motor syndrome (shortened and widened gait and widened landing foot splay) and hearing loss caused by toluene. Brainstem auditory response amplitudes were decreased by *n*-hexane, co-exposure to toluene did not block this effect.

Co-exposure to approximately equal concentrations of xylene or toluene (Nylen et al.1989) has also prevented *n*-hexane-induced testicular atrophy in Sprague-Dawley rats. The protective effects of xylene and toluene on peripheral neuropathy and testicular atrophy caused by *n*-hexane may result from competition for metabolism, resulting in a slowing of *n*-hexane conversion to 2,5-hexanedione.

2.9 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to *n*-hexane than will most persons exposed to the same level of *n*-hexane in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters may result in reduced detoxification or excretion of *n*-hexane, or compromised function of target organs affected by *n*-hexane. Populations who are at greater risk due to their unusually high exposure to *n*-hexane are discussed in Section 5.7, Populations With Potentially High Exposure.

No population has been identified which is unusually susceptible to toxic effects resulting from *n*-hexane exposure. It is possible that individuals with diminished peripheral nerve function may be more susceptible to *n*-hexane neurotoxicity than the general population. This group would include diabetics, alcoholics and the aged.

2.10 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to *n*-hexane. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to *n*-hexane. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to *n*-hexane.

R.H. Dreisbach, ed.1987. Handbook of Poisoning. Appleton and Lange, Norwalk.

L.M. Haddad and J.F. Winchester, eds.1990. Clinical Management of Poisoning and Drug Overdose, 2nd. edition. W.B. Saunders, Philadelphia.

C.K. Aaron and M.A. Howland, eds.1994. Goldfrank’s Toxicologic Emergencies, 5th edition. Norwalk.

Treatment information specific to *n*-hexane exposure was not located. Treatment of acute exposure to *n*-hexane would be similar to that for other aliphatic hydrocarbons.

2.10.1 Reducing Peak Absorption Following Exposure

Because of the high volatility of liquid *n*-hexane, the most likely route of exposure is via inhalation. In this case, removal of the individual from the source of the *n*-hexane halts absorption. Residual *n*-hexane in alveolar air would leave the lung within a few minutes. In the case of ingestion, the major danger with *n*-hexane (and other liquid aliphatic hydrocarbons) is not absorption from the gastrointestinal tract, but rather the risk of chemical pneumonitis after aspiration into the lungs (Ervin and Manske 1990). Since this type of aspiration can occur during vomiting, induction of emesis is not recommended. An exception would be in the case where the *n*-hexane contained other potentially toxic agents (e.g., pesticides, organosoluble metal compounds). Careful gastric lavage may be necessary in this situation (Klein and Simon 1986). No binding agents have been identified for the aliphatic hydrocarbons. In the case of dermal exposure, removal of *n*-hexane from the skin with large amounts of soap and water would reduce absorption by this route.

2.10.2 Reducing Body Burden

Neither *n*-hexane nor its metabolites are retained to any significant extent by the body, so methods to reduce the body burden would not be necessary in the treatment of toxic effects caused by *n*-hexane exposure.

2.10.3 Interfering with the Mechanism of Action for Toxic Effects

n-Hexane toxicity appears to be caused by the action of one of its metabolites, 2,5-hexanedione, on the cytoskeletal structures of axons in peripheral nerve. The damage in the individual axon is cumulative as long as exposure continues and eventually affects action potential conduction, producing clinical signs of peripheral neuropathy. The initial step in this process appears to be pyrrolidation of protein followed by cross-linking. While this is a potential step for interfering with the mechanism of action for toxic effects, no methods currently exist that would accomplish this. Pyrrole-to-pyrrole crosslinking can be inhibited in vitro by thiol-containing compounds including *N*-acetylcysteine and glutathione (Zhu et al.1995). Future research might identify a way to deliver these inhibitory compounds to the site of pyrrole crosslinking. Additionally, any method that would reduce peak serum concentrations of 2,5-hexanedione after *n*-hexane exposure would also be useful, but none were identified.

2.11 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 *n*-hexane is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of *n*-hexane.

