CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of *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. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

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

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 by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to *n*-hexane, but may not be inclusive of the entire body of literature. A systematic review of the scientific evidence of the health effects associated with exposure to *n*-hexane was also conducted; the results of this review are presented in Appendix C.

Animal inhalation studies are presented in Table 2-1 and Figure 2-2, animal oral studies are presented in Table 2-2 and Figure 2-3; animal dermal studies are presented in Table 2-3.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. Effects have been classified into "less serious LOAELs" or "serious LOAELs (SLOAELs)." "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 endpoint 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 endpoints. 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.

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

n-Hexane is a hydrocarbon produced from crude oil that is a component of many solvents used in industry, such as certain glues and adhesives, and it is also present in gasoline. Although pure *n*-hexane is used in laboratories, most commercial/industrial hexanes are a mixture of hexane isomers and aliphatic hydrocarbons. Major uses for solvents containing *n*-hexane include as cleaning agents in the printing, textile, furniture, and shoemaking industries, and for extracting vegetable oils from crops such as soybeans. Most of the available occupational health information comes from workers in these industries, and exposures are often to various mixtures of industrial solvents. 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.

This profile addresses the toxicological and toxicokinetics database of *n*-hexane. Animal studies utilizing pure reagent-grade *n*-hexane were evaluated, while animal studies using commercial-grade hexane were not included. Although the available occupational data is presumed to evaluate exposure to commercial-grade hexanes (and possibly other potentially neurotoxic compounds), these data provide critical information on the symptoms and progression of *n*-hexane-induced neurotoxicity.

The health effects of *n*-hexane have been evaluated in epidemiological studies, case reports, and experimental animal studies. The primary route of exposure to *n*-hexane is by inhalation due to its volatility, and it is also the most studied route in experimental animal studies. A number of case reports/ case series have extensively documented the clinical signs and altered neurological function in humans exposed to *n*-hexane, commonly referred to as peripheral neuropathy. Although useful in determining the clinical pathology of the disease, case reports and series typically lack the necessary exposure information for dose response. For the purposes of this profile, data from case reports and case series are only included to fill in gaps in outcomes with no other human data. Outcomes with sufficient information from other sources (i.e., neurological effects) do not include discussion of case reports/series. Animal inhalation data are available for each of the health effect categories and often lend support to the human data when available.

Human studies. Most of the available literature on human exposure comes from occupational epidemiology studies and case reports/series examining workers with peripheral neuropathy. 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 peripheral neuropathy that started with numbness in the feet and hands (sometimes referred to as glove and stocking neuropathy), 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 most patients over the course of several months to 2 years. Due to the volatility of *n*-hexane and its use in occupational settings, most of the available human data are from inhalation exposure and assumed to be chronic in duration.

Animal studies. Information is readily available for experimental animals exposed to *n*-hexane primarily by the inhalation and oral routes and for acute and intermediate durations. The majority of these studies have evaluated neurotoxicity as the primary outcome, although data are available for all of the health categories included in this profile. Studies on the potential carcinogenicity of *n*-hexane are limited, which may be related to the lack of available chronic-duration studies and the neurotoxic effects observed at shorter durations.

Overview of Health Effects. As discussed in Chapter 1, the neurological, respiratory, reproductive, and developmental systems appear to be sensitive targets of toxicity following exposure to *n*-hexane. Based on data in humans and animals, neurological effects are the primary outcome following inhalation or oral

exposure to *n*-hexane. Additional outcomes have also been reported, although some may be secondary effects resulting from overt or underlying neurological effects. The outcomes examined in human and animal studies of *n*-hexane are presented in Figure 2-1. A systematic review was conducted on the available human and animal studies for the most sensitive effects following exposure to *n*-hexane: neurological, developmental, and respiratory outcomes.

- **Neurological effects:** Neurological effects following inhalation or oral exposure are a known health effect for humans based on a high level of evidence in human studies and a high level of evidence in animal studies. Muscle weakness (particularly in the lower extremities), numbness, and decreased sensation have been reported in workers exposed to *n*-hexane, and alterations in nerve conduction and evoked potentials have been observed through further testing. Animal studies have shown similar results, with reduced motor activity, limb weakness, and paralysis being accompanied by changes in nerve conduction and nerve histopathology.

- **Respiratory effects:** Respiratory effects following inhalation exposure to *n*-hexane are a suspected health effect based on inadequate evidence in human studies and a high level of evidence in animal studies. Higher incidences of self-reported respiratory symptoms have been observed in a study of workers exposed to *n*-hexane. Animal studies have observed nasal and lung lesions following intermediate-duration inhalation exposure.

- **Developmental effects:** Developmental effects following inhalation exposure to *n*-hexane are a suspected health effect based on inadequate evidence in human studies and high evidence in animal studies. In humans, positive associations have been observed between low birth weight or alterations in the neonatal immune system and maternal exposure to *n*-hexane. Developmental effects observed in rodent inhalation and oral exposure studies include decreased fetal or litter weights and decreased number of live fetuses.

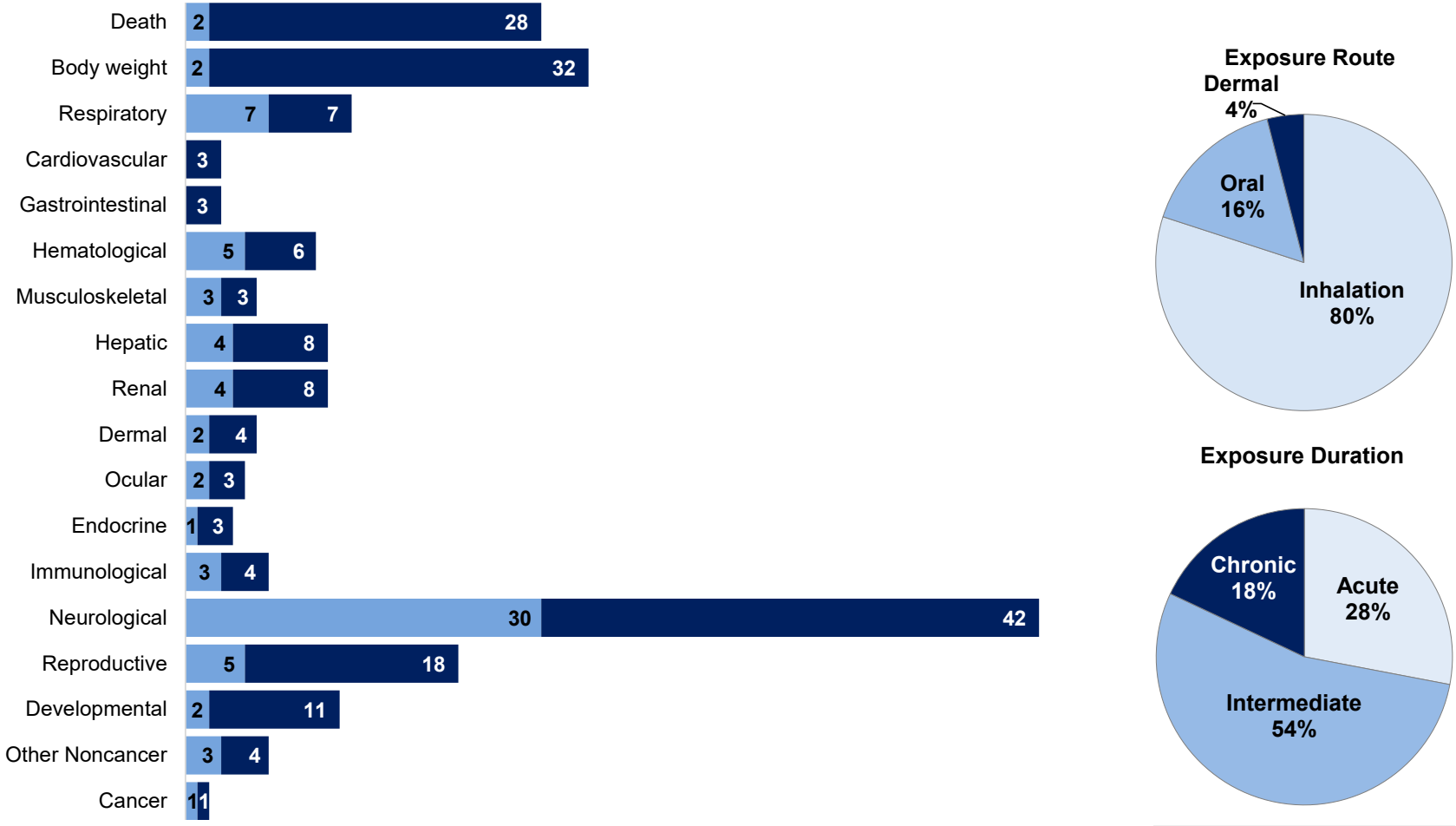
Additional effects following *n*-hexane exposure that did not undergo a full systematic review include body weight and reproductive outcomes. Although these are not the most sensitive endpoints, there is evidence to support hazard identification.

- **Body weight effects:** Although very few studies have identified weight changes in humans following exposure to *n*-hexane, decreased body weight is a common effect observed in animal studies, particularly after intermediate-duration inhalation exposure. These decreases are often accompanied by decreased food consumption and/or overt neurological effects.

- **Reproductive effects:** Human studies have identified the female reproductive system as a potential target, with reports of longer menstrual cycles, increased risk of preeclampsia, and higher risk of spontaneous abortions in women exposed to *n*-hexane. Animal studies have observed effects in the male reproductive system, including decreased testis weight and histopathology in the testes and epididymis.

Figure 2-1. Overview of the Number of Studies Examining *n*-Hexane Health Effects*

Most studies examined the potential neurological, body weight, and reproductive effects of *n*-hexane
Fewer studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 76 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
API 1979									
1	Rat (Hybrid) 17–20 F	10 days GDs 6–15 6 hours/day (WB)	0, 93, 409	BW, CS, DX, FI, GN, LE	Bd wt Develop	409 409			
Bus et al. 1979									
2	Rat (Fischer-344) 7 F	5 days GDs 8–12 6 hours/day (WB)	0, 1,000	DX	Develop	1,000			
Bus et al. 1979									
3	Rat (Fischer-344) 6–9 F	5 days GDs 12–16 6 hours/day (WB)	0, 1,000	DX	Develop	1,000			
Bus et al. 1979									
4	Rat (Fischer-344) 3–8 F	9 days GDs 8–16 6 hours/day (WB)	0, 1,000	DX	Develop		1,000		Decreased litter weight (13.9% at 3 weeks after birth)
Chalansonnet et al. 2013									
5	Rat (Sprague-Dawley) 8 M	10 days 6 hours/day	0, 1,000	LE, CS	Neuro	1,000			

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
De Martino et al. 1987									
6	Rat (Sprague-Dawley) 8 M	1–2 weeks 6 days/week 16 hours/day	0, 5,000	BW, CS, FI, LE, HP, NX	Bd wt			5,000	Decreased body weight (20–30%)
					Neuro		5,000		Decreased motor conduction velocity
					Repro			5,000	Testicular lesions (spermatocyte necrosis, exfoliation of spermatids, and Sertoli cell vacuolization)
De Martino et al. 1987									
7	Rat (Sprague-Dawley) 6 M	24 hours	0, 5,000	CS, BW, FI, HP, RX	Repro			5,000	Testicular lesions (focal degeneration of spermatocytes and mild exfoliation of elongated spermatids)
De Martino et al. 1987									
8	Rat (Sprague-Dawley) 3 M	2–8 days 16 hours/day	0, 5,000	CS, BW, FI, HP, RX	Repro			5,000	Testicular lesions (degeneration of spermatocytes, exfoliation of elongated spermatids, and Sertoli cell vacuolization)
NIEHS 1987									
9	Rat (Sprague-Dawley) 40 F	14 days 20 hours/day GDs 6–19 (WB)	0, 200, 1,000, 5,000	LE, BW, DX	Bd wt	1,000		5,000	Decreased body weight (10%), decreased extragestational body weight gain (44%)
					Neuro	5,000			
					Develop	200[b]		1,000	Decreased fetal body weight (7.5% in male offspring)
NIEHS 1988a									
10	Mouse (B6C3F1) 20 M	5 days 20 hours/day (WB)	0, 200, 1,000, 5,000	LE, CS, BW, GN	Bd wt	5,000			
					Neuro	5,000			
					Repro	5,000			

Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NIEHS 1988b									
11	Mouse (Swiss CD-1) 20 M	5 days 20 hours/day (WB)	0, 200, 1,000, 5,000	LE, CS, BW, RX	Bd wt	5,000			
					Neuro	5,000			
					Repro	5,000			
NIEHS 1988c									
12	Mouse (Swiss)	12 days GDs 6–17 20 hours/day	0, 200, 1,000, 5,000	LE, CS, BW, DX	Bd wt	5,000			
					Neuro	5,000			
					Develop	1,000		5,000	Decreased number of live fetuses per litter, increased incidence of late resorptions
INTERMEDIATE EXPOSURE									
Altenkirch et al. 1982									
13	Rat (Wistar) 5 M	9 weeks 7 days/week 22 hours/day (WB)	0, 500, 700	BW, CS, GN, HP, LE	Neuro			500	Clinical signs (narcosis, paralysis), multifocal giant axonal swellings, primarily in the calf muscles, breakdown of axons, and myelin degradation
Altenkirch et al. 1982									
14	Rat (Wistar) 5 M	40 weeks 7 days/week 8 hours/day (WB)	0, 700	BW, CS, GN, HP, LE	Bd wt	700			
					Neuro		700		Axonal swelling in the spinal cord

Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
API 1978									
15	Rat (Sprague-Dawley) 12 M, 12 F	26 weeks 5 days/week 6 hours/day (WB)	0, 6, 26, 129	BW, CS, BC, HE, LE	Bd wt	129			
					Hemato	129			
					Hepatic	129			
					Renal	129			
API 1978									
16	Rat (Sprague-Dawley) 12 M, 12 F	26 weeks 7 days/week 21 hours/day (WB)	0, 5, 27, 126	BW, CS, BC, HE, LE	Bd wt	126			
					Hemato	126			
					Hepatic	126			
					Renal	126			
API 1981									
17	Rat (Sprague-Dawley) 20 M	6 months 7 days/week 22 hours/day (WB)	0, 500	BW, CS, GN, HP, LE, OW	Bd wt			500	Decreased body weight (30%)
					Resp	500			
					Cardio	500			
					Gastro	500			
					Musc/skel			500	Skeletal muscle atrophy
					Hepatic	500			
					Renal		500		Increased kidney weight, chronic nephropathy
					Dermal	500			
					Ocular	500			
					Endocr	500			
					Immuno	500			
					Neuro			500	Abnormal gait, peripheral nerve atrophy
					Repro	500			

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Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Cavender et al. 1984									
18	Rat (Fischer-344) 15 M, 15 F	13 weeks 5 days/week 6 hours/day (WB)	0, 3,000, 6,500, 10,000	LE, CS, FI, BW, GN, HP, OP, OF, UR, HE, BC, OW	Bd wt	10,000 F 6,500 M	10,000 M		Decreased body weight (11%)
					Resp	10,000			
					Hemato	10,000			
					Hepatic	10,000			
					Renal	10,000			
					Ocular	10,000			
					Immuno	10,000			
					Neuro	10,000 F 3,000 M	6,500 M		Axonopathy in the sciatic nerve
					Repro	10,000 M			
De Martino et al. 1987									
19	Rat (Sprague-Dawley) 3–8 M	3–6 weeks 6 days/week 16 hours/day	0, 5,000	BW, CS, FI, LE, HP, NX	Bd wt			5,000	Decreased body weight (20-30%) with decreased food consumption
					Neuro			5,000	Decreased motor conduction velocity, peripheral neuropathy, and paralysis
					Repro			5,000	Testicular lesions (spermatocyte necrosis, exfoliation of spermatids, and Sertoli cell vacuolization)
Frontali et al. 1981									
20	Rat (Sprague-Dawley) 6–9 M	7 weeks 5 days/week 9 hours/day (WB)	0, 500, 1,500, 5,000	BW, CS, HP	Neuro	5,000			
Frontali et al. 1981									
21	Rat (Sprague-Dawley) 6–9 M	14 weeks 5 days/week 9 hours/day (WB)	0, 500, 1,500, 5,000	BW, CS, HP	Neuro	1,500		5,000	Tibial nerve axonal degeneration

Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Frontali et al. 1981									
22	Rat (Sprague-Dawley) 6–9 M	30 weeks 5–6 days/week 9–10 hours/day (WB)	0, 500, 2,500	BW, CS, HP	Neuro	500		2,500	Tibial nerve axonal degeneration
Howd et al. 1983									
23	Rat (Fischer-344) 10 M	11 weeks 7 days/week (4 weeks) 6 days/week (7 weeks) 24 hours/day (WB)	0, 1,000	LE, CS, BW, OW, NX	Bd wt			1,000	Decreased body weight (34%)
					Resp	1,000			
					Hepatic	1,000			
					Renal	1,000			
					Neuro			1,000	Decreased hindlimb and forelimb strength, and ataxia, increased action potential latency, and increased brainstem auditory-evoked response
					Repro	1,000			
Weanling rats									
Howd et al. 1983									
24	Rat (Fischer-344) 10 M	11 weeks 7 days/week (4 weeks) 6 days/week (7 weeks) 24 hours/day (WB)	0, 1,000	LE, CS, BW, OW, OF, NX	Death			1,000	Increased mortality (50%)
					Bd wt			1,000	Decreased body weight (54%)
					Resp	1,000			
					Hepatic	1,000			
					Renal	1,000			
					Neuro			1,000	Decreased hindlimb and forelimb strength, and ataxia, increased action potential latency, and increased brainstem auditory-evoked response
					Repro	1,000			
Young adult rats									

Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Huang et al. 1989									
25	Rat (Wistar) 8 M	16 weeks 7 days/week 12 hours/day (WB)	0, 500, 1,200, 3,000	BI, BW, CS, HP, LE, NX	Bd wt	500	1,200		Decreased body weight (12%)
					Neuro	500	1,200		Decreased grip strength and motor nerve conduction velocity, paranodal swelling, demyelination, and remyelination of the peripheral nerve
Ichihara et al. 1998									
26	Rat (Wistar) 8 M	20 weeks 6 days/week 12 hours/day	0, 2,000	LE, CS, BW, NX	Bd wt	2,000			
					Neuro		2,000		Decreased motor conduction velocity
Li et al. 2014, 2015									
27	Rat (Wistar) 5 F	20 days GDs 1–20 4 hours/day	0, 100, 500, 2,500, 12,500	LE, CS, DX	Neuro	2,500	12,500		Irritability, aggression
					Develop	2,500		12,500	Decreased live pups/litter, decreased percentage of secondary follicles, increased atretic follicles, alterations in estrus cycle in female offspring
Nylen and Hagman 1994									
28	Rat (Sprague-Dawley) 18–36 M	61 days 18 hours/day (WB)	0, 1,000	BW, OF	Neuro		1,000		Decreased motor conduction velocity and auditory brainstem response, increased flash evoked potential latency

Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Nylen et al. 1989									
29	Rat (Sprague-Dawley) 12–18 M	28–61 days 7 days/week 18–21 hours/day	0, 1,000	GN, HP	Musc/skel			1,000	Hindlimb muscular atrophy
					Repro			1,000	Testicular atrophy
Nylen et al. 1994									
30	Rat (Sprague-Dawley) 18 M	28 days 21 hours/day (WB)	0, 1,000	BW, OF	Neuro		1,000		Decreased motor conduction velocity and flash evoked potential amplitude
Pryor and Rebert 1992									
31	Rat (Fischer-344) 12 M	9 weeks 7 days/week 14 hours/day (WB)	0, 4,000	NX, OF, LE	Bd wt			4,000	Decreased terminal body weight (49%)
					Neuro			4,000	Moderate to severe hindlimb flaccid paralysis; decreased grip strength, decreased motor conduction velocity, decreased auditory brainstem response amplitude
Pryor et al. 1983									
32	Rat (Fischer-344) 11–12 M	14 weeks 7 days/week 14 hours/day	0, 2,000	CS, BW, NX	Bd wt		2,000		Decreased body weight (19%)
					Neuro		2,000		Decreased limb grip strength, startle response, and motor activity; increased evoked potentials latencies

Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Rebert and Sorenson 1983									
33	Rat (Fischer-344) 8 M	10-11 weeks 5 days/week 24 hours/day (WB)	0, 500, 1,000, 1,500	NX, BW, LE	Death			1,500	LC_{50} (4/8 died during 6-week recovery period)
					Bd wt	500		1,000	Decreased body weight (23%)
					Neuro		500	1,000	LOAEL: Decreased fore- and hindlimb grip strength; increased latency of evoked potentials at 1,000 ppm SLOAEL: Increased latency of evoked potentials
					Other noncancer	1,500			
Schaumburg and Spencer 1976									
34	Rat (Sprague-Dawley) 8 NS	42–162 days 7 days/week 24 hours/day (WB)	400–600	GN, HP	Neuro			400	Central and peripheral neuropathy, foot-drop, waddling gait, limb weakness, swollen axons, axonal degeneration
Stoltenburg-Didinger et al. 1990									
35	Rat (Wistar) 8–20 F	21 days GDs 1–21 7 days/week 23 hours/day	0, 500	OF, OW, BW, DX, CS	Neuro	500			
					Develop			500	Decreased fetal weight (22% at 9 days after birth)
Stoltenburg-Didinger et al. 1990									
36	Rat (Wistar) 8–20 F	63 days GD 1–PND 42 7 days/week 23 hours/day	0, 800	OF, OW, BW, DX, CS	Neuro		800		Hindlimb weakness
Takeuchi et al. 1980									
37	Rat (Wistar) 7 M	16 weeks 7 days/week 12 hours/day (WB)	0, 3,040	LE, CS, BW, HP, LE	Death			3,040	Increased mortality (29%)
					Bd wt			3,040	Decreased body weight (33%) and body weight gain (79%)

Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Musc/skel			3,040	Muscular atrophy, denervation, irregular fibers, and disordered myofilaments
					Neuro			3,040	Gait disturbances, decreased motor and mixed nerve conduction velocity, axonal swelling, neurofilament accumulation, denervated neuromuscular junctions
API 1980									
38	Mouse (CD-1) 12 M	8 weeks 5 days/week 6 hours/day (WB)	0, 99, 396	RX	Repro	396			
Liu et al. 2012									
39	Mouse (ICR) 5 F	5 weeks 7 days/week 4 hours/day	0, 850, 4,300, 21,500	BW, RX	Bd wt	4,300	21,500		15% lower terminal body weight
					Repro	4,300	21,500		Decreased duration of diestrus
NTP 1991 (this study was also published as Dunnick et al. 1989)									
40	Mouse (B6C3F1) 8 M, 8 F	13 weeks 5 days/week 6 hours/day (WB)	0, 580, 1,109, 4,421, 10,000	CS, BW, NX	Neuro	4,421	10,000		Decreased locomotor activity

Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NTP 1991 (this study was also published as Dunnick et al. 1989)									
41	Mouse (B6C3F1) 10 M, 10 F	13 weeks 5 days/week 6 hours/day (WB)	0, 580, 1,109, 4,421, 10,000	NX, BW, CS, GN, HP, LE	Bd wt	10,000 F 4,421 M	10,000 M		Decreased body weight (17%)
					Resp	1,109 F 4,421 M	4,421 F	10,000	LOAEL: Lesions in the nasal cavity (multifocal regeneration and metaplasia in the olfactory epithelium) SLOAEL: Lesions in the nasal cavity (multifocal erosion, regeneration, inflammation, metaplasia in the olfactory and respiratory epithelium), respiratory irritation (sneezing)
					Cardio	10,000			
					Gastro	10,000			
					Hemato	10,000 F 4,421 M	10,000 M		Increased number of segmented neutrophils
					Hepatic	10,000			
					Renal	10,000			
					Dermal	10,000			
					Endocr	10,000			
					Immuno	10,000			
					Neuro	4,421 F 10,000 M	10,000 F		Paranodal swellings in tibial nerve
					Repro	10,000			
NTP 1991 (this study was also published as Dunnick et al. 1989)									
42	Mouse (B6C3F1) 8 M, 8 F	13 weeks 5 days/week 22 hours/day (WB)	0, 1,099	CS, BW, NX	Neuro	1,099 M	1,099 F		Decreased locomotor activity

Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
NTP 1991 (this study was also published as Dunnick et al. 1989)									
43	Mouse (B6C3F1) 10 M, 10 F	13 weeks 5 days/week 22 hours/day (WB)	0, 1,099	NX, BW, CS, GN, HP, LE	Bd wt	1,099 F	1,099 M		Decreased body weight (10%)
					Resp		1,099[c]		Lesions in the nasal cavity (multifocal regeneration and metaplasia in olfactory epithelium)
					Cardio	1,099			
					Gastro	1,099			
					Hemato	1,099			
					Hepatic	1,099			
					Renal	1,099			
					Dermal	1,099			
					Endocr	1,099			
					Immuno	1,099			
					Neuro		1,099		Paranodal swellings in tibial nerve
Lungarella et al. 1984									
44	Rabbit (New Zealand) 12 M	24 weeks 5 days/week 8 hours/day (WB)	0, 3,000	LE, CS, BW, HE, OP, GN, HP	Death			3,000	Increased mortality (17%)
					Bd wt	3,000			
					Resp			3,000	Upper respiratory tract irritation (nasal discharge and salivation), respiratory difficulties (gasping, lung rales, mouth breathing), histopathology (centrilobular emphysema, pulmonary fibrosis, goblet cell metaplasia, epithelial desquamation)
					Hemato	3,000			
					Ocular		3,000		Ocular irritation (lacrimation, hyperemia of the conjunctiva)
					Neuro	3,000			

Table 2-1. Levels of Significant Exposure to *n*-Hexane – Inhalation (ppm)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
					Cancer			3,000	CEL: Lung tumors (papillary tumors in the bronchiolar epithelium)
CHRONIC EXPOSURE									
Imai and Omoto 1999									
45	Rat (F344/Jcl) 6 M	415 days 6 days/week 4 hours/day	0, 1,000	LE, CS, BW, FI, OW, HP	Bd wt	1,000			
					Repro		1,000		Leydig cell hyperplasia and benign Leydig cell tumors

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an acute-duration inhalation minimal risk level (MRL) of 6 ppm. The NOAEL of 200 ppm was converted to a $NOAEL_{HEC}$ of 167 ppm and then divided by a total uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability); see Appendix A for details.
[c]Used to derive an intermediate-duration inhalation MRL of 0.4 ppm. The LOAEL of 1,099 ppm was converted to a $LOAEL_{HEC}$ of 111 ppm and then divided by a total uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustments, and 10 for human variability); see Appendix A for details.

BC = blood chemistry; Bd wt or BW = body weight; Cardio = cardiovascular; CEL = Cancer Effect Level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; F = female(s); FI = food intake; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; HE = hematology; HEC = human equivalent concentration; Hemato = hematological; HP = histopathology; Immuno = immunological; LC_{50} = median lethal concentration; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurological function; OF = organ function; OP = ophthalmology; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive function; SLOAEL = serious lowest-observed-adverse-effect level; UR = urinalysis; (WB) = whole-body

Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation
Acute (≤14 days)

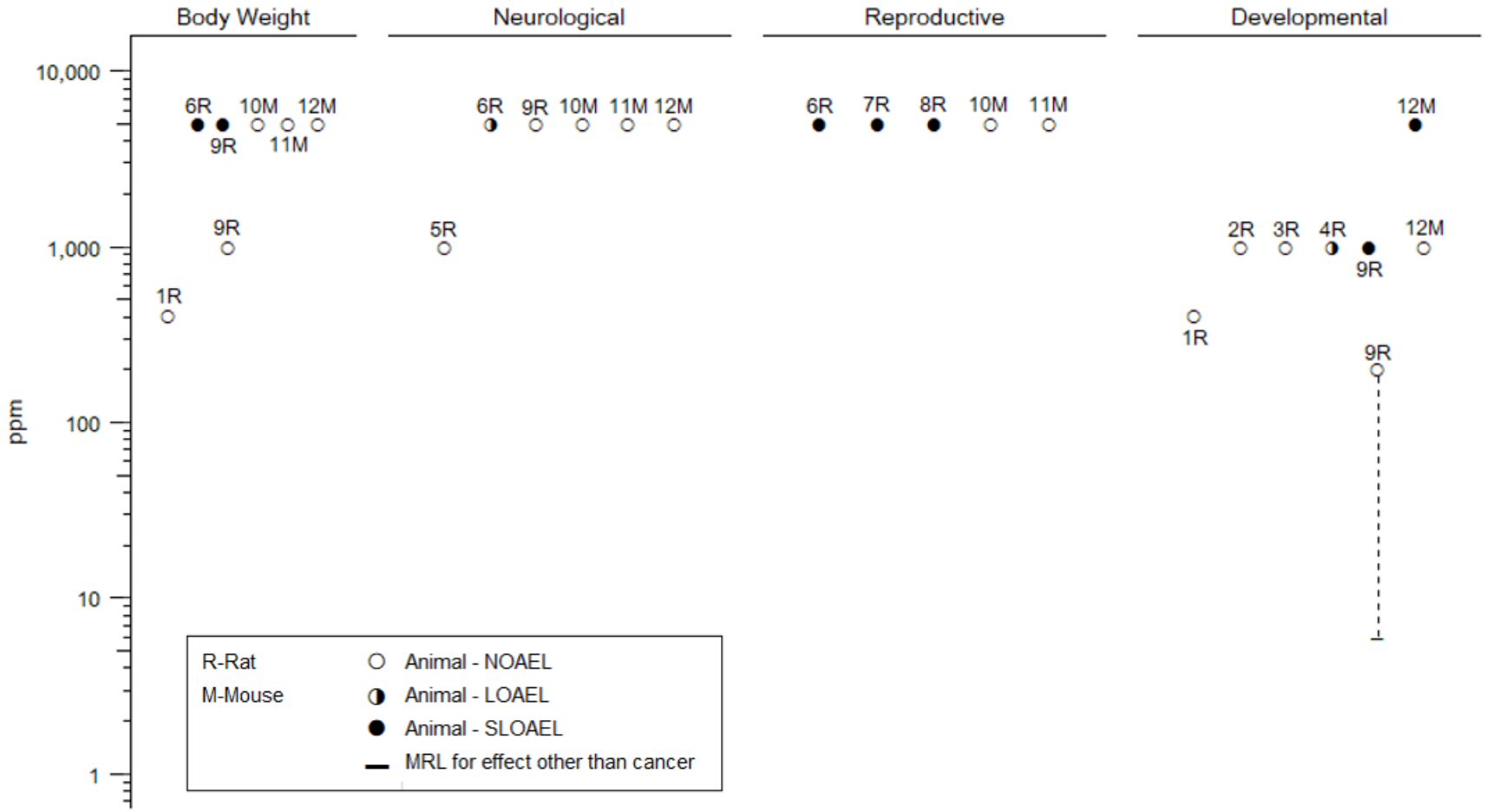


Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation
Intermediate (15–364 days)

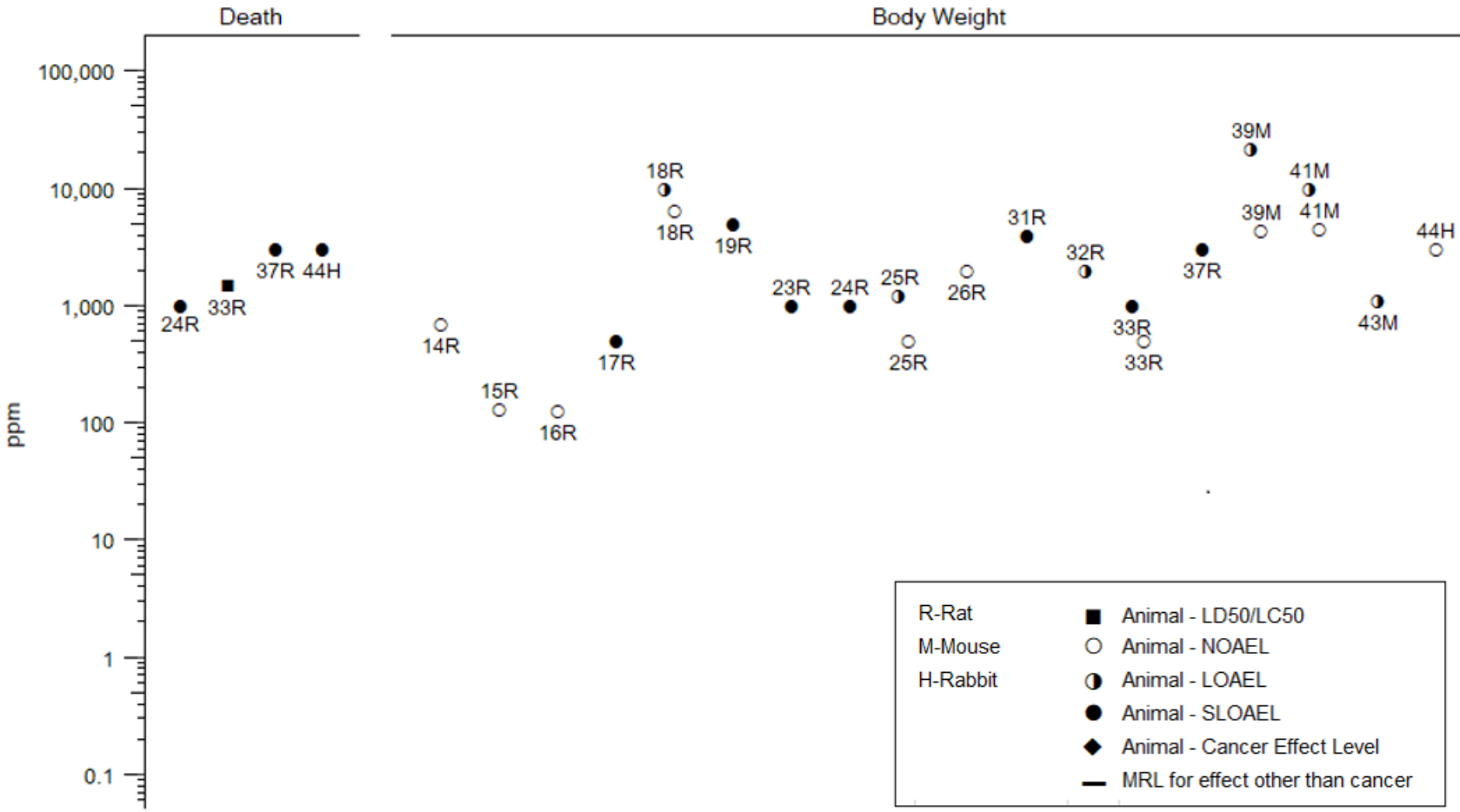


Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation
Intermediate (15–364 days)

