

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 2-hexanone. 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.

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 2-hexanone, but may not be inclusive of the entire body of literature.

Animal inhalation studies are presented in Table 2-1 and Figure 2-2, and animal oral studies are presented in Table 2-2 and Figure 2-3; only one dermal study was identified for 2-hexanone.

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. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be

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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 C). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

The toxicity of 2-hexanone has been evaluated in laboratory animals; limited information on the effects of 2-hexanone in humans comes from studies in workers (Figure 2-1). In humans, almost all available data were obtained from a screening study of 1,157 workers exposed to 2-hexanone in a fabric finishing plant. The primary focus of this study was on neurological effects. Unfortunately, reliable exposure data are not available for this study; furthermore, exposure to other chemicals also occurred. Note that significant exposure of the general population to 2-hexanone is not likely because it is no longer manufactured, processed, or used for commercial purposes in the United States.

Animal data are available for each health effect category and exposure duration category. In animals, most data are from inhalation studies. Endpoints were evaluated for all systems. The most examined endpoints were neurological effects (approximately 25%) and body weight (approximately 12%). Chronic-duration oral and inhalation studies in animals did not report any evidence of cancer. Note that animal studies were conducted with commercial 2-hexanone, with purity ranging from 70-96% (Topping et al. 2001); contaminants may include methyl isobutyl ketone (MiBK). This is of concern because MiBK potentiates the neurotoxicity of 2-hexanone through induction of hepatic microsomal cytochrome P-450 enzymes, resulting in increased production of the 2-hexanone active metabolite, 2,5-hexanedione (ATSDR 1999).

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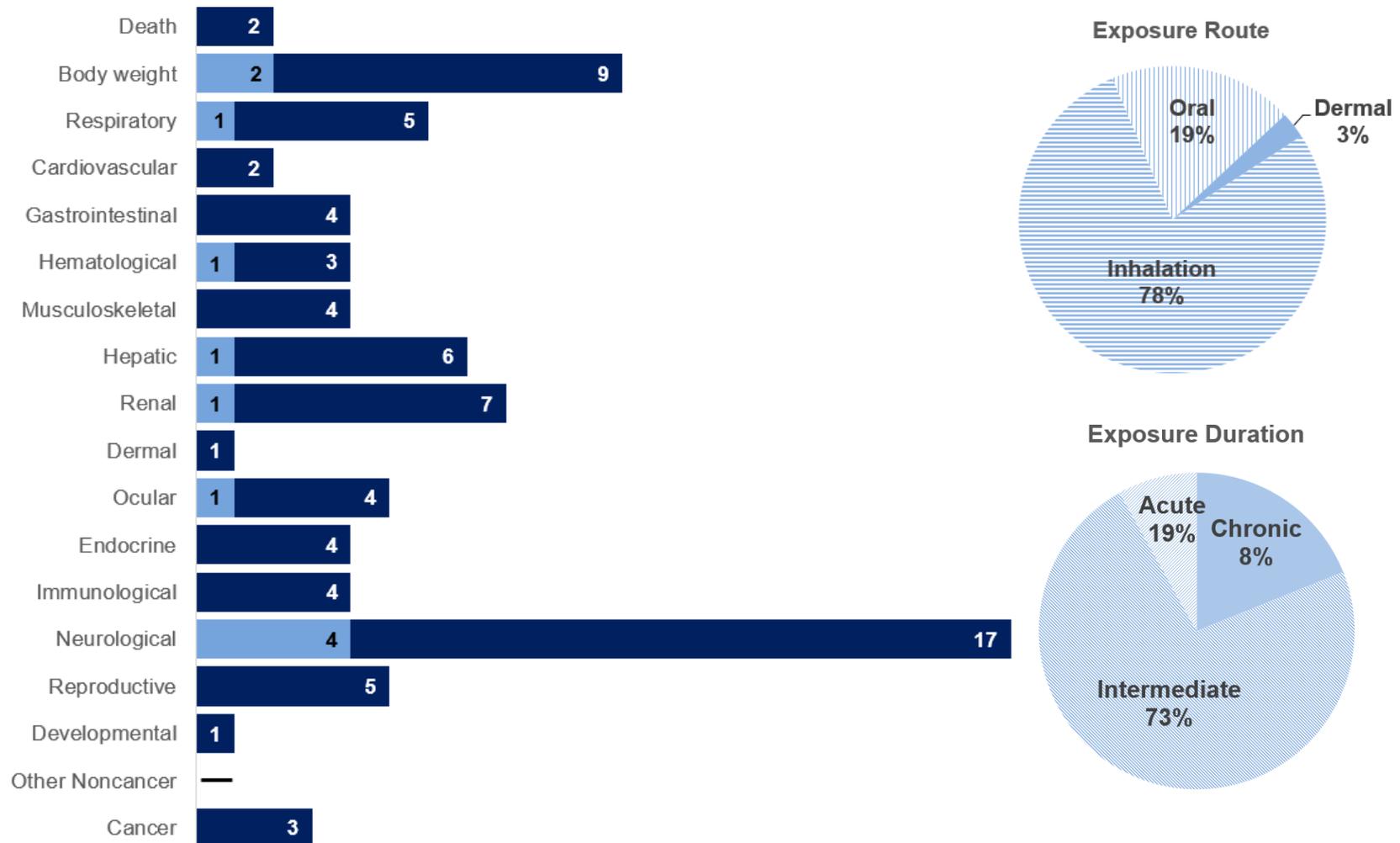
Available data in humans and animals suggest that the nervous system, musculoskeletal system, and effects on body weight are the most sensitive endpoints of 2-hexanone toxicity:

- **Nervous System Endpoints:** Toxicity to the nervous system is the most sensitive target of 2-hexanone. In humans, neurological effects attributed to 2-hexanone include peripheral neuropathy characterized by axon and myelin disruption, axonal swellings involving motor and sensory nerves, alterations in nerve conduction velocity, ataxia, and sensory deficits. In laboratory animals, inhalation and oral exposure to 2-hexanone results in effects to the peripheral nervous system similar to those reported in humans. Toxicity to the central nervous system has also been observed in animal studies.
- **Musculoskeletal System Endpoints.** Muscle weakness has been observed in workers exposed to 2-hexanone, with findings accompanied by electromyographic abnormalities. In animals, 2-hexanone induced skeletal muscle pathology, including skeletal muscle atrophy, following repeated inhalation or oral exposure. Alterations generally consisted of atrophy and degenerative changes. These effects are considered secondary to neurological damage.
- **Body Weight Endpoint.** In both humans and animals, decreased body weight (humans) and decreased body weight gain (animals) have been observed following 2-hexanone exposure. However, due to lacking information on food intake, the cause of decreased body weight has not been determined.
- **Other Endpoints.** Other effects observed in animal studies included male reproductive effects (atrophy of the testicular germinal epithelium), developmental effects (reduced pup weight and hyperactivity), hematological effects (decreased white blood cell count), and renal effects (tubular degeneration). However, these do not appear to be sensitive targets of 2-hexanone toxicity.

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Figure 2-1. Overview of the Number of Studies Examining 2-Hexanone Health Effects

Most studies examined the potential neurological and body weight effects of 2-hexanone
Fewer studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 37 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints.

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Table 2-1. Levels of Significant Exposure to 2-Hexanone – Inhalation

Figure key ^a	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
ACUTE EXPOSURE									
1	Guinea pig (NS) NS	1 minute	0, 1,000, 2,300	CS	Resp	1,000	2,300		Nasal irritation
Schrenk et al. 1936									
2	Guinea pig (NS) NS	810 minutes	0, 1,000, 2,300, 6,500, 20,000	LE, CS, GN, HP	Death Neuro	1,000	2,300	6,500	Death in 450 minutes Incoordination after 90 minutes of exposure
Schrenk et al. 1936									
INTERMEDIATE EXPOSURE									
3	Monkey 8 M	25–41 weeks 5 day/week 6 hours/day	0, 100, 1,000	BW, CS, NX	Bd wt Neuro	100 M	100 M	1,000 M	Reduced nerve conduction velocity at 100 ppm; 36% reduction in motor nerve conduction velocity
Johnson et al. 1977									
4	Rat (Wistar) 40 NS	6 months 5 days/week 8 hours/day	0, 50	CS, HP	Hepatic Renal Neuro	50 50		50	Sciatic nerve demyelination in 32/40 rats
Duckett et al. 1979									
5	Rat (Sprague-Dawley) 6 M	6 months 7 days/week 22 hours/day	0, 100	BW, CS, HP	Bd wt Neuro	100 M		100 M	Peripheral and central histopathology
Egan et al. 1980									

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Figure key ^a	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
6	Rat 10 M	25–29 weeks 5 days/week 6 hours/day	0, 100, 1,000	BW, CS, NX	Bd wt	100 M	1,000 M		Decreased terminal body weight
					Neuro			100 M	45% reduced nerve conduction velocity
Johnson et al. 1977									
7	Rat 5 M	11 weeks 72 hours/week 18 hours/day	0, 700	BC, BW, CS, HP	Bd wt	700 M			Decreased terminal weight
					Hemato			700 M	40% decrease in WBCs
					Neuro			700 M	Severe neuropathy
				Repro			700 M	Decreased testes weight, histopathology	
Katz et al. 1980									
8	Rat 4 NS	12 weeks 24 hours/day 7 days/week	0, 400	CS, HP	Neuro			400	Neuropathy
Mendell et al. 1974									
9	Rat 25 F	GDs 0–21 6 hours/day	0, 500, 1,000, 2,000	CS, BW, NX, MX, TG	Develop			1,000 F	Behavioral effects in offspring
Peters et al. 1981									
10	Rat 12 NS	6–9.5 weeks 24 hours/day	0, 225, 400	CS, HP	Neuro			225	Paralysis, histopathology
Saida et al. 1976									
11	Rat 6 NS	4 months 5 days/week 6 hours/day	0, 1,300	BW, CS, HP	Neuro			1,300	Nerve degeneration
Spencer et al. 1975									
12	Cat 4 NS	>8 weeks 24 hours/day 7 days/week	0, 400	CS, HP	Neuro			400 F	Neuropathy, histopathology
Mendell et al. 1974									