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

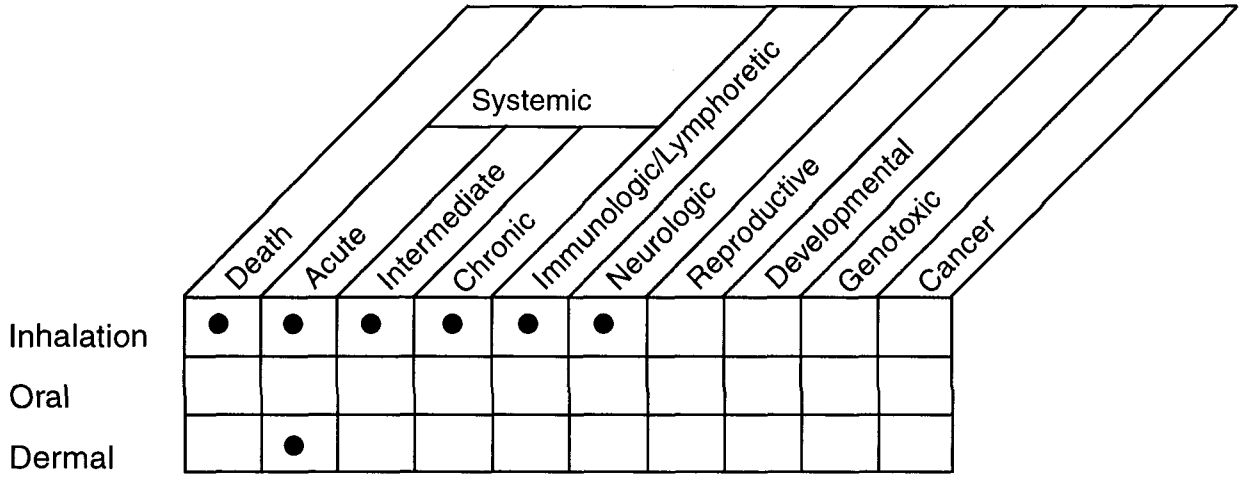
2.11.1 Existing Information on Health Effects of *n*-Hexane

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to *n*-hexane are summarized in Figure 2-8. The purpose of this figure is to illustrate the existing information concerning the health effects of *n*-hexane. 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* Toxicol*ogical Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

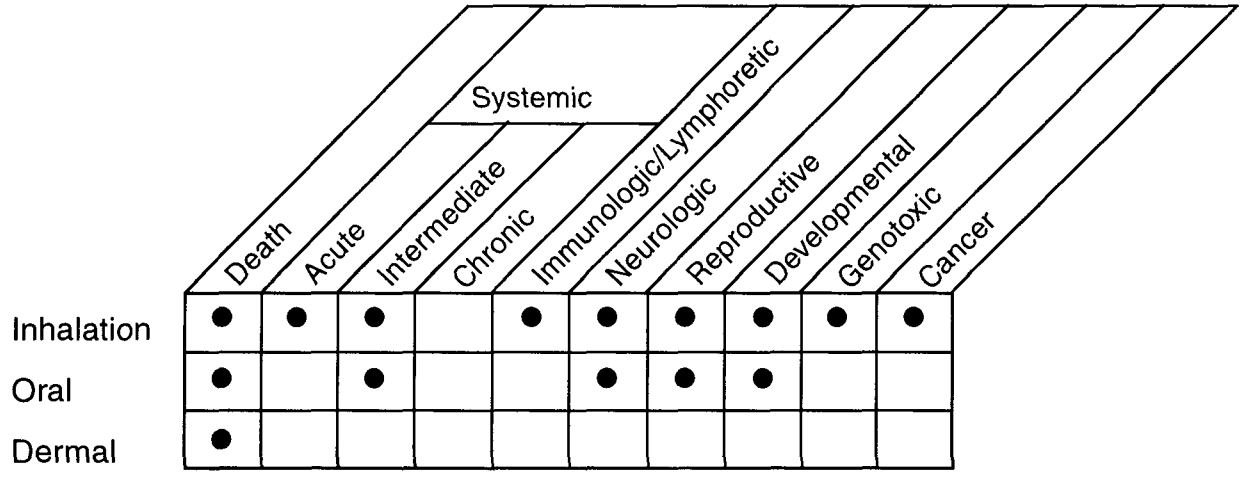
2.11.2 Identification of Data Needs

Acute-Duration Exposure. *n*-Hexane appears to be of low acute toxicity. *n*-Hexane in air is not irritating to humans (Nelson et al.1943) and the only human health effect reported after acute-duration exposure is dermal irritation with undiluted liquid *n*-hexane (Wahlberg 1984). No reports of oral toxicity to *n*-hexane in humans were located. Oral LD_{50} values in Sprague-Dawley rats range from 15,840 to

