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 chlordane. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

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

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 chlordane, 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. 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. 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 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. Levels of exposure associated with cancer (Cancer Effect Levels, CELs) of chlordane are indicated in Table 2-2 and Figure 2-3.

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.

As illustrated in Figure 2-1, human studies predominantly evaluated neurological and cancer endpoints. Available human and animal data suggest the following sensitive targets of chlordane toxicity:

- Hepatic endpoint: Inhalation and oral exposure of animals resulted in liver weight increases and histopathologic effects including hepatocellular hypertrophy and dose-related incidence and severity of degenerative and regenerative liver lesions.

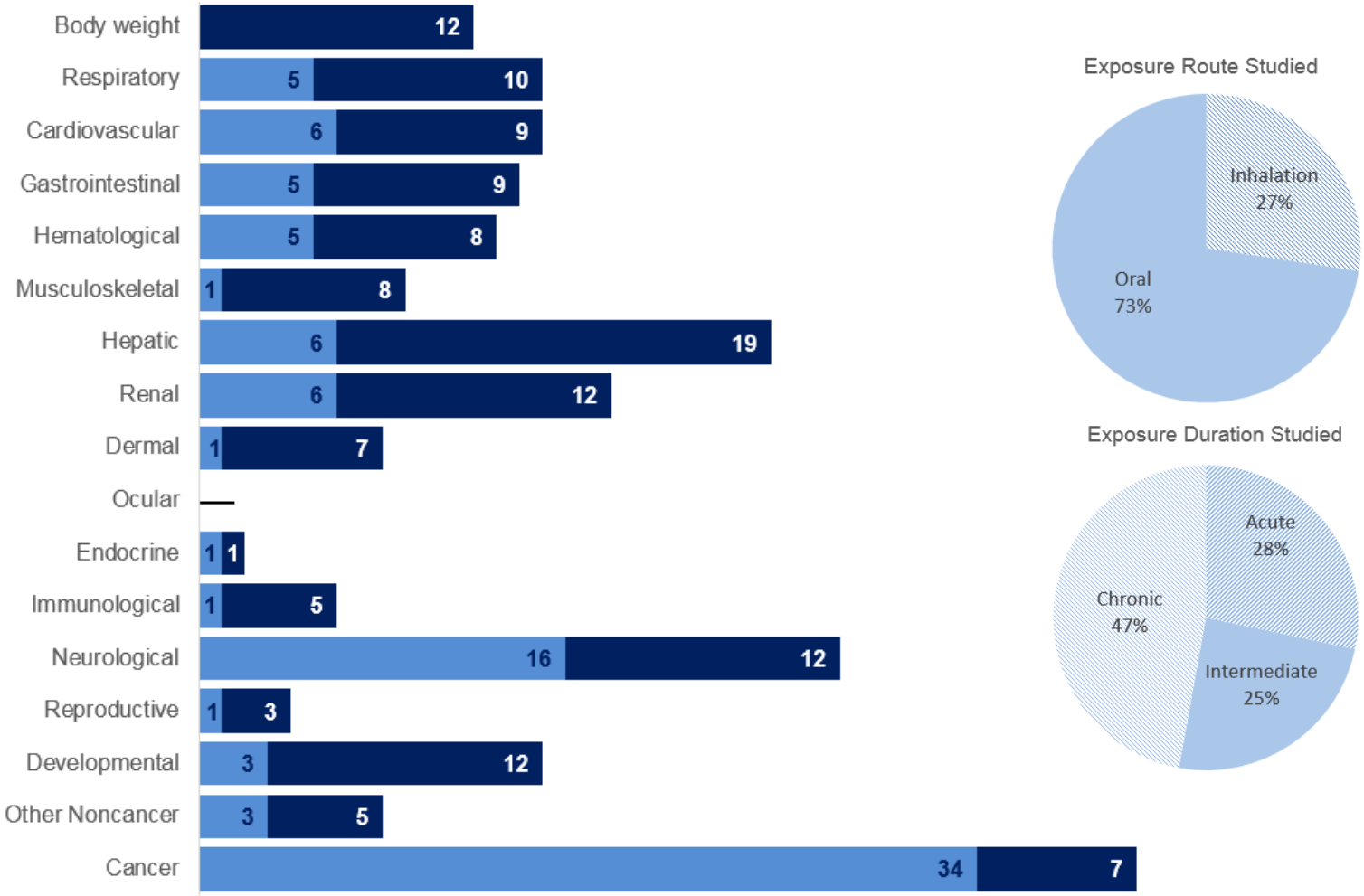
- Neurological endpoint: Central nervous system effects including headache, dizziness, muscle tremors, and convulsions have been reported in humans exposed by inhalation following its former use as a pesticide and following ingestion of chlordane-containing substances. Effects such as tremors and convulsions were observed in laboratory animals following relatively high level inhalation or oral exposure to chlordane.

- Developmental endpoint: Limited human data have not provided reliable associations between chlordane exposure and developmental outcomes. However, results from oral animal studies suggest that pre- and postnatal exposure may adversely affect neurobehavioral development and the immune system; one study reported chlordane treatment-related decreased postnatal survival.

- Hematological endpoint: Limited human data have not provided associations between chlordane exposure and hematological effects. One 90-day inhalation study of rats reported 8% increased leukocyte count and 26% decreased platelets in females (but not males) intermittently exposed at a concentration as low as 1 mg/m^3. One oral study in mice reported increased leukocyte count following single gavage dosing of chlordane at 8 mg/kg/day. However, these effects have not been substantiated in other animal studies.

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

Most studies examined the potential hepatic, renal, neurological, and cancer effects of chlordane
More studies evaluated health effects in **animals** than **humans**, **except for neurological and cancer endpoints**
(counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 106 studies include those finding no effect. Most studies examined multiple endpoints.

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
ACUTE EXPOSURE									
1	Rat (Wistar) 10 M, 10 F	Up to 28 days 5 days/week 8 hours/day	0, 5.8, 28.2, 154, 413		Death			154	Terminated after 11 exposures due to deaths by day 5; at 413 mg/m^3, terminated after 3 exposures
					Bd Wt		154		Unspecified body weight loss
					Resp	154		413	Epithelial degeneration and cellular debris in bronchi and alveoli
					Hemato	154			
					Hepatic		154	413	Increased blood ALT, AST, SGDH, bile acids, cholesterol, liver enlargement and discoloration, centrilobular hepatocyte enlargement; effects were more severe at 413 mg/m^3
					Neuro			154	Abnormal respiratory movements, salivation, convulsions
					Other noncancer		154		Increased height of thyroid follicular cells

EPA 1987f; Khasawinah et al. 1989; Chlordane technical

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
INTERMEDIATE EXPOSURE									
2	Rat (Wistar) 10 M, 10 F	28 days 5 days/week 8 hours/day	0, 5.8, 28.2		Resp	28.2			
					Cardio	28.2			
					Gastro	28.2			
					Hemato	28.2			
					Musc/skel	28.2			
					Hepatic	5.8	28.2		Centrilobular hepatocellular hypertrophy; decreased blood glucose, increased blood total protein, albumin, and globulin
					Renal	28.2			
					Dermal	28.2			
					Immuno	5.8	28.2		Decreased thymus weight in females
					Neuro	5.8	28.2		Hypersensitivity to touch in females
					Other noncancer	5.8	28.2		Increased heighted of follicular epithelium of thyroid

EPA 1987f; Khasawinah et al. 1989; Chlordane technical

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
3	Rat (Wistar) 15 M, 15 F	90 days 5 days/week 8 hours/day	0, 0.1, 1.0, 10		Resp	10			
					Cardio	10			
					Gastro	10			
					Hemato	0.1	1		8% increased leukocytes, 26% decreased platelets in females
					Musc/skel	10			
					Hepatic	0.1[b,c]	1		Centrilobular hypertrophy, hepatocellular vacuolization, increased P-450, decreased albumin, decreased albumin/ globulin ratio
					Renal	10			
					Dermal	10			
					Other noncancer	1	10		Increased height of follicular cells of the thyroid in males
EPA 1987f; Khasawinah et al. 1989; Chlordane technical									
4	Monkey (cyno-molgus) 6 M, 6 F	90 days 5 days/week 8 hours/day	0, 0.1, 1.0, 10		Resp	10			
					Cardio	10			
					Gastro	10			
					Hemato	10			
					Musc/skel	10			
					Hepatic	10			
					Renal	10			
					Dermal	10			
					Other noncancer	10			
EPA 1987f; Khasawinah et al. 1989; Chlordane technical									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
CHRONIC EXPOSURE									
5	Human	1–15 years 8 hours/day 5 days/week			Resp	0.0017			
					Cardio	0.0017			
					Gastro	0.0017			
					Hemato	0.0017			
					Hepatic	0.0017			
					Renal	0.0017			
					Other noncancer	0.0017			

Fishbein et al. 1964

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an intermediate-duration inhalation minimal risk level (MRL) of 0.0002 mg/m^3; concentration adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 to extrapolate from animals to humans and 10 for human variability).
[c]The NOAEL of 0.1 mg/m^3 for hepatic effects identified in an intermediate-duration study (EPA 1987f; Khasawinah et al. 1989) was used to derive a chronic-duration inhalation MRL of 0.00002 mg/m^3; the concentration was adjusted for an intermittent exposure and divided by an uncertainty factor of 1,000 (10 to extrapolate from intermediate duration to chronic duration exposure, 10 to extrapolate from animals to humans, and 10 for human variability).

ALT = alanine aminotransferase; AST = aspartate aminotransferase; Bd Wt = body weight; Cardio = cardiovascular; F = female(s); Gastro = gastrointestinal; Hemato = hematological; Immuno = immunological; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; Resp = respiratory; SGDH = serum glutamic dehydrogenase

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

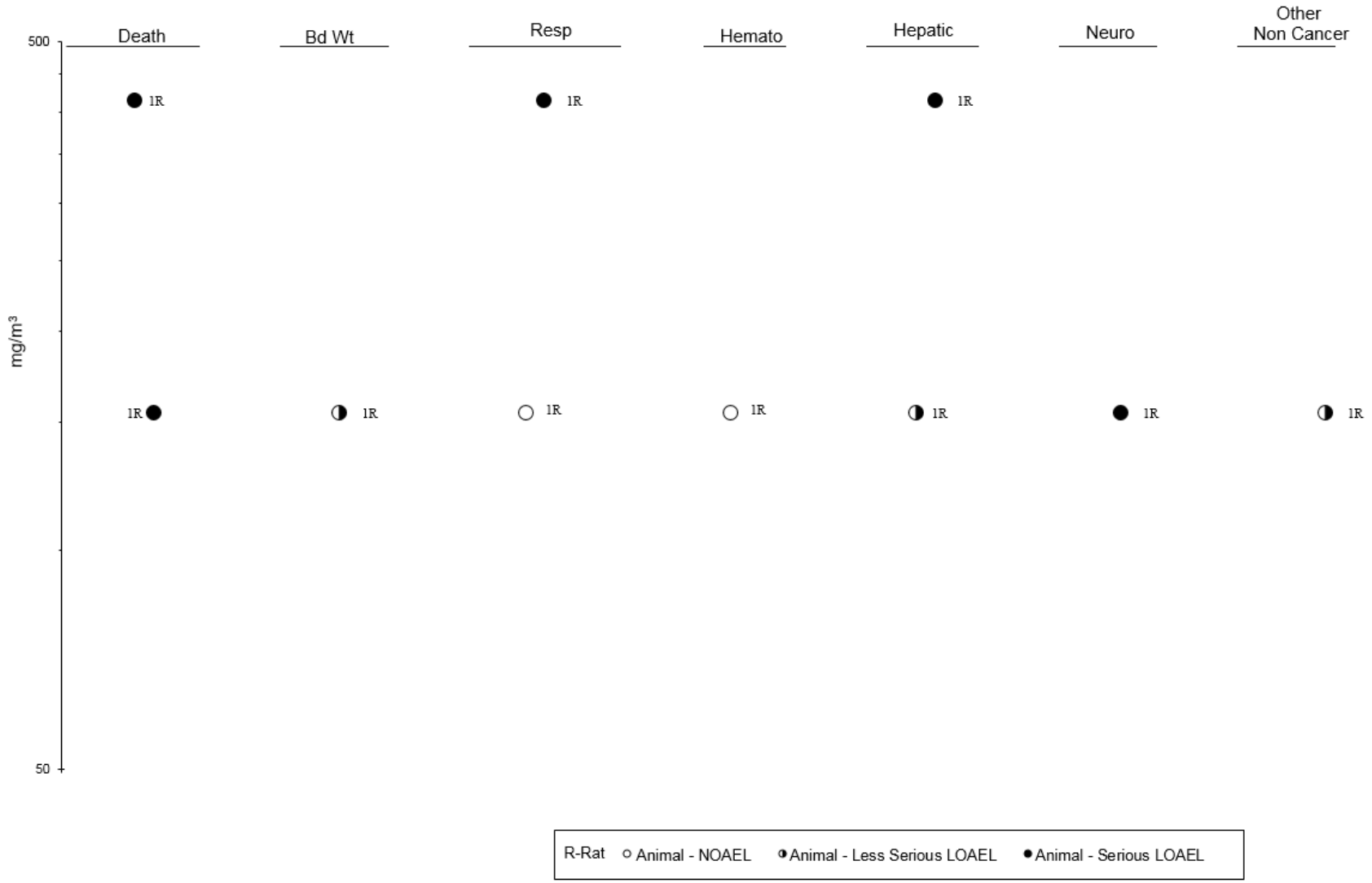


Figure 2-2. Levels of Significant Exposure to Chlordane – Inhalation
Intermediate (15-364 days)