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Table 2-1. Levels of Significant Exposure to 2-Hexanone – Inhalation

Figure key ^a	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
CHRONIC EXPOSURE									
13	Rat (Sprague-Dawley)	72 weeks 5 days/week 6 hours/day	0, 100, 330	CS, BW, WI, GN, HP	Bd wt Resp Gastro Hemato Musc/skel Renal Ocular Endocr Immuno Neuro Repro	330 M 330 M 330 M 330 M 100 M 330 M 330 M 330 M 330 M 100 M 330 M		330 M	Degenerative changes in skeletal muscle fibers Peripheral neuropathy
Krasavage and O'Donoghue 1977									
14	Cat (domestic)	2 years 7 days/week 6 hours/day	0, 100, 330	CS, BW, HE, GN, HP	Bd wt Resp Cardio Gastro Hemato Musc/skel Hepatic Renal Ocular Endocr Immuno	330 F 330 F			

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Table 2-1. Levels of Significant Exposure to 2-Hexanone – Inhalation

Figure key ^a	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effects
					Neuro	100 F		330 F	Axonal degeneration of central and peripheral nervous systems
					Repro	330 F			

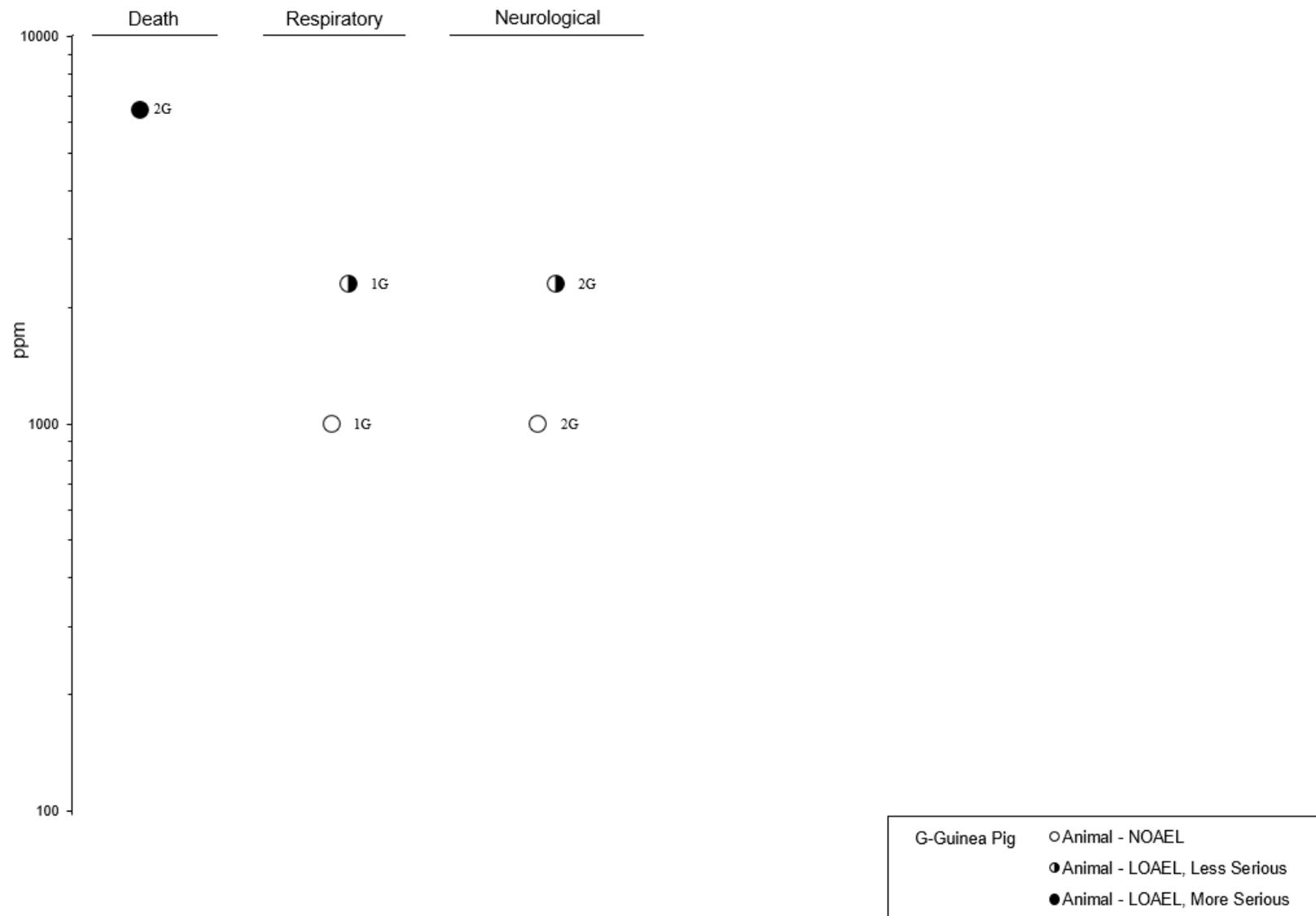
O'Donoghue and Krasavage 1979

^aThe number corresponds to entries in Figure 2-2; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-2. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

Bd wt or BW = body weight; Cardio = cardiovascular; CS = clinical signs; Endocr = endocrine; F = female(s); FX = fetal toxicity; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; Hemato = hematological; HP = histopathology; Immuno = immunological; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = muscular/skeletal; MX = maternal toxicity; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurological function; Repro = reproductive; Resp = respiratory; TG = teratogenicity; WBC = white blood cell; WI = water intake

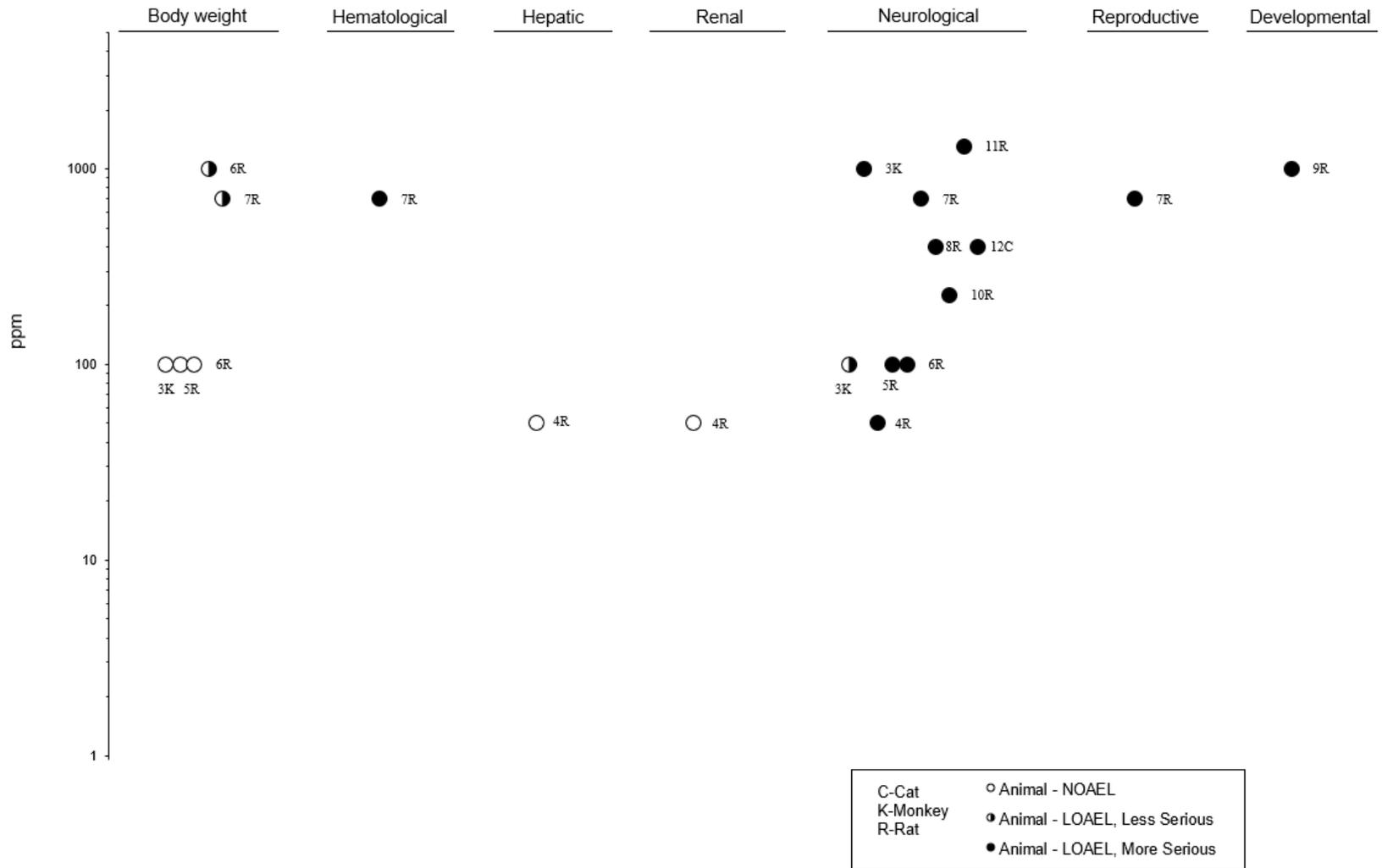
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Figure 2-2. Levels of Significant Exposure to 2-Hexanone – Inhalation
Acute (≤ 14 days)