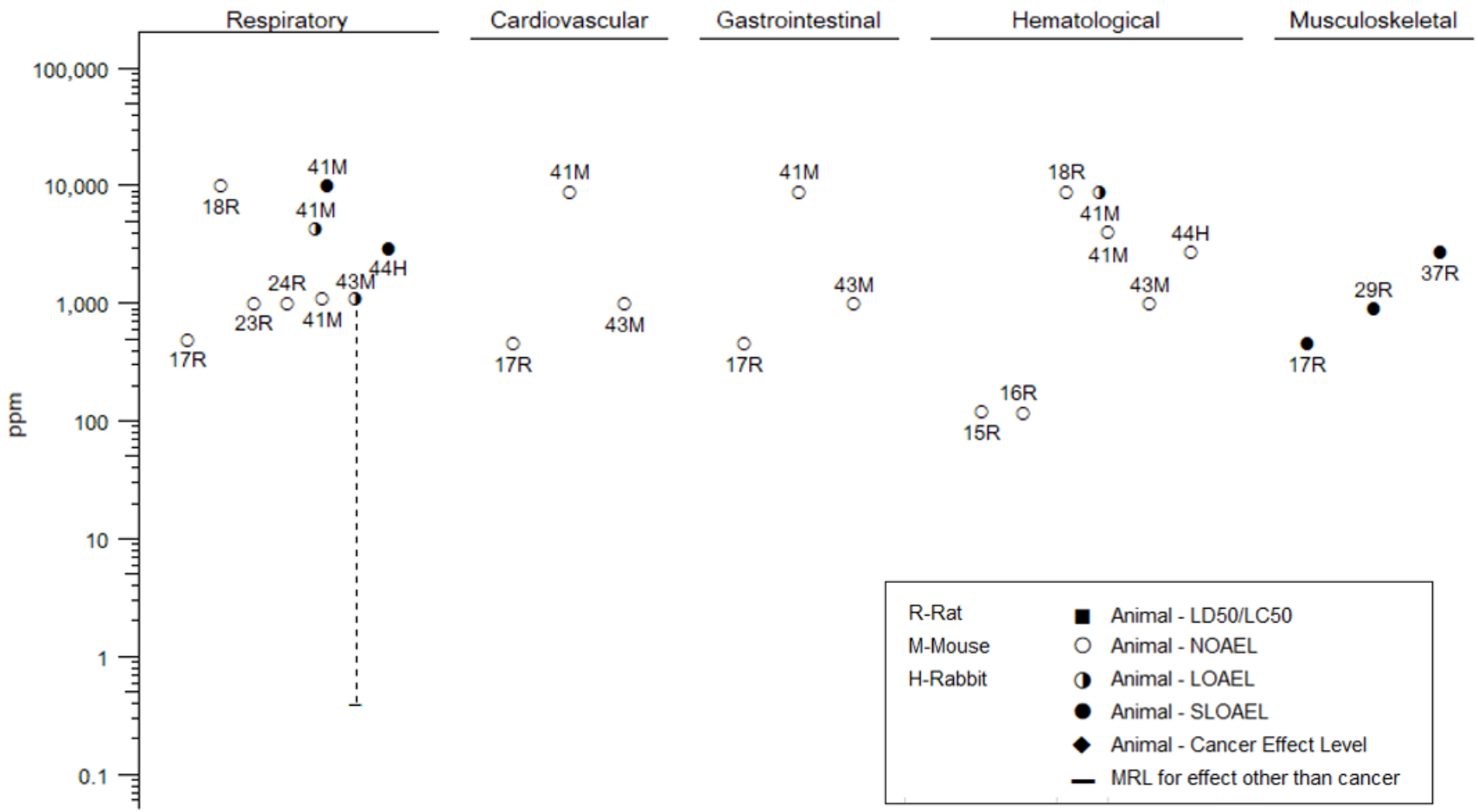


Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation
Intermediate (15–364 days)

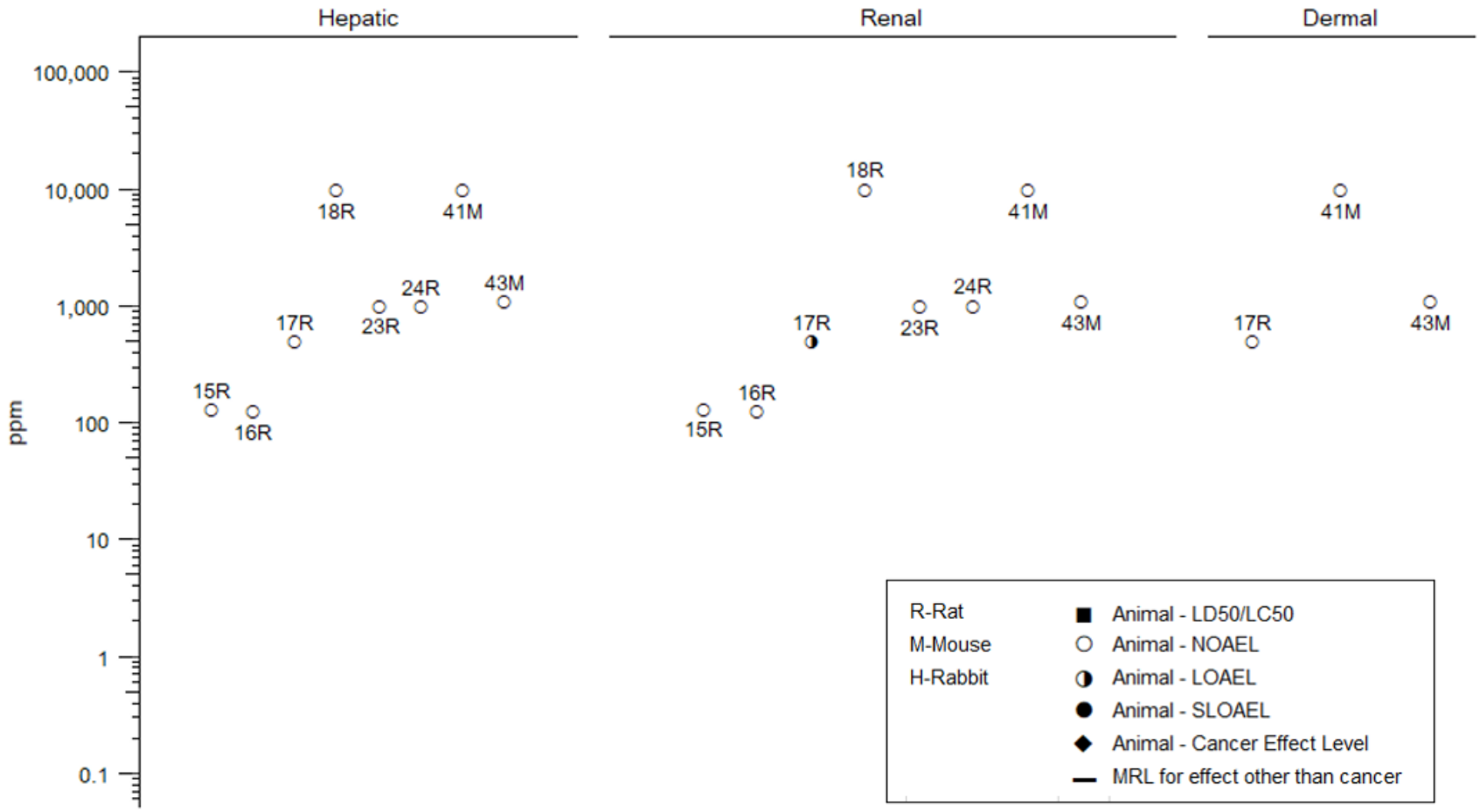


Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation
Intermediate (15–364 days)

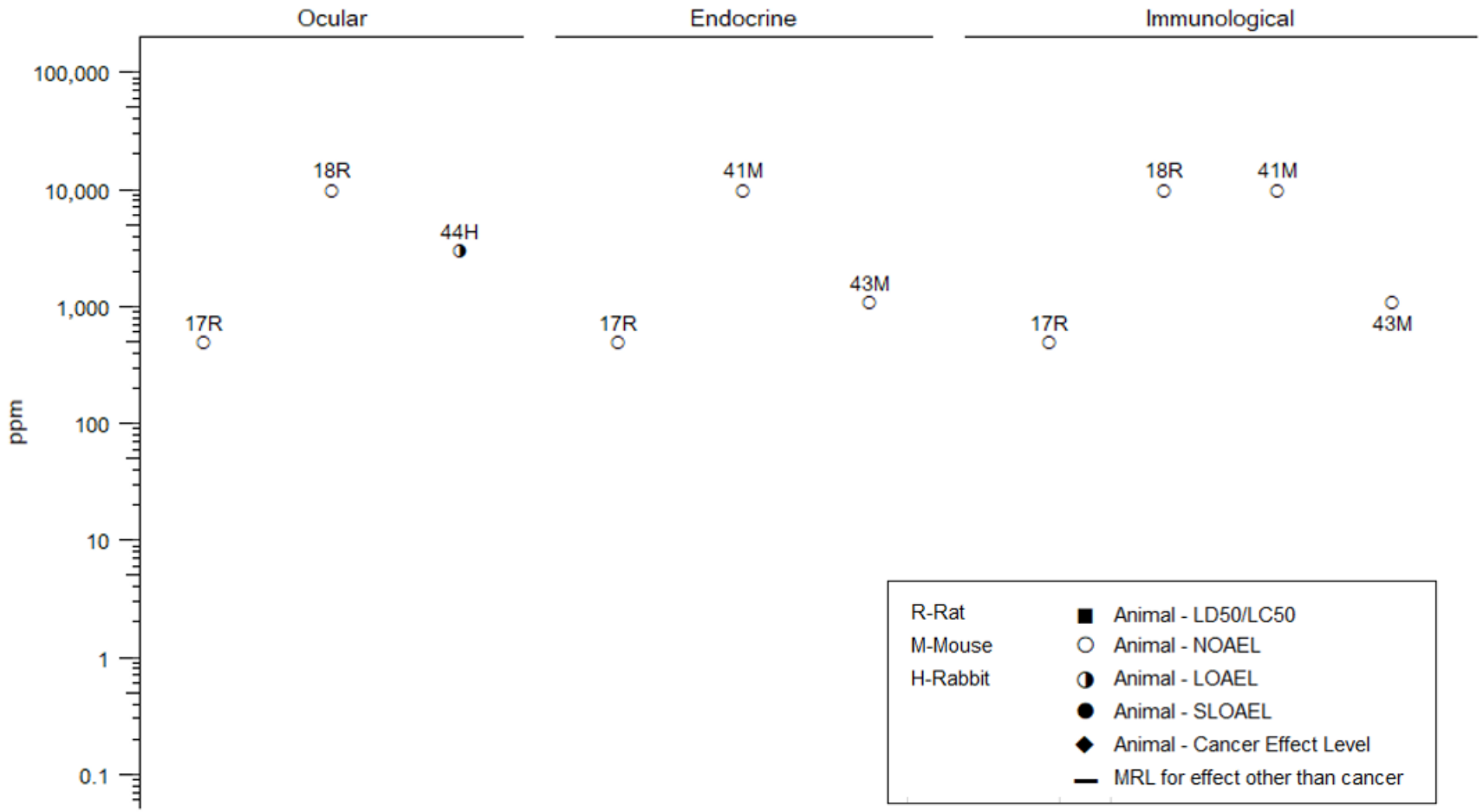


Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation
Intermediate (15–364 days)

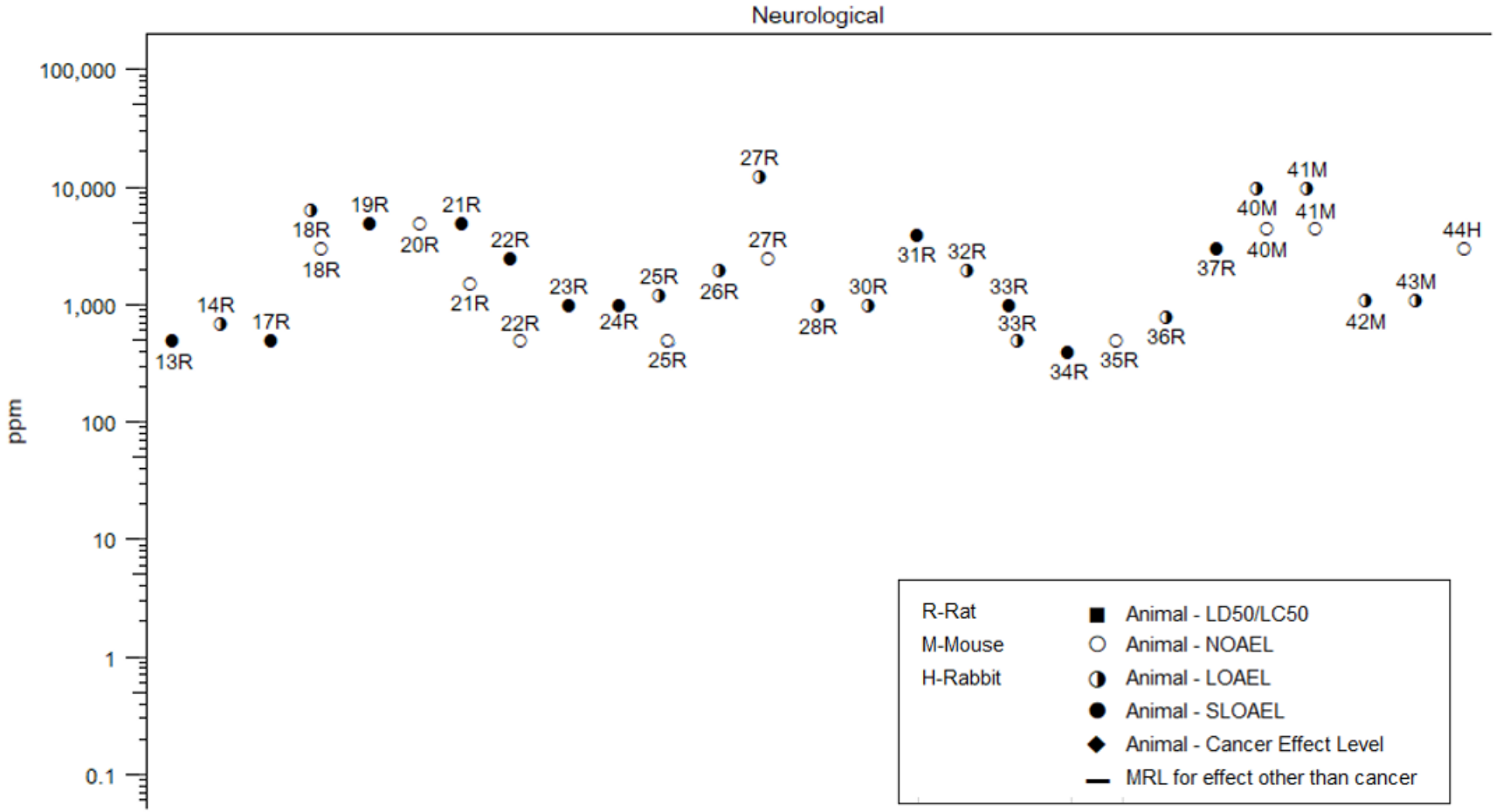


Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation
Intermediate (15–364 days)