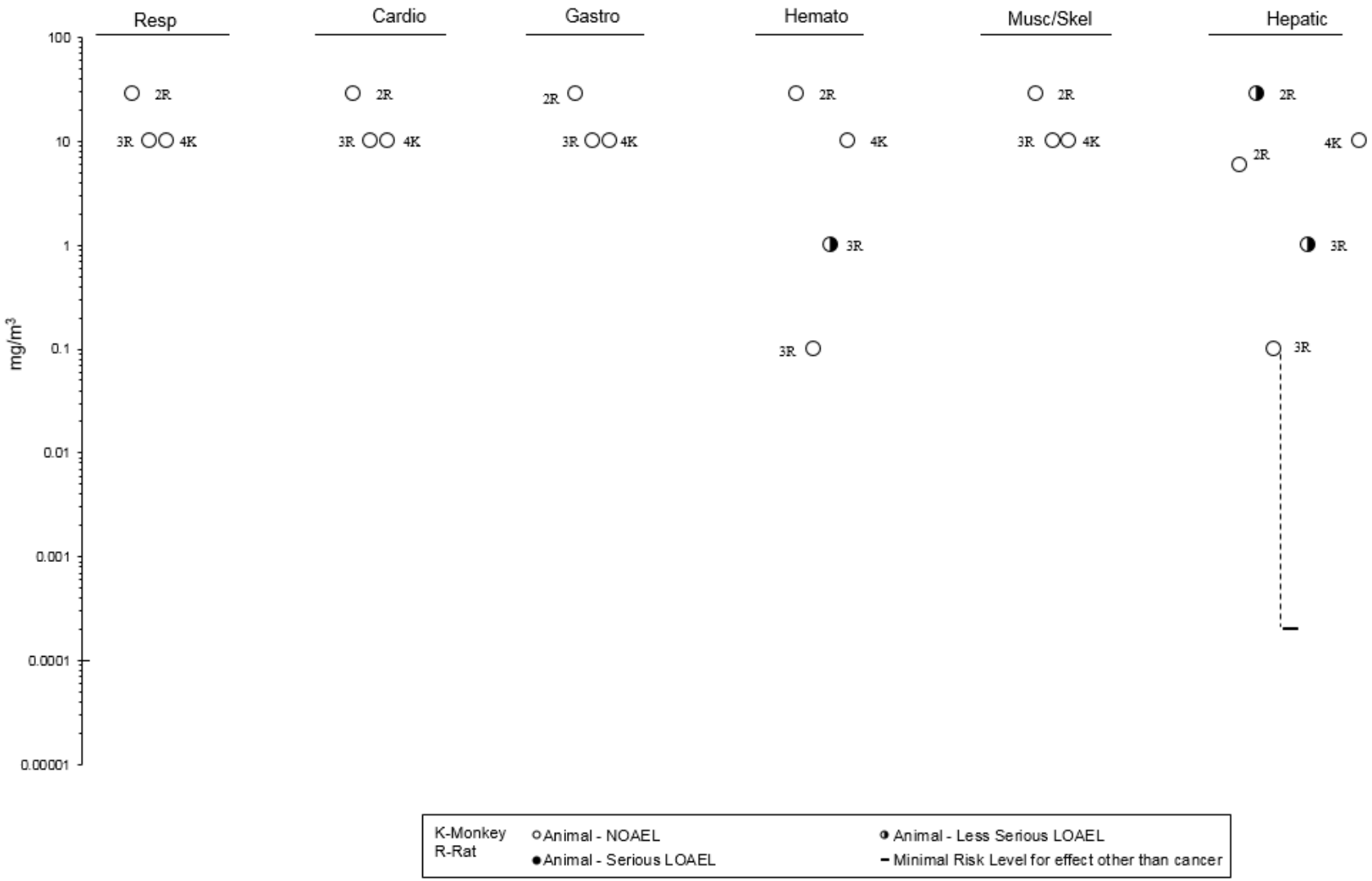


Figure 2-2. Levels of Significant Exposure to Chlordane – Inhalation
Intermediate (15-364 days)

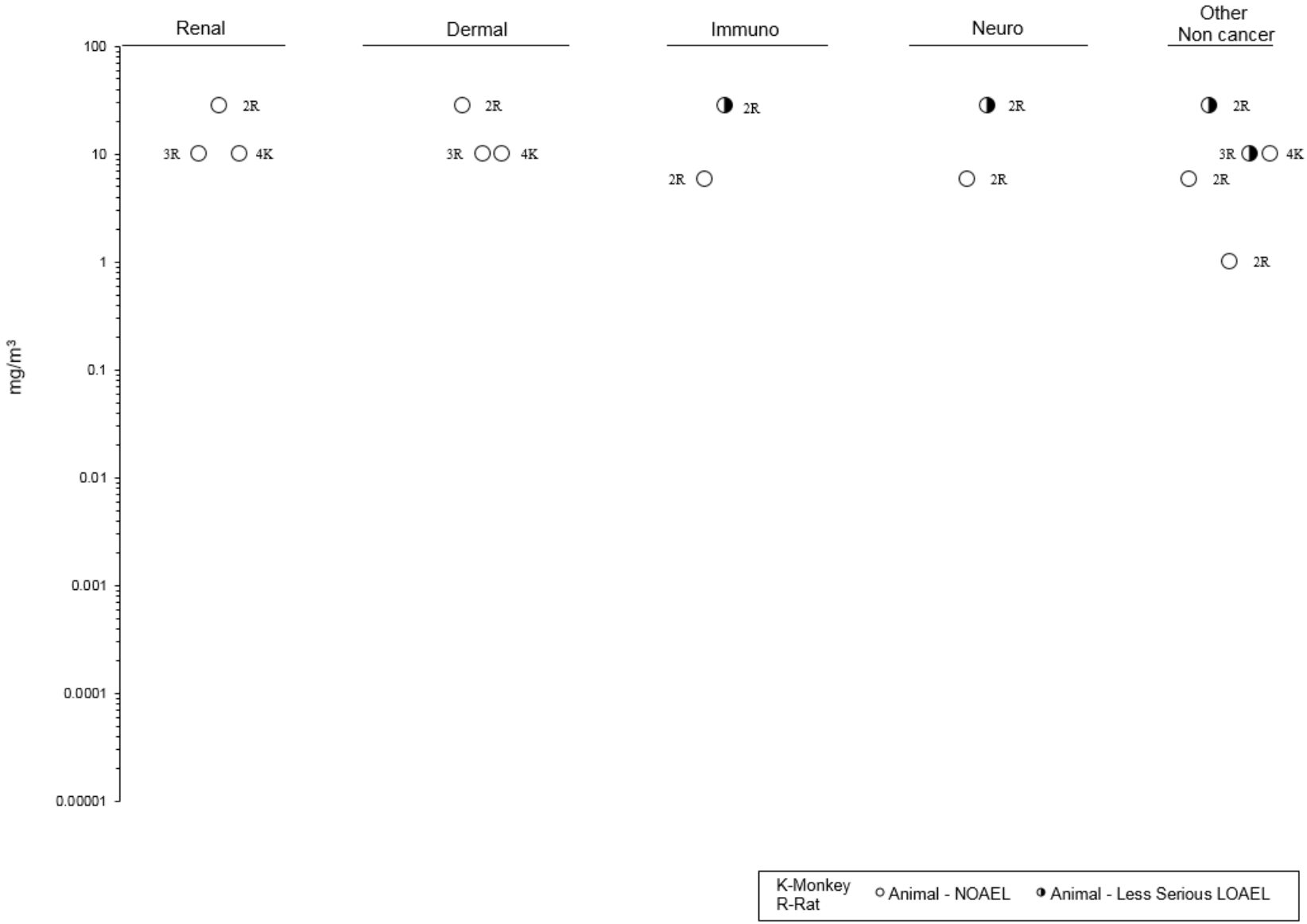


Figure 2-2. Levels of Significant Exposure to Chlordane – Inhalation
Chronic (≥365 days)

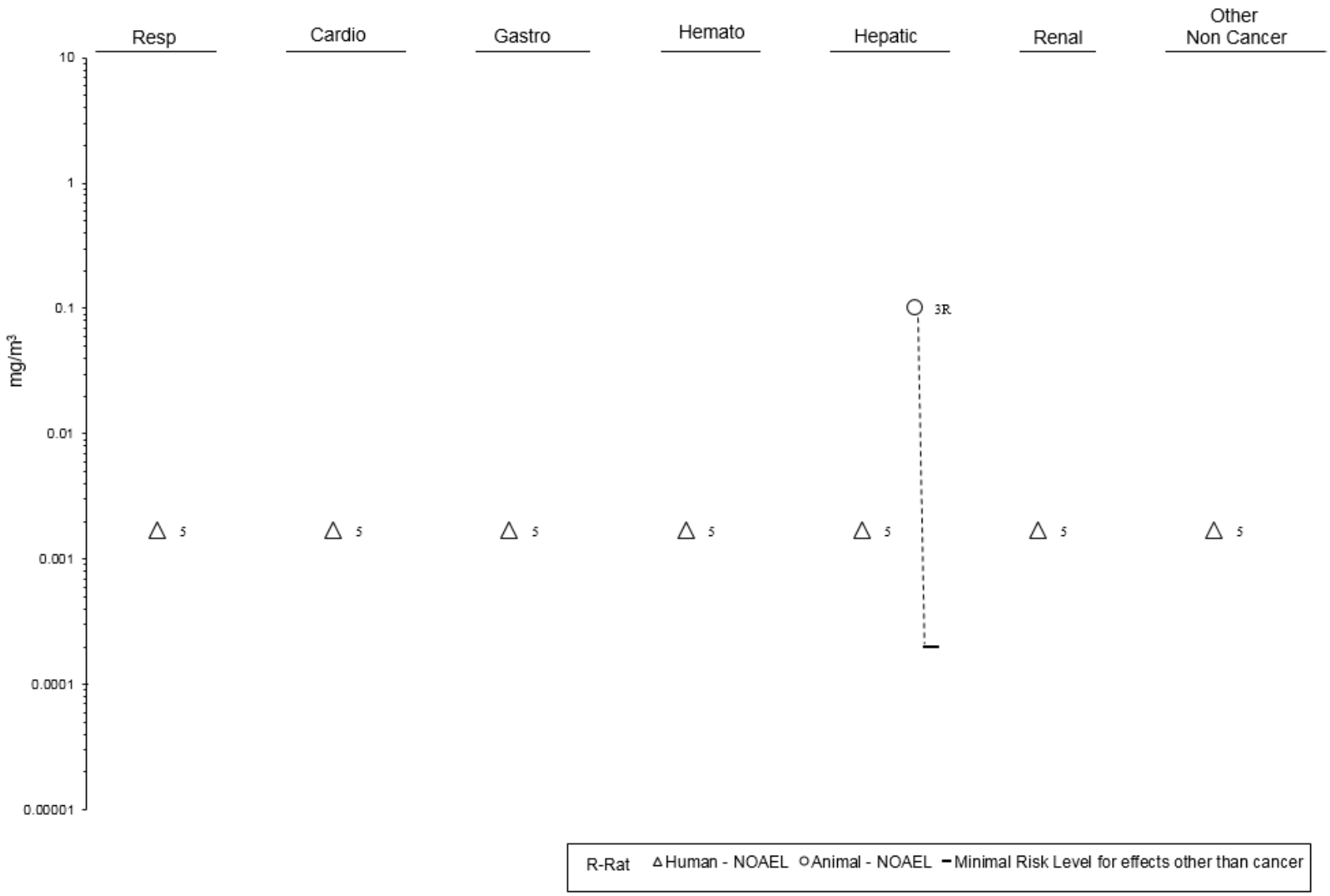


Table 2-2. Levels of Significant Exposure to Chlordane – Oral

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)	Effect
ACUTE EXPOSURE									
1	Rat (Albino) 3–15 M 5–15 F	Once (GO)	0, 200, 300, 400, 500, 600, 700, 800		Death			590	LD_{50}
					Neuro			200	Convulsions
Ambrose et al. 1953a; Chlordane technical									
2	Rat (Albino) 5 (sex NS)	Up to 15 days 1 time/day (GO)	6.25, 12.5, 25, 50, 100, 200		Death			50	50 mg/kg/day: 2/5 died by day 12 100 mg/kg/day: 5/5 died by day 9 200 mg/kg/day: 5/5 died by day 4
					Neuro	25		50	Convulsions and death in two rats on days 9 and 12
Ambrose et al. 1953a; Chlordane technical									
3	Rat (Albino) 5 M 5 F	Up to 7 days (F)	135		Death			135	100% mortality by day 7
Ambrose et al. 1953a; Chlordane technical									
4	Rat (NS)	Once (G)			Death			283	LD_{50}
Ben-Dyke et al. 1970; Chlordane technical									
5	Rat (Wistar)	Once (G)			Death			137	LD_{50}
Boyd and Taylor 1969; Chlordane technical									
6	Rat (NS)	Once (GO)			Death			420	LD_{50}
Deichmann and Keplinger 1970; Chlordane technical									