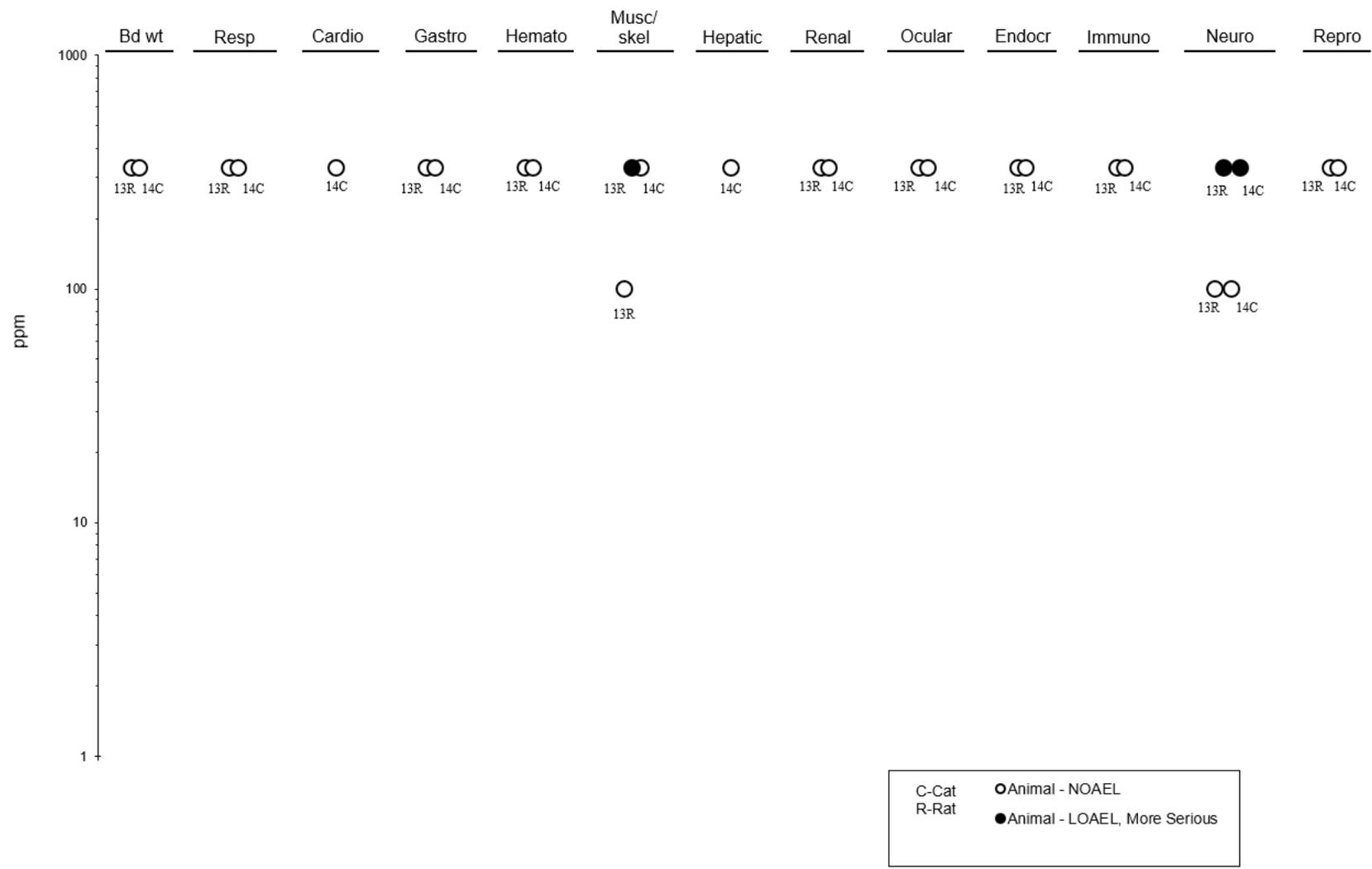
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Figure 2-2. Levels of Significant Exposure to 2-Hexanone – Inhalation
Intermediate (15-364 days)



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



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Table 2-2. Levels of Significant Exposure to 2-Hexanone – Oral

Figure key ^a	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
ACUTE EXPOSURE									
1	Rat (Fischer-344) 6 M	Once (GO)	0, 1,500	BI, HP	Hepatic	1,500			
					Renal		1,500		Tubular degeneration
Brown and Hewitt 1984									
2	Rat (Wistar) 5 NS	Once (G)	0, 2, 110, 113, 160	LE	Death			2,590	LD ₅₀
Smyth et al. 1954									
INTERMEDIATE EXPOSURE									
3	Rat (Wistar) 60 M	40 weeks 1 time/day (GW)	0, 400	OF, BW, CS	Hepatic Renal Neuro	400 400	400		Hindlimb weakness
Eben et al. 1979									
4	Rat 5 M	90 days 5 days/week 1 time/day (G)	0, 660	CS, HP	Bd wt Neuro			660 660	39% reduced terminal body weight Paralysis, histopathology
Krasavage et al. 1980									
5	Rat (Wistar) 5 F	120 days <i>ad libitum</i> (W)	0, 480, 1,010	LE, CS, BW, FI, WI, OW, GN, HP	Bd wt Resp Cardio Gastro Musc/skel Hepatic Renal Ocular Endocr Immuno	1010 1010 1,010 1,010 1,010 1,010 1,010 1,010 1,010	480 480		46% reduction in terminal body weight Skeletal muscle atrophy

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Table 2-2. Levels of Significant Exposure to 2-Hexanone – Oral

Figure key ^a	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Neuro			480	Peripheral neuropathy
					Repro	1,010			
Union Carbide 1977									
6	Guinea pig (English short hair) 5 NS	24 weeks (W)	0, 124, 310	BW, WI, CS	Neuro			310	40% reduction in locomotor activity
Abdel-Rahman et al. 1978									
CHRONIC EXPOSURE									
7	Rat (Sprague-Dawley) 10 M	13 months <i>ad libitum</i> (W)	0, 143, 266, 560	CS, BW, OW, FI, HP	Bd wt	143	266	560	14% reduced final weight at 266 mg/kg/day; 36% reduced final weight at 560 mg/kg/day
					Resp	560			
					Cardio	560			
					Gastro	560			
					Musc/skel	143	266		Skeletal muscle myofiber atrophy
					Hepatic	560			
					Renal	560			
					Ocular	560			
					Endocr	560			
					Immuno	560			

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Table 2-2. Levels of Significant Exposure to 2-Hexanone – Oral

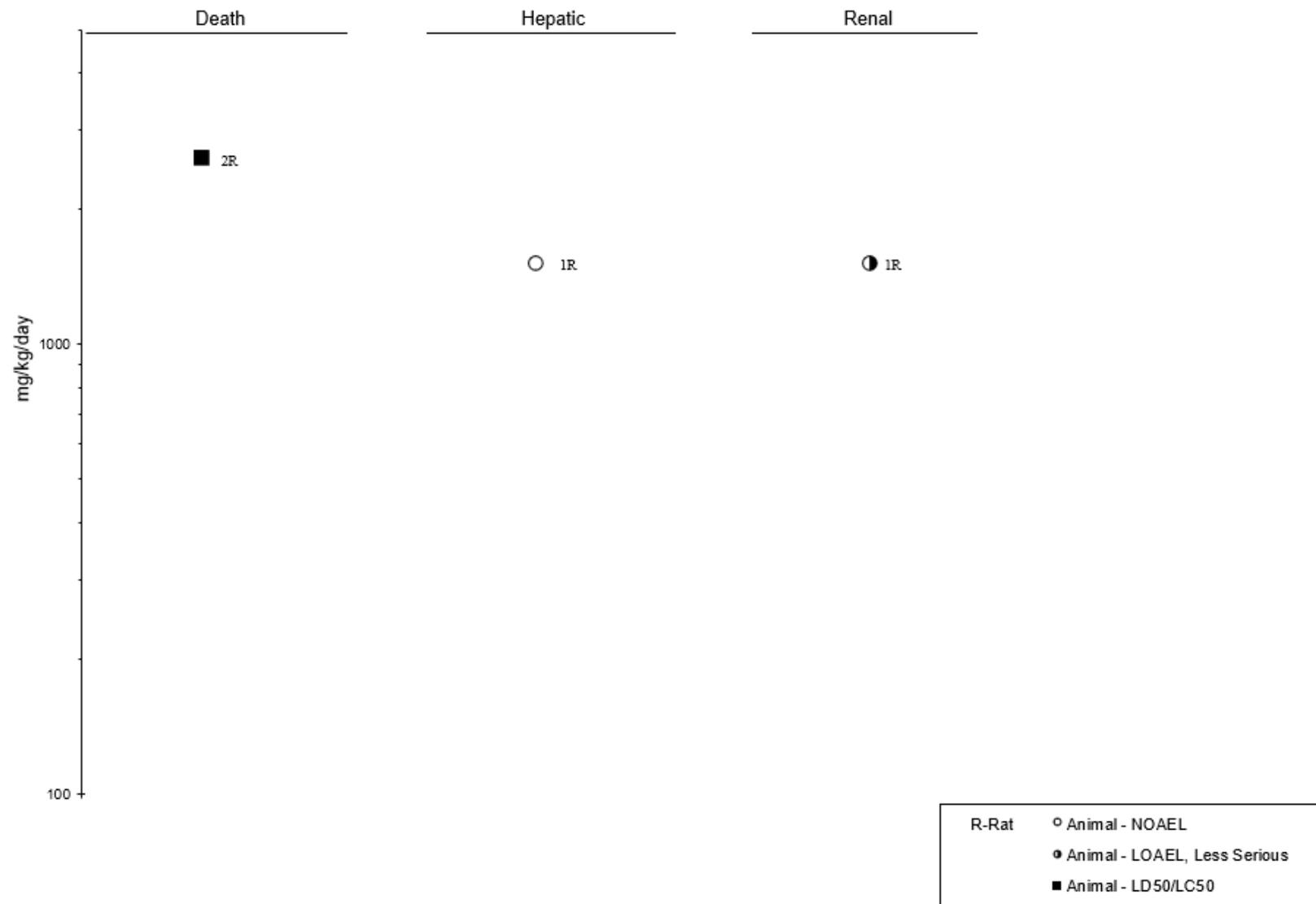
Figure key ^a	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Neuro		143 ^b	266	Peripheral nerve axonal swelling at 143 mg/kg/day; axonal and myelin degeneration at 266 mg/kg/day
					Repro	560			
O'Donoghue et al. 1978									

^aThe number corresponds to entries in Figure 2-3; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

^bUsed to derive a chronic-duration oral MRL of 0.05 mg/kg/day based on LOAEL of 143 mg/kg/day, an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability), and a modifying factor of 3.