Figure 2-8. Existing Information on Health Effects of *n*-Hexane



Human



Animal

● Existing Studies

32,340 mg/kg, depending on age (Kimura et al.1971). No deaths have been reported in animals after acute-duration inhalation exposure at concentrations up to the practical upper limit for experimental *n*-hexane exposure of 10,000 ppm (Dunnick et al.1989; NTP 1991). Respiratory effects (rales, gasping, mouth breathing) have been reported in rabbits during acute-duration inhalation exposure at 3,000 ppm (Lungarella et al.1984) and a reduction in motor nerve conduction velocity in rats after a 1-week exposure to 5,000 ppm *n*-hexane (De Martino et al.1987). Reproductive effects in male rats (altered sperm morphology) have been reported after a 24-hour exposure to 5,000 ppm (De Martino et al.1987). Existing data are insufficient to derive an acute-duration MRL for any route of exposure. Acute-duration inhalation studies may be useful to establish threshold levels and dose-response relationships for the reproductive and neurological effects seen in the De Martino et al. (1987) study. Although no thorough studies of acute inhalation effects have been done, except for developmental end points, the intermediate and chronic database would not suggest any particular end points of concern besides neurological and reproductive effects. Oral exposure is an unlikely route for human exposure due to the volatility of *n*-hexane, so acute-duration oral studies are not needed as critically. However, with deeply buried waste or leaking underground storage tanks, private drinking well water (municipal treatment is likely to volatilize all *n*-hexane at the plant) could become contaminated with *n*-hexane, so oral drinking water studies might be appropriate. The solubility of *n*-hexane in water is very low (9.5 mg/L), so it is questionable whether test animals would ingest enough *n*-hexane to result in any toxic response. Calculations would need to be made on the fluid intake of the animal model before it can be determined if such studies can be conducted. In rat gavage studies, doses ≥1,000 mg/kg/day were necessary to produce neurological effects (Krasawage et al.1980; Ono et al.1981). An acute-duration dermal study in animal models may be useful to obtain toxicokinetic data for this exposure route. There is virtually no data on dermal toxicity, although, again because of the volatility of *n*-hexane, exposure by this route is unlikely and acute-duration toxicity studies are not needed as critically. There is little pharmacokinetic data (one study on *in vitro* skin permeability and one study measuring 2,5-hexanedione levels after oral exposure) on oral or dermal routes of exposure, nor does the Perbellini PBPK model address these routes of exposure, so there is little basis for extrapolating from the target organs of inhalation exposure to the identification of target organs of oral and dermal exposure. However, intermediate-duration toxicity data that does exist would suggest that acute oral exposure targets of particular concern are also the nervous and male reproductive systems.

Intermediate-Duration Exposure. Case studies of occupational exposure to *n*-hexane by the inhalation route show that neurotoxicity can develop in humans over this duration period (Altenkirch et al. 1977; Wang et al.1986; Yamamura 1969). Peripheral neuropathy (both sensory and motor) was the major

finding; muscle wasting and atrophy were also observed. No reports of human neurotoxicity after oral or dermal exposure to *n*-hexane were located. Repeated exposures in animals show that a similar form of neurotoxicity can also be produced experimentally, with the rat being the most sensitive species (Altenkirch et al.1982; De Martino et al.1987; Dunnick et al.1989; Frontali et al.1981; Huang et al.1989; IRDC 1981; NTP 1991; Schaumburg and Spencer 1976; Takeuchi et al.1980). *n*-Hexane neurotoxicity can also be induced over the intermediate duration via the oral route in rats at 4,000 mg/kg/day (Krasavage et al. 1980). In another oral exposure study at 1,251 mg/kg/day, decreases in nerve conduction velocity, but no clinical signs of peripheral neuropathy, were observed (Ono et al.1981). Deaths were observed in pregnant mice receiving 2,200-2,830 mg/kg/day, although developmental effects were not observed (Marks et al. 1980).

Reproductive effects (testicular atrophy, degeneration) have been observed in male rats after intermediateduration inhalation exposure at 1,000 ppm *n*-hexane and at 5,000 ppm (De Martino et al.1987; Nylen et al.1989). Atrophy of the testicular germinal epithelium was also observed in an oral exposure in rats at 4,000 mg/kg/day (Krasavage et al.1980). Severe neurotoxicity occurred in all these studies. These effects were not observed in studies in rats using lower concentrations of *n*-hexane or in mice exposed via inhalation to up to 10,000 ppm for 13 weeks (Dunnick et al.1989; NTP 1991). A decrease in ventricular fibrillation potential has been observed in perfused hearts from rats exposed subcutaneously for an intermediate duration to *n*-hexane (Khedun et al.1996).

The critical effect of intermediate-duration exposure to *n*-hexane in humans is neurotoxicity, specifically peripheral neuropathy. No inhalation MRL was derived for this duration because the reports of neurological effects in humans were predominantly case reports with inadequate documentation of exposure levels or comparison with unexposed groups. A large database on neurological effects in rats exists for this duration; however, the design of these experiments precluded documentation of clear dose-response relationships within a single study. Because of the limited database for oral exposure to *n*-hexane and the lack of toxicokinetic data for this route, no MRL was derived for oral exposure to *n*-hexane.

Intermediate-duration inhalation studies establishing a threshold and dose-response for neurological and male reproductive effects in the rat would be useful since this is an area of potential concern for humans exposed to *n*-hexane occupationally or near hazardous waste sites. The only other effects documented after inhalation exposure in animal models for this duration are respiratory effects, and these occur at *n*-hexane levels far above any plausible human exposure; thus, studies on this end point are not necessary. An

intermediate-duration inhalation exposure experiment that measures ventricular fibrillation potential may be useful to determine if results seen after subcutaneous exposure (Khedun et al.1996) can be reproduced by an exposure route relevant to humans.