Table 2-2. Levels of Significant Exposure to Chlordane – 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)	Effect
7	Rat (NS)	Once (GO)			Death			335	LD_{50}
Gaines 1960; Chlordane technical									
8	Rat (NS)	Once (GO)			Death			430	LD_{50}
Gaines 1960; Chlordane technical									
9	Rat (NS)	Once (G)			Death			457	LD_{50}
Lehman 1951; Chlordane technical									
10	Rat (Fischer 344); 8 F	Once (GO)	0, 16, 52, 156, 291	BH, LE, OF	Neuro	52	156	291	≥156: Piloerection, reactivity to handling 291: Clonic-tonic convulsions, tiptoe gait, increased forelimb grip strength
Moser et al. 1995; Chlordane technical									
11	Rat (Fischer 344); 8 F	Up to 14 days (GO)	0, 5, 16, 52, 156	BH, BW, LE, OF	Death			52	100% mortality
					Bd wt	16	52		Body weight loss
					Neuro	16		52	Increased excitability prior to death
Moser et al. 1995; Chlordane technical									
12	Rat (Fischer 344); 19–23 F	GDs 6–19 1 time/day (G)	0, 21, 28	BW, CS, DX, FX, LE, MX, TG	Bd wt			21	33% depressed maternal weight gain
					Develop			21	>30% postnatal pup loss/litter
Narotsky and Kavlock 1995; Chlordane technical									

Table 2-2. Levels of Significant Exposure to Chlordane – 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)	Effect
13	Rat (NS)	4 days 1 time/day (GO)			Hepatic		100		Increased serum cholesterol, gamma-GPT; reduced blood sugar, increased relative liver weight and lipid content; increased lipid peroxidation; hepatocellular hypertrophy
Ogata and Izushi 1991; Chlordane technical									
14	Rat (NS)	Once (G)			Musc/skel		260		Hypertonicity of skeletal muscles
Santolucito and Whitcomb 1971; Chlordane technical									
15	Rat (NS)	Once (GO)			Death			350	LD_{50}
					Resp	200			
					Cardio	200			
					Gastro	200			
					Hepatic		200		Increased serum AST and LDH, depressed liver AST, LDH, ChE, G6PDH, hypertrophy, dilatation of centrilobular sinuses, and congestion.
					Renal		200		Congestion, tubular dilation
					Neuro		200		Congestion in the brain
					Other noncancer (adrenal)	200			
Truhaut et al. 1974, 1975; Chlordane technical									

Table 2-2. Levels of Significant Exposure to Chlordane – 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)	Effect
16	Rat (NS)	GDs 7–17 1 time/day (GO)			Death			80	Death in 4/8 dams
					Develop	80			
Usami et al. 1986; Chlordane technical									
17	Mouse (NS)	7 days (3rd trimester) 1 time/day			Develop		1[b]		Altered conditioned avoidance response, open field test, and electroshock seizure threshold
Al-Hachim and Al-Baker 1973; Chlordane technical									
18	Mouse (NS)	7 days 1 time/day (GO)			Death			300	4/10 died
Balash et al. 1987; Chlordane technical									
19	Mouse (NS)	GDs 8–12 1 time/day (GO)			Death			50	3/25 died
					Develop	50			
Chernoff and Kavlock 1982; Chlordane technical									
20	Mouse (NS)	Once (GO)			Death			390	LD_{50}
					Resp	200			
					Cardio	200			
					Gastro	200			
					Hepatic		200		Hepatic hypertrophy, congestion, dilation of centrilobular sinuses, elevated liver ALT and AST and serum ALT and LDH
					Renal		200		Congestion and tubular dilation
					Neuro		200		Congestion in the brain

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

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					Other noncancer (adrenal)	200			
Truhaut et al. 1975; Chlordane technical									
21	Hamster	Once (GO)			Death			1,720	LD_{50}
					Resp	1,200			
					Cardio	1,200			
					Gastro		1,200		Atrophy of gastric mucosa
					Hepatic		1,200		Decreased liver LDH and increased G6PDH, congestion, dilatation of centrilobular sinuses, and hypertrophy
					Renal		1,200		Congestion and tubular dilatation
					Neuro		1,200		Congestion in the brain
					Other noncancer (adrenal)	1,200			
Truhaut et al. 1974, 1975; Chlordane technical									
22	Rat (NS)	2 weeks ad lib (F)			Death			40 F 80 M	4/5 deaths in females; 5/5 deaths in males
NCI 1977; Chlordane analytical									
23	Rat (NS)	Once (GO)			Neuro		100	200	Paralysis, convulsions
Hrdina et al. 1974; *cis*-Chlordane									

Table 2-2. Levels of Significant Exposure to Chlordane – 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)	Effect
24	Rat (NS)	Once (GO)			Hepatic		200		Increased blood glucose and urea, decreased liver glycogen, increased gluconeogenesis
					Renal		200		Increased kidney gluconeogenic enzymes, cyclic adenosine monophosphatase, and adenyl cyclase activity
Kacew and Singhal 1973; *cis*-Chlordane									
25	Rat (NS)	Once (GO)			Death			83	LD_{50}
Podowski et al. 1979; *cis*-Chlordane									
26	Mouse (NS)	14 days 1 time/day (GO)			Bd wt	8			
					Hemato	4	8		52% increased lymphocytes
					Immuno	8			
Johnson et al. 1986; *trans*-Chlordane									
INTERMEDIATE EXPOSURE									
27	Rat (Albino) 5 M, 5 F	Up to 163 days (F)	67		Death			32	100% mortality
Ambrose et al. 1953a; Chlordane technical									
28	Rat (NS)	15 days 7 days/week (GO)			Bd wt	25	50		Unspecified body weight loss
					Hepatic		6.25		Intracytoplasmic bodies
Ambrose et al. 1953a; Chlordane technical									

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

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29	Rat (NS)	Weaning through mating, gestation and lactation (F)			Repro			16	Decreased fertility and survivability
Ambrose et al. 1953a; Chlordane technical									
30	Rat (Sprague-Dawley); 7 M, 7 F	28 days 1 time/day (GO)	0, 0.25, 2.5, 25	BC, BW, CS, DI, EA, FI, GN, HE, HP, LE, OF, OW, TM, UR, WI	Bd wt	25			
					Hemato	25			
					Hepatic	M: 0.25 F: 2.5	M: 2.5 F: 25		Increased liver weight, hepatocellular hypertrophy
					Renal	F: 25	M: 0.25		Epithelial hyperplasia, protein and cell debris in tubule luminae
					Endocr	M: 0.25 F: 2.5	M: 2.5 F: 25		Aspherical follicles in thyroid
Bondy et al. 2000; Chlordane technical									
31	Rat (NS)	12 weeks ad lib (F)	0, 2.15, 4.3, 8.6		Neuro	4.3		8.6	Convulsions
Drummond et al. 1983; Chlordane technical									
32	Rat (NS)	2–9 months ad lib (F)			Hepatic		0.125		Centrilobular hypertrophy, cytoplasmic inclusion bodies
					Renal	1.25			
Ortega et al. 1957; Chlordane technical									

Table 2-2. Levels of Significant Exposure to Chlordane – 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)	Effect
33	Rat (NS)	90 days ad lib (F)			Repro		19.5		360% increase in androgen receptor content of the ventral prostate gland
Shain et al. 1977; Chlordane technical									
34	Rat (NS) 7 M, 7 F	28 days 1 time/day (GO)	0, 0.25, 2.5, 25	BW, CS, OF, OW	Bd wt Immuno	25 25			
Tryphonas et al. 2003; Chlordane technical									
35	Mouse (NS)	30 days 1 time/day (GO)			Repro		100		Reduced size of seminiferous tubules, degeneration in spermatogenic epithelium.
Balash et al. 1987; Chlordane technical									
36	Mouse (NS)	18 days GDs 1–18 (F)			Develop	4	8		Decreased delayed hypersensitivity response in offspring
Barnett et al. 1985a; Chlordane technical									
37	Mouse (NS)	19 days GDs 1–19 (F)			Develop		4		Decreased delayed hypersensitivity, mixed lymphocyte reactivity
Barnett et al. 1985b; Chlordane technical									
38	Mouse (NS)	18 days 7 days/week (F)			Immuno Develop	8	4		Decreased liver cell-colony forming capacity
Barnett et al. 1990a; Chlordane analytical									
39	Mouse (NS)	18 days GDs 1–18 (F)			Develop		8		Decreased liver cell-colony forming capacity
Barnett et al. 1990b; Chlordane analytical									

Table 2-2. Levels of Significant Exposure to Chlordane – 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)	Effect
40	Mouse (BALB-C)	GDs 1–18 1 time/day (peanut butter)	0, 8	CS, DX, MX	Develop		8		Depressed numbers of bone marrow colony forming units-granulocyte/ macrophage in female offspring
Blyler et al. 1994; Chlordane analytical									
41	Mouse (NS)	19 days GDs 1–19 (F)			Develop			8.0	Death of 55% of offspring
Cranmer et al. 1984; Chlordane analytical									
42	Mouse (NS)	6 weeks ad lib (F)			Death			20.8	2/5 deaths in males
NCI 1977; Chlordane analytical									
43	Mouse (NS)	19 days GDs 1–19 (F)			Develop	0.16		8	Decreased cell-mediated immunity response
Spyker-Cranmer et al. 1982; Chlordane analytical									
44	Mouse (NS)	18 days GDs 1–18 1 time/day (GO)			Develop		8.0		Decreased 5'-nucleotidase activity in macrophages; activation of macrophages to inflammatory state in mice exposed prenatally
Theus et al. 1992; Chlordane analytical									
45	Rat (NS)	10–20 week 2 times/week (F)			Hepatic		0.1		Increased cytochrome P-450 content at 10 weeks; decreased microsomal protein at 20 weeks
Mahon et al. 1978; *cis*- and *trans*-Chlordane									

Table 2-2. Levels of Significant Exposure to Chlordane – 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)	Effect
CHRONIC EXPOSURE									
46	Rat (Albino) 5 M, 5 F	Up to 407 days (F)	0, 0.48, 0.97, 1.9, 3.9, 7.75, 15.5		Bd wt	7.75	15.5		11–18% decrease in body weight
					Hemato	15.5			
					Hepatic	1.9	3.9		Increased liver weight
Ambrose et al. 1953a; Chlordane technical									
47	Rat (Fischer 344) 80 M, 80 F	30 months ad lib (F)	M: 0, 0.045, 0.23, 1.18 F: 0, 0.055, 0.27, 1.41		Bd wt	M 1.18 F 1.41			
					Resp	M 1.18 F 1.41			
					Cardio	M 1.18 F 1.41			
					Gastro	M 1.18 F 1.41			
					Hemato	M 1.18 F 1.41			
					Musc/skel	M 1.18 F 1.41			
					Hepatic	M 1.18 F 0.055[c]	F 0.273		Hepatocellular hypertrophy
					Renal	M 1.18 F 1.41			
					Dermal	M 1.18 F 1.41			
EPA 1985a; Khasawinah and Grutsch 1989a; Chlordane technical									

Table 2-2. Levels of Significant Exposure to Chlordane – 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)	Effect
48	Mouse (ICR) 80 M, 80 F	24 months ad lib (F)	M: 0, 0.12, 0.65, 1.65 F: 0, 0.14, 0.65, 1.65		Bd wt	1.65			
					Resp	1.65			
					Cardio	1.65			
					Gastro	1.65			
					Hemato	1.65			
					Musc/skel	1.65			
					Hepatic	M 0.12 F 0.14	M 0.65 F 0.65		Hepatocellular hypertrophy in males and females
					Renal	1.65			
					Dermal	1.65			
					Cancer			1.65	CEL: hepatocellular adenoma and hemangioma in males
EPA 1985a; Khasawinah and Grutsch 1989b; Chlordane technical									
49	Mouse (CD-1)	18 months ad lib (F)			Death			6.5	86–86% mortality
					Cancer			3.25	CEL: hepatocellular carcinoma
IRDC 1973; Epstein 1976; Chlordane technical									
50	Mouse (B6C3F1) 210 M	Up to 2 years (F)	0, 9.4	BW, CS, GN, HP, LE, OW	Bd wt	9.4			
					Hepatic		9.4		Increased liver weight, hepatocellular hypertrophy
					Cancer			9.4	CEL: liver tumors
Malarkey et al. 1995; Chlordane technical									