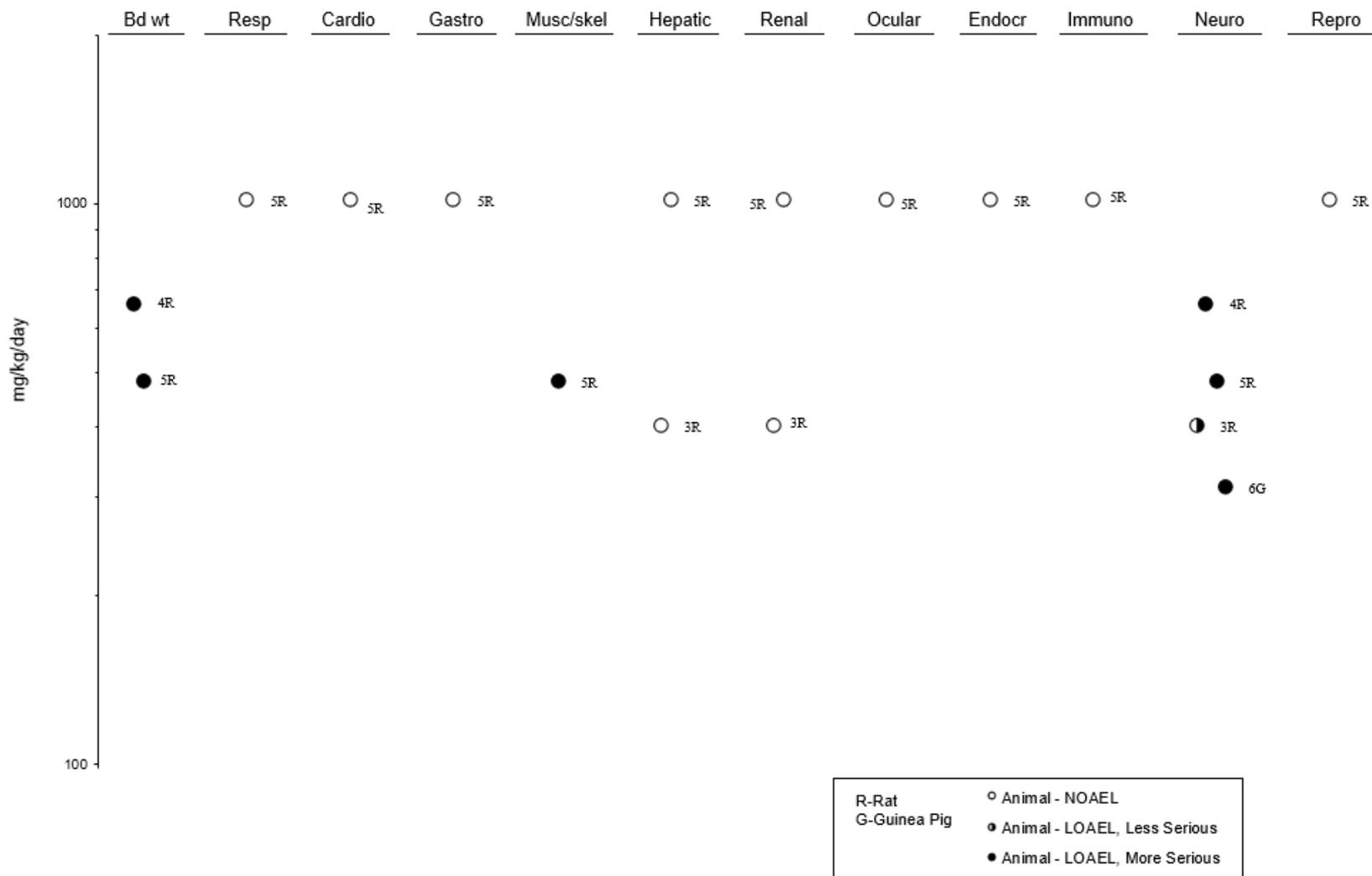
Bd wt or BW = body weight; Cardio = cardiovascular; CS = clinical signs; Endocr = endocrine; F = female(s); FI = food intake; (G) = gavage; (GO) = gavage in oil; (GW) = gavage in water; Gastro = gastrointestinal; GN = gross necropsy; HP = histopathology; Immuno = immunological; LC₅₀ = lethal concentration, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; Musc/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; Repro = reproductive; Resp = respiratory; (W) = water; WI = water intake

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

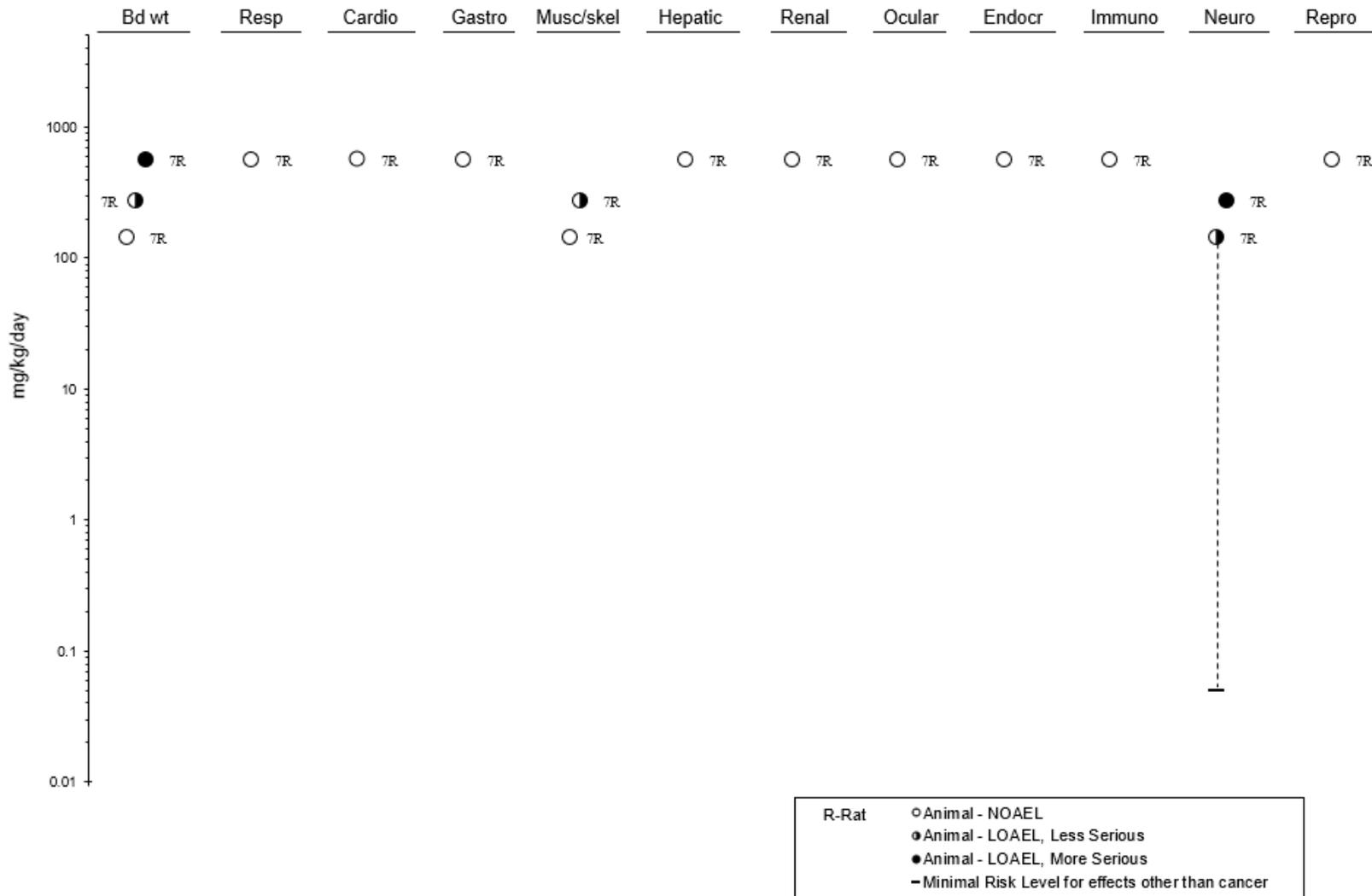
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Figure 2-3. Levels of Significant Exposure to 2-Hexanone – Oral
Intermediate (15-364 days)



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Figure 2-3. Levels of Significant Exposure to 2-Hexanone – Oral
 Chronic (≥365 days)



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2.2 DEATH

No studies were located regarding death in humans following inhalation exposure to 2-hexanone. Death occurred in guinea pigs following exposure to 6,500 ppm of commercial-grade 2-hexanone for 540 minutes or to 20,000 ppm for 70 minutes (Schrenk et al. 1936). Death was preceded by incoordination, narcosis, and gasping-type respiration.