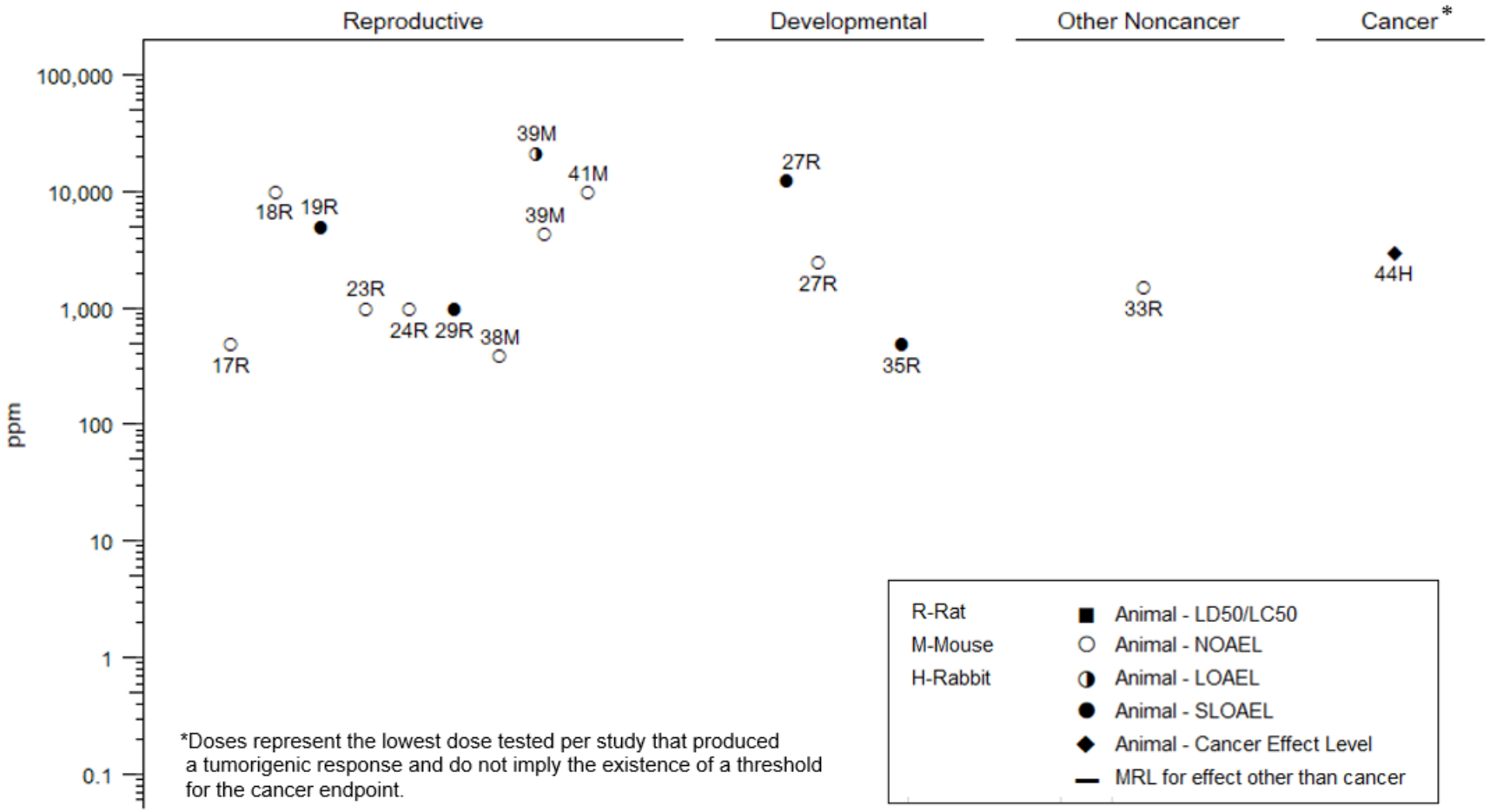


Figure 2-2. Levels of Significant Exposure to *n*-Hexane – Inhalation
Chronic (≥365 days)

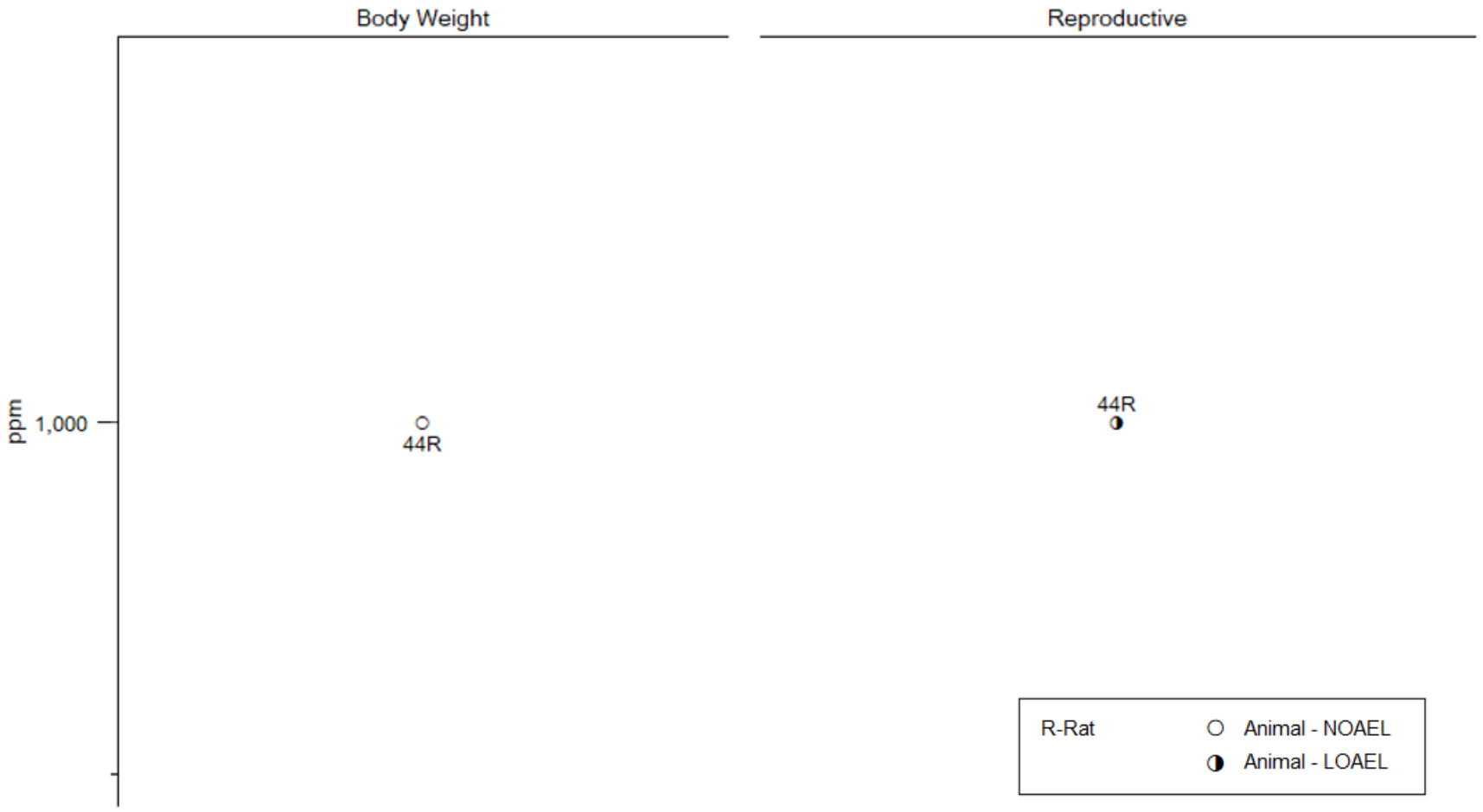


Table 2-2. Levels of Significant Exposure to *n*-Hexane – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE									
Kimura et al. 1971									
1	Rat (Sprague-Dawley) 6–12 B	Once (G)		LE	Death			15,840	LD_{50}
Linder et al. 1992									
2	Rat (Sprague-Dawley) 6 M	1 day 2 times/day (G)	0, 20,000	BW, HP, RX, OW	Repro	20,000			
Linder et al. 1992									
3	Rat (Sprague-Dawley) 6 M	5 days 2 times/day (G)	0, 10,000	BW, HP, OF, OW	Repro	10,000			
Marks et al. 1980									
4	Mouse (CD-1) 24–35 F	10 days GDs 6–15 3 times/day (GO)	0, 2,170, 2,830, 7,920, 9,900	CS, DX, LE	Death			2,830	Increased mortality in dams (9%, not statistically significant)
					Develop	2,830	7,920		Decreased fetal weight (6%)
Marks et al. 1980									
5	Mouse (CD-1) 6–14 F	10 days GDs 6–15 1 time/day (GO)	0, 260, 660, 1,320, 2,200	CS, DX, LE	Develop	2,200			

Table 2-2. Levels of Significant Exposure to *n*-Hexane – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
INTERMEDIATE EXPOSURE									
Krasavage et al. 1980									
6	Rat (COBS) 5 M	90–120 days 5 days/week 1 time/day (G)	0, 570, 1,140, 4,000	BW, CS, HP	Bd wt		570		Decreased body weight (15%)
					Neuro	1,140		4,000	Hindlimb paralysis, axonal swelling, myelin retraction
					Repro	1,140		4,000	Testicular atrophy of the germinal epithelium
Li et al. 2018									
7	Rat (Wistar) 10 M	10 weeks 6 times/week (GO)	0, 1,000, 2,000, 3,000	CS, BW, NX	Bd wt	1,000	2,000	3,000	LOAEL: Decreased body weight (19%) SLOAEL: Decreased body weight (26%)
					Neuro	1,000	2,000		Abnormal gait, decreased ability to stay on rotating rod
Li et al. 2020a									
8	Rat (Wistar) 10 M	7 weeks 1 time/day (GO)	0, 3,000	CS, BW, HP	Bd wt			3,000	Decreased body weight (23%)
					Neuro			3,000	Paralysis, abnormal gait, nerve damage
Li et al. 2020b									
9	Rat (Wistar) 8 M	10–24 weeks (GO)	0, 500, 1,000, 2,000, 4,000	CS, BW, NX	Bd wt	500	1,000	4,000	LOAEL: Decreased body weight (19%) SLOAEL: Decreased body weight (28%)
					Neuro	500	1,000	2,000	LOAEL: Transient paralysis, abnormal gait, decreased rotarod latencies, decreased motor conduction velocity SLOAEL: Paralysis after 14 weeks of exposure

Table 2-2. Levels of Significant Exposure to *n*-Hexane – Oral (mg/kg/day)

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
Ono et al. 1981									
10	Rat (Wistar) 5–7 M	8 weeks 1 time/day (GO)	0, 1,000	BW, CS, NX	Bd wt	1,000			
					Neuro		1,000		Decreased motor and mixed nerve conduction velocity
Wang et al. 2017									
11	Rat (Wistar) 12 M	8 weeks 1 time/day (GO)	0, 3,000	CS	Neuro		3,000		Decreased grip strength, abnormal gait
Gao et al. 2019									
12	Mouse (Kunming) 10 M, F	20 days (G)	0, 43.5, 86.5, 173.0	BW, NX	Neuro		43.5[b]		Impaired performance on a test of memory

[a]The number corresponds to entries in Figure 2-3.
[b]Used to derive an intermediate-duration oral risk level (MRL) of 0.1 mg/kg/day. The LOAEL of 43.5 mg/kg/day was divided by a total uncertainty factor of 300 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability); see Appendix A for details.

B = both males and females; Bd wt or BW = body weight; CS = clinical signs; Develop = developmental; DX = developmental toxicity; F = female(s); (G) = gavage; GD = gestation day; (GO) = gavage in oil; HP = histopathology; LD_{50} = median lethal dose; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Neuro = neurological; NOAEL = no-observed-adverse-effect level; NX = neurological function; OF = organ function; OW = organ weight; Repro = reproductive; RX = reproductive function; SLOAEL = serious lowest-observed-adverse-effect level

Figure 2-3. Levels of Significant Exposure to *n*-Hexane – Oral
Acute (≤14 days)

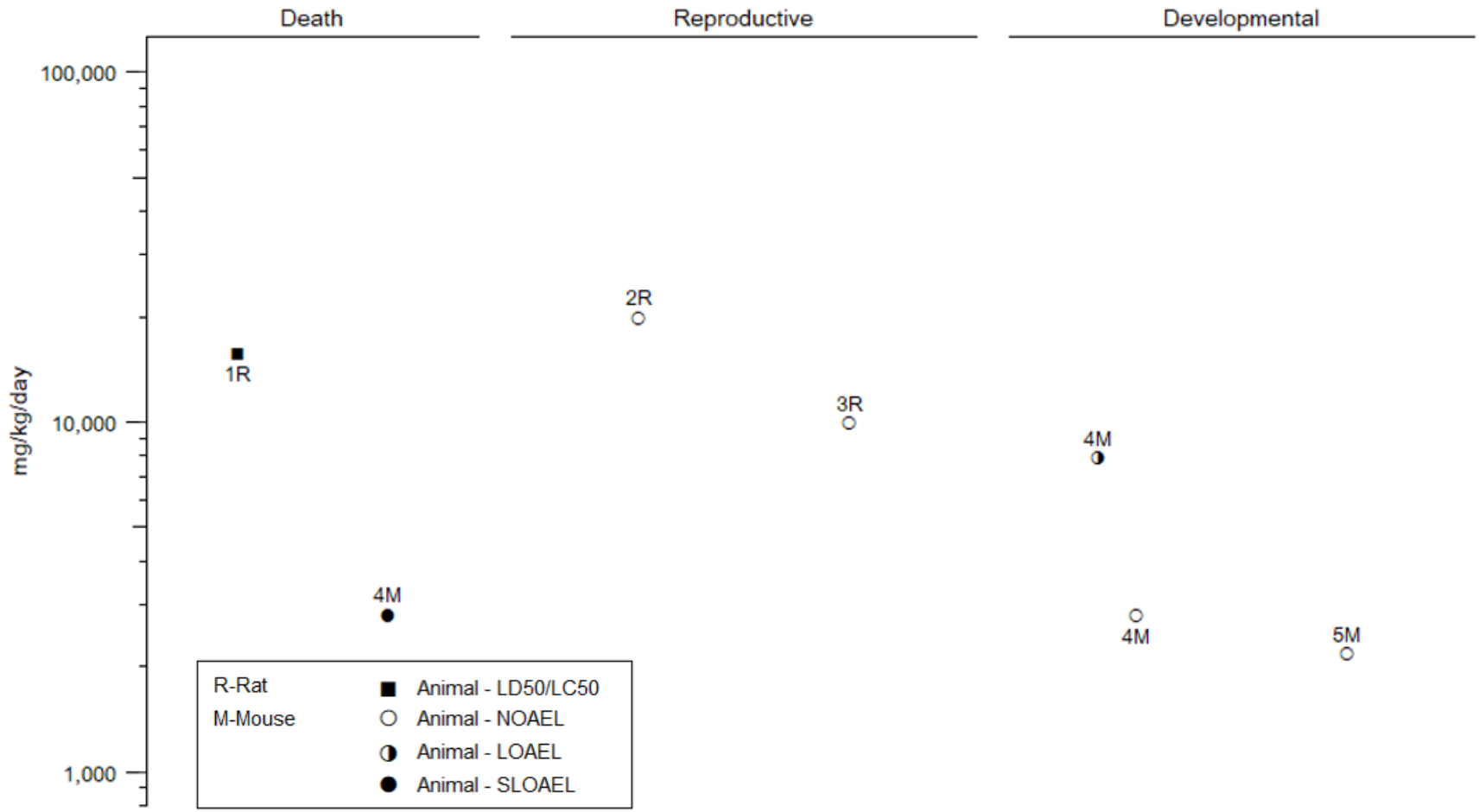


Figure 2-3. Levels of Significant Exposure to *n*-Hexane – Oral
Intermediate (15–364 days)