Table 2-2. Levels of Significant Exposure to Chlordane – 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)	Effect
51	Mouse (B6D2F1) 160 M	Up to 2 years (F)	0, 9.4	BW, CS, GN, HP, LE, OW	Bd wt	9.4			
					Hepatic		9.4		Increased liver weight, hepatocellular hypertrophy
					Cancer			9.4	CEL: liver tumors
Malarkey et al. 1995; Chlordane technical									
52	Rat (NS)	80 weeks ad lib (F)	M: 0, 16.07, 32.13 F: 0, 11.08, 22.15		Death			F 11.08	18% decreased survival
					Bd wt	M 32.13 F 22.15			
					Resp	M 32.13 F 22.15			
					Cardio	M 32.13 F 22.15			
					Gastro	M 32.13 F 22.15			
					Musc/skel	M 32.13 F 22.15			
					Hepatic	M 32.13 F 22.15			
					Renal	M 32.13 F 22.15			
					Dermal	M 32.13 F 22.15			
					Neuro	M 32.13 F 11.08		22.15	Tremors in females
NCI 1977; Chlordane analytical									

Table 2-2. Levels of Significant Exposure to Chlordane – 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)	Effect
53	Mouse (NS)	80 weeks ad lib (F)	M: 0, 5.13, 9.64 F: 0, 5.20, 11.02		Death			M 5.13	40% decreased survival of males
					Bd wt	M 9.64 F 11.02			
					Resp	M 9.64 F 11.02			
					Cardio	M 9.64 F 11.02			
					Gastro	M 9.64 F 11.02			
					Musc/skel	M 9.64 F 11.02			
					Hepatic	M 9.64 F 11.02			
					Renal	M 9.64 F 11.02			
					Dermal	M 9.64 F 11.02			
					Neuro	M 5.13 F 5.20		M 9.64 F 11.02	Tremors
					Cancer			M: 5.13 F: 11.02	CEL: hepatocellular carcinoma

NCI 1977; Chlordane analytical

Table 2-2. Levels of Significant Exposure to Chlordane – 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)	Effect
54	Mouse (C57B1/ 10J) 100 M	24 months (F)	0, 8.6	GN, HP	Cancer			8.6	CEL: liver tumors

Barrass et al. 1993; Chlordane (form not specified)

[a]The 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.
[b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.001 mg/kg/day; dose divided by an uncertainty factor of 1,000 (10 for use of LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).
[c]Used to derive intermediate- and chronic-duration oral MRLs of 0.0006 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ad lib = ad libitum; ALT = alanine aminotransferase; AST = aspartate aminotransferase; Bd wt or BW = body weight; BC = biochemistry; BH = behavioral; Cardio = cardiovascular; CEL = cancer effect level; ChE = cholinesterase; CS = clinical signs; Develop = developmental; DI = distribution; DX = developmental toxicity; EA = enzyme activity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; FX = fetal toxicity; G6PDH = glucose-6-phosphate dehydrogenase; (G) = gavage-not specified; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; (GO) = gavage in oil vehicle oil; GPT = glutamyl transpeptidase; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LD_{50} = lethal dose, 50% kill; LDH = lactate dehydrogenase; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OW = organ weight; Resp = respiratory; TG = teratogenicity; TM = tissue metabolites; UR = urinalysis; WI = water intake

Figure 2-3. Levels of Significant Exposure to Chlordane – Oral

Acute (≤14 days)

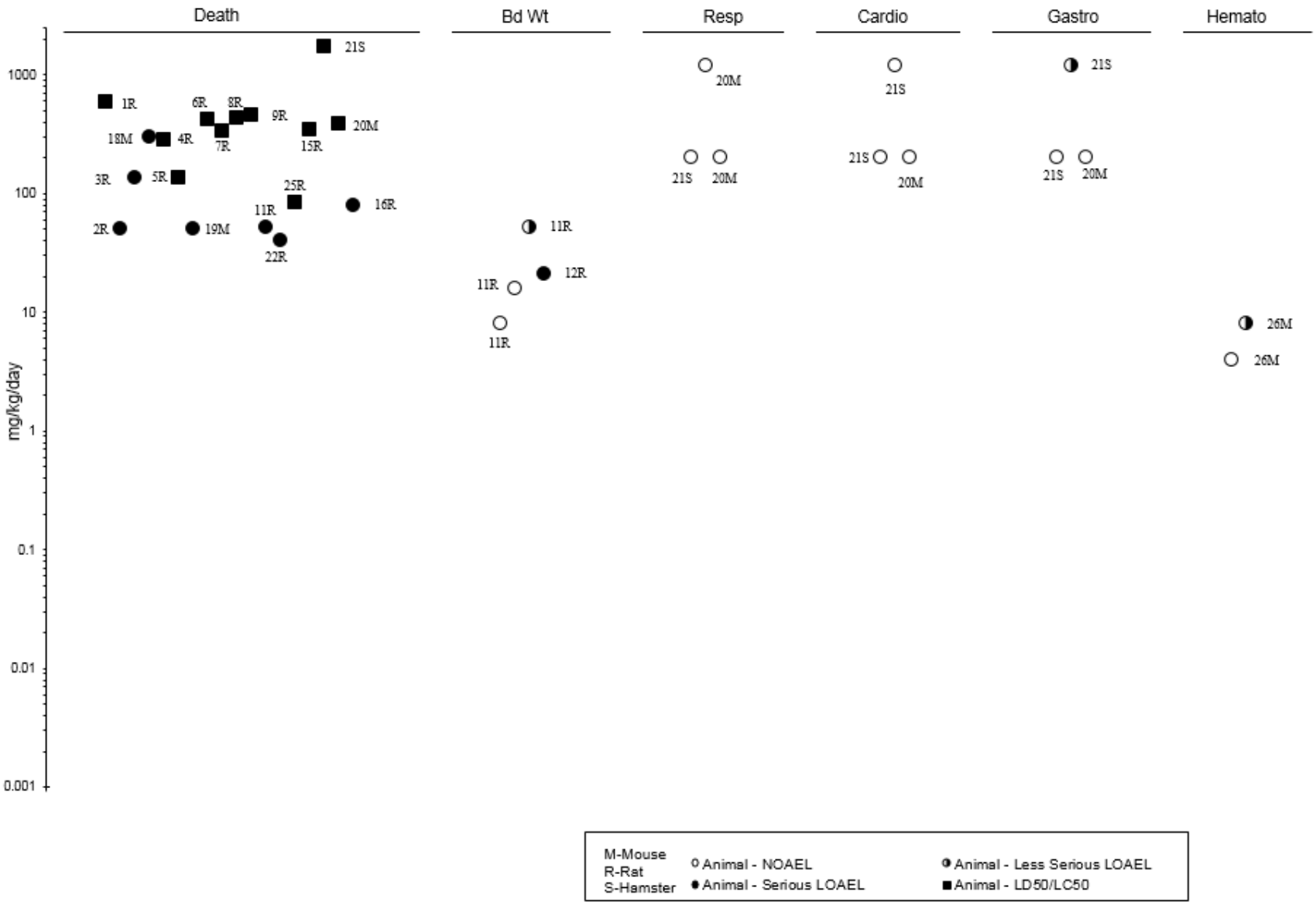

Figure 2-3. Levels of Significant Exposure to Chlordane – Oral
Acute (≤14 days)

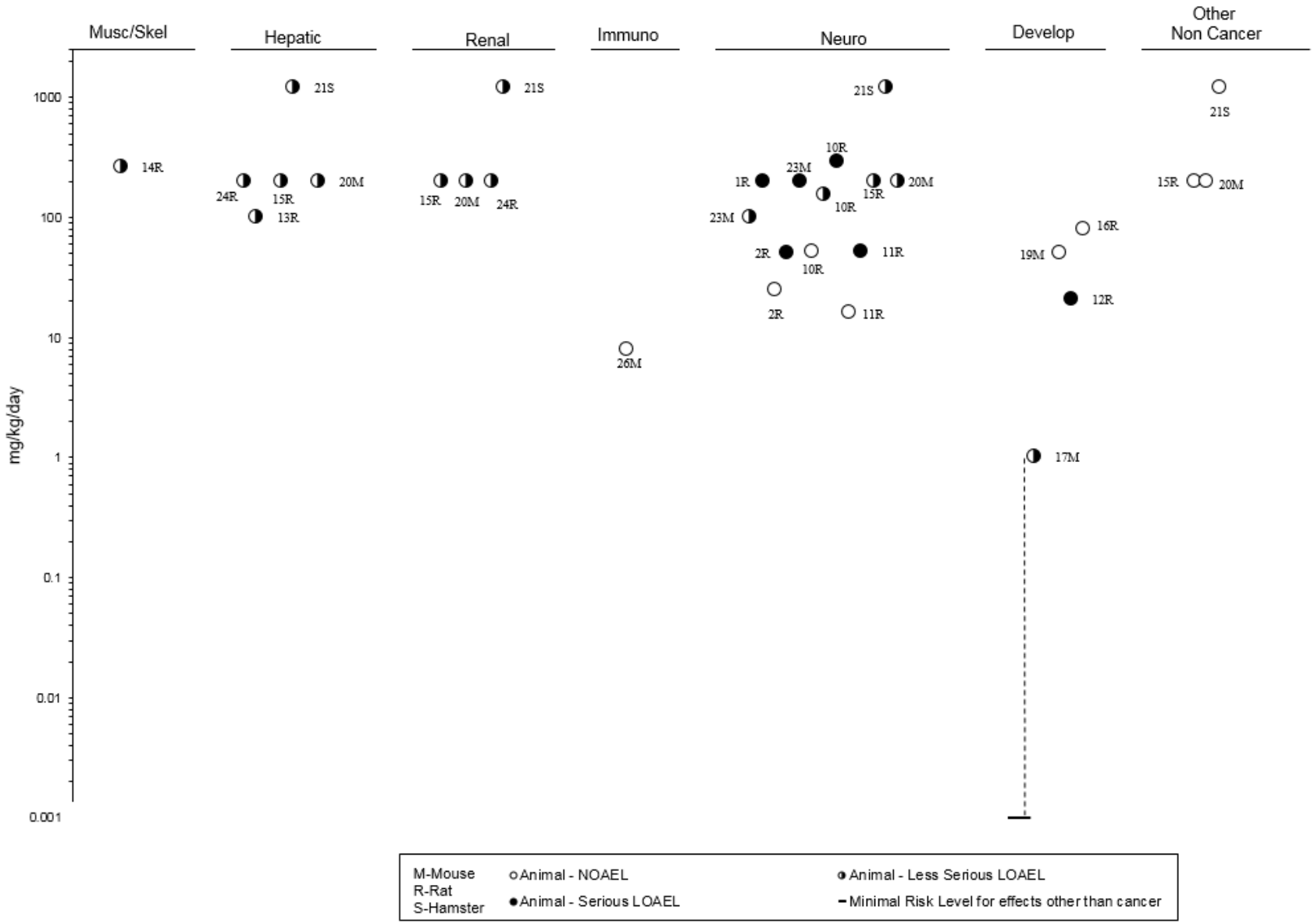

Figure 2-3. Levels of Significant Exposure to Chlordane – Oral
Intermediate (15-364 days)