An LD₅₀ of 2,590 mg/kg was calculated for a gavage administration of 2-hexanone (purity not stated) to Wistar rats. No information about the cause of death or sex of the animals was reported (Smyth et al. 1954).

2.3 BODY WEIGHT

Little information is available regarding the effects of 2-hexanone on body weight in humans. A 1973 outbreak of distal polyneuropathy involving 86 of 1,157 employees was reported in a plant that had been using 2-hexanone for about 10 months in the production of plastic-coated and color-printed fabrics (Allen et al. 1975; Billmaier et al. 1974) (neurological effects associated with this exposure are discussed in Section 2.15). Clinical evaluations indicated that of 10 workers whose body weight was recorded, weight loss ranging from 3 to 60 pounds was observed in the eight workers found to have moderate to severe neurological impairment (Allen et al. 1975). It is not clear whether the affected individuals had decreased appetites and/or food consumption levels in conjunction with their weight loss. Of the milder cases, no significant weight change could be correlated with the presence of the disorder. Atmospheric sampling conducted after this incident indicated that 2-hexanone levels averaged 9.2 ppm in front of the printing machines and 36 ppm behind the machines. After the use of 2-hexanone was discontinued, weight gain was uniformly noted in those who had lost weight.

Results of studies in laboratory animals show weight loss or decreased body weight gain following intermediate-duration inhalation exposure and intermediate- and chronic-duration oral exposure. However, the toxicological significance of these observations is uncertain, particularly for inhalation exposures, because information regarding food consumption was not reported. No acute-duration studies evaluating the effects of 2-hexanone were identified.

Intermediate-duration inhalation studies have reported decreases in body weight in animals exposed to 2-hexanone. A NOAEL of 100 ppm was reported in rats in two studies (Egan et al. 1980; Johnson et al.

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1977). In the former study, which tested pure 2-hexanone in rats exposed for 22 hours/day, 7 days/week, for 6 months, 100 ppm was the only concentration tested, whereas Johnson et al. (1977), who tested a commercial-grade 2-hexanone of unknown purity in rats exposed for 6 hours/day, 5 days/week for 15 weeks, reported a LOAEL of 1,000 ppm in rats. These rats displayed progressive weight loss, which became statistically significant at 20 weeks. In rats exposed to 700 ppm (only level tested) pure 2-hexanone for 16 or 20 hours/day for 72 hours/week for 11 weeks, terminal body weights were decreased by approximately 44%, compared to controls (Katz et al. 1980). Johnson et al. (1977) also tested monkeys and reported that exposure to 1,000 ppm 2-hexanone induced a progressive nonsignificant loss of body weight beginning 4 months after exposure started; 100 ppm did not induce significant effects.

A developmental study in rats showed decreased weight gain following 2-hexanone inhalation exposure. Weight gain decrements of 10 and 14% relative to controls were reported in groups of 25 pregnant rats exposed to 1,000 or 2,000 ppm 2-hexanone, respectively, 6 hours/day during 21 days of gestation (Peters et al. 1981). No body weight effects were seen in dams exposed to 500 ppm. However, no statistical analysis was performed on these results. Rats in the 2,000 ppm exposure group were observed to eat less than controls, but no quantitative data were presented.

Reductions in weight gain were reported in rats in intermediate- and chronic-duration oral exposure studies. A group of five rats given doses of 660 mg/kg/day pure 2-hexanone by gavage 5 days/week over 90 days weighed about 61% of control rats by 10 weeks of exposure (Krasavage et al. 1980). Treated rats consumed approximately 18% less food (g/rat/day) than control rats, which would suggest that factors other than the reduced food consumption played a role in the reduced weight gain. Similar results were reported in a 120-day drinking water study in rats (Union Carbide 1977). In the chronic study, rats dosed with 266 or 560 mg/kg/day pure 2-hexanone weighed 14 and 36% less than control rats, respectively, after 13 months of treatment (O'Donoghue et al. 1978), and doses of 143 mg/kg/day did not significantly affect weight gain; no data on food consumption were provided in this study. No effects on body weight were observed following chronic-duration oral exposure of rats and cats. Exposure of rats and cats to ≤ 330 ppm 2-hexanone (purity unknown) for 72 weeks or 2 years, respectively, did not result in significant alterations in body weight (Krasavage and O'Donoghue 1977; O'Donoghue and Krasavage 1979).

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2.4 RESPIRATORY

Acute inhalation exposure to high concentrations of 2-hexanone produces irritation in humans and animals; however, longer-duration exposure to lower concentrations does not appear to cause respiratory irritation. These effects are likely to occur by direct contact of the chemical with mucosal surfaces rather than a systemic mode of action.

In humans, nasal irritation was observed in an early study in which men were exposed to $\geq 2,300$ ppm 2-hexanone (commercial-grade) vapors for 25–60 seconds. Study subjects considered the contaminated air extremely disagreeable due to a strong odor (Schrenk et al. 1936).

Limited additional data are available in animals regarding respiratory effects of 2-hexanone. Nasal irritation was observed in guinea pigs exposed to 2,300 ppm 2-hexanone after 1 minute of exposure; no such signs were reported in guinea pigs exposed to 1,000 ppm 2-hexanone (Schrenk et al. 1936). In mice, inhalation exposure to high concentrations of 2-hexanone showed a mixed pattern of sensory and pulmonary irritation. In cannulated mice, the concentration of 2-hexanone that reduced the respiratory rate by 50% (RD₅₀) during the first 10 minutes of exposure was 6,183 ppm (Hansen and Nielsen 1994). Intermittent whole-body exposure of rats or cats to ≤ 330 ppm 2-hexanone vapors (purity unknown) 6 hours/day, 5 days/week for 72 weeks or 2 years, respectively, did not induce treatment-related gross or microscopic alterations in the trachea or lungs (Krasavage and O'Donoghue 1977; O'Donoghue and Krasavage 1979).

One study evaluating respiratory effects of oral exposure to 2-hexanone was identified. Exposure to $\leq 1,010$ mg/kg/day 2-hexanone (assumed to be pure) for 120 days (Union Carbide 1977) or ≤ 560 mg/kg/day pure 2-hexanone for 13 months via the drinking water did not induce gross or microscopic lesions in the lungs or trachea of rats (O'Donoghue et al. 1978).

2.5 CARDIOVASCULAR

Little data are available regarding cardiovascular effects of 2-hexanone. No significant gross or microscopic alterations were reported in the heart of rats or cats exposed following intermittent whole-body inhalation exposure to ≤ 330 ppm 2-hexanone vapors (purity unknown) for 72 weeks or 2 years, respectively (Krasavage and O'Donoghue 1977; O'Donoghue and Krasavage 1979). Oral exposure of rats to $\leq 1,010$ mg/kg/day 2-hexanone for 120 days (Union Carbide 1977) or ≤ 560 mg/kg/day pure

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2-hexanone for 13 months via the drinking water did not induce gross or microscopic lesions in the heart (O'Donoghue et al. 1978).

2.6 GASTROINTESTINAL

Gastrointestinal effects of 2-hexanone have not been well-investigated. Intermittent, whole-body inhalation exposure of rats or cats to ≤ 330 ppm 2-hexanone vapors (purity unknown) for 72 weeks or 2 years, respectively, did not induce treatment-related gross or microscopic alterations in the gastrointestinal tract (Krasavage and O'Donoghue 1977; O'Donoghue and Krasavage 1979). In rats exposed to $\leq 1,010$ mg/kg/day 2-hexanone for 120 days or ≤ 560 mg/kg/day pure 2-hexanone for 13 months through the drinking water, no treatment-related gross or microscopic lesions in the gastrointestinal tract were observed (O'Donoghue et al. 1978; Union Carbide 1977).

2.7 HEMATOLOGICAL

Limited information is available regarding hematological effects in humans following inhalation exposure to 2-hexanone. In a study of workers exposed to 2-hexanone in a plant producing plastic-coated and color-printed fabrics in Ohio who developed polyneuropathy, hematological tests results were reported to be within normal limits, but quantitative data were not shown and specific tests were not reported (Allen et al. 1975).

The available data are insufficient to determine if exposure of laboratory animals produces hematological effects. A significant reduction in total leukocyte counts to about 60% of control values was observed in rats intermittently exposed to 700 ppm (16 or 20 hours/day for 72 hours/week) pure 2-hexanone after 8 weeks of an 11-week study (Katz et al. 1980). Hemoglobin concentration, hematocrit, and differential white cell counts were similar to control values. Although the decrease in total white blood cell counts suggested an effect on bone marrow, the investigators found no microscopic evidence of such damage. Therefore, the clinical significance of their findings is uncertain.

Chronic-duration intermittent whole-body exposure of rats or cats to ≤ 330 ppm 2-hexanone vapors (purity unknown) did not induce alterations in the bone marrow of the animals (Krasavage and O'Donoghue 1977; O'Donoghue and Krasavage 1979); no hematological tests were conducted in these studies that could have helped interpret the findings of the intermediate-duration study of Katz et al. (1980).

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No information was located regarding hematological effects in animals following oral exposure to 2-hexanone.

2.8 MUSCULOSKELETAL

Results of animal studies indicate that effects on skeletal muscles are secondary to neurological effects.