Oral exposure is an unlikely route for human exposure due to the volatility of *n*-hexane, so intermediateduration oral studies are not needed as critically. However, with deeply buried waste or leaking underground storage tanks, private drinking weil water (municipal treatment is likely to volatilize all *n*-hexane at the plant) could become contaminated with *n*-hexane, so oral drinking water studies might be appropriate. The solubility of *n*-hexane in water is very low (9.5 mg/L), so it is questionable whether test animals would ingest enough tz-hexane to result in any toxic response. Intermediate-duration oral studies may be useful to establish threshold levels and dose response relationships for the reproductive and neurological effects already observed. There is little pharmacokinetic data (one study on *in vitro* skin permeability) for dermal exposure, nor does the Perbellini PBPK model address this route of exposure, so there is little basis for extrapolating from the target organs of inhalation exposure to the identification of dermal exposure targets. There is virtually no data on dermal toxicity, although, again because of the volatility of *n*-hexane, exposure by this route is unlikely and intermediate-duration toxicity studies are not needed as critically.

Chronic-Duration Exposure and Cancer. For chronic-duration exposure, case studies of occupational exposure to *n*-hexane by the inhalation route show that neurotoxicity can develop in humans over this duration period (Yamamura 1969; Wang et al.1986). Peripheral neuropathy (both sensory and motor) was the major finding; muscle wasting and atrophy were also observed. Subclinical effects on nerve conduction velocity and evoked potential response have also been reported over this duration (Mutti et al. 1982a, 1982b; Sanagi et al.1980; Seppalainen et al.1979). An MRL of 0.6 ppm has been derived for this duration exposure based on a study of workers exposed to approximately 58 ppm *n*-hexane for an average of 6 years. A small decrease in motor nerve conduction velocity was observed in these workers compared to an age-matched control group. No clinical signs of toxicity were evident. No reports of human neurotoxicity after oral or dermal exposure to *n*-hexane were located. Both animal and human neurological studies examining both central and peripheral end points are a data need for chronic inhalation exposure. No animal studies exist for chronic inhalation exposure, and all the human studies involve co-exposure to other chemicals. A chronic-duration inhalation exposure experiment that measures ventricular fibrillation potential may be useful to determine if results seen after subcutaneous exposure (Khedun et al.1996) can be reproduced by an exposure route relevant to humans. No chronic-duration exposure studies in animals

were located for any route, thus the database was insufficient to derive an oral MRL for chronic-duration exposure. Oral exposure is an unlikely route for human exposure due to the volatility of *n*-hexane, so chronic-duration oral studies are not needed as critically. However, with deeply buried waste or leaking underground storage tanks, private drinking well water (municipal treatment is likely to volatilize all *n*-hexane at the plant) could become contaminated with *n*-hexane, so oral drinking water studies might be appropriate. The solubility of *n*-hexane in water is very low (9.5 mg/L), so it is questionable whether test animals would ingest enough *n*-hexane to result in any toxic response. There is no data on dermal toxicity, although, again because of the volatility of *n*-hexane, exposure by this route is unlikely and chronicduration toxicity studies are not needed as critically.

There is currently only limited information on the carcinogenic potential of *n*-hexane. No epidemiological studies were located that address this question in humans. Neither structure activity relationships nor mutagenicity assays point to a concern. Papillary tumors, but not the incidence, have been reported in the bronchiolar epithelium of rabbits after a 24-week exposure to 3,000 ppm *n*-hexane (Lungarella et al. 1984). A 2-year study of inhalation exposure with commercial hexane (51.5% *n*-hexane) (Bio/Dynamics 1995a, 1995b) resulted in a significant increase in liver neoplasms in female mice, but no increase at any site in male mice or rats of either sex (Bio/Dynamics 1995a, 1995b). It is unclear what components of the hexane mixture caused the neoplasms. Replication of this study, perhaps with *n*-hexane rather than commercial hexane, and further studies on the mechanism of this effect in female mice (e.g., precancerous changes in the liver) may clarify the significance of this finding for human exposure to *n*-hexane.

Genotoxicity. No information is available on the genotoxicity of *n*-hexane in humans. The database on *n*-hexane in animals, mammalian cells, and microorganisms indicates little potential for genotoxicity. *n*-Hexane was negative in dominant lethal tests in mice by the inhalation route at up to 396 ppm (Litton Bionetics 1980) and at 5,000 ppm (Mast et al.1989b). In an inhalation study, morphological alterations in sperm were noted in one study on rats at 5,000 ppm *n*-hexane (De Martino et al.1987). In other *in vivo* tests, subcutaneous injection of *n*-hexane had no significant effect on the incidence of sister chromatid exchange or chromosomal aberrations in mouse bone marrow; inhalation exposure had no effect on micronuclei incidence in mouse erythrocytes (NTP 1991). Results have generally been negative for *n*-hexane in bacterial tester strains such as *E. coli, B. subtilis,* and *S. typhirnurium* both with and without metabolic activation (Houk et al.1989; Ishidate et al.1984; McCarroll et al.198 la, 1981b; Mortelmans et al.1986; NTP 1991). Negative results were also obtained in mammalian cells, except for one observation of polyploidy in Chinese hamster CHL cells (Ishidate et al.1984; NTP 1991; Perocco et al.1983). Only a

single report was located on the genotoxicity of *n*-hexane metabolites; induction of chromosome loss was observed in yeast with 2,5-hexanedione (Mayer and Goin 1994). It is also unclear if incubation with liver microsomes (S9 fraction) in *in vitro* genotoxicity tests results in similar metabolites to those observed in humans *in vivo*.