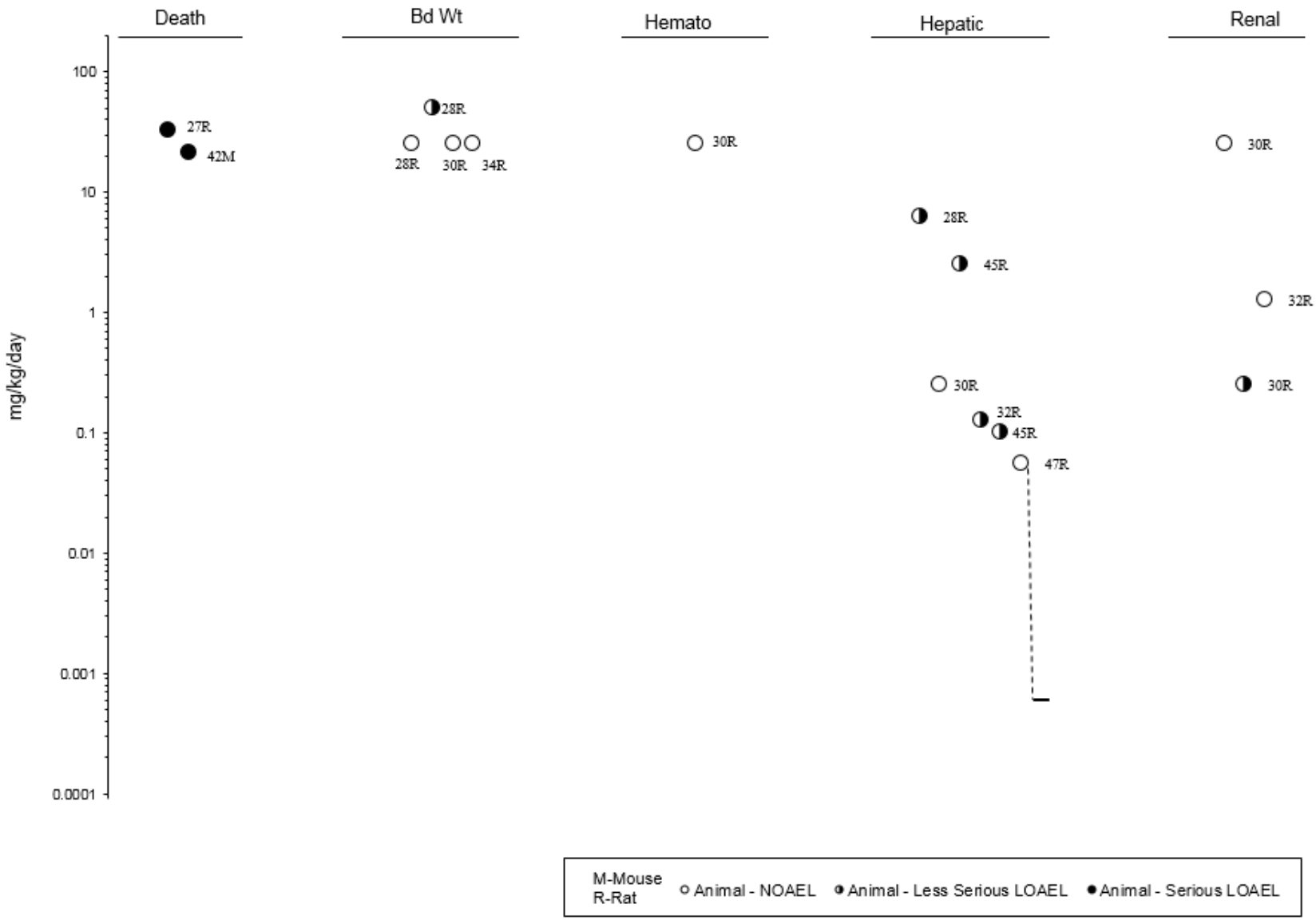

Figure 2-3. Levels of Significant Exposure to Chlordane – Oral
Intermediate (15-364 days)

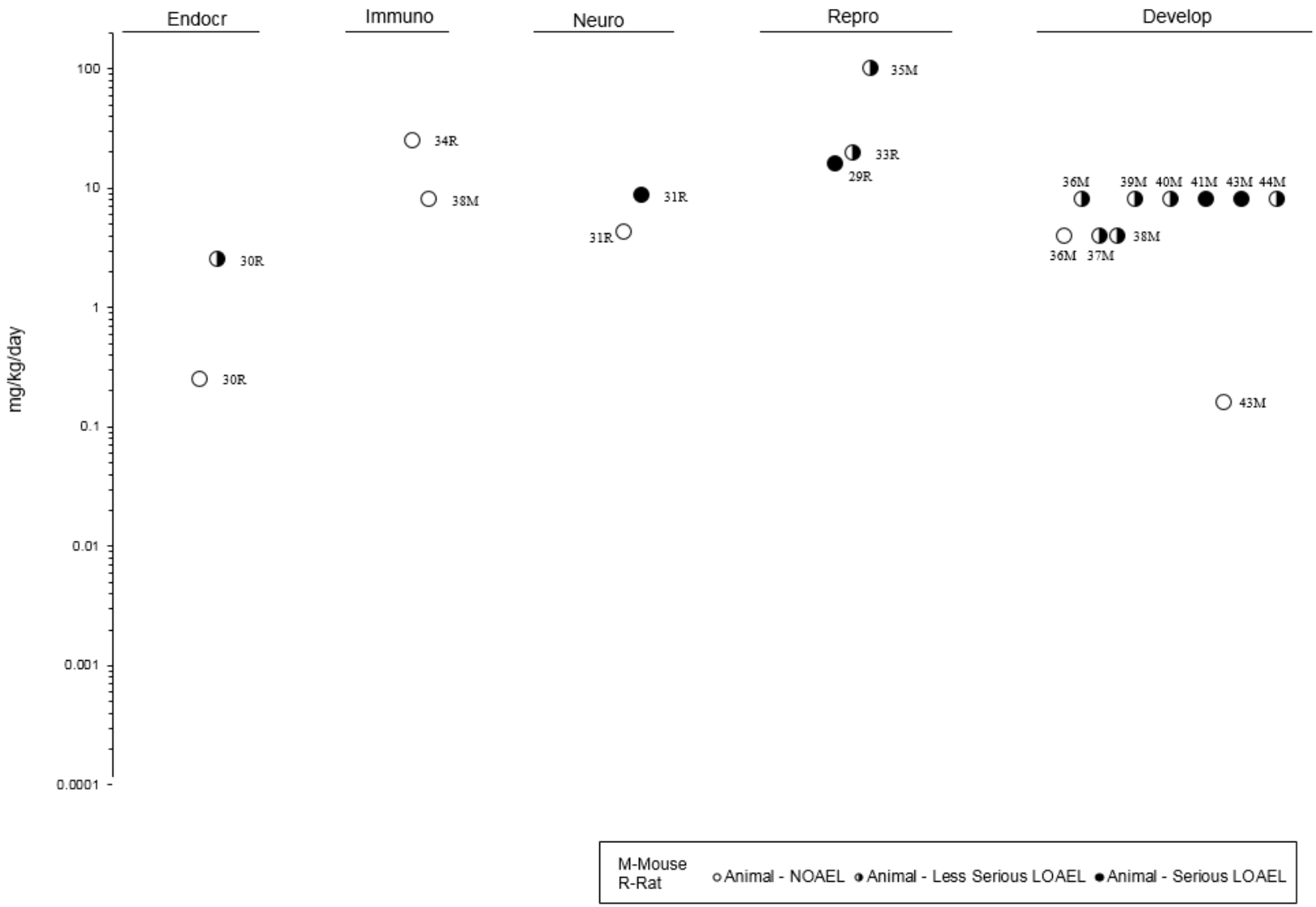

Figure 2-3. Levels of Significant Exposure to Chlordane – Oral
Chronic (≥365 days)

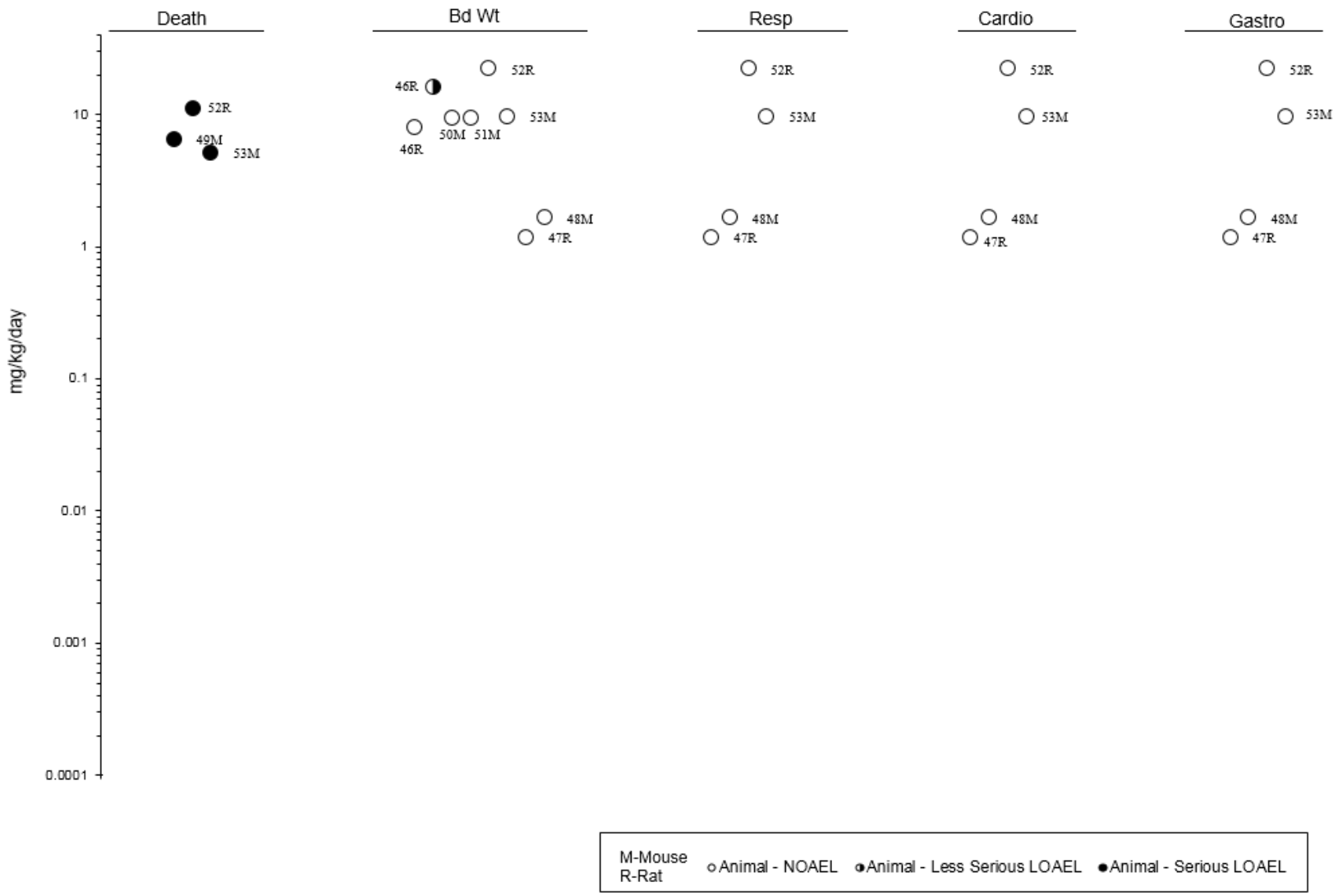

Figure 2-3. Levels of Significant Exposure to Chlordane – Oral
Chronic (≥365 days)