Intermittent whole-body exposure of rats to 330 ppm 2-hexanone vapors (unknown purity) for 72 weeks induced degenerative changes in hindlimb skeletal muscles that resulted in muscle weakness (Krasavage and O'Donoghue 1977). This effect, however, was attributed to damage to the nerves innervating the muscles (see Section 3.2.1.4). No such effect was reported in rats exposed to 100 ppm 2-hexanone. Cats similarly exposed to ≤ 330 ppm 2-hexanone for 2 years did not develop skeletal muscle alterations (O'Donoghue and Krasavage 1979).

Skeletal muscle pathology of neurogenic origin was reported in rats following exposure to ≥ 480 mg/kg/day 2-hexanone for 120 days (Union Carbide 1977). Similar findings were reported in rats dosed with ≥ 266 mg pure 2-hexanone/kg/day for 13 months via the drinking water (O'Donoghue et al. 1978). Gross pathology was limited to atrophy of skeletal muscles of the hind limbs and lumbar muscles. Light microscopy showed significant treatment-related alterations of neurogenic skeletal muscle atrophy in proximal and distal hind limb musculature of high-dose rats. Alterations in rats treated with 266 mg/kg/day 2-hexanone were similar but less severe; no significant alterations were reported at 143 mg/kg/day 2-hexanone.

2.9 HEPATIC

The limited data available suggest that the liver is not a primary target for 2-hexanone. However, few studies have evaluated the potential for 2-hexanone to induce hepatotoxicity.

In the Allen et al. (1975) study of workers exposed to 2-hexanone, clinical tests (liver enzymes, total bilirubin, serum albumin, total protein, serum cholesterol) performed on most workers suspected of a neuropathy were, for the most part, within normal values. Few details were reported.

In rats, no effect on hexobarbital-induced sleep times was observed following continuous inhalation exposure to 225 ppm 2-hexanone (purity not stated) for 7 days (Couri et al. 1977). Results indicate that

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2-hexanone exposure under these conditions does not affect the hepatic microsomal enzyme activities associated with this response. No histopathological effects were seen in the liver in rats exposed to 50 ppm 2-hexanone (purity not stated) for 6 months (Duckett et al. 1979) or in rats (Krasavage and O'Donoghue 1977) and cats (O'Donoghue and Krasavage 1979) exposed chronically to ≤ 330 ppm 2-hexanone (purity not reported).

A single gavage dose of 1,500 mg/kg pure 2-hexanone did not produce histological changes in livers of rats (Brown and Hewitt 1984). In a 40-week study of rats administered gavage doses of 400 mg/kg/day pure 2-hexanone, periodic assessments of serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) showed values within normal limits (Eben et al. 1979). 2-Hexanone (purity unknown) did not affect liver morphology in rats administered doses of $\leq 1,010$ mg/kg/day for 120 days (Union Carbide 1977). The lack of histopathology was also confirmed in a 13-month drinking water study in rats that received doses of ≤ 560 mg/kg/day pure 2-hexanone (O'Donoghue et al. 1978).

2.10 RENAL

The only relevant information regarding renal effects in humans is that blood urea nitrogen (BUN) appeared to be low (no quantitative data were provided) in some workers studied by Allen et al. (1975) who had signs of neuropathy. However, the difference between subjects affected with neuropathy and not affected was not significant and there was no correlation between BUN values and severity of the neuropathy.

Based on studies in laboratory animals, the kidney does not appear to be a primary target for 2-hexanone. No histopathological effects were seen in the kidneys of rats exposed to 50 ppm 2-hexanone (purity not stated) for 6 months (Duckett et al. 1979) or in rats (Krasavage and O'Donoghue 1977) or cats (O'Donoghue and Krasavage 1979) exposed chronically to ≤ 330 ppm 2-hexanone (purity not reported).

A single gavage dose of 1,500 mg/kg 2-hexanone produced tubular degeneration in rats (Brown and Hewitt 1984), but no quantitative data were provided. 2-Hexanone (unknown purity) did not induce gross or microscopic changes in the kidneys of rats when given doses of $\leq 1,010$ mg/kg/day for 120 days (Union Carbide 1977). In rats administered 400 mg/kg/day pure 2-hexanone by gavage for 40 weeks, periodic assessments of plasma urea and creatinine, as indices of kidney function, showed no effects of exposure (Eben et al. 1979). Exposure of rats for 13 months to ≤ 560 mg/kg/day pure 2-hexanone in the drinking water did not induce gross or microscopic alterations in the kidneys (O'Donoghue et al. 1978).

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2.11 DERMAL

No studies were located regarding dermal effects in animals following inhalation or in humans or animals following oral exposure to 2-hexanone. Application of undiluted 2-hexanone to the skin of rabbits for 24 hours resulted in Grade 1 (least severe) irritation (Smyth et al. 1954).

2.12 OCULAR

An early study by Schrenk et al. (1936) reported that men exposed to $\geq 2,300$ ppm 2-hexanone (commercial-grade) vapors for 25–60 seconds complained of irritation of the eyes (Schrenk et al. 1936).

Guinea pigs exposed to 2,300 ppm 2-hexanone vapor showed signs of eye irritation after 1 minute of exposure and lacrimation after 10 minutes of exposure; no such signs were reported in guinea pigs exposed to 1,000 ppm 2-hexanone. These effects are likely due to direct contact of 2-hexanone vapors with the eye surface. A long-term exposure study reported that no treatment-related ocular effects were reported in rats or cats exposed whole-body to ≤ 330 ppm 2-hexanone vapors (purity not reported) (Krasavage and O'Donoghue 1977; O'Donoghue and Krasavage 1979).

No treatment-related alterations in the eyes were observed in rats exposed via drinking water to $\leq 1,010$ mg/kg/day 2-hexanone for 120 days (Union Carbide 1977) or ≤ 560 mg/kg/day pure 2-hexanone for 13 months (O'Donoghue et al. 1978).

Ocular instillation of 2-hexanone in rabbits resulted in Grade 3 (moderate) corneal necrosis (Smyth et al. 1954).

2.13 ENDOCRINE

No information on potential endocrine effects of 2-hexanone in humans was identified.

In animals, no treatment-related histological alterations occurred in the adrenals, thyroid, or parathyroid glands of rats or cats exposed whole-body to ≤ 330 ppm 2-hexanone vapors (purity not reported) for 72 weeks or 2 years, respectively (Krasavage and O'Donoghue 1977; O'Donoghue and Krasavage 1979). Drinking water exposure of rats to $\leq 1,010$ mg/kg/day 2-hexanone for 120 days (Union Carbide 1977) or

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to ≤ 560 mg/kg/day pure 2-hexanone for 13 months did not induce gross or microscopic alterations in adrenals, thyroid, or parathyroid glands, or the pancreas (O'Donoghue et al. 1978).

2.14 IMMUNOLOGICAL

No studies were located regarding immunological effects of 2-hexanone in humans.

The available studies in laboratory animals do not provide sufficient information to assess possible adverse immunological effects due to exposure to 2-hexanone. Intermittent whole-body exposure of rats or cats to ≤ 330 ppm 2-hexanone vapors (unknown purity) for 72 weeks or 2 years, respectively, did not induce gross or microscopic alterations in the spleen, thymus, or lymph nodes (Krasavage and O'Donoghue 1977; O'Donoghue and Krasavage 1979). No tests of immunocompetence were conducted in these studies. Oral exposure of rats to $\leq 1,010$ mg/kg/day 2-hexanone for 120 days (Union Carbide 1977) or to ≤ 560 mg pure 2-hexanone/kg/day in the drinking water for 13 months (O'Donoghue et al. 1978) did not induce gross or microscopic alterations in the spleen or thymus.

2.15 NEUROLOGICAL

Studies in humans and animals show that the neurological system is the primary target for 2-hexanone. Neurological effects observed in animals are consistent with those reported in workers exposed to 2-hexanone.

In humans, the most important effect associated with inhalation exposure to 2-hexanone is neurological dysfunction, most commonly observed as peripheral neuropathy. Reported effects in human studies include a peripheral neuropathy characterized by axon and myelin disruption and axonal swellings involving motor and sensory nerves and resulting in alterations in nerve conduction velocity, ataxia, sensory deficits, and skeletal muscle weakness accompanied by electromyographic abnormalities. Widespread attention was brought to this phenomenon after a 1973 outbreak of distal neuropathy in an Ohio fabric finishing plant that had introduced the use of 2-hexanone into its processing operations approximately 10 months before the first cases of neuropathy were reported. The time worked in the print department by persons with peripheral neuropathy ranged from 5 weeks to 27 years. The screening of 1,157 employees resulted in the detection of 86 verified cases of neuropathy (Allen et al. 1975; Billmaier et al. 1974). Eleven of these cases were moderate to severe with both motor and sensory involvement; 38 were mild with sensory signs prevailing; and 37 were considered minimal, without clinical

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manifestations but with characteristic electrodiagnostic abnormalities. General characteristics of the neuropathy included muscle weakness, sensory loss (inability to discriminate pain, touch, temperature, or vibration) in the hands and feet, and diminution or loss of reflexes. Electromyographic testing generally indicated that nerve conduction velocities were slower, especially in the ulnar, peroneal, tibial, and sural nerves, and the distal latencies (times to response) were prolonged in parallel to the reduction of the nerve conduction velocity. Other abnormalities included waves and fibrillations, especially in the more severe cases, and a decrease in the number and an increase in the size of motor unit potentials. No histological evidence of nerve damage was obtained in any of these patients. Atmospheric sampling conducted after this incident indicated that 2-hexanone levels in the processing plant averaged 9.2 ppm in front of the printing machines, 36 ppm behind them, and 6.1 ppm in the wind-up area. The operators spent 60–80% of their time in front of the printing machines (mean 9.2 ppm). After the use of 2-hexanone was discontinued, marked improvement was seen in the affected employees during the next few months, including all of the moderate-to-severe cases and most of the mild and minimal cases. It should be noted that significant exposure to methyl ethyl ketone also occurred. While methyl ethyl ketone does not induce neuropathy by itself, it has been shown to potentiate the effects induced by 2-hexanone (Saida et al. 1976). Also worth mentioning is that dermal and oral exposures were likely to have occurred due to practices such as eating in/on work areas or washing the hands in solvent.