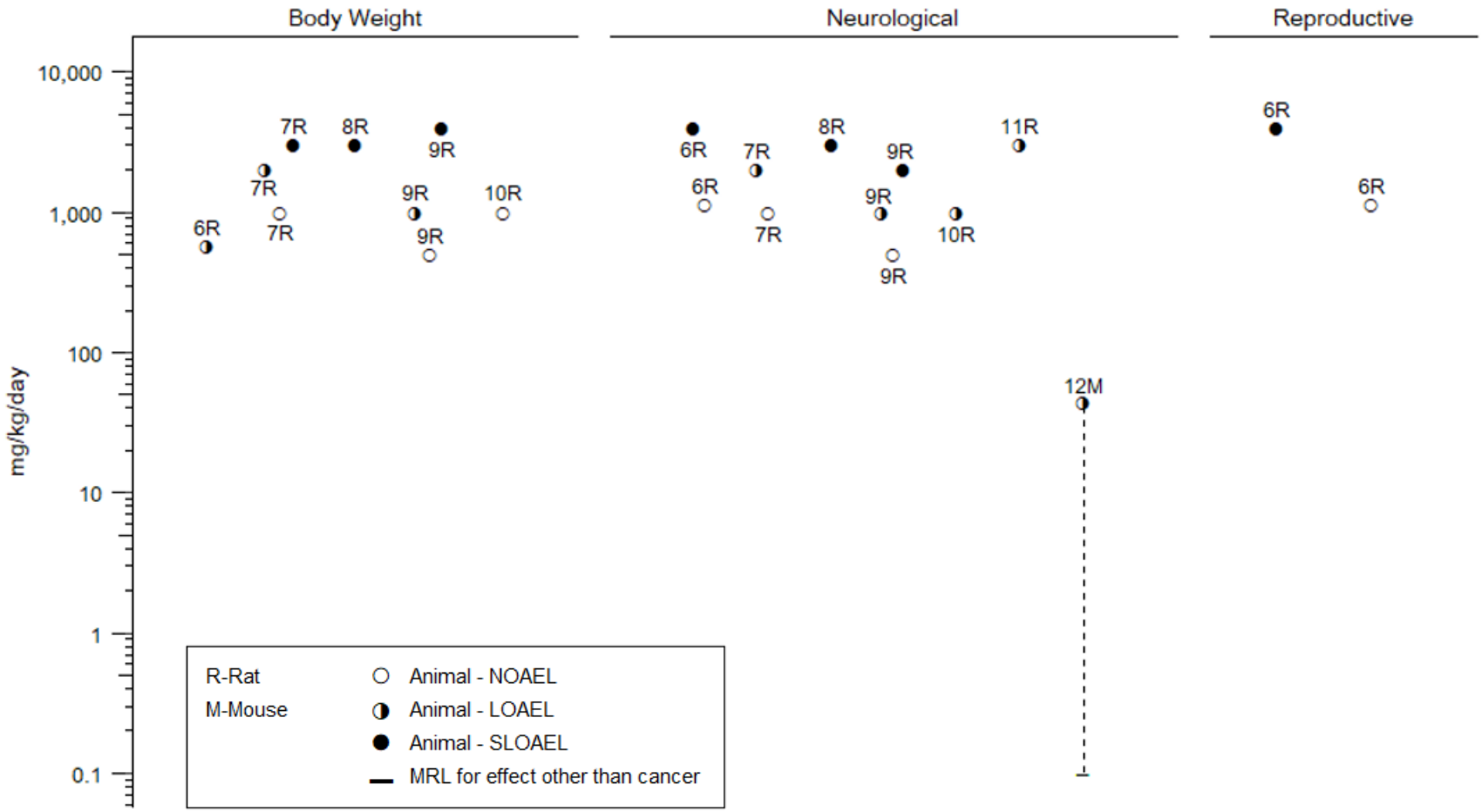


Table 2-3. Levels of Significant Exposure to *n*-Hexane – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
Iyadomi et al. 2000								
Mouse (BALB/c) 5 F	Once	0, 80 µL	OF	Dermal		80		Increased ear thickness
Wahlberg and Boman 1979								
Guinea pig (NS) 30 NS	Once	2 mL	BW, CS, LE	Bd wt	2			

Bd wt or BW = body weight; CS = clinical signs; F = female(s); LE = lethality; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function

2.2 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. All-cause mortality was increased in a cohort of shoe manufacturing workers compared to U.S. referent rates, but not compared to state or county reference rates (Lehman and Hein 2006). Excess deaths from lung cancer and ischemic heart disease (IHD) were the main contributors to the overall mortality elevation (compared to U.S. rates). In a population-based study from Canada, positive associations were observed between increased *n*-hexane exposure and non-accidental causes and nonmalignant respiratory mortality (Villeneuve et al. 2013). When adjusting for nitrogen dioxide (NO_2), only nonaccidental causes remained significant. No associations were observed between *n*-hexane and mortality from cardiovascular disease or cancer (including lung cancer).

No studies were located describing death in test animals after acute-duration inhalation exposure to *n*-hexane. No exposure-related deaths were observed in male or female mice or in female rats exposed up to 5,000 ppm for as long as 14 days (API 1979; Chalansonnet et al. 2013; NIEHS 1987, 1988a, 1988b 1988c).

Longer-duration inhalation studies have shown mixed results with regard to mortality. Increased mortality (up to 50%) was observed in several intermediate-duration inhalation studies in rats and rabbits with concentrations as low as 1,000 ppm *n*-hexane (Howd et al. 1983; Lungarella et al. 1984; Rebert and Sorenson 1983; Takeuchi et al. 1980). In contrast, no exposure-related deaths were observed in rats or mice exposed up to 10,000 ppm for as long as 40 weeks (Altenkirch et al. 1982; API 1978, 1981; Cavender et al. 1984; Howd et al. 1983; Huang et al. 1989; Ichihara et al. 1998; NTP 1991; Pryor et al. 1983) or in rats chronically exposed to 1,000 ppm (Imai and Omoto 1999).

Several oral LD_{50} (lethal dose, 50% kill) values are available for *n*-hexane: 15,840 mg/kg was reported for 14-day-old rats, 32,340 mg/kg for young adult rats (80–160 g), and 28,710 mg/kg for older adult rats (300–470 g) (Kimura et al. 1971). No exposure-related deaths were observed in mice following acute-duration exposure up to 2,830 mg/kg/day or in rats exposed for up to 4,000 mg/kg/day 120 days (Bouakkaz et al. 2018; Krasavage et al. 1980; Marks et al. 1980).

Topical application of a single 2-mL dose of undiluted *n*-hexane had no effect on survival in exposed guinea pigs observed for 35 days after exposure (Wahlberg and Boman 1979).

2.3 BODY WEIGHT

Data on body weight effects in humans exposed to *n*-hexane are very limited. In an offset printing factory in Hong Kong, weight loss of >5 pounds was reported in employees who developed peripheral neuropathy after exposure to solvents containing *n*-hexane, and in an additional 5 out of 26 asymptomatic workers who were considered to have subclinical peripheral neuropathy (Chang et al. 1993). In a cross-sectional study conducted in Portugal, exposure to indoor *n*-hexane was associated with an increased risk of obesity in children (mean age of 9 years) (Paciencia et al. 2019).

Body weight effects were often observed in animal studies, but these effects were typically accompanied by decreased food consumption and are thought to be a secondary effect following injury to the primary neurological targets of *n*-hexane. In acute-duration inhalation studies, exposure to *n*-hexane at ≥5,000 ppm resulted in decreased body weight in female rats (NIEHS 1987), but not at concentrations ≤1,000 ppm (API 1979; NIEHS 1987), and not in male or female mice (NIEHS 1988a, 1988b, 1988c). Body weight decreases were also observed following intermediate-duration exposure to *n*-hexane. Concentrations ≥1,000 ppm resulted in 10–79% decreases in body weight and/or body weight gain in rats and mice (Altenkirch et al. 1982; API 1981; Cavender et al. 1984; De Martino et al. 1987; Howd et al. 1983; Huang et al. 1989; NTP 1991; Pryor et al. 1983; Rebert and Sorenson 1983; Takeuchi et al. 1980). In contrast, several intermediate- and chronic-duration studies have not found changes in body weights in similarly exposed rats, mice, and rabbits (Altenkirch et al. 1982; API 1978; Cavender et al. 1984; Ichihara et al. 1998; Imai and Omoto 1999; Lungarella et al. 1984).

Body weight changes are also a common occurrence following oral exposure to *n*-hexane. Intermediate-duration studies have reported decreased body weights in rats with daily doses ≥570 mg/kg/day (Krasavage et al. 1980; Li et al. 2020a, 2020b). Daily oral doses of 1,000 mg/kg/day for 8 weeks of *n*-hexane had no effect on body weight in male rats (Ono et al. 1981).

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).

2.4 RESPIRATORY

Data on potential respiratory effects in humans are limited. In a controlled human study, vapor concentrations up to 500 ppm *n*-hexane (purity not listed) did not cause irritation to the nose or throat in 10 volunteers exposed for 3–5 minutes in an inhalation chamber (Nelson et al. 1943). Self-reported respiratory symptoms including cough, phlegm, bronchitis, and chest tightness were more frequent in solvent-exposed chemical plant workers compared to controls (Mustajbegovic et al. 2000). No association was observed between self-reported breathing difficulty and total months working with solvents in shoe workers (Nijem et al. 2000), although a second study showed that self-reported breathing difficulty was correlated with years of exposure in varnishing workers (Nijem et al. 2001).

Ambient concentrations of *n*-hexane were associated with lower forced expiratory volume (FEV_1) and forced vital capacity (FVC) scores in children (mean age of 8 years) living near petrochemical plants in Argentina (Wichmann et al. 2009). In a cross-sectional study, no association was observed between ambient *n*-hexane concentrations and hospital visits for children (≤16 years of age) with wheezy episodes (Buchdahl et al. 2000). Similarly, no association was observed between indoor *n*-hexane concentrations and the incidence of rhinitis in children (mean age 9 years) (Paciencia et al. 2020).

Respiratory effects have been reported in mice and rabbits following intermediate-duration inhalation studies, while rats may be less sensitive to these effects. Increased lung weights have been observed in male rats exposed to concentrations ≥1,000 ppm (Howd et al. 1983), but no histopathological changes in the lungs or nasal cavities were observed (API 1981; Cavender et al. 1984). In mice, histopathological effects in the nasal cavity (multifocal regeneration and metaplasia in the olfactory epithelium), have been observed at *n*-hexane concentrations ≥4,421 ppm for 6 hours/day, 5 days/week or 1,099 ppm for 22 hours/day, 5 days/week for 13 weeks (NTP 1991). Sneezing was observed in mice exposed to 10,000 ppm starting at 4 weeks of exposure (NTP 1991). Male rabbits exposed to 3,000 ppm for 24 weeks showed signs of respiratory tract irritation (nasal discharge) and breathing difficulties (gasping, lung rales, mouth breathing) (Lungarella et al. 1984). Additionally, histopathological examination revealed pulmonary fibrosis, centriacinar emphysema, and epithelial desquamation.

Increased lung weights were also observed in male rats administered 600 mg/kg/day *n*-hexane via gavage for 8 weeks (Bouakkaz et al. 2018). Histopathological evaluation of the lungs revealed lesions comparable to acute interstitial pneumonia, including multifocal bronchopneumonia, fibronecrotic lesions, alveoli filled with red blood cells, and inflammatory cells. The presence of erythrocytes in the

lungs was graded as a severe effect at this dose by the study authors. The nature of these lesions suggests an injury to the lungs, which could potentially occur with an error in gavage dosing.

2.5 CARDIOVASCULAR

No studies were located on cardiovascular effects of *n*-hexane in humans.

Histopathological examination of the heart and aorta revealed no treatment related lesions in male rats exposed up to 500 ppm *n*-hexane for 22 hours/day, 7 days/week for 6 months (API 1981). Differences in relative heart weights were reported in B6C3F1 mice exposed for 13 weeks to *n*-hexane at concentrations of 1,099 ppm for 22 hours/day, although no histopathological changes were observed (NTP 1991).

2.6 GASTROINTESTINAL

No studies were located on gastrointestinal effects of *n*-hexane in humans.

Histopathological examination of gastrointestinal tissues revealed no treatment-related lesions in male rats exposed to up to 500 ppm *n*-hexane for 22 hours/day, 7 days/week for 6 months (API 1981). Likewise, no gastrointestinal alterations were reported in mice exposed for 13 weeks to *n*-hexane at concentrations up to 10,000 ppm for 6 hours/day or 1,099 ppm for 22 hours/day (NTP 1991).

2.7 HEMATOLOGICAL

No exposure-related differences in blood parameters (e.g., complete blood counts) were reported in offset printers (Chang et al. 1993) or tungsten carbide alloy workers (Sanagi et al. 1980) occupationally exposed to *n*-hexane. White blood cell counts were unaffected by *n*-hexane exposure in 35 workers compared to 23 unexposed controls (Karakaya et al. 1996). No differences in complete blood or platelet counts were observed in *n*-hexane-exposed male shoe repairers compared to controls (Tomei et al. 1999). Decreased hematocrit (females only) and mean corpuscular volume (males only) were observed in shoe workers in Bosnia and Herzegovina compared to controls, although the study authors stated that the values still fell within the laboratory's reference range (Umicevic et al. 2022).

Hematological parameters were within normal limits in rats exposed up to 10,000 ppm for 13 weeks (Cavender et al. 1984). Similarly, no exposure-related changes were observed in male and female rats

exposed up to 129 ppm for 6 months (API 1978). In male mice exposed to 10,000 ppm for 6 hours/day for 13 weeks, increased number of segmented neutrophils was observed, which the study authors attributed to chronic active inflammation in the nasal mucosa (NTP 1991). No other biologically relevant changes were observed in female mice similarly exposed up to 10,000 ppm for 6 hours/day for 13 weeks or in male and female mice exposed to 1,099 ppm *n*-hexane for 22 hours/day for 13 weeks. No changes in hematological parameters were observed in male rabbits exposed to 3,000 ppm *n*-hexane for 24 weeks (Lungarella et al. 1984).

Increases in white blood cells were observed in male rats administered 300 mg/kg/day *n*-hexane via gavage for 8 weeks compared to vehicle controls (Bouakkaz et al. 2018). These animals presented with signs of acute interstitial pneumonia.

2.8 MUSCULOSKELETAL

Muscle wasting and atrophy have been reported in humans with severe neurotoxicity occupationally exposed to *n*-hexane (Yamamura 1969). Three women who worked as cabinet finishers and presented with signs of neuropathy had normal levels of creatine phosphokinase (data not provided), while electromyograms showed fibrillation in the proximal and distal muscles (Herskowitz et al. 1971). Additionally, histopathological evaluation of the muscle showed angulation of fibers and target fibers, both signs of denervation. Muscle weakness and denervation were also reported in offset printers occupationally exposed to *n*-hexane (Chang et al. 1993).

Hindlimb muscular atrophy characterized as “severe” was reported in male rats exposed to 986 ppm *n*-hexane for up to 61 days (Nylen et al. 1989). Skeletal muscle atrophy was observed in male rats exposed to 500 ppm *n*-hexane for 22 hours/day for 6 months (API 1981). Electron microscopy of the gastrocnemius and soleus muscles in male rats exposed to 3,040 ppm *n*-hexane for 12 hours/day for 16 weeks, revealed atrophy, denervation, irregular fibers, disordered myofilaments, zigzagging of the Z-band, and numerous invaginations of the plasma membrane (Takeuchi et al. 1980).

2.9 HEPATIC

There is a limited amount of information on potential hepatic effects in workers. Mean concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, and alkaline phosphatase (AP) were higher among *n*-hexane-exposed male shoe repairers compared to controls, although no

differences in gamma-glutamyl transferase (GGT), cholesterol, or triglycerides were observed (Tomei et al. 1999). Increased bilirubin, AST, and GGT were observed in male and female shoe workers in Bosnia and Herzegovina compared to controls, although the study authors stated that most values still fell within the laboratory's reference range (Umicevic et al. 2022). Serum AST and ALT activities were within normal limits in a group of shoe and leather workers (Murata et al. 1994).

Based on data from animal studies, the liver does not appear to be a primary target of *n*-hexane toxicity. Increased liver weights have been observed in mice and rats exposed to *n*-hexane concentrations ≥1,000 ppm, but histopathological changes have either not been observed or not been evaluated (API 1981; Cavender et al. 1984; Howd et al. 1983; NTP 1991). No change in liver function parameters (ALT, AST, AP) were observed in male and female rats exposed up to 10,000 ppm for 13 weeks (Cavender et al. 1984) or in male or female rats exposed up to 129 ppm for as long as 6 months (API 1978).

2.10 RENAL

No exposure-related differences in kidney function tests (e.g., urinalysis, blood urea nitrogen) were reported in offset printers (Chang et al. 1993), tungsten carbide alloy workers (Sanagi et al. 1980), or male shoe repairers (Tomei et al. 1999). Decreased creatinine levels were observed in female shoe workers in Bosnia and Herzegovina compared to controls, although the study authors stated that it still fell within the laboratory's reference range (Umicevic et al. 2022).

An increase in relative kidney weights along with an increased incidence and severity of chronic nephropathy were noted in male rats exposed to 500 ppm *n*-hexane for 22 hours/day for 6 months (API 1981). The study 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. Relative kidney weights were also increased in rats and mice exposed to *n*-hexane at ≥1,000 ppm, but no histopathological lesions were observed (Cavender et al. 1984; Howd et al. 1983; NTP 1991).

Urine pH was decreased in male rats exposed to 10,000 ppm for 13 weeks, but not in females and no other changes in urine parameters were noted in either sex (Cavender et al. 1984). Decreased blood urea nitrogen was noted in female rats, but not male rats, exposed to 126 ppm *n*-hexane for 21 hours/day, 7 days/week for 6 months (API 1978). This difference was not observed at 3 months or in female rats

exposed for 6 hours/day, 5 days/week for 6 months. The toxicological significance of these changes in urinary parameters is unknown.

2.11 DERMAL

Coldness, reddishness, or roughness of the skin in the distal extremities was observed in workers with peripheral neuropathy after occupational inhalation exposure to *n*-hexane (Yamamura 1969). Application of 1.5 mL analytical-grade *n*-hexane to the volar forearm of a male volunteer caused an initial increase blood flow (expressed as a relative, dimensionless value), which returned to control approximately 60 minutes after application (Wahlberg 1984). A slight transient erythema was observed after 10–20 minutes of exposure and a stinging and/or burning sensation was reported by the volunteer.

Histopathological examination of the skin after intermediate-duration inhalation exposure revealed no treatment-related lesions in rats exposed to 500 ppm (API 1981) or in mice exposed to *n*-hexane at concentrations up to 10,000 ppm for 6 hours/day for 13 weeks or 1,099 ppm for 22 hours/day for 13 weeks (NTP 1991).