Reproductive Toxicity. Reproductive effects have not been examined in humans after exposure to *n*-hexane. Dominant lethal tests in mice showed no effect on male fertility (Litton Bionetics 1980). No effects were seen on reproductive tissues in male rats after intermediate-duration inhalation exposure up to 500 ppm (IRDC 1981) or in either sex of mice after intermediate-duration inhalation exposure to up to 10,000 ppm *n*-hexane (Dunnick et al. 1989; NTP 1991). However, inhalation exposure in male rats to higher concentrations of *n*-hexane showed effects after acute-duration exposure to 5,000 ppm (spermatid) and spermatocyte degeneration and exfoliation) and testicular atrophy after intermediate-duration exposure to 1,000 ppm (De Martino et al. 1987; Nylen et al. 1989). Atrophy of the testicular germinal epithelium in rats was also noted after intermediate-duration oral exposure at 4,000 mg/kg/day (Krasavage et al. 1980). A study of end points of testicular function should be don in an occupationally exposed group of humans to determine if the effects seen in animals also occur in humans. Animal inhalation studies to more accurately determine the dose-response and threshold levels for testicular effects should also be conducted. oral exposure is an unlikely route for human exposure due to the volatility of *n*-hexane, so oral reproductive studies are not needed as critically. However, with deeply buried waste or leaking underground storage tanks, private drinking well water (municipal treatment is likely to volatilize all *n*-hexane at the plant) could become contaminated with *n*-hexane, so oral drinking water studies might be appropriate. The solubility of *n*-hexane in water is very low (9.5 mg/L), so it is questionable whether test animals would ingest enough *n*-hexane to result in any toxic response. Again, because of the volatility of *n*-hexane, exposure by the dermal route is unlikely and reproductive toxicity studies are not needed as critically.

Developmental Toxicity. Developmental effects have not been examined in humans after exposure to *n*-hexane. Development effects were not observed in acute-duration inhalation animal studies with *n*-hexane except for a temporary decrease in pup weight gain in offspring from pregnant rats exposed via inhalation to 1,000 ppm during gestation days 8-16 (Bus et al., 1979). No effects on offspring were seen in pregnant rats exposed via inhalation to 409 ppm *n*-hexane during gestation days 6-15 (Litton Bionetics 1979). Similar results were seen when pregnant mice were orally exposed to up to 2,830 mg/kg/day *n*-hexane during gestation days 6-15 (Marks et al. 1980). In pregnant female Wistar rats exposed to

500 ppm *n*-hexane for 23 hours a day throughout gestation (21 days), reduced body weight of offspring was reported ranging from 22% at postnatal day 9 to 13% at postnatal day 25. Delayed histogenesis of the cerebellar cortex in the offspring of exposed dams was also reported in this study during the first 30 postnatal days (Stoltenburg-Didinger et al.1990). The number of offspring examined in this study was not reported and statistical analysis of body weights was not performed, so these results need to be confirmed. Reduced fetal weight was seen in this study at 7,920 mg/kg/day, but there was also maternal toxicity at this dose. Developmental studies via the inhalation route in a species other than rats (e.g., rabbits) may be useful to assess the potential developmental toxicity of *n*-hexane exposure in humans. There is also a need for developmental studies in animal models where assessment of neurological, reproductive, and possibly other end points continues up to sexual maturity after exposure to *n*-hexane in *utero* and during maturation. Development of these systems continues after birth, the peripheral nervous system is a known target organ for *n*-hexane neurotoxicity, and reproductive effects have been observed in animal studies. Oral exposure is an unlikely route for human exposure due to the volatility of *n*-hexane, so oral developmental studies are not needed as critically. However, with deeply buried waste or leaking underground storage tanks, private drinking well water (municipal treatment is likely to volatilize all *n*-hexane at the plant) could become contaminated with *n*-hexane, so oral drinking water studies might be appropriate. The solubility of *n*-hexane in water is very low (9.5 mg/L), so it is questionable whether test animals would ingest enough *n*-hexane to result in any toxic response. Again, because of the volatility of *n*-hexane, exposure by the dermal route is unlikely and developmental toxicity studies are not needed as critically.