Mallov (1976) reported three cases of severe peripheral neuropathy among 26 spray painters at one work site. In two cases, exposure to 2-hexanone was the most likely cause of the condition; in the third case, exposure to 2-hexanone was the probable cause. Davenport et al. (1976) also reported a case of peripheral neuropathy in a subject exposed to 2-hexanone at work; in this case, there was also exposure to other compounds, including MiBK. In both case studies, workers were exposed for several months, but <1 year.

In all animal species studied (monkeys, cats, and rats), the clinical observations generally indicated a progression from weakness and ataxia to complete paralysis of the limbs. These clinical observations were accompanied or preceded by morphological changes in the peripheral nerves, including an increase in the number of neurofilaments in the nerve fibers, axonal swelling, and inpouchings and thinning of the myelin sheath. Studies in animals also show involvement of the central nervous system. Studies that have examined the metabolic disposition of 2-hexanone have shown that the chemical entity responsible for the neurotoxic effects of 2-hexanone is 2,5-hexanedione, a metabolite of 2-hexanone in rats, guinea pigs, and humans (Abdel-Rahman et al. 1978; DiVincenzo et al. 1976, 1978; Eben et al. 1979).

Comparative studies of the relative neurotoxicities of 2-hexanone, 2,5-hexanedione, and other compounds

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have concluded that 2,5-hexanedione is a more potent neurotoxicant than 2-hexanone (Abou-Donia et al. 1982; Krasavage et al. 1980). Comparative studies also have shown the relative species sensitivity to 2-hexanone as cat > dog > primate > rat (Abdo et al. 1982; Mendell et al. 1974).

Limited data regarding acute inhalation exposure were located. Schrenk et al. (1936) reported that exposure of guinea pigs to 2,300 ppm commercial-grade 2-hexanone for 90 minutes produced incoordination; no adverse clinical signs were seen in guinea pigs exposed to 1,000 ppm 2-hexanone for up to 810 minutes. In another study, severe neurotoxicity was reported in rats as a result of 7 days of continuous inhalation exposure to 225 ppm 2-hexanone of unknown purity (Couri et al. 1977). No further details were provided in this study.

Intermediate-duration inhalation studies provide data on neurotoxicity of 2-hexanone in rats, cats, and monkeys. Evaluation of the data, however, is complicated because several studies tested only one exposure level, which caused neurotoxicity, so NOAELs were not defined. In addition, the true LOAEL was probably lower than the exposure level tested. Furthermore, in most studies, the purity of the compound tested was not stated and it could have varied between 70 and 98% 2-hexanone. Intermediate-duration inhalation studies in rats reported neuropathies that affected axons and the myelin sheath; axonal swelling was commonly seen (Duckett et al. 1979; Egan et al. 1980; Mendell et al. 1974; Saida et al. 1976; Spencer et al. 1975). These effects can lead to nerve degeneration. The lowest LOAEL was histopathological effects in rats at 50 ppm 2-hexanone (unknown purity) (Duckett et al. 1979). Histopathological changes were usually accompanied by signs such as weakened hindlimbs (Katz et al. 1980) and forelimbs (Spencer et al. 1975), hindlimb dragging (Mendell et al. 1974), and even paralysis (Saida et al. 1976). In general, the higher the exposure concentration, the earlier the effects appeared. Electrophysiological tests conducted in one study showed significantly decreased motor nerve conduction velocity in the sciatic-tibial nerve after intermittent exposure to ≥ 100 ppm commercial-grade (unknown purity) 2-hexanone for 29 weeks (Johnson et al. 1977). The latter study also reported impaired operant behavioral performance in rats exposed to 1,000 ppm 2-hexanone. Alterations in the central nervous system were also reported in rats after 4 months of exposure to 100 ppm pure 2-hexanone 22 hours/day (Egan et al. 1980); lesions included giant axonal swellings in the medulla oblongata and cerebellum. Similar findings were reported in cats and monkeys exposed repeatedly to 2-hexanone for intermediate durations. In monkeys, 100 ppm commercial-grade 2-hexanone (unknown purity) was a LOAEL for reduced conduction velocity in the sciatic-tibial nerve (Johnson et al. 1977). In cats, continuous exposure to 400–600 ppm 2-hexanone (unknown purity) induced hind limb dragging followed by forelimb weakness and eventual paralysis (Mendell et al. 1974). Morphological evaluations showed axonal

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swelling and demyelination of nerve fibers. In both cats and monkeys, recovery occurred months after exposure to 2-hexanone ceased.

Chronic-duration inhalation studies in rats and cats showed equivocal clinical and morphological signs of neuropathy in rats exposed intermittently (6 hours/day, 5 days/week) to 330 ppm 2-hexanone vapors for 72 weeks (Krasavage and O'Donoghue 1977) and clear morphological signs of neuropathy in cats similarly exposed to 330 ppm 2-hexanone for 2 years (O'Donoghue and Krasavage 1979). No signs of neuropathy were reported in rats or cats exposed to 100 ppm 2-hexanone. In neither study was the purity of 2-hexanone stated. Poor reporting of the results made it difficult to establish a NOAEL and LOAEL in the rat study; however, rats exposed to 330 ppm showed degenerative changes in skeletal muscle that were most likely due to neuropathy in the innervating nerve fibers. No clinical neurological signs were reported in the cats, but all cats in the 330 ppm exposure group showed lesions in the peripheral and central nervous system at and below the levels of the cerebellum and pons. In the periphery, sciatic nerve axons showed organelle accumulations with rare focal discrete "giant" axonal swelling that also involved the myelin. The sensory portion of the peripheral nervous system was least affected. Neuropathological effects in the central nervous system were generally minor; swollen terminals were found in the posterior cerebellar peduncles, folial white matter, nucleus gracilis, fasciculus gracilis, spino-cerebellar tracts, medullary reticular formation, and all levels of the spinal cord. Detailed examination of tibial nerve fibers showed a higher percentage of demyelinated, re-myelinated, swollen, and degenerative fibers in the high-exposure group than in controls and low-exposure groups.

No studies were located regarding neurological effects in humans after oral exposure to 2-hexanone, but based on results from oral studies in animals and on what is known regarding the toxicokinetics of 2-hexanone in humans and in animals, adverse neurological effects will likely occur in humans following high oral exposure to this chemical.

Oral exposures of laboratory animals provide evidence of neurotoxicity of 2-hexanone. Intermediate- and chronic duration studies showed that 2-hexanone causes the same type of neurological effects observed after inhalation exposure, which is not unexpected since both routes of exposure give rise to the toxic entity, 2,5-hexanedione. No information on acute-duration oral studies in animals was identified.

Intermediate-duration oral studies provided LOAELs for clinical signs and morphological alterations in the peripheral nervous system, but NOAELs were not identified. In rats, doses of 400 mg/kg/day 2-hexanone induced transient weakness of the hindlimbs on weeks 17–28 of a 40-weeks study (Eben et al.

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1979), and doses of ≥ 480 mg/kg/day 2-hexanone induced clinical signs such as muscle weakness and hindlimb dragging (Krasavage et al. 1980; Union Carbide 1977). In these two studies (Krasavage et al. 1980; Union Carbide 1977), microscopic examination of peripheral nerves showed evidence of neuropathy involving both axons and the myelin sheath. No histological examinations were conducted by Eben et al. (1979). Both Eben et al. (1979) and Krasavage et al. (1980) used high-purity 2-hexanone. A 40% decrease in locomotor activity was reported in groups of five guinea pigs given 2-hexanone of unknown purity in drinking water at dosage levels of approximately 310 mg/kg/day 2-hexanone during a 24-week study (Abdel-Rahman et al. 1978). Reduced pupillary responses to light (measured by changes in pupillary diameter) were also reported in this study. However, no information was provided regarding effects that may have occurred at a lower exposure level of approximately 124 mg/kg/day 2-hexanone.

In the single oral chronic-duration (13-month) study available for review, the lowest dose of 2-hexanone (high purity) tested, 143 mg/kg/day, caused axonal swellings in peripheral nerves of rats (O'Donoghue et al. 1978). Clinical neurological signs were seen in rats dosed with ≥ 266 mg/kg/day 2-hexanone and first appeared on day 42 in the rats dosed with 560 mg/kg/day and on day 77 in rats dosed with 266 mg/kg/day. Signs included decreased extension of hindlimbs, hindlimb weakness, waddling gait, dragging of hind paws, and loss of tone in hindlimb musculature with grossly observable atrophy of hindlimb musculature and axial muscles of the lumbar area. Histological examinations showed that rats from all treated groups had "giant" axonal neuropathy, with a LOAEL value of 143 mg/kg/day. Axonal swelling and giant axonopathy were common in peripheral nerves and spinal cord, less common in dorsal root ganglia, and rare in the brain. Myelin alterations were also seen in peripheral nerves. Neurogenic skeletal muscle atrophy occurred in proximal and distal hindlimb musculature. Alterations in the 266 mg/kg/day group were similar but less severe than in the 560 mg/kg/day group. Less severe changes were seen in peripheral nerves in the 143 mg/kg/day group of rats; fewer giant axons were evident, but myelin changes were more common. Spinal lesions and neurogenic muscle atrophy were minimal.