Dermal application of 80 µL to the front and back of the ears of female mice resulted in increased ear thickness within 2 hours of exposure (Iyadomi et al. 2000). Peak ear thickness was observed at 6 hours and was still significantly thicker than controls 24 hours post-exposure. There were no differences in ear thickness 48 or 72 hours post-exposure, as compared to controls.

2.12 OCULAR

In a controlled human study, vapor concentrations up to 500 ppm *n*-hexane did not cause irritation to the eyes, nose, or throat in 10 volunteers exposed for 3–5 minutes in an inhalation chamber (Nelson et al. 1943). Maculopathy and color discrimination defects were identified in 11 out of 15 workers exposed to *n*-hexane from glues or solvents used in vegetable oil extraction, although these workers were also exposed to numerous other chemicals (Raitta et al. 1978; Seppalainen et al. 1979).

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/day for 6 months (API 1981). No ophthalmologic differences were reported in male and female rats exposed to up to 10,000 ppm for 13 weeks compared to controls (Cavender et al. 1984). Ocular

irritation (lacrimation, hyperemia of the conjunctiva) was observed in male rabbits exposed to 3,000 ppm *n*-hexane for 24 weeks (Lungarella et al. 1984).

2.13 ENDOCRINE

The data on the potential endocrine effects of *n*-hexane are very limited. Three women who worked as cabinet finishers and presented with signs of neuropathy had normal thyroxine levels (data not provided) (Herskowitz et al. 1971).

Histopathological examination of endocrine tissues after intermediate-duration inhalation exposure revealed no treatment-related lesions in male rats exposed to 500 ppm *n*-hexane daily for 22 hours/day for 6 months (API 1981). Similar results were seen in mice exposed for 13 weeks to *n*-hexane at concentrations up to 10,000 ppm for 6 hours/day or at 1,099 ppm for 22 hours/day (NTP 1991).

2.14 IMMUNOLOGICAL

No differences were observed in natural killer (NK) cell cytotoxic capacity or in serum interleukin-2 (IL-2) and interferon-gamma (IFN-γ) levels in two male shoe factory workers compared to controls (Yücesoy et al. 1999). Serum immunoglobulins (IgG, IgM, and IgA) were reduced in male workers compared to unexposed controls, and Ig levels were correlated with urinary 2,5-hexanedione concentrations but not with workplace *n*-hexane concentrations (Karakaya et al. 1996). The reductions also remained well within the normal ranges for immunoglobulins in blood (Jackson et al. 1997), so the toxicological significance of these findings cannot be assessed. A study of outpatient clinic visits for urticaria found associations between higher levels of *n*-hexane in the air on the fourth lag day and the number of clinic visits (Tseng and Lu 2024). When the subjects were grouped by sex or age, the association was only found in subjects under 65 years of age. The study did not include an adjustment for confounding exposure to other volatile organic compounds (VOCs); associations were also found between urticaria clinic visits and several VOCs including benzene, ethyl benzene, toluene, xylene, 1,3,5-trimethylbenzene, and methylcyclohexane

No changes in spleen weight or histopathology were observed in male and female rats exposed to up to 10,000 ppm for 13 weeks (Cavender et al. 1984). No treatment-related lesions were observed in the lymph nodes, thymus, bone marrow, or spleen of rats exposed to 500 ppm for 6 months (API 1981) or in

mice exposed for 13 weeks to concentrations up to 10,000 ppm for 6 hours/day or 1,099 ppm for 22 hours/day (NTP 1991).

2.15 NEUROLOGICAL

The neurotoxicity of *n*-hexane was first observed in the shoe industries of Japan and Italy in the 1960s and 1970s (Abbritti et al. 1976; Cianchetti et al. 1976; Sobue et al. 1978; Yamamura 1969). 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 either not measured at all or not 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, such as acetone, methyl ethyl ketone (MEK), and toluene.

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). The most common physical symptoms included numbness in the distal portions of the extremities, muscle weakness, and hypoactive reflexes, while electromyography exams showed reductions in motor nerve conduction velocities and nerve biopsies revealed demyelination and axonal degeneration. Since then, numerous case reports/series have evaluated workers/patients exposed to organic solvents and exhibiting the characteristic signs of *n*-hexane-induced peripheral neuropathy (e.g., Abbritti et al. 1976; Altenkirch et al. 1977; Carelli et al. 2007; Hageman et al. 1999; Herskowitz et al. 1971; Kanavouras et al. 2011; Pastore et al. 2002; Paulson and Waylonis 1976; Pezzoli et al. 1995; Pradhan and Tandon 2015; Puri et al. 2007; Sendur et al. 2009; Smith and Albers 1997; Sun et al. 2020; Thulasirajah et al. 2020; Valentino 1996; Vanacore et al. 2000; Yokoyama et al. 1990).

Several occupational epidemiology studies are available that have evaluated a number of symptoms commonly observed with solvent-induced polyneuropathy (Table 2-4). Some of the most reported clinical signs include weakness in the extremities, absent or decreased reflexes, weight loss, muscle pain, and headaches (Chang 1987; Chang and Yip 1987; Chang et al. 1993; Huang et al. 1991; Nijem et al. 2000; Sanagi et al. 1980). Several studies have demonstrated subclinical alteration in neurological function after inhalation exposure to *n*-hexane. Abnormal nerve conduction tests (conduction velocity, distal latency, potential amplitude) have been observed in workers with and without outward clinical

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects

Reference, study type, and population	Exposure/biomarker	Outcome evaluated	Result
Bates et al. 2016, 2019 Cross-sectional, 831 automotive workers (San Francisco Bay area, California)	Estimated from MSDS Q1: 0 mg/m^3-years Q2: >0–<32 mg/m^3 -years Q3: ≥32 mg/m^3-years	Psychomotor speed, memory, fine motor function, mood	↔
		Clinical signs	↔ Ankle reflexes, touch sensation, vibration sensation
		MCV	↔ Peroneal
		SSPL	↓ (Q2 only)
Beckman et al. 2016 Cross-sectional, 689 automotive workers (San Francisco, California)	Estimated from MSDS Q1: 0 ppm-years Q2: >0–<9.6 ppm-years Q3: ≥9.6 ppm-years	Color vision	↔
Boggess et al. 2016 Case-control, 30 children with autism spectrum disorders, 30 age-matched controls (Pennsylvania)	Serum hexane concentration 11.7 µg/g in cases versus 9.44 µg/g in controls	Autism Diagnostic Observation Schedule	↔
Chang and Yip 1987 Cross-sectional, 75 printing factory workers, 72 controls (Taiwan) 25 polyneuropathy (S), 5 subclinical (AS), and 45 unaffected workers (HW)	Not reported	Clinical signs	↑ Weakness in extremities ↑ Absent/decreased reflexes
		MCV	↓ Median, ulnar, peroneal, tibial (S, AS, HW)
		MAP amplitude	↓ Median (S, AS, HW) ↓ Ulnar, peroneal, tibial (S, AS)
		MAP distal latency	↓ Median, ulnar, peroneal, tibial (HW, AS, S) (decreased latency)
		SCV	↓ Median, ulnar, sural (S, AS)
		SAP amplitude	↓ Median, ulnar, sural (S, AS, HW)
		SAP onset latency	↓ Median, ulnar, sural (S, AS) (decreased latency)

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects

Reference, study type, and population	Exposure/biomarker	Outcome evaluated	Result
Chang 1987 Cross-sectional, 34 printing factory workers, 22–25 controls (Taiwan) 22 polyneuropathy (S), 5 subclinical (AS), and 7 unaffected workers (HW)	Not reported	Clinical signs	↑ Weakness in extremities ↑ Absent/decreased reflexes ↔ Headaches, sleep disorders, mental changes
		MCV	↓ Median, ulnar, peroneal, tibial
		SCV	↓ Median, ulnar, sural
		Patterned visual evoked potentials	↑ Latency (N1, P1, N2, N1–N2) ↓ Amplitude (N1–P1, P1–N2)
		Brainstem auditory evoked potentials	↑ Latency (wave III, wave V, I–III, III–V, I–V) ↔ Latency (wave I)
		Somatosensory evoked potentials	↑ Latency (scalp median, scalp peroneal, neck median)
Chang et al. 1993 Cross-sectional, 56 printing press workers, 20 controls (Hong Kong) 10 healthy workers (HW), 26 asymptomatic (AS) and 20 symptomatic (S) neuropathic workers	Background 63 ppm (30–110 ppm) Personal samplers 132 ppm (80–210 ppm) 2.6 years (1 month–12 years) (Work schedule 12 hours/day, 6 days/week)	SAP amplitude	↓ Median (HW, AS, S) ↓ Ulnar (S) ↓ Sural (AS, S)
		MAP amplitude	↓ Median (S) ↓ Ulnar (AS, S) ↓ Posterior tibial (S) ↓ Common peroneal (S)
		SAP distal latency	↑ Median (AS, S) ↑ Ulnar (S) ↑ Sural (S)
		MAP distal latency	↑ Median (AS, S) ↑ Ulnar (AS, S) ↑ Posterior tibial (S) ↑ Common peroneal (S)
		Motor conduction velocity	↓ Median (AS, S) ↓ Ulnar (AS, S) ↓ Posterior tibial (AS, S) ↓ Common peroneal (AS, S)
		Clinical signs	↑ Weight loss >5 pounds (AS, S) ↑ CNS symptoms[a] (S)

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects

Reference, study type, and population	Exposure/biomarker	Outcome evaluated	Result
Chen et al. 2023a, 2023b 2,610 Deepwater Horizon disaster oil spill workers (United States) 1,646 workers <50 years of age 964 workers ≥50 years of age	Cumulative exposure: Q3: 397–1,239 ppb-days Q4: 1,241–62,299 ppb-days	Vibrotactile threshold	↑, Q3 ≥50 years ↔, Q4 all participants, <50 years
		Visual contrast sensitivity performance	↔
		Postural sway (eyes open and eyes closed)	↔, Q4 all participants, <50 years, ≥50 years
		Inability to maintain single leg stance	↔, Q4 all participants, <50 years, ≥50 years
Goldman et al. 2012 Case-control, 99 pairs of twins (World War II veterans) discordant for Parkinson's disease	Estimated from job history Low 2.5 ppm Medium 25 ppm High 100 ppm	Parkinson disease	↔
Gong et al. 2003 Cross-sectional, 182 furniture workers, 96 unexposed controls (Japan)	Mean 0.29 ppm Maximum 12.9 ppm	Color vision	↑ Color confusion index ↓ Visual contrast sensitivity
		Visual evoked potential	↔ Latency, amplitude
Governa et al. 1987 Cross-sectional, 40 shoe factory workers (Italy)	Urinary 2,5-HD 6.8 mg/L (0.5–19 mg/L)	Electroneuromyographic abnormalities	↑

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects

Reference, study type, and population	Exposure/biomarker	Outcome evaluated	Result
Huang et al. 1991 Cross-sectional, 44 ball manufacturing workers, 52 controls (Taiwan)	High 86 and 110 ppm (cement coating, nylon fiber winding) Low 75 ppm (gas injection, outer layer production)	Clinical signs	↑ Muscle pain, weakness
		MCV	↓ Median, ulnar, peroneal, tibial
		Motor amplitude	↓ Median, ulnar, peroneal, tibial (high only)
		Motor DL	↑ Median, ulnar, peroneal, tibial
		SCV	↓ Median, ulnar, sural (high) ↓ Median (low)
		Sensory amplitude	↓ Median, ulnar, peroneal, tibial (high only)
		Sensory DL	↑ Median, ulnar, peroneal, tibial
Issever et al. 2002 Case-control, 26 workers diagnosed with *n*-hexane-induced polyneuropathy, 50 unexposed controls (Turkey)	Not conducted	Color vision	↑ Hue error test scores
Ithnin et al. 2011 Cross-sectional, 17 locomotive depot workers, 17 controls (Malaysia)	0.01–0.03 ppm	Neurobehavioral tests	↓ Santa Ana Manual Dexterity (non-dominant hand) ↑ Pursuit aiming test 1 ↔ Reaction time, digital symbol, trail making
Juarez-Perez et al. 2014 Cross-sectional, 77 paint factory workers, 84 controls (Mexico)	0.96 ppm median (0.25–19 ppm)	Hearing impairments	↑ Hearing loss in all frequencies
		Brainstem auditory-evoked potentials	↑ Latency
Murata et al. 1994 Cross-sectional, 30 shoe and leather workers, 25 unexposed controls (Japan)	Urinary 2,5-HD 1.39 mg/L (0–3.18 mg/L)	Electrocardiographic parameters	↓ R-R interval variability; parasympathetic activity ↔ Heart rate; sympathetic activity
		Nerve conduction velocity	↓ DCV, SCV (median, forearm) ↔ MCV (median), SCV (median, hand)
		Correlation with 2,5-HD levels	↔

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects

Reference, study type, and population	Exposure/biomarker	Outcome evaluated	Result
Mutti et al. 1982a Cross-sectional, 95 shoe factory workers, 52 controls (Italy)	Mild (M) exposure group 49/69 ppm (median/mean) High (H) exposure group 103/134 ppm (median/mean) 9.1 years (1–25 years)	Clinical signs (all exposed)	↑ Sleepiness, dizziness, weakness, paraesthesia, hypoesthesia ↔ Headache, muscular cramps, neurasthenic syndrome, sleep disturbances
		Motor conduction velocity	↓ Median, peroneal (M, H) ↔ Ulnar
		MAP amplitude	↓ Median, ulnar, peroneal (M, H)
		MAP duration	↑ Median (H) ↑ Ulnar (M, H) ↔ Peroneal (M, H), median (M)
Mutti et al. 1982b Cross-sectional, 15 shoe factory workers, 15 controls (Italy)	127/195 ppm (median/mean) 4.5 years (2–8 years)	Motor conduction velocity	↓ Median, ulnar, peroneal
		Distal sensory conduction velocity	↓ Median, ulnar
		Sensory nerve action potential latency	↑ Median, ulnar
		MAP duration; MAP amplitude; distal latency	↔ Median, ulnar, peroneal
		Sensory nerve action potential amplitude	↔ Median, ulnar
Neghab et al. 2012 Cross-sectional, 27 AS shoemakers, 20 controls (Iran)	Breathing zone 33 ppm (5–85 ppm) Urinary 2,5-HD 0.23 mg/L (0.12–0.36 mg/L) 26 years (17–44 years)	MCV, MAP, DL	↔ Median, ulnar, posterior tibial, peroneal
		SCV, DL	↔ Median, ulnar, sural
		SAP	↓ Median, sural ↔ Ulnar
		Correlation between SAP and 2,5-HD	↑
Nijem et al. 2000 Cross-sectional, 103 shoe workers (West Bank)	Not reported	Clinical signs	↑ Headaches, mental irritability
		Correlation with years of exposure	↔

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects

Reference, study type, and population	Exposure/biomarker	Outcome evaluated	Result
Nijem et al. 2001 Cross-sectional, 167 shoe factory workers (West Bank)	Not reported	Correlation with years of exposure	↔ Headaches, mental irritability ↑ Tingling limbs (plastic work), sore eyes (cleaning, plastic work)
Park et al. 2009 Cross-sectional, 41 solvent workers, 90 nonexposed controls (South Korea)	0.11–1.41 ppm across 4 plants	Postural sway (eyes open)	↑ Sway (area, length)
		Postural sway (eyes closed)	↔ Sway (area, length)
Pastore et al. 1994 Cross-sectional, 20 “healthy” workers with urinary 2,5-HD of >5 mg/L, 49–141 controls from previous studies (Spain)	Urinary 2,5-HD 11 mg/L (5–24 mg/L) 8 years (1.5–23 years)	Sensory nerve conduction velocity	↔ Median, ulnar, sural
		MNCV, latency	↔ Tibial
		Sensory nerve action potential amplitude	↓ Median, sural, ulnar
Raitta et al. 1978; Seppalainen et al. 1979 Cross-sectional, 15 adhesive and oil extraction workers, 10 unexposed controls (Finland)	Highest concentrations varied from 1,500 to 3,250 ppm 12 years (5–21 years)	Visual evoked potentials	↓ Amplitude (N1, P2, N2, P3, N3) ↑ Amplitude (P1) ↑ Latency (P1, N1) ↓ Latency (P2) ↔ Latency (N0, N2, P3, N3)
		Electroretinograms	↓ Amplitude ↔ Latency (a wave) ↓ Latency (b wave)
		Visual acuity, visual fields, intraocular pressure, biomicroscopic examination findings	↔
		Color vision defects	↑ (12/15 examined)