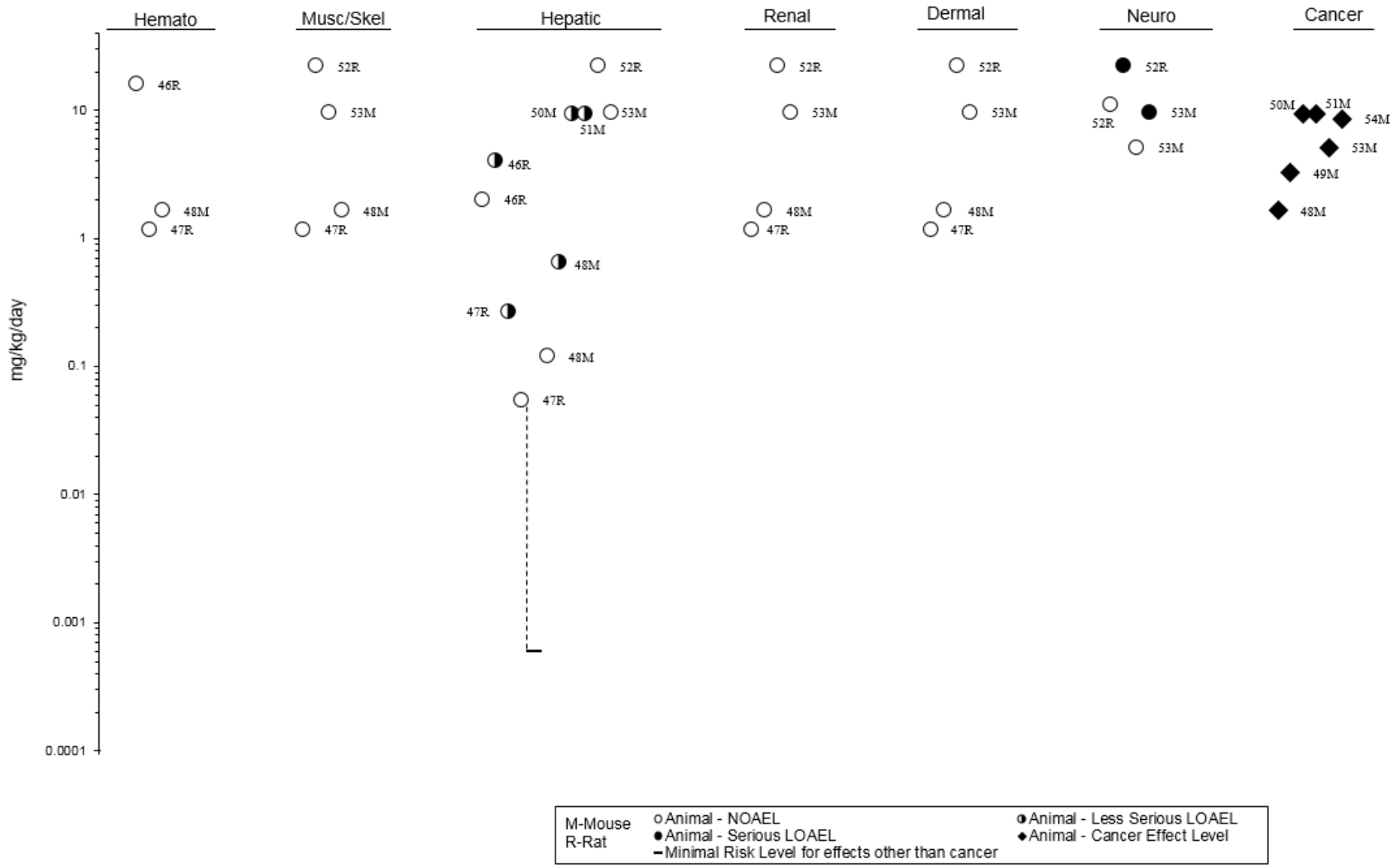

Table 2-3. Levels of Significant Exposure to Chlordane – Dermal

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)	Effect
ACUTE EXPOSURE								
Rat (NS)	1–4 days 1 time/day		Death				217	1/5 died
Ambrose et al. 1953a; Chlordane technical								
Rat (NS)	Once		Death				840 M 530–690 F	LD_{50}
Gaines 1960; Chlordane technical								
Rabbit (NS)	Once		Death				1,150	LD_{50}
Ingle 1965; Chlordane technical								
INTERMEDIATE EXPOSURE								
Guinea pig (NS)	90 days		Dermal			168		Hyperkeratosis
Datta et al. 1977; Chlordane technical								

F = female(s); LD_{50} = lethal dose; 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; NS = not specified

2.2 DEATH

Available epidemiological studies that evaluated mortality among chlordane-exposed workers have serious limitations, including unquantified exposure concentrations and exposure to other pesticides. Retrospective cohort mortality studies of workers in chlordane and other organochlorine manufacturing plants reported no increase in mortality rate and no increase in any specific cause of death attributed to chlordane exposure (MacMahon et al. 1988; Shindell and Ulrich 1986). Wang and MacMahon (1979b) reported no increase in mortality rate in a prospective study of pesticide applicators. In a retrospective mortality study of 1,403 men employed for 23 months at two plants, a significantly increased risk of death from cerebrovascular disease was found, but the authors could not definitively attribute this excess to chlordane exposure (Wang and MacMahon 1979a). In another retrospective mortality study of a cohort of 327 workers exposed for ≥6 months, an increased risk of death due to stomach cancer (standardized mortality ratio [SMR] 303; 95% confidence limit [CL] 61–885) was reported (Ditraglia et al. 1981). However, the study included only three cases of stomach cancer. Furthermore, an 11-year follow-up study of this cohort found no statistically significant excess risk of death from any cause (Brown 1992).

Most cases of acute human oral exposure to chlordane involved accidental ingestion by children (Aldrich and Holmes 1969; Curley and Garrettson 1969; EPA 1980a). The estimated doses of chlordane ingested were 11.0 mg/kg; complete recovery generally followed medical intervention. In humans, an estimated acute oral lethal dose of chlordane (WHO 1984) was between 25 and 50 mg/kg, but documentation was not provided and the method of estimation was not discussed. A man who accidentally ingested an unknown quantity of chlordane developed convulsions shortly after the ingestion and subsequently died (Kutz et al. 1983). No studies were located regarding lethality in humans following longer-term oral exposure to chlordane.

Only one report was found regarding mortality in humans following dermal exposure to chlordane. The usefulness of this report is limited, however, because the individual was exposed to chlordane, DDT, Velsicol AR50, and triton X-100 mixed together in the form of a suspension. After a woman spilled the suspension on the front of her clothes, she became confused, developed convulsions, and died within minutes after exposure (Derbes et al. 1955). At autopsy, the brain, lungs, and kidneys were found to have nonspecific pathological changes. Deaths were not reported in a compilation of cases and personal reports of acute human dermal exposure to chlordane (EPA 1980a).

In a range-finding study, male and female rats (10/sex/group) were exposed by inhalation to chlordane technical for 8 hours/day at 0, 5.8, 28.2, 154, or 413 mg/m^3 for up to 28 days (Khasawinah et al. 1989). The 154 and 413 mg/m^3 exposure levels were terminated after 11 and 3 exposures, respectively, due to unspecified numbers of mortalities; females were reported to die earlier than males. All rats exposed for 28 days at 28.2 mg/m^3 survived. There was no mortality in rats or monkeys exposed to technical chlordane at 10 mg/m^3 for 8 hours/day, 5 days/week for 90 days (EPA 1987f; Khasawinah et al. 1989).

Acute oral LD_{50} values for technical-grade chlordane in the rat range from 137 to 590 mg/kg (Ambrose et al. 1953a; Ben-Dyke et al. 1970; Boyd and Taylor 1969; Deichmann and Keplinger 1970; Gaines 1960). Acute oral LD_{50} values for mice (Truhaut et al. 1974) and hamsters (Truhaut et al. 1974, 1975) were 390 and 1,720 mg/kg, respectively. Truhaut et al. (1975) speculated, on the basis of different activities of liver microsomal enzymes in rats, mice, and hamsters, that species differences in LD_{50} values may reflect differences in the rate of metabolism of the constituents of chlordane. The *cis*-chlordane isomer appears to be somewhat more lethal than chlordane technical; a rat acute oral LD_{50} of 83 mg/kg/day was reported for this isomer (Podowski et al. 1979).

Daily oral dosing of rats or mice for 5–14 days at 50–156 mg/kg/day was lethal (Ambrose et al. 1953a; Chernoff and Kavlock 1982; Moser et al. 1995; Usami et al. 1986). All rats dosed at 32 mg/kg/day died during up to 163 days of treatment (Ambrose et al. 1953a). Decreased survival was observed in mice treated at 6.5 mg/kg/day for up to 18 months (IRDC 1973; Epstein 1976). Oral exposure of female rats to analytical-grade chlordane for up to 80 weeks at 11.08 mg/kg/day resulted in 18% decreased survival; however, survival was not affected in male rats treated at up to 32.13 mg/kg/day (NCI 1977). Oral exposure of male mice to analytical-grade chlordane for up to 80 weeks at 5.13 mg/kg/day resulted in 40% decreased survival; however, survival was not affected in female mice treated at up to 11.02 mg/kg/day (NCI 1977).

Acute dermal LD_{50} values for rats (Gaines 1960) and rabbits (Ingle 1965) treated with technical-grade chlordane were 530–840 and 1,150 mg/kg, respectively.

2.3 BODY WEIGHT

Body weight loss (magnitude not specified) was reported in rats intermittently exposed to chlordane technical by inhalation for up to 12 days at 154 mg/m^3 (EPA 1987f; Khasawinah et al. 1989). Body weight loss was also reported in rats administered chlordane technical by daily gavage for up to 14 days at

52 mg/kg/day; however, this dose level also resulted in 100% mortality (Moser et al. 1995). In a study of rats administered chlordane technical in the food for up to 80 weeks (NCI 1977), body weights of high-dose males (32.13 mg/kg/day) and high-dose females (22.15 mg/kg/day) were slightly less than those of controls throughout most of the study. However, body weights were never less than 90% of control body weights; therefore, the effect is not considered adverse. There were no treatment-related effects on body weight in similarly-treated male and female mice at doses up to 9.64 and 11.02 mg/kg/day, respectively (NCI 1977).

2.4 RESPIRATORY

Physical examination of library workers acutely exposed to high but unquantified concentrations of chlordane from a spill revealed no indication of respiratory effects (NIOSH 1984a). Chest pains, dyspnea, and shortness of breath were reported in a compilation of cases and personal reports of humans accidentally exposed to chlordane by inhalation (EPA 1980a); exposures frequently involved a mixture of chemicals (such as other related and nonrelated pesticides) and vehicles (including petroleum distillates). Therefore, these effects cannot be attributed to chlordane alone. Results of a questionnaire indicated increases, compared with the National Center for Health Statistics 1979 National Health Interview Survey, in sore throat and respiratory infections in humans shortly after their homes were treated for termites (Menconi et al. 1988). Chronic exposure in pesticide treated homes was associated with bronchitis and sinusitis, which increased in incidence with higher concentrations of pesticides in the air. Because aldrin and heptachlor were included with chlordane in the analysis for pesticides in the indoor air, these effects cannot be attributed unequivocally to chlordane exposure. Other limitations of the Menconi et al. (1988) study include self-selection of respondents. Respiratory effects generally were not found in occupational exposure studies (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951). In a retrospective mortality study of a cohort of 327 workers exposed for ≥6 months at a chlordane manufacturing plant (Ditraglia et al. 1981) and 11 years of follow-up (Brown 1992), there was no increased risk of death from noncancer respiratory disease.

In a series of experiments, rats exposed to 413 mg technical chlordane/m^3 for 3 days had epithelial degeneration and cellular debris in the bronchi and alveoli (EPA 1987f; Khasawinah et al. 1989). Respiratory tract lesions were not observed in rats intermittently exposed to technical chlordane at ≤28.2 mg/m^3 for 28 days or rats or monkeys intermittently exposed at 10 mg/m^3 for 90 days (EPA 1987f; Khasawinah et al. 1989).

No signs of chlordane-induced respiratory tract effects were seen in oral or dermal animal studies that evaluated the respiratory system (EPA 1985a; Khasawinah and Grutsch 1989a, 1989b; NCI 1977; Truhaut et al. 1974, 1975; Velsicol Chemical Co. 1983a, 1983b).

2.5 CARDIOVASCULAR

Tachycardia was among the symptoms attributed to chlordane exposure in a compilation of cases and personal reports of accidental human inhalation exposure to high concentrations of chlordane (EPA 1980b). Cardiovascular effects were not reported in library workers acutely exposed to high but unquantified concentrations of chlordane from a spill (NIOSH 1984a). Cardiovascular effects were not found in occupational exposure studies (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951). Equivocal evidence of increased risk of cerebrovascular disease was reported in workers involved in the manufacture of chlordane (Wang and MacMahon 1979a).

No signs of chlordane-induced cardiovascular effects were seen in inhalation or oral animal studies that evaluated the cardiovascular system (EPA 1985a; Khasawinah and Grutsch 1989a, 1989b; NCI 1977; Truhaut et al. 1974, 1975).

2.6 GASTROINTESTINAL

Gastrointestinal effects (cramps, diarrhea, nausea) were a consistent observation in a compilation of cases and personal reports of accidental human inhalation exposure to high concentrations of chlordane (EPA 1980a). NIOSH (1984a) also reported gastrointestinal symptoms (nausea, diarrhea) in 4 of 13 humans within 4 days of inhalation and/or dermal exposure as a result of 1% chlordane being spilled in a subterranean library room. The greatest prevalence of symptoms occurred in those directly involved in the cleanup, where potential for exposure to higher concentrations was greatest. Concentrations in air, taken ≈4.5 months after the spill, ranged from 0.0001 to 0.0003 mg/m^3. Because air concentration data are not available for the first 4 days of exposure, concentrations associated with the observed effects cannot be estimated. Occupational exposure, however, has not been associated with gastrointestinal effects. Alvarez and Hyman (1953) reported no gastrointestinal effects in a group of 24 workers involved in chlordane manufacture. Both inhalation and dermal exposure occurred. Princi and Spurbeck (1951) reported no effects in workers involved in the manufacture of insecticides (chlordane, aldrin, and dieldrin) when air concentrations of total chlorinated hydrocarbons were ≤10 mg/m^3; exposure was by inhalation and skin contact for 11–36 months. Fishbein et al. (1964) reported no gastrointestinal effects in

production workers exposed to chlordane concentrations of 0.0012–0.0017 mg/m^3 over a period of 1–15 years.

Atrophy of gastric mucosa was reported in hamsters following single gavage dosing of chlordane technical at 1,200 mg/kg (Truhaut et al. 1974, 1975). No signs of chlordane-induced gastrointestinal effects were seen in other inhalation or oral animal studies that evaluated the gastrointestinal system (EPA 1985a; Khasawinah and Grutsch 1989a, 1989b; NCI 1977; Truhaut et al. 1974, 1975).