Immunotoxicity. One report of immunological effects in humans after exposure to *n*-hexane was located (Karakaya et al.1996) describing a reduction in immunoglobulin levels in a group of 35 workers compared to a control group of 23. The reductions correlated with 2,5-hexanedione in urine but not with workplace *n*-hexane concentrations (23-2 15 ppm). The reductions also remained well within the normal ranges for immunoglobulins in blood, so the toxicological significance of these findings can not be assessed without confirmatory studies (Jackson et al.1997). Cell counts (lymphocytes, neutrophils, monocytes, eosinophils) were unaffected by *n*-hexane exposure. No reports on dermal sensitization after exposure to *n*-hexane in humans were found in the literature. The animal database is limited to intermediate-duration inhalation studies where immunologic/lymphoreticular tissues were examined histopathologically. No adverse effects were observed in the examined tissues (Cavender et al.1984; Dunnick et al.1989; IRDC 1981; NTP 1991). An increase in lymphocytes in the blood was noted after exposure of mice to 10,000 ppm *n*-hexane; however, this was attributed to inflammation of the respiratory tract in these

animals (Dunnick et al.1989; NTP 1991). A battery of immunological-function tests after inhalation exposure to *n*-hexane in rats may provide information on whether immunological effects may be a concern for humans exposed near hazardous waste sites. Oral exposure is an unlikely route for human exposure due to the volatility of *n*-hexane, so oral immunological studies are not needed as critically. However, with deeply buried waste or leaking underground storage tanks, private drinking well water (municipal treatment is likely to volatilize all *n*-hexane at the plant) could become contaminated with *n*-hexane, so oral drinking water studies might be appropriate. The solubility of *n*-hexane in water is very low (9.5 mg/L), so it is questionable whether test animals would ingest enough *n*-hexane to result in any toxic response. Again, because of the volatility of *n*-hexane, exposure by the dermal route is unlikely and immunological toxicity studies are not needed as critically.

Neurotoxicity. The major public health concern regarding *n*-hexane exposure is the potential for the development of neurotoxicity. Occupational studies have documented that human exposure to *n*-hexane can result in a peripheral neuropathy that in severe cases can lead to paralysis (Altenkirch et al.1977; Yamamura 1969; Wang et al.1986). The dose-duration relationship has not been well characterized in humans, but concentrations of 500 ppm and above and exposure for 6 months or more have been associated with human neurotoxicity. Clinical neurotoxicity can be reproduced in rats, but not in other test species, via the inhalation and oral routes (Altenkirch et al.1982; De Martino et al.1987; Dunnick et al. 1989; Frontali et al.1981; Huang et al.1989; IRDC 1981; Krasavage et al.1980; NTP 1991; Schaumburg and Spencer 1976; Takeuchi et al.1980). Other data needs are the determination of threshold levels for neurotoxicity for acute- and intermediate-duration inhalation exposure in the rat model, and the effect of age on susceptibility to *n*-hexane. There are no chronic-duration neurotoxicity studies in animals; such an inhalation study should evaluate both peripheral and central targets. Oral exposure is an unlikely route for human exposure due to the volatility of *n*-hexane, so oral neurological studies are not needed as critically. However, with deeply buried waste or leaking underground storage tanks, private drinking well water (municipal treatment is likely to volatilize all *n*-hexane at the plant) could become contaminated with *n*-hexane, so oral drinking water studies might be appropriate. The solubility of *n*-hexane in water is very low (9.5 mg/L), so it is questionable whether test animals would ingest enough *n*-hexane to result in any toxic response. Again, because of the volatility of *n*-hexane, exposure by the dermal route is unlikely and neurological toxicity studies are not needed as critically.

The molecular mechanism responsible for the axonal swelling, demyelination, and axonal degeneration seen in human *n*-hexane neurotoxicity has not been completely proven, although it is believed to be related to the

pyrrolidation of neuronal proteins by the neurotoxic metabolite 2,5-hexanedione. Whether neurofilament cross-linking is key to the neurofilament accumulation, axonal swellings, and ultimate axonal degeneration observed in *n*-hexane neurotoxicity or is incidental remains to be elucidated (Graham et al.1995). The puzzling observation that the active *n*-hexane metabolite 2,5-hexanedione actually speeds rather than slows axonal transport (Pyle et al.1993) deserves better explanation and perhaps replication. Further studies in the rat model to answer this important question would be helpful in human risk assessment.

Epidemiological and Human Dosimetry Studies. Epidemiological information is available for the effects caused by occupational exposure to *n*-hexane. A complicating factor in these studies is that workers are almost always exposed to many other chemicals besides *n*-hexane. Epidemiological studies that followed populations exposed to *n*-hexane either in the workplace or near hazardous waste sites would be useful in assessing adverse effects in humans. Of particular importance are reproductive effects in males and whether any relationship exists between *n*-hexane exposure and chronic degenerative neurological diseases. Human dosimetry studies would be useful in associating *n*-hexane levels with the reported effects.

Biomarkers of Exposure and Effect.

Exposure. The presence of the *n*-hexane metabolite 2,5-hexanedione in the urine is a reasonably reliable marker for exposure to *n*-hexane and has been correlated with air concentrations in the workplace. This is not a specific marker since 2-hexanone is also metabolized to 2,5-hexanedione. The levels of this metabolite in the urine associated with neurotoxicity are not known. A more sensitive marker for exposure may be the presence of pyrolidated proteins in the blood or hair, a result of the reaction of 2,5-hexanedione with the side-chain amino group of lysine (Graham et al.1995; Johnson et al.1995). These methods have only been tested after oral exposure to 2,5-hexanedione in the rat model. It would be very useful to know if measurement of pyrrole adducts or cross-linked proteins is also feasible after inhalation exposure to *n*-hexane in the rat model. Further development and validation of this method in an occupationally exposed population may then be useful.