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects

Reference, study type, and population	Exposure/biomarker	Outcome evaluated	Result
Sanagi et al. 1980 Cross-sectional, 14 tungsten carbide alloy workers, 14 controls (Japan)	58 ppm 8-hour TWA 6.2 years (1–12 years)	Clinical signs	↑ Headache, hearing deficit, muscle weakness, dysesthesia in limbs ↔ Vertigo, muscle pain, numbness in limbs
		Neurological tests	↓ Muscle strength (jumping on one foot), vibration sensation on the radial processes ↔ Grip, position sense, coordination
		Motor conduction velocity	↔ Median ↓ Tibial
		Residual latency	↔ Median ↑ Tibial
		MAP; MNCV; distal sensory conduction velocity	↔ Median, tibial
Sliwinska-Kowalska et al. 2005 Cross-sectional, 1,117 yacht, ship, paint and lacquer, plastic and shoe workers, 157 (no solvent or noise exposure) and 66 (no solvent exposure) controls (Poland)	Not reported	Hearing loss	↑ Solvent exposed, *n*-hexane and toluene exposed
Talbott et al. 2015 Case-control, 217 children diagnosed with autism (Pennsylvania)	0.00004 ppm (50^{th} percentile)	Autism Spectrum Disorders	↔
Tsai et al. 1997 Cross-sectional, 298 paint factory workers (Taiwan)	0–62.35 ppm	Neurobehavioral tests	↑ Continuous performance test, pattern comparison test, pattern memory test latencies ↔ Finger tapping, associate learning, switching attention, mood scales

Table 2-4. Results of Epidemiological Studies Evaluating Exposure to *n*-Hexane and Neurological Effects

Reference, study type, and population	Exposure/biomarker	Outcome evaluated	Result
Verberk et al. 2004 Case-control, 30 male patients diagnosed with chronic solvent encephalopathy, 41 controls (Netherlands)	Not reported	Pattern-reversal visual evoked potentials	↓ Low and low/high contrast (N75–P100)
		Pattern-onset visual evoked potentials	↔
		P300	↑ Latency ↓ Amplitude
Wang et al. 1986 Cross-sectional, 57 press proofing workers (Taiwan) (54 examined)	Low 0–23 ppm Mid 11–93 ppm High (AS) 34–41 ppm High (AS) 22–190 ppm 5.8 years (2 months–25 years)	MCV	↓ Median (all groups) ↓ Ulnar, peroneal (low, mid, high symptomatic) ↓ Tibial (high symptomatic)
		SCV	↓ Median, ulnar, sural (high symptomatic)
		Nerve histopathology	↑ Axonal degradation, changes in the myelin sheath
Yokoyama et al. 1997 Cross-sectional, 29 shoe workers, 22 unexposed controls (Japan)	40 ppm (13–100 ppm) 28 years (3–42 years)	Clinical signs	↔
		Postural sway (eyes open)	↑ Sway (2–4 Hz, anterior posterior); sway path; power of sway ↔ Sway (0–1, 1–2, 2–4 Hz medio-lateral; 0–1, 1–2 Hz anterior-posterior)
		Postural sway (eyes closed)	↑ Sway (0–1 Hz, anterior posterior; 0–1 Hz mediolateral); sway path; power of sway ↔ Sway (1–2, 2–4 Hz medio-lateral; 1–2, 2–4 Hz anterior-posterior)

[a]Symptoms included headache, deteriorating memory, drunken feeling, and vertigo.

↑ = increase; ↔ = no change; ↓ = decrease; 2,5-HD = 2,5-hexanedione; AS = asymptomatic; CNS = central nervous system; DCV = distribution of nerve conduction velocities; DL = distal latency; HW = healthy worker; Hz = frequency; MAP = motor action potential; MCV = motor nerve conduction velocity; MNCV = mixed nerve conduction velocity; MSDS = Material Safety Data Sheet; Q = quartile; S = symptomatic; SAP = sensory action potential; SCV = sensory nerve conduction velocity; SSPL = sural sensory peak latency; TWA = time-weighted average

signs of polyneuropathy (Chang 1987; Chang and Yip 1987; Chang et al. 1993; Huang et al. 1991; Mutti et al. 1982a, 1982b; Neghab et al. 2012; Raitta et al. 1978; Seppalainen et al. 1979). Additional studies have evaluated alterations in vision (Chen et al. 2023a, 2023b; Gong et al. 2003; Issever et al. 2002; Raitta et al. 1978; Seppalainen et al. 1979; Verberk et al. 2004), hearing loss/ototoxicity (Juarez-Perez et al. 2014; Sliwinska-Kowalska et al. 2005), postural sway (Chen et al. 2023a, 2023b; Park et al. 2009; Yokoyama et al. 1997), vibrotactile threshold (Chen et al. 2023a, 2023b), and a variety of neurobehavioral outcomes (Ithnin et al. 2011; Tsai et al. 1997).

Neurological outcomes are commonly evaluated in rodents following inhalation exposure to *n*-hexane (Table 2-5). No clinical signs of neurotoxicity have been observed in acute-duration inhalation studies in rats and mice following exposure up to 5,000 ppm for as long as 14 days (Chalansonnet et al. 2013; NIEHS 1987, 1988a, 1988b, 1988c), although motor conduction velocity was decreased in rats exposed to 5,000 ppm *n*-hexane for 6 days and for 2–4 weeks (De Martino et al. 1987). 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. Clinical signs of neurotoxicity (abnormal gait, hindlimb weakness, irritability), altered nerve conduction tests (decreased motor conduction velocity), and histopathology (axonal swellings, myelin degradation, denervated neuromuscular junctions) have all been reported (Altenkirch et al. 1982; API 1981; Cavender et al. 1984; Frontali et al. 1981; Howd et al. 1983; Huang et al. 1989; Ichihara et al. 1998; Li et al. 2014; NTP 1991; Pryor et al. 1983; Rebert and Sorenson 1983; Schaumburg and Spencer 1976; Takeuchi et al. 1980). No signs of peripheral neurotoxicity, such as hindlimb weakness or foot dragging, were observed in male rabbits exposed by inhalation to 3,000 ppm *n*-hexane for 24 weeks (Lungarella et al. 1984).

Exposure to *n*-hexane may also result in hearing loss (ototoxicity). Rats exposed to 1,000 ppm *n*-hexane for 21 or 61 days showed a decrease in auditory sensitivity (decreased brainstem auditory response) (Nylen and Hagman 1994; Nylen et al. 1994), although this response had disappeared 3 months after exposure (Nylen et al. 1994). Alterations in the brainstem auditory responses in rats have also been measured in several other rat studies at concentrations ≥1,000 ppm (Howd et al. 1983; Pryor and Rebert 1992; Pryor et al. 1983; Rebert and Sorenson 1983).

Similar effects have also been observed in rodents following oral exposure to *n*-hexane. Decreased motor nerve conduction velocity was reported in male rats orally exposed to 1,000 mg/kg/day for 8 weeks, although no changes in behavior were observed (Ono et al. 1981). Abnormal gait and decreased ability to

Table 2-5. Results of Rodent Studies Evaluating Inhalation Exposure to *n*-Hexane and Neurological Effects

Reference species (strain), sex	Exposure, duration	Total hours	Outcome evaluated	Result
Acute-duration exposure				
Chalansonnet et al. 2013 Rats (SD), M	1,000 ppm 10 days, 6 hours/day	60	Clinical signs	↔
NIEHS 1988a Mice (B6C3F1), M	5,000 ppm 5 days, 20 hours/day	100	Clinical signs	↔
NIEHS 1988b Mice (CD-1), M	5,000 ppm 5 days, 20 hours/day	100	Clinical signs	↔
Intermediate-duration exposure				
Altenkirch et al. 1982 Rats (Wistar), M	500 ppm 9 weeks, 7 days/week, 22 hours/day	1,386	Clinical signs	↑ Narcosis, limb weakness, paralysis
			Histopathology	↑ Scattered multifocal giant axonal swellings, breakdown of axons, myelin degradation
	700 ppm 40 weeks, 7 days/week, 8 hours/day	2,240	Clinical signs	↔
			Histopathology	↑ Axonal swellings
API 1981 Rats (Albino), M	500 ppm 6 months, 7 days/week, 22 hours/day	3,960	Clinical signs	↑ Abnormal gait
			Histopathology	↑ Peripheral nerve atrophy ↔ Axonal degeneration, lesions in the brain, spinal cord, or neuroganglia

Table 2-5. Results of Rodent Studies Evaluating Inhalation Exposure to *n*-Hexane and Neurological Effects

Reference species (strain), sex	Exposure, duration	Total hours	Outcome evaluated	Result
Cavender et al. 1984 Rats (F-344), M/F	10,000 ppm 13 weeks, 5 days/week, 6 hours/day	390	Clinical signs	↔ Posture, gait, tone and symmetry of facial muscles, reflexes
			Absolute brain weight	↓ 10% (males only)
			Histopathology	↔ Brain ↑ Sciatic nerve (axonopathy, paranodal axonal swelling)
De Martino et al. 1987 Rats (SD), M	5,000 ppm 6 days, 16 hours/day	96	Motor conduction velocity	↓
	5,000 ppm 4–6 weeks, 6 days/week, 16 hours/day	384–576	Clinical signs	↑ Polyneuropathy
Frontali et al. 1981 Rats (SD), M	5,000 ppm 14 weeks, 5 days/week, 9 hours/day	630	Clinical signs	↔
			Histopathology	↑ Giant axonal degeneration (tibial nerve branches), paranodal and internodal swellings ↔ Optic nerve, medulla oblongata
	2,500 ppm 30 weeks, 6 days/week, 10 hours/day	1,800	Clinical signs	↔
			Histopathology	↑ Giant axonal degeneration (tibial nerve branches), paranodal and internodal swellings ↔ Optic nerve, medulla oblongata

Table 2-5. Results of Rodent Studies Evaluating Inhalation Exposure to *n*-Hexane and Neurological Effects

Reference species (strain), sex	Exposure, duration	Total hours	Outcome evaluated	Result
Howd et al. 1983 Rats (F-344), M Weanling (21 days old) and Adults (80 days old)	1,000 ppm 11 weeks, 6–7 days/week, 24 hours/day	1,680	Clinical signs	↑ Ataxia, difficulty walking, flaccid hindlimbs ↓ Grip strength
			Compound action potential	↓ Amplitude ↑ Latency
			Brainstem auditory-evoked response	↑ Latency
			Absolute brain weight	↓
			Relative brain weight	↓ (adults only)
			Histopathology	NC
Huang et al. 1989 Rats (Wistar), M	1,200 ppm 16 weeks, 7 days/week, 12 hours/day	1,344	Clinical signs	↓ Speed of movement ↔ Paralysis
			Sensory motor tests	↓ Grip strength
			Motor nerve conduction velocity	↓
			Histopathology	↑ Paranodal swellings, demyelination, remyelination
Ichihara et al. 1998 Rats (Wistar), M	2,000 ppm 20 weeks, 6 days/week, 12 hours/day	1,440	Motor nerve conduction velocity	↓
			Distal latency	↔
Li et al. 2014 Rats (Wistar), F	12,500 ppm 20 days, 4 hours/day	80	Clinical signs	↑ Irritability, attack tendency
Lungarella et al. 1984 Rabbits (New Zealand), M	3,000 ppm 24 weeks, 5 days/week, 8 hours/day	600	Clinical signs	↔

Table 2-5. Results of Rodent Studies Evaluating Inhalation Exposure to *n*-Hexane and Neurological Effects

Reference species (strain), sex	Exposure, duration	Total hours	Outcome evaluated	Result
NIEHS 1988c Mice (CD-1), F	5,000 ppm 12 days, 20 hours/day	240	Clinical signs	↔
NIEHS 1987 Rats (SD), F	5,000 ppm 14 days, 20 hours/day	280	Clinical signs	↔
NTP 1991 Mice (B6C3F1), M/F	10,000 ppm 13 weeks, 5 days/week, 6 hours/day	390	Sensory motor tests	↓ Motor activity (females only)
			Histopathology	↑ Paranodal swellings (tibial nerve) ↔ Paranodal swellings (spinal cord), axonal degeneration, demyelination, brain
			Relative brain weight	↑ (males only)
	1,099 ppm 13 weeks, 5 days/week, 22 hours/day	1,430	Sensory motor tests	↓ Motor activity (females only)
			Histopathology	↑ Paranodal swellings (tibial nerve) ↔ Paranodal swellings (spinal cord), axonal degeneration, demyelination, brain
			Relative brain weight	↑ (males only)
Nylen et al. 1994 Rats (SD), M	1,000 ppm 28 days 21 hours/day	588	Motor conduction velocity	↓
			Auditory brainstem response	↑ Latency, amplitude
			Flash evoked potential	↓ Amplitude
Nylen and Hagman 1994 Rats (SD), M	1,000 ppm 61 days 18 hours/day	1,098	Motor conduction velocity	↓
			Auditory brainstem response	↑ Latency
			Flash evoked potential	↑ Latency
Pryor and Rebert 1992 Rats (F-344), M	4,000 ppm 9 weeks 14 hours/day	882	Sensory motor tests	↓ Grip strength
			Motor conduction velocity	↓
			Auditory brainstem response	↓ Amplitude

Table 2-5. Results of Rodent Studies Evaluating Inhalation Exposure to *n*-Hexane and Neurological Effects

Reference species (strain), sex	Exposure, duration	Total hours	Outcome evaluated	Result
Pryor et al. 1983 Rats (F-344), M	2,000 ppm 14 weeks, 7 days/week, 14 hours/day	1,372	Sensory motor tests	↓ Motor activity, startle response, grip strength, conditioned avoidance response
			Auditory evoked response	↔ Latency
			Brainstem auditory-evoked potential	↓ Amplitude ↔ Latency
			Visual evoked response	↑ Latency
			Compound action potential	↑ Latency
			Histopathology	↔ Tibial and sciatic nerves
Rebert and Sorenson 1983 Rats (F-344), M	1,000 ppm 11 weeks, 5 days/week, 24 hours/day	1,320	Sensory motor tests	↓ Grip strength
			Compound action potential	↑ Latency
			Somatosensory evoked response	↑ Latency
			Brainstem auditory-evoked response	↑ Latency
			Auditory evoked response	↑ Latency
			Visual evoked response	↑ Latency
Schaumburg and Spencer 1976 Rats (SD), NS	400–600 ppm 162 days, 7 days/week, 24 hours/day	3,888	Clinical signs	↑ Unsteady, waddling gait, distal hindlimb weakness with footdrop, distal weakness of the upper extremities
			Histopathology	↑ Axonal dilation and swellings, myelinated fiber degeneration
Stoltenburg-Didinger et al. 1990 Rats (Wistar), F	500 ppm 21 days, 23 hours/day	483	Clinical signs	↔
	800 ppm 63 days, 23 hours/day	1,449	Clinical signs	↑ Hindlimb weakness

Table 2-5. Results of Rodent Studies Evaluating Inhalation Exposure to *n*-Hexane and Neurological Effects

Reference species (strain), sex	Exposure, duration	Total hours	Outcome evaluated	Result
Takeuchi et al. 1980 Rats (Wistar), M	3,000 ppm 16 weeks, 7 days/week, 12 hours/day	1,344	Clinical signs	↑ Unsteady gait, footdrop
			Nerve conduction velocity	↓ Motor, mixed
			Distal latency	↑
			Histopathology	↑ Paranodal swellings, denervated neuromuscular junctions

↑ = increase; ↔ = no change; ↓ = decrease; F = female; M = male; NS = not specified; SD = Sprague-Dawley

stay on a rotating rod were observed in rats receiving gavage doses of 2,000 mg/kg/day *n*-hexane for 10 weeks, although all animals were normal in appearance (Li et al. 2018). In addition to abnormal gait, decreased grip strength was observed in male rats administered 3,000 mg/kg/day *n*-hexane for 8 weeks via gavage (Wang et al. 2017).

Clinical signs of neurotoxicity (hindlimb weakness or paralysis) were observed in male rats administered 4,000 mg/kg/day *n*-hexane via gavage for 90 days, along with tibia1 nerve alterations (multifocal axonal swellings, adaxonal myelin infolding, paranodal myelin retraction) (Krasavage et al. 1980). Abnormal gait, paralysis, decreased rotarod latency, and decreased motor nerve conduction were observed in male rats exposed to 3,000 mg/kg/day *n*-hexane for 7 weeks (Li et al. 2020a). Histopathological examination of the sciatic nerve revealed damage including loss of myelin and vacuolization. Similarly, transient paralysis, abnormal gait, decreased rotarod latency, and decreased motor nerve conduction were observed in male rats exposed to 1,000 mg/kg/day *n*-hexane for 24 weeks (Li et al. 2020b).

While nonmammalian species are not commonly used in toxicology studies, the chicken has proven to be a valuable model for human neurotoxicity caused by organophosphates, which is clinically similar to that caused by *n*-hexane (Abou-Donia and Lapadula 1990). During a continuous 90-day exposure, chickens exposed to 1,000 ppm *n*-hexane developed mild ataxia and histologic changes in the spinal cord (no changes were seen in peripheral nerves). However, chickens exposed continuously to 1,008 ppm for 30 days showed no effects (Abou-Donia et al. 1991). Chickens orally exposed to 2,000 mg/kg showed mild leg weakness followed by full recovery after 2–4 days (Abou-Donia et al. 1982). Similarly, oral exposure to 100 mg/kg/day for 90 days resulted in leg weakness, but no other serious signs of neurotoxicity (Abou-Donia et al. 1982).