2.7 HEMATOLOGICAL

A questionnaire survey revealed that 4% of persons living in homes treated with chlordane to control termites reported anemia as a chronic effect (Menconi et al. 1988). The effect cannot be attributed to chlordane alone, because the quantitative amounts of aldrin and heptachlor were combined with the chlordane measurement in the analysis of indoor air. A number of anecdotal reports of blood dyscrasia associated with organochlorine pesticides (chlordane, lindane, DDT) suggest that there may be an unusually susceptible subpopulation (Ellenhorn and Barceloux 1988). Several cases of blood dyscrasia (aplastic anemia, hemolytic anemia, thrombocytopenic purpura, acute disseminated hemorrhages, pernicious anemia, megaloblastic anemia) were observed in persons exposed to chlordane or heptachlor in their home or garden or as a result of their profession as pest control operators (Epstein and Ozonoff 1987; Infante et al. 1978). The usefulness of these reports is limited because exposure to chlordane was unquantified, the individuals were exposed to other chemicals, and there were other confounding factors. No effects on hemoglobin concentrations or sedimentation rates were found in a group of 24 men employed in chlordane manufacture, where exposure was both via inhalation and dermal contact (Alvarez and Hyman 1953). No effects on typical hematological parameters were found in 34 workers exposed to chlordane at unspecified concentrations (Princi and Spurbeck 1951) or in 15 workers exposed to 0.0012–0.0017 mg/m^3 (Fishbein et al. 1964).

Limited information is available regarding chlordane-induced hematological effects in animals. Among male and female rats intermittently exposed to chlordane technical at 1.0 mg/m^3 for 90 days, females (but not males) exhibited 8% increased leukocyte counts and 26% decreased platelet counts (EPA 1987f; Khasawinah et al. 1989). However, no hematological effects were observed in rats intermittently exposed at ≤28.2 mg/m^3 for 28 days or 154 mg/m^3 for 11 days, or in monkeys similarly exposed to ≤10 mg/m^3 for 90 days (EPA 1987f; Khasawinah et al. 1989). Increased lymphocyte count (52% greater than controls) was reported in mice administered *trans*-chlordane by gavage for 14 days at 8 mg/kg/day (Johnson et al.

1986). However, there were no effects on hematology among rats administered chlordane technical by gavage for 38 days at 25 mg/kg/day (Bondy et al. 2000), other male and female rats receiving chlordane technical from the diet for 30 months at 1.18 and 1.41 mg/kg/day, respectively, or male and female mice receiving chlordane technical from the diet for 24 months at 1.65 mg/kg/day (EPA 1985a; Khasawinah and Grutsch 1989a, 1989b).

2.8 MUSCULOSKELETAL

Information regarding chlordane-related musculoskeletal effects is limited. In a study of 115 men from the general Swedish male population, persistent organochlorine serum concentrations, including oxychlordane (a chlordane metabolite) and *trans*-nonachlor (a component of chlordane technical) were not associated with bone mineral density (Glynn et al. 2000). No musculoskeletal effects were reported in a compilation of cases and personal reports of acute human exposure (EPA 1980a).

Hypertonicity of skeletal muscles was noted in rats administered chlordane technical once by gavage at 260 mg/kg (Santolucito and Whitcomb 1971). However, these results are not interpreted as indicating that oral exposure to chlordane was associated with musculoskeletal effects because there was no effect on the mechanical response of the muscle measured with a strain-gauge transducer *in situ*. Furthermore, no significant increase in the serum level of creatine phosphokinase was found in rats treated orally with 100 mg/kg/day technical chlordane for 4 days (Ogata and Izushi 1991). Comprehensive histopathological examination performed on rats receiving analytical-grade chlordane from the diet for 80 weeks at up to 32.13 and 22.15 mg/kg/day (males and females, respectively) (NCI 1977) or chlordane technical from the diet for 30 months at up to 1.18 and 1.41 mg/kg/day (males and females, respectively) (EPA 1985a; Khasawinah and Grutsch 1989a) revealed no evidence of effects in the musculoskeletal system. Comprehensive histopathological examinations performed on mice receiving analytical-grade chlordane from the diet for 80 weeks at up to 9.64 and 11.02 mg/kg/day (males and females, respectively) (NCI 1977) or chlordane technical from the diet for 24 months at up to 1.65 mg/kg/day (EPA 1985a; Khasawinah and Grutsch 1989b) revealed no evidence of effects in the musculoskeletal system.

2.9 HEPATIC

Hepatic effects were not reported in library workers acutely exposed to high but unquantified concentrations of chlordane from a spill (NIOSH 1984a). Jaundice, reflecting liver effects, was sometimes reported in cases of inhalation exposure to chlordane in a compilation of cases and personal reports of accidental exposure (EPA 1980a). When reported, jaundice was frequently associated with

continued exposure such as living in a house in which chlordane had been used to control termites (EPA 1980a). Occupational exposures to chlordane have not generally been associated with liver effects (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951). However, elevated serum levels of triglycerides, lactic acid dehydrogenase, and gamma-glutamyl transferase were measured in pesticide applicators (Ogata and Izushi 1991). There is little information concerning hepatic effects in humans following oral exposure to chlordane. A compilation of cases and personal reports of human exposure (EPA 1980a) did not suggest that liver effects are a predominant part of the clinical picture for acute exposure. Results of various liver function and damage tests were within normal limits in a 20-month-old male who ingested an unknown amount of technical grade (74%) chlordane (Curley and Garrettson 1969). Evaluations were made at 20 hours to 3 days after exposure.

Biochemical evidence of liver damage (increased serum aspartate transaminase [AST], alanine transaminase [ALT], glutamate dehydrogenase [GDH], bile acids, and cholesterol), as well as hepatocellular enlargement and vacuolation, were observed in rats intermittently exposed to chlordane technical by inhalation at 154 mg/m^3 for 11 exposures or 413 mg/m^3 for 3 exposures (EPA 1987f; Khasawinah et al. 1989). Serum chemistry changes indicative of liver damage occurred in females, and increased liver weight occurred in males intermittently exposed at 28.2 mg/m^3 for 28 days (EPA 1987f; Khasawinah et al. 1989). Both sexes exposed to 28.2 mg/m^3 had centrilobular hepatocyte enlargement. Lesions in the rats exposed to 154 mg/m^3 for 11 exposures included hepatocellular enlargement and vacuolation; frank necrosis occurred in rats exposed to 413 mg/m^3 for 3 exposures. A 90-day inhalation study in male and female rats exposed intermittently to technical chlordane at 0, 0.1, 1.0, or 10 mg/m^3 reported mild liver lesions (hepatocellular enlargement or vacuolization) and slight changes in serum chemistry at ≤1.0 mg/m^3 and increased liver weight in both sexes at 10 mg/m^3 (EPA 1987f; Khasawinah et al. 1989). The lowest concentration, 0.1 mg/m^3, was judged a NOAEL. In monkeys exposed by the same protocol, no effects occurred at 1.0 mg/m^3, but 10 mg/m^3 was associated with increased mean liver weight. The NOAEL of 0.1 mg/m^3 in rats was used to derive intermediate- and chronic-duration inhalation MRLs for chlordane technical as described in the footnote to Table 2-1 and Appendix A.

Liver effects from acute oral exposure to chlordane include liver microsomal enzyme induction, alterations in the activities of mitochondrial enzymes, histochemical and histomorphological alterations, and increased liver weight. In a 14-day feeding study in rats, Den Tonkelaar and Van Esch (1974) reported significant liver drug metabolizing enzyme induction (aniline hydroxylase, aminopyrine demethylase, and hexobarbital oxidase) at dietary concentrations equivalent to 0.50–2.5 mg/kg/day, but not at 0.25 mg/kg/day. Liver microsomal enzyme induction is considered an adaptative effect rather than

an adverse effect. It should be noted, however, that in the case of exposure to chlordane, which induces enzymes associated with its own metabolism, some of the metabolites of chlordane are potentially more toxic than the parent compound. A single oral dose of 200 mg/kg *cis*-chlordane in rats significantly decreased liver glycogen and significantly increased the activities of hepatic enzymes associated with gluconeogenesis (i.e., pyruvate carboxylase, fructose-1,6-diphosphatase, and glucose-6-phosphatase) when measured 1 hour after treatment (Kacew and Singhal 1973; Singhal and Kacew 1976). These investigators also found that the activity of adenyl cyclase increased in the livers of these rats and that levels of hepatic cyclic adenosine monophosphate (cAMP) correspondingly increased. Rats treated by gavage with 100 mg/kg/day technical chlordane for 4 days had increased liver weight and liver lipid content, hypertrophy, and increased serum triglyceride, cholesterol, and gamma-glutamyl transferase (Ogata and Izushi 1991). There were no effects on activities of serum AST, ALT, creatinine phosphokinase, or lactate dehydrogenase (LDH). Liver toxicity characterized by hypertrophy, dilatation of centrilobular sinuses, and congestion by increased serum ALT and LDH and decreased serum cholinesterase, and by decreased liver AST, LDH, cholinesterase, and glucose-6-phosphate dehydrogenase occurred in rats given a single oral dose of 200 mg/kg (Truhaut et al. 1974, 1975). In mice given 200 mg/kg, hepatic hypertrophy, congestion, and dilatation of centrilobular sinuses were also seen (Truhaut et al. 1975). In addition, serum ALT and LDH were increased, as were hepatic ALT and AST. In hamsters given a single gavage dose of 1,200 mg/kg, serum cholinesterase was depressed, while hepatic LDH was decreased and hepatic glucose-6-phosphate dehydrogenase was increased (Truhaut et al. 1974, 1975). Hamsters also had congestion, dilatation of centrilobular sinuses, and hypertrophy. In mice treated by gavage for 2 weeks, liver weight increased at 8 mg/kg/day, but not at 4 mg/kg/day (Johnson et al. 1986); liver histopathology was not performed in this study.

Intracytoplasmic bodies were found in the liver cells of rats administered chlordane technical by gavage for 15 days at ≥6.25 mg/kg/day (Ambrose et al. 1953a). Centrilobular hypertrophy and cytoplasmic inclusions were found in rats exposed to technical chlordane in the diet for 2–9 months at doses ≥0.125 mg/kg/day (Ortega et al. 1957). No histopathological liver lesions or increased levels of serum ALT or alkaline phosphatase were found in rats exposed to chlordane in the diet at 0.1 mg/kg/day for 10–20 weeks (Mahon et al. 1978). However, cytochrome P-450 content was significantly increased at 10 weeks, and microsomal protein content was significantly decreased at 20 weeks, when compared with controls.

In a study of male and female rats administered technical chlordane by daily gavage for 28 days, significantly increased liver weights (59–87% higher than controls) and histopathologic liver lesions

(hypertrophy and changes in the appearance of cytoplasm in males and females; anisokaryosis in females) were observed at 25 mg/kg/day (Bondy et al. 2000). Males also exhibited 13% increased liver weight at a dose level of 2.5 mg/kg/day; there were no apparent effects on the liver at 0.25 mg/kg/day.

In a 30-month oral study of chlordane technical in rats, no liver effects occurred in males at up to 1.18 mg/kg/day, but regional liver hypertrophy occurred in females at 0.27 mg/kg/day (EPA 1985a, Khasawinah and Grutsch 1989a). The NOAEL for liver effects in the female rats was 0.055 mg/kg/day; this NOAEL served as the basis for deriving intermediate- and chronic-duration oral MRLs for chlordane as described in the footnote in Table 2-2 and Appendix A. Significantly increased liver weight, liver cell inclusion bodies, and hepatocellular hypertrophy were found in rats given technical chlordane in the diet at doses ≥4 mg/kg/day for 407 days (Ambrose et al. 1953a). These lesions were not observed at 2 mg/kg/day. A 24-month oral study of chlordane technical in mice identified NOAELs of 0.12 and 0.14 mg/kg/day (males and females, respectively) and a LOAEL of 0.65 mg/kg/day for histopathologic liver lesions in both sexes (hepatocellular swelling and degeneration in males and females and necrosis in males) (EPA 1985a; Khasawinah and Grutsch 1989b). In an unpublished 18-month dietary study in mice (IRDC 1973), which was reviewed by Epstein (1976), significantly increased liver weights and hepatocytomegaly were observed at all dose levels tested (0.65–6.5 mg/kg/day).

Malarkey et al. (1995) reported chlordane-induced hepatocellular hypertrophy, frequent micronucleate hepatocytes, and hepatoproliferative lesions composed predominantly of acidophilic hepatocytes in nearly 100% of male B6C3F1 and B6D2F1 mice administered technical chlordane in the diet for a lifetime at an estimated dose of 9.4 mg /kg/day. Centrilobular hypertrophy was detected within 50 days after the initiation of treatment, persisted in virtually all treated mice, and declined in prevalence and severity as tumor development progressed. Barrass et al. (1993) reported chlordane-induced hepatocellular hypertrophy in male C57B1/10J mice that received chlordane in the diet for up to 2 years at an estimated dose of 8.6 mg/kg/day.