Effect. There are currently no subtle or sensitive biomarkers of effects associated specifically with exposure to *n*-hexane. Electroneurographic testing, however, may prove useful in the detection of nerve conduction abnormalities in their early stages before they are accompanied by clinical manifestations. In a study of 15 women who had been exposed to *n*-hexane in a shoe factory, all nerve conduction velocities

(motor and sensory) were significantly slowed in exposed workers compared to controls (Mutti et al. 1982b). None of these women had clinical signs of peripheral neuropathy. Two studies suggest that the most sensitive electrophysiological biomarker of effect in *n*-hexane exposed workers may be the amplitude of the sensory nerve action potential, while amplitude of the motor nerve action potential, nerve conduction velocities, and distal latencies are less sensitive (Chang et al.1993; Pastore et al.1994). Further studies correlating electrophysiological studies with biomarkers of *n*-hexane exposure would be useful.

Pyrrolidation and crosslinking of proteins can be considered biomarkers of either exposure or effect and are discussed in the previous subsection.

Absorption, Distribution, Metabolism, and Excretion. Toxicokinetic information is available for the inhalation route in humans and animals but is almost totally lacking for the oral and dermal routes. Inhaled *n*-hexane is readily absorbed in the lungs. In humans, approximately 20-30% of inhaled *n*-hexane is absorbed systemically. Absorption takes place by passive diffusion through epithelial cell membranes. Inhaled *n*-hexane distributes throughout the body; based on blood-tissue partition coefficients, preferential distribution would be in the order: body fat>>liver, brain, muscle>kidney, heart, lung>blood. *n*-Hexane is metabolized by mixed function oxidases in the liver to a number of metabolites including the neurotoxicant 2,5-hexanedione. Approximately 10-20% of absorbed *n*-hexane is excreted unchanged in exhaled air, and 2,5-hexanedione is the major metabolite recovered in urine. *n*-Hexane metabolites in the urine and *n*-hexane in exhaled air do not account for total intake, suggesting that some of the metabolites of *n*-hexane enter intermediary metabolism. Saturation of metabolism occurs in rats at ≥3,000 ppm, far above any plausible human exposure. Further studies in animals via the oral and dermal routes are necessary to assess whether significant toxicity is likely to occur in humans exposed by these routes. A PBPK model exists for *n*-hexane which successfully predicts blood levels of *n*-hexane and urinary excretion of 2,5-hexanedione (Perbellini et al.1986, 1990a) in exposed humans. Thus, further kinetic studies in humans (e.g., metabolism in human liver homogenates) are not necessary.

Comparative Toxicokinetics. The toxicokinetic studies available indicate that the rat is a good model for human neurotoxicity observed after occupational exposure to *n*-hexane. Mild signs can be produced in chickens and mice, but these do not progress to the serious neurotoxicity observed in humans and rats. Toxicokinetic data from other species (absorption, distribution, metabolism, excretion) could provide insight on the molecular mechanism(s) of the species specificity of *n*-hexane toxicity and would be valuable for predicting toxic effects in humans.

Methods for Reducing Toxic Effects. No information was located on mitigating the specific effects of *n*-hexane intoxication. Since the mechanism of absorption is passive diffusion, removal from exposure stops absorption. Distribution is via partitioning based on physicochemical properties. Toxicity is due to the neurotoxic metabolite 2,5-hexanedione. Methods to reduce this metabolite in the blood would be useful. The specific mechanism of action is unknown, although there is strong evidence that pyrrolidation of proteins by 2,5-hexanedione followed by crosslinking is involved. Methods to prevent this reaction may be helpful; none currently exist. Pyrrole-to-pyrrole crosslinking can be inhibited *in vitro* by thiol-containing compounds including *N*-acetylcysteine and glutathione (Zhu et al.1995). Future research in the rat model might identify a way to deliver these inhibitory compounds to the site of pyrrole crosslinking. Clinical experience with *n*-hexane neurotoxicity is that once exposure ceases, recovery occurs over several months to a year. Supportive treatment would be similar to that for peripheral neuropathies caused by other conditions, for example diabetes.