The neurotoxicity of *n*-hexane is believed to ultimately result from the effects the *n*-hexane metabolite, 2,5-hexanedione, on peripheral nerves. Another potential metabolite, 2-hexanone, has also caused neurotoxicity in humans (Allen et al. 1975). The other metabolites of *n*-hexane can also produce neurotoxicity in rats via their subsequent metabolism to 2,5-hexanedione (Krasavage et al. 1980). 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 1976; 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.

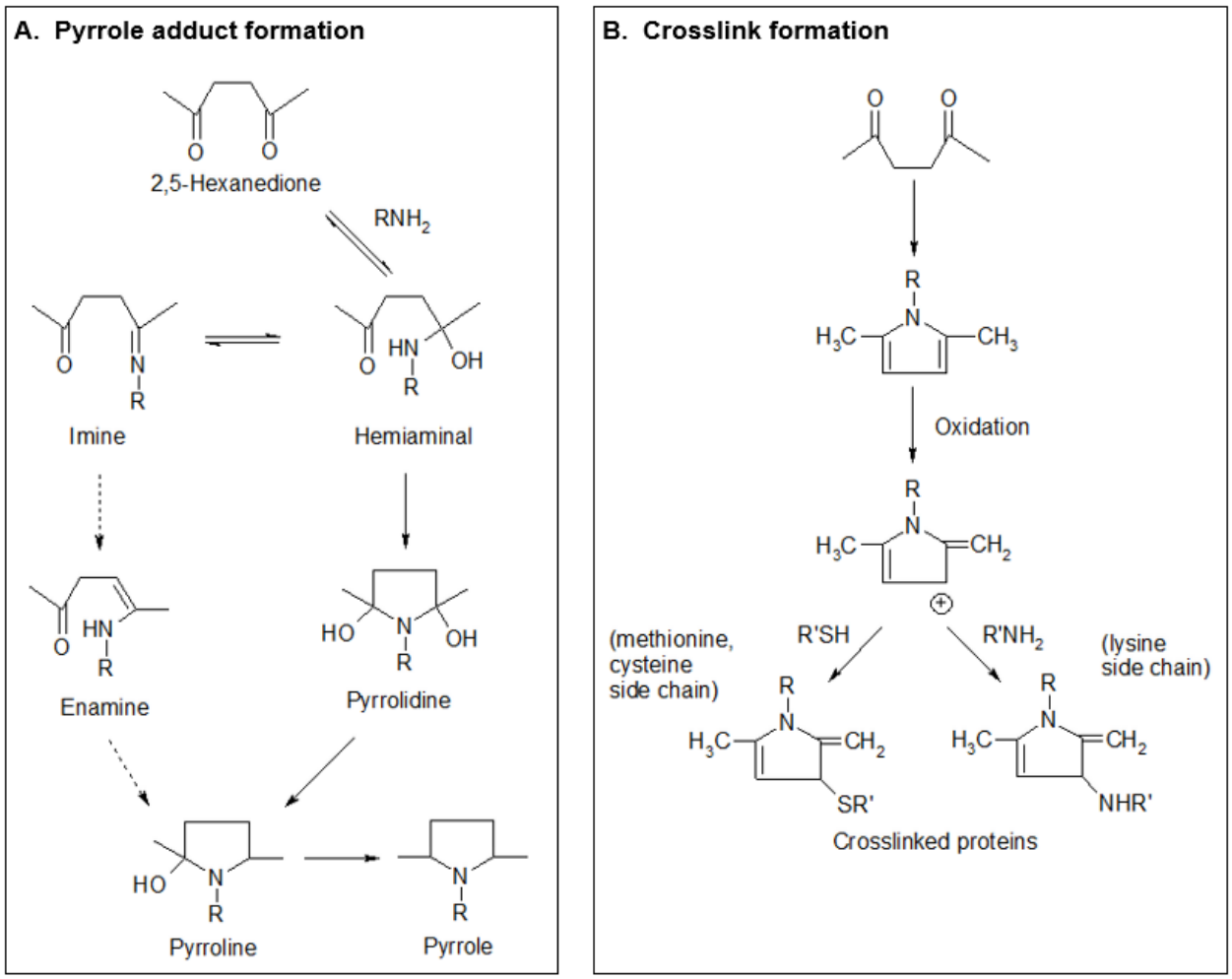
The sequence of events in *n*-hexane-induced neuropathy has been described in rats (Spencer and Schaumburg 1977a). The process appears to begin by increases in the number of 10 nm axonal neurofilaments and accumulation in swellings on the proximal sides of the nodes of Ranvier in distal regions of large, 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 suggests that it could react with lysine side-chain amino groups in proteins to form pyrroles (see Figure 2-4). *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 (Genter 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 that could form pyrroles but was resistant to oxidation. This analogue (3-acetyl-2,5-hexanedione) caused pyrrolidation of protein *in vivo*, but not neurotoxicity.

Figure 2-4. Reaction of 2,5-Hexanedione with Protein



Source: Graham et al. 1995

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).

2.16 REPRODUCTIVE

Women shoemakers reported longer menstrual cycles and longer times to get pregnant compared to controls (Ruiz-García et al. 2020). Additionally, serum FSH concentrations were inversely associated with *n*-hexane exposure and urinary 2,5-hexanedione levels. A cross-sectional study examining female shoemakers in Portugal found similar results, with increased self-reporting of longer menstrual cycles and increase difficulty of getting pregnant (Sallmen et al. 2008). A case-control study examining 108 women diagnosed with spontaneous abortion found a higher risk associated with higher solvent exposure in the shoe and leather industry (estimated by self-reported time and methods for using solvents) but not with occupation as a shoemaker (Agnesi et al. 1997).

Increased risk of preeclampsia was observed in asthmatic mothers exposed to ambient *n*-hexane during the second trimester or whole pregnancy, while mothers without asthma only showed an increased risk with the whole pregnancy average exposure (Mendola et al. 2016). Exposure to *n*-hexane was correlated with increased risk of preeclampsia during the first 20 weeks of gestation, but not when evaluated over the whole pregnancy (Nobles et al. 2019). No association was observed between ambient *n*-hexane concentrations and gestational hypertension during any window of exposure.

One study evaluated the reproductive toxicity of *n*-hexane in female mice. A 3-week exposure to 21,500 ppm *n*-hexane resulted in a decreased duration of diestrus in ICR mice (Liu et al. 2012). In super-ovulated mice, there were decreases in mature ovarian follicles and increases in primordial follicles and atretic follicles at 21,500 ppm and a decrease in the number of ovulated ova at ≥850 ppm (Liu et al. 2012).

Several acute- and intermediate-duration studies have examined the potential reproductive effects following *n*-hexane exposure in male rodents. Mice exposed up to 5,000 ppm *n*-hexane for 20 hours/day for 5 consecutive days had no changes in mouse sperm morphology or fertility compared to controls (NIEHS 1988a, 1988b). The fertility of male mice was unaffected by exposure up to 396 ppm *n*-hexane for 8 weeks (API 1980). Histopathological examination of mice exposed for 13 weeks to *n*-hexane at concentrations up to 10,000 ppm for 6 hours/day or 1,099 ppm for 22 hours/day revealed no treatment-related lesions in any of the reproductive tissues examined (seminal vesicles, prostate, testis, epididymis, ovary, uterus) (NTP 1991).

Increased testes weights were reported in male rats exposed to 10,000 ppm *n*-hexane 13 weeks (Cavender et al. 1984), but testes weights were decreased in rats following exposure to 1,000 ppm *n*-hexane for 11 weeks (Howd et al. 1983). Histopathological lesions were either not found or not examined; therefore, the toxicological significance of this finding is uncertain. No treatment-related lesions were observed in the seminal vesicles, prostate, testis, or epididymis of male rats exposed to 500 ppm *n*-hexane 22 hours/day for 6 months (API 1981).

Bilateral testicular damage and reduced testicular size and weight was reported in male rats exposed to 1,000 ppm *n*-hexane for 18–21 hours/day for 28 or 61 days (Nylen et al. 1989). Severe testicular atrophy and loss of nerve growth factor-immunoreactive cell population were also observed. Testicular lesions were observed in male rats exposed to 5,000 ppm *n*-hexane for 24 hours or for 16 hours/day, 6 days/week for up to 6 weeks (De Martino et al. 1987). These lesions included exfoliation of spermatids and spermatocytes, degeneration of spermatocytes, and vacuolization of Sertoli cells, with some animals reaching aplasia. Morphological alterations in sperm (multinucleated round spermatids and spermatocytes) were also observed (De Martino et al. 1987). In contrast, sperm abnormalities were not observed in B6C3F1 mice exposed to up to 5,000 ppm *n*-hexane for 20 hours/day for 5 days (NIEHS 1988a). Analysis of sperm obtained 5 weeks post-exposure showed no significant effects on morphology compared to the control group. In a chronic-duration study, Leydig cell hyperplasia and benign Leydig cell tumors were observed in male rats exposed to 1,000 ppm for 415 days (Imai and Omoto 1999).

No changes in testicular histopathology were reported in male rats administered via gavage 20,000 mg/kg for 1 day or 10,000 mg/kg/day for 5 days (Linder et al. 1992). A decrease was noted in total sperm head counts per gram of testis following 1 day at 20,000 mg/kg, but this was not observed in rats receiving gavage doses of 10,000 mg/kg/day for 5 days, and no other changes in sperm motility or morphology

were reported. Varying stages of testicular atrophy of the germinal epithelium was observed in rats administered 4,000 mg/kg/day *n*-hexane via gavage for up to 120 days (Krasavage et al. 1980).

The metabolite, 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.

2.17 DEVELOPMENTAL

A positive association was observed between ambient *n*-hexane exposure (represented as a unitless exposure intensity) and low birth weight in a case-control study in Texas (Gong et al. 2018). In a study examining the effects of *n*-hexane on the neonatal immune system, the percentage of T cells producing IL-2 in cord blood was increased with increased maternal *n*-hexane exposure, although no difference was observed in T cells producing IFN-γ, tumor necrosis factor-alpha (TNF-α), or IL-4 (Lehmann et al. 2002).

Several acute- and intermediate-duration studies have examined the potential developmental effects of *n*-hexane exposure in rodents. No differences in fetal parameters (e.g., number of implantations, resorptions, fetal sex ratio, fetal malformations) were observed in rats exposed up to 409 ppm for 6 hours/day during GDs 6–15 (API 1979) or in rats exposed up to 5,000 ppm for 20 hours/day over GDs 6–19 compared to controls (NIEHS 1987). Decreased fetal body weights were observed for male offspring (7.5%) at 1,000 ppm and in both male (15%) and female (14%) offspring at 5,000 ppm (NIEHS 1987). Additionally, an increased incidence of reduced ossification in the sternebrae 1–4 was observed in the offspring of rats exposed to 5,000 ppm. Decreased litter weight (13.9% at postnatal day [PND] 21) was observed in the offspring of rats exposed to 1,000 ppm *n*-hexane for 6 hours/day on GD 8–16, although litter weights were similar to controls at week 7 and there were no developmental effects observed in the offspring of rats exposed to the same concentration on GDs 8–12 or 12–16 (Bus et al. 1979).

The number of live fetuses per litter were decreased, while the number of late resorptions per litter was increased in pregnant mice exposed to 5,000 ppm *n*-hexane for 20 hours/day during GDs 6–17 (NIEHS

1988c). Fetal weights of female offspring were decreased 6% at 5,000 ppm, but this was not observed in male offspring, and no differences were observed in the incidence of malformations in either sex. In another study, reduced pup body weight (22% at PND 9) was observed in the offspring of female rats exposed to 500 ppm *n*-hexane for 23 hours/day throughout gestation (21 days) (Stoltenburg-Didinger et al. 1990). A third study reported a decreased ratio of live pups per litter, decreased proportion of ovarian secondary follicles, and increased proportion of atretic follicles in the female offspring of rats exposed to 12,500 ppm on GDs 1–20 (Li et al. 2014). No difference in the day of vaginal opening or ovarian pathology were observed, although slight changes in the estrus cycle were measured.

In a set of oral exposure studies, no differences were observed in total number of implants, number of resorptions, fetal deaths, sex ratio, number of stunted fetuses, live fetuses per dam, fetal weight, or incidence of malformed fetuses (visceral or skeletal) in the offspring of female mice administered doses up to 2,200 mg/kg/day via gavage on GDs 6–15 (Marks et al. 1980). Fetal weights were decreased 6% in the offspring of female mice receiving gavage doses 3 times daily at 7,920 mg/kg/day on GDs 6–15, but no other developmental effects were observed.

2.18 OTHER NONCANCER

No exposure-related differences in metabolic function (e.g., fasting blood glucose, electrolytes) were reported in offset printers (Chang et al. 1993), cabinet finishers (Herskowitz et al. 1971), or sandal makers (Yamamura 1969) occupationally exposed to *n*-hexane.

No effect in body temperature was observed in rats following exposure up to 1,500 ppm *n*-hexane for 11 weeks (Rebert and Sorenson 1983). No change in metabolic parameters were observed in male or female rats exposed up to 10,000 ppm for 13 weeks (Cavender et al. 1984). No exposure-related differences in mean-fasting glucose were observed in male or female rats exposed up to 129 ppm for as long as 6 months (API 1978).

2.19 CANCER

EPA (IRIS 2005) concluded that there is inadequate information to assess the carcinogenic potential of *n*-hexane. HHS and IARC have not assessed the carcinogenicity of *n*-hexane.

A case-control study evaluating the occurrence of intracranial tumors among employees at a petrochemical research facility found a positive association between self-reported *n*-hexane exposure and glioma cases, although no association was observed using project-based exposure estimates (Beall et al. 2001). Additionally, an analysis separating cases by duration of chemical use did show a positive association for *n*-hexane and longer duration (>48 months) of potential use. This study is limited by the small number of cases, the number of various co-exposures, and the observed correlations with other compounds/agents, including ionizing radiation.

Papillary tumors derived from Clara cells in the bronchiolar epithelium were observed in male rabbits exposed to 3,000 ppm *n*-hexane for 24 weeks (Lungarella et al. 1984), although the incidence was not reported, making the significance of this finding difficult to determine.

2.20 GENOTOXICITY

Genotoxic effects have not been examined in humans after *n*-hexane exposure. The database on *n*-hexane in animals, mammalian cells, and microorganisms is limited (see Tables 2-6 and 2-7) but indicates little potential for genotoxicity. *n*-Hexane was negative in a dominant lethal test in mice by the inhalation route at up to 396 ppm *n*-hexane ppm for 6 hours/day, 5 days/week for 8 weeks (API 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/day for 5 days (NIEHS 1988b).

Table 2-6. Genotoxicity of *n*-Hexane *In Vitro*

		Results		
		Activation		
Species (test system)	Endpoint	With	Without	Reference
Nonmammalian cells				
Escherichia coli WP2, WP2 uvr A, CM611, WP67, WP100, WP110, p3478		–	–	McCarroll et al. 1981a
Bacillus subtilis H17, M45		–	–	McCarroll et al. 1981b
Salmonella typhimurium TA98, TA100, TA1535, TA1537,	Reverse mutation	–	–	Mortelmans et al. 1986
S. typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	Ishidate et al. 1984
S. typhimurium TA98, TA100	Reverse mutation	–	–	Houk et al. 1989
S. typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation	–	–	NTP 1991[a]

Table 2-6. Genotoxicity of *n*-Hexane *In Vitro*

Species (test system)	Endpoint	Results: Activation With	Results: Activation Without	Reference
Saccharomyces cerevisiae	Chromosome loss	–	–	Mayer and Goin 1994
Mammalian cells				
Human (lymphocytes)	Unscheduled DNA synthesis	–	–	Perocco et al. 1983
Hamster (CHO)	Chromosomal aberration	–	–	NTP 1991[a]
Hamster (CHO)	Sister chromatid exchange	+	–	NTP 1991[a]
Hamster (CHL)	Polyploidy	ND	+	Ishidate et al. 1984

[a]Unpublished NTP data reported in NTP (1991).

– = negative results; CHL = Chinese hamster lung; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; ND = not detectable; NTP = National Toxicology Program

Table 2-7. Genotoxicity of *n*-Hexane *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Rat	Sperm morphology	+	De Martino et al. 1987
Mouse (bone marrow)	Sister chromatid exchange	–	NTP 1991[a]
Mouse	Dominant lethal mutation	–	API 1980
Mouse	Chromosomal exchange	–	NTP 1991[a]
Mouse	Micronuclei formation	–	NTP 1991[a]
Mouse	Sperm morphology	–	NIEHS 1988a
Mouse	Dominant lethal mutation	–	NIEHS 1988a

[a]Unpublished NTP data reported in NTP (1991).

– = negative result; + = positive result; NTP = National Toxicology Program

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/day, 5 days/week for 13 weeks or in mice exposed to 1,000 ppm for 22 hours/day for 13 weeks (NTP 1991). In an *in vivo* mouse bone marrow cytogenetics assay, doses up to 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. 1981a, 1981b; 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 *Saccharomyces cerevisiae* (Mayer and Goin 1994).

Negative results were also obtained in mammalian cells except for one observation of polyploidy in Chinese hamster lung 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).