Mechanisms of Neurotoxicity. The neurotoxicity of 2-hexanone is caused by its active metabolite, 2,5-hexanedione. The mechanism of toxicity of γ -diketones (such as 2,5-hexanedione) has been extensively studied, not only with respect to 2-hexanone, but with a wider focus on γ -diketones in general, as this chemical is also a metabolite of other substances that induce neuropathy such as *n*-hexane. Because of the extensive nature of the literature that covers 2-hexanone, *n*-hexane, as well as 2,5-hexanedione itself, the summary below has been extracted from reviews and the reader is referred to references cited therein for more detailed information (LoPachin and DeCaprio 2004, 2005; LoPachin and Gavin 2015; LoPachin et al. 2000). The two main features of 2-hexanone toxicity are the appearance of

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giant neurofilamentous axonal swellings and axonal atrophy. As discussed in these reviews, studies have reached different conclusions regarding whether the principal neuropathological manifestation of 2,5-hexanedione toxicity is distal swelling of myelinated fiber (central-peripheral distal axonopathy) or axonal atrophy. However, it appears that axonal atrophy is the most significant component of 2,5-hexanedione-induced neuropathy.

Ruling out axonal swelling as the main feature of 2,5-hexanedione intoxication was based on some of the following observations. Studies showed that the *in vivo* neurotoxic potencies of various chemicals whose metabolism lead to the production of 2,5-hexanedione were correlated with the corresponding serum concentration of 2,5-hexanedione. Yet, the frequency of axonal swellings in the nerves examined did not correlate with the concentration of 2,5-hexanedione in serum. In fact, the relative frequency of swollen axons was inversely related to the serum concentration of 2,5-hexanedione and to the manifestation of neurotoxicity. This was shown to occur in both the peripheral and central nervous systems. Studies also showed that axonal swellings appeared during the later stages of 2,5-hexanedione intoxication, indicating lack of temporal association with the expression of neurological deficits. Overall, these results suggested that 2,5-hexanedione induction of neurological dysfunction was not dependent on axonal swelling and that this phenomenon could represent a secondary response to neurotoxic injury or stress.

2,5-Hexanedione-induced axonal atrophy is characterized by reduction in axon cross-sectional area without a significant change in perimeter length and degree of myelination. Morphological evaluations have shown that axon atrophy is associated with faster anterograde slow axonal transport in both peripheral nerves and in white central matter tracts of rats exposed to 2,5-hexanedione, which would lead to proximal axon atrophy and secondary distal accumulation of neurofilaments and swelling. However, subsequent studies that conducted spatio-temporal analyses showed that atrophy was widespread in the central and peripheral nervous systems and that it developed as an early consequence of 2,5-hexanedione intoxication. Observations that reductions in axon perimeter can develop in the absence of axonal swelling supported the view that axonal atrophy is the principal lesion that develops as an early consequence of 2,5-hexanedione intoxication regardless the dose or route of exposure. Further support for axonal atrophy being the main neuropathological feature of 2,5-hexanedione intoxication is the fact that reduced axon diameter is associated with reduced nerve conduction velocity.

The mechanism by which 2,5-hexanedione induces axonal atrophy has not been completely elucidated; however, studies have shown that adduction with cytoskeletal proteins plays a key role.

2,5-Hexanedione, a diketone electrophile, reacts covalently with nucleophilic lysine ϵ -amino groups to

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form 2,5-dimethylpyrrole adducts on neurofilaments and other proteins. This is thought to interfere with turnover and maintenance of the axonal cytoskeleton, and some have suggested that, following formation, pyrrole adducts undergo oxidative reactions that yield cross-linked neurofilament proteins. However, since virtually all proteins, neuronal and non-neuronal, contain one or more lysine ϵ -amino groups, *in vivo* exposure to 2,5-hexanedione would result in multiple physiological systems being affected; however, this does not seem to be the case. In addition, *in vitro* and *in vivo* studies showed that only a very small fraction of the total available lysyl ϵ -amino groups on neurofilament proteins were converted to pyrrole adducts, so a specific mechanism needed to be involved. Further *in vitro* experiments showed that the adducted lysine residues were primarily located within the KSP (lysine-serine-proline) repeat on the C-terminal regions of neurofilament-M and neurofilament-H subunit proteins.

Exactly how neurofilament protein adduction can lead to axonal atrophy is not totally understood. Results from some studies suggested that 2,5-hexanedione might reduce phosphorylation of neurofilaments, an important determinant of cytoskeletal protein turnover and axon diameter. Reduced phosphorylation would prevent neurofilaments from associating with the cytoskeletal polymer or cause premature dissociation of integrated neurofilaments. In turn, depletion of neurofilaments by anterograde transport of hypophosphorylated neurofilaments would lead to loss of axon diameter. More recent data have shown that 2,5-hexanedione can affect components of the axon cytoskeleton other than neurofilament subunit proteins. Studies in rats treated orally with 2,5-hexanedione showed that 2,5-hexanedione impaired binding of microtubule associated proteins (e.g., MAP1A, tau) to recognition sites on microtubules. Presumably, this disruption was caused by 2,5-hexanedione adduct formation with ϵ -amino groups on lysine residues that mediate such protein-protein interactions. Based on the critical role in cytoskeletal physiology, MAPs could represent a relevant target of γ -diketone axonopathy. Studies also suggested that higher molecular weight neurofilament derivatives were not a consequence of 2,5-hexanedione cross-linking of these proteins, because they also appeared in nervous tissues of untreated animals. Rather, these derivatized neurofilaments likely represented baseline levels of proteins that were cross-linked by normal activities of axon transglutaminases that increase cytoskeletal stability. The elevated content of higher molecular weight neurofilament complexes in 2,5-hexanedione-treated rats was thought to represent excess fragmentation of the stationary cytoskeleton possibly as a result of 2,5-hexanedione-impaired polymer maintenance (LoPachin and DeCaprio 2004, 2005; LoPachin and Gavin 2015; LoPachin et al. 2000).

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2.16 REPRODUCTIVE

No studies were located regarding reproductive effects in humans after exposure to 2-hexanone.

A few studies evaluated the effects of 2-hexanone on the reproductive system of animals. Although some studies showed testicular damage, these effects have not been rigorously evaluated. Based on exposure levels producing adverse effects, environmental levels of 2-hexanone (mostly water levels reported in the past, ppb range) are unlikely to represent a reproductive risk for humans.

Intermediate-duration inhalation exposure to a high concentration of 2-hexanone caused testicular damage in rats. Marked and significant reductions in absolute and relative testes weight and atrophy of testicular germinal epithelium were observed in male rats exposed to 700 ppm 2-hexanone (96.1% pure) for 11 weeks (Katz et al. 1980); no other exposure level was tested in this study. Chronic-duration exposure of male rats or female cats to ≤ 330 ppm 2-hexanone (unknown purity) did not induce compound-related gross or microscopic alterations in the reproductive organs of either species (Krasavage and O'Donoghue 1977; O'Donoghue and Krasavage 1979).

Oral studies in laboratory animals also show that oral exposure to 2-hexanone may induce adverse reproductive effects; however, limited information is available. Male rats that were given 2-hexanone (96.1% pure) at 660 mg/kg/day by gavage in a 90-day study were reported to develop atrophy of the germinal epithelium of the testes (Krasavage et al. 1980). However, no quantitative data were presented, so this effect is not listed in oral LSE table (Table 2-2). The only additional relevant information regarding reproductive effects of 2-hexanone in animals is that treatment of female rats with $\leq 1,040$ mg/kg/day 2-hexanone for 120 days (Union Carbide 1977) or male rats with ≤ 560 mg/kg/day 2-hexanone (96.1% pure) via drinking water for 13 months did not induce gross or microscopic alterations in the reproductive organs (O'Donoghue et al. 1978).

2.17 DEVELOPMENTAL

No studies were located regarding developmental effects in humans after exposure to 2-hexanone.

Only one study evaluating developmental effects of 2-hexanone in laboratory animals was identified. Intermittent inhalation exposure of groups of 25 pregnant rats to 2,000 ppm 2-hexanone (unknown purity) 6 hours/day during the entire gestation period resulted in a significant reduction in the number of pups per

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litter and in neonatal weight (40%); no such effects were reported in rats exposed to 1,000 ppm (Peters et al. 1981). In this study, behavioral alterations consisting of reduced activity in the open field, increased activity in the running wheel, and deficits in avoidance conditioning were reported in the offspring of exposed dams at all ages (newborn, weanling, puberty, and adult) except geriatric in which results were similar to those of controls. Behavioral tests in most cases indicated that maternal exposure to 2-hexanone was associated with hyperactivity in the young and decreased activity in the geriatric stage, which the authors speculated to be due to premature aging resulting from the earlier hyperactivity. It is not clear whether these effects are the result of transplacental exposure to 2-hexanone or of postnatal exposure to 2-hexanone and/or its metabolites via the milk of the exposed dams.

No firm conclusions can be made regarding developmental effects of 2-hexanone based on a single animal study; further information would be necessary.

2.18 CANCER

Three chronic-duration studies conducted histopathological assessments of comprehensive tissues: two inhalation studies in rats (Krasavage and O'Donoghue 1977) and cats (O'Donoghue and Krasavage 1979) and one oral study in rats (O'Donoghue et al. 1978). No evidence of cancer was reported in any of these studies.

Neither the HHS nor IARC have classified 2-hexanone regarding its carcinogenicity (IARC 2019; NTP 2016). The EPA stated that “there is inadequate information to assess the carcinogenic potential” of 2-hexanone (EPA 2009a).

2.19 GENOTOXICITY

No studies were located regarding the potential genotoxic effects in humans or animals following any route of exposure to 2-hexanone. One study was located that provided data on genotoxicity of 2-hexanone in an *in vitro* system. The study reported that 2-hexanone was mutagenic in *Salmonella typhimurium* 1535 [pSK 1002] as assessed by the SOS/*umu* Test (Nakajima et al. 2006).