Children's Susceptibility. There are no populations of children identified that have been specifically exposed to *n*-hexane, although several teenagers have developed peripheral neuropathy after *n*-hexane exposure by solvent abuse (Altenkirch et al.1977) and in the workplace (Yamamura et al.1969). These reports did not indicate any difference in susceptibility or clinical signs between teenagers and adults. Animal studies provide limited further information; only 2 studies were located where the responses to *n*-hexane were compared between young animals and adults. In a study in rats directly comparing the effects of *n*-hexane exposure in weanlings (21 days old) and young adults (80 days old) (Howd et al.1983), peripheral neuropathy occurred in both groups, although onset was more rapid in the young adult group. No deaths were observed over the 11-week exposure period and 3-week recovery period in weanling rats. In young adults, however, 5 of 10 rats died as the result of severe neuropathy. An oral LD_{50} study showed 14-day-old rats were more susceptible to the acute effects of a large dose of *n*-hexane than young adults (Kimura et al.1970). LD_{50} values for *n*-hexane were 15,840 mg/kg for 14-day-olds and 32,340 mg/kg for the young adults. Clinical signs and time to death were not reported. The Howd et al. (1983) study used relatively high concentrations of *n*-hexane (1,000 ppm) and should be repeated at lower levels (≥500 ppm) to determine if the differences in susceptibility still exist at levels to which humans are more likely to be exposed.

Data needs relating to development are discussed in detail above under Developmental Toxicity.

There is no experimental evidence available to assess whether the toxicokinetics of *n*-hexane differ between children and adults. Experiments in the rat model comparing kinetic parameters in weanling and mature animals after exposure to *n*-hexane would be useful. These experiments should be designed to determine the concentration-time dependence (area under the curve) for blood levels of the neurotoxic *n*-hexane metabolite 2,5-hexanedione. *n*-Hexane and its metabolites cross the placenta in the rat (Bus et al.1979); however, no preferential distribution to the fetus was observed. *n*-Hexane has been detected, but not quantified, in human breast milk (Pellizzari et al.1982), and a milk/blood partition coefficient of 2.10 has been determined experimentally in humans (Fisher et al.1997). However, no pharmacokinetic experiments are available to confirm that *n*-hexane or its metabolites are actually transferred to breast milk. Based on studies in humans, it appears unlikely that significant amounts of *n*-hexane would be stored in human tissues at likely levels of exposure, so it is unlikely that maternal stores would be released upon pregnancy or lactation. A PBPK model is available for the transfer of *n*-hexane from milk to a nursing infant (Fisher et al.1997); the model predicted that *n*-hexane intake by a nursing infant whose mother was exposed to 50 ppm at work would be well below the EPA advisory level for a 10-kg infant. However, this model cannot be validated without data on *n*-hexane content in milk under known exposure conditions.

There is no experimental evidence adequate to evaluate whether metabolism of *n*-hexane is different in children. Similarly, there is no information available from animal experiments. The initial step in *n*-hexane metabolism in animals is a hydroxylation step catalyzed by a P-450 enzyme. Since some of these enzymes are developmentally regulated, it would be of interest to know: (1) if there are specific P-450 isozymes involved in *n*-hexane hydroxylation and, (2) if so, are these isozymes known to be developmentally regulated?

Due to the lack of reports of *n*-hexane toxicity in children (except a few case reports of teenagers) there is no data available as to whether children differ in their susceptibility to *n*-hexane toxicity compared to adults. Weanling rats (21 days old) were more resistant to the development of *n*-hexane peripheral neuropathy than young adults (80 days old) during an exposure to 1,000 ppm *n*-hexane (Howd et al.1983). The authors suggested that the relative resistance of the weanling rats may have been due to shorter, smaller-diameter axons, or to a greater rate of growth and repair in their peripheral nerves compared to those of adults. While this is a plausible explanation for the results in rats, it would be speculative to predict on this basis that children would be less sensitive than adults. If cases of clinical *n*-hexane neurotoxicity occur in the future in adults in a setting where children are likely to have been exposed (e.g. home use of *n*-hexane containing products) thorough neurological and electrophysiological examinations

should be performed on the children. Additionally, both immediate and long-term health effects caused by *n*-hexane in neonatal and juvenile animals could be investigated, possibly in some of the same studies examining postnatal exposures and developmental effects which are discussed in a previous data needs section.

Child health data needs relating to exposure are discussed in Section 5.8.1, Data Needs: Exposures of Children.

2.11.4 Ongoing Studies

Dr. Doyle Graham of Vanderbilt University is conducting a series of mechanistic investigations on *n*-hexane neurotoxicity (FEDRIP 1996). The specific aims are:

(1) to determine the specificity and generality of the gamma-diketone structure in the genesis of the neurotoxicity of alkanes;

(2) to define the mechanism of pyrrole synthesis and the relationship between the rate of pyrrole formation and neurotoxicity;

(3) to determine whether pyrrole autoxidation and crosslinking are necessary steps in the pathogenetic sequence;

(4) to determine the structure of the crosslinking adducts;

(5) to determine whether neurofilamentous crosslinking is progressive during chronic intoxication;

(6) to determine the role of axonal constrictions at nodes of Ranvier in the development of axonal swellings;

(7) to determine the mechanism of degeneration of the distal axon; and

(8) to determine what steps in the proposed pathogenetic scheme for *n*-hexane neurotoxicity have parallels in the pathogenesis of the neuropathies caused by beta,beta'-iminodipropionitrile, carbon disulfide and acrylamide.