NCI (1977) reported no compound-related liver lesions in male or female rats receiving analytical-grade chlordane (72% *cis* and 23% *trans* isomers) from the diet for up to 80 weeks at doses as high as 32.13 and 22.15 mg/kg/day, respectively. NCI (1977) reported no compound-related nonneoplastic liver lesions in similarly-treated male or female mice at doses as high as 9.64 and 11.02 mg/kg/day, respectively.

Results from several studies provide evidence for mechanisms of chlordane-induced liver toxicity. The primary effect, induction of hepatic cytochrome P-450 and other microsomal protein, is accompanied by a

large increase in the volume of the smooth endoplasmic reticulum, which results in hepatocellular enlargement and hypertrophy (Khasawinah et al. 1989). These effects appear to be reversible. In mice treated repeatedly over several weeks, the body burden of the chlordane isomers decreased, and the body burden of oxychlordane increased with time (Hirasawa and Takizawa 1989). This suggests that chlordane induces its own metabolism, probably to intermediates that bind to and disrupt the function of vital cellular macromolecules (Brimfield and Street 1981). The components and metabolites of chlordane may exert their effects by altering the permeability of the mitochondrial membrane, inhibiting mitochondrial oxidative phosphorylation (Ogata et al. 1989). Also, chlordane may induce production of superoxide (Suzaki et al. 1988), which may result in lipoperoxidation, a known mechanism of toxicity to the liver. In support, Bagchi and coworkers (Bagchi et al. 1995; Stohs et al. 1997) reported 3-fold increased lipid peroxidation in the liver of female rats administered chlordane by gavage.

2.10 RENAL

Evidence of altered renal function was not reported in library workers acutely exposed to high but unquantified concentrations of chlordane from a spill (NIOSH 1984a), or in a compilation of cases and personal reports of accidental exposure (EPA 1980a). No kidney effects were found in occupational studies of chlordane manufacture (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951). Few data were located regarding renal effects in humans after oral exposure to chlordane. A compilation of cases and personal reports (EPA 1980a) did not mention kidney effects as a part of the clinical picture of acute human exposure. In one case report, no apparent renal effects were observed in an 18-year-old girl 24–48 hours after an acute exposure to 32 mg/kg of chlordane (Dadey and Kammer 1953). Because the patient vomited after ingestion, the dose of 32 mg/kg does not reflect the dose available for absorption.

The kidney was evaluated in a series of intermediate-duration studies that employed inhalation exposure to chlordane technical (EPA 1987f; Khasawinah et al. 1989). Increased kidney weight (magnitude not specified), in the absence of histopathologic kidney lesions, was reported for male (but not female) rats intermittently exposed for 28 days at 28.2 mg/m^3; therefore, the 28.2 mg/m^3 level is considered a NOAEL. Elevated kidney weights (9–11% higher than controls) were reported in both sexes of rats intermittently exposed for 90 days at 10 mg/m^3, in the absence of histopathologic kidney lesions; therefore, the 10 mg/m^3 level is considered a NOAEL. No kidney effects were observed in monkeys exposed for 90 days at 10 mg/m^3.

In acute gavage studies of chlordane technical, Truhaut et al. (1974, 1975) reported congestion of the kidneys in rats and mice treated at 200 mg/kg and hamsters treated at 1,200 mg/kg, but kidney weight was unaffected. A single oral dose of 200 mg/kg *cis*-chlordane significantly increased kidney gluconeogenic enzymes, kidney basal and fluoride-stimulated adenyl cyclase, and cyclic AMP (Kacew and Singhal 1973). No histopathological renal lesions were found in rats exposed to chlordane technical for 2–9 months at 1.25 mg/kg/day (Ortega et al. 1957). No histopathological lesions of the kidney were observed in male or female rats receiving analytical-grade chlordane from the diet for up to 80 weeks at doses as high as 32.13 and 22.15 mg/kg/day, respectively, or in male or female mice at doses up to 9.64 and 11.02 mg/kg/day, respectively (NCI 1977). No histopathological lesions of the kidney, no blood chemistry alterations suggesting kidney effects, and no effects on urinalysis were reported in male or female rats receiving chlordane technical from the diet for up to 30 months doses as high as 1.18 and 1.41 mg/kg/day, respectively, or male or female mice treated for up to 24 months at doses as high as 1.65 mg/kg/day (EPA 1985a, Khasawinah and Grutsch 1989b). Histological examination of the kidneys of rats receiving chlordane technical from the diet for up to 407 days at doses as high as 16 mg/kg/day revealed no lesions (Ambrose et al. 1953a).

Bondy et al. (2000) reported significantly increased mean absolute (but not relative) kidney weight in male (but not female) rats administered chlordane technical by gavage for 28 days at 25 mg/kg/day; clinical chemistry analysis revealed significantly increased blood urea nitrogen (BUN) and significantly decreased serum creatine kinase. There was clear evidence of histopathologic kidney lesions at 25 mg/kg/day. There was some evidence for treatment-related kidney lesions in male rats at 0.25 and 2.5 mg/kg/day as well (incidences of 2/7 and 3/7, respectively, compared to 0/7 controls).

2.11 DERMAL

Dermatitis was reported to occur in persons living in homes treated with chlordane at a greater frequency than in a reference population (Menconi et al. 1988). The effects, however, cannot be attributed to chlordane alone, because aldrin and heptachlor were included in the analysis for chlordane in the indoor air. A compilation of cases and personal reports (EPA 1980a) did not mention dermal effects as a part of the clinical picture of acute human exposure.

No dermal effects were found in rats intermittently exposed to technical chlordane by inhalation for 28 days at up to 28.2 mg/m^3 (EPA 1987f; Khasawinah et al. 1989). There was no histopathological evidence of chlordane-induced dermal effects in male or female rats receiving chlordane technical from

the diet for 30 months at up to 1.18 and 1.41 mg/kg/day, respectively (EPA 1985a; Khasawinah and Grutsch 1989a), or male or female mice receiving chlordane technical from the diet for up to 24 months at doses up to 1.65 mg chlordane/kg/day (EPA 1985a; Khasawinah and Grutsch 1989b). There was no histopathological evidence of treatment-related dermal effects following dietary administration of analytical-grade chlordane for up to 80 weeks to male and female rats at up to 32.13 and 22.15 mg/kg/day, respectively, or male or female mice treated at up to 9.64 and 11.02 mg/kg/day, respectively (NCI 1977).

2.12 OCULAR

A compilation of cases and personal reports (EPA 1980a) did not mention ocular effects as a part of the clinical picture of acute human exposure.

No ocular effects were found in rats intermittently exposed to technical chlordane by inhalation for 28 days at up to 28.2 mg/m^3 (EPA 1987f; Khasawinah et al. 1989). No ophthalmoscopic or histopathological changes were observed in the eyes or skin of rats or monkeys similarly exposed at 10 mg/m^3 for 90 days (EPA 1987f; Khasawinah et al. 1989). There was no histopathological evidence of chlordane-induced ocular effects in male or female rats receiving chlordane technical from the diet for 30 months at doses up to 1.18 and 1.41 mg/kg/day, respectively (EPA 1985a; Khasawinah and Grutsch 1989a), or male or female mice receiving chlordane technical from the diet for up to 24 months at doses up to 1.65 mg/kg/day (EPA 1985a; Khasawinah and Grutsch 1989b). There was no histopathological evidence of treatment-related dermal effects following dietary administration of analytical-grade chlordane for up to 80 weeks to male and female rats at doses up to 32.13 and 22.15 mg/kg/day, respectively, or male or female mice similarly treated at doses up to 9.64 and 11.02 mg/kg/day, respectively (NCI 1977).

2.13 ENDOCRINE

Nagayama et al. (2007) reported approximately 2-fold higher concentrations of selected organochlorine substances (polychlorinated biphenyls [PCBs], dioxin-like compounds, DDT, hexachlorocyclohexanes, chlordane, and hexachlorobenzene) in the breast milk of Japanese mothers who gave birth to neonates with cretinism (congenital hypothyroidism usually owing to maternal hypothyroidism; n=22) compared with a group of 102 mothers who gave birth to normal neonates. After adjustments for parity and mother's age, separate significant associations were noted for hexachlorobenzene, DDT, chlordane, and hexachlorocyclohexanes.

Increased height of thyroid follicular epithelial cells was observed in male rats exposed to chlordane technical by inhalation at 154 mg/m^3, 8 hours/day, 5 days/week for up to 11 exposures (EPA 1987f; Khasawinah et al. 1989) and in other male rats similarly exposed at 28.2 mg/m^3 for 28 days; thyroid weight was increased in a group of male rats exposed at 5.8 mg/m^3 (EPA 1987f; Khasawinah et al. 1989). In similarly-designed 90-day inhalation experiments in rats and monkeys, a slightly increased height in the follicular cells of the thyroid was found in rats exposed at 10 mg/m^3 (EPA 1987f; Khasawinah et al. 1989). In studies that employed single gavage dosing of chlordane technical, no histopathological lesions of the adrenal were found in rats or mice dosed at 200 mg/kg or hamsters dosed at 1,200 mg/kg (Truhaut et al. 1974, 1975).

2.14 IMMUNOLOGICAL

Chlordane caused statistically significant immune alterations in humans who had been exposed to chlordane aerosols in the home or in the workplace for periods ranging from 3 days to 15 months (average exposure period, 5.84 months) (McConnachie and Zahalsky 1992). The length of time from exposure to testing ranged from 4 months to 10 years, and the mean interval was 2.4 years. Impaired proliferative responses to all three plant mitogens tested suggested that chlordane exposure was associated with immune deficiency. Eleven of 12 subjects tested for autoimmunity demonstrated an increased titer of a form of autoantibody.

Tryphonas et al. (2003) administered technical chlordane, *trans*-nonachlor, or *cis*-nonachlor to male and female Sprague-Dawley rats by gavage for 28 days at doses up to 25 mg/kg/day. Significantly increased serum IgM was observed in high-dose female rats. Both the *trans*- and *cis*-nonachlor-treated groups exhibited more pronounced immunological effects than did those treated with technical chlordane.

Reduced thymus weight was observed in female rats, but not male rats, intermittently exposed by inhalation to chlordane technical for 28 days at 28.2 mg/m^3 (EPA 1987f; Khasawinah et al. 1989). There was no effect on thymus weight among male or female rats exposed for 90 days at 10 mg/m^3 and no histological lesions in thymus or lymph nodes of similarly-exposed monkeys. However, immune function was not assessed.

In single-dose gavage treatment with chlordane technical, no histopathological lesions of the spleen were found in rats or mice treated at 200 mg/kg or in hamsters treated at 1,200 mg/kg, but tests of immune

function were not performed (Truhaut et al. 1974, 1975). In a 14-day gavage study, no definitive evidence of immune dysfunction was observed in mice treated at 8 mg/kg/day, but leukocytosis associated with lymphocytosis were noted (Johnson et al. 1986). Oral treatment of adult mice for 18 days with 8 mg/kg/day had no effect on granulocyte-macrophage and spleen colony forming stem cell populations in the bone marrow (Barnett et al. 1990a). No effects on spleen weight or spleen histology were found in rats receiving technical chlordane from the diet for 2–9 months at 0.125 or 1.25 mg/kg/day (Ortega et al. 1957) or in other rats treated for up to 407 days at up to 16 mg/kg/day (Ambrose et al. 1953a). However, only six rats/sex/group were used and immune function was not assessed.

Miyagi et al. (1998) examined the effect of chlordane on chemotaxis of monkey neutrophils and monocytes *in vitro*. Chlordane was found to inhibit chemotaxis of neutrophils and monocytes toward interleukin-8 and RANTES (chemokines), respectively, suggesting that chlordane might alter leukocyte-related immune functions.

2.15 NEUROLOGICAL

Central nervous system effects including ataxia, headache, dizziness, irritability, excitability, confusion, incoordination, muscle tremors, seizures, convulsions, and coma have been described in a compilation of cases and personal reports of humans accidentally exposed by inhalation to unquantified concentrations of chlordane and following acute oral exposure to insecticidal formulations of chlordane (EPA 1980a).

EPA (1986d) reported three cases of optic neuritis that may have been due to chlordane exposure in homes treated for termites. Humans experienced neurological symptoms (headache, fatigue, sleeping disturbance, blurred vision, weakness, fainting, confusion) shortly after their homes were treated for termites (Menconi et al. 1988). Chronic exposure in the treated homes was associated with migraines and neuritis/neuralgia, which increased in incidence with higher concentrations of pesticide in the air. Because aldrin and heptachlor were included in the analysis for chlordane in indoor air, these effects cannot be attributed unequivocally to chlordane. NIOSH (1984a) reported neurological symptoms (headache, dizziness, blurred vision, irritability, paresthesia, muscle dysfunction) in 4 of 13 humans within 4 days of inhalation and/or dermal exposure as a result of 1% chlordane being spilled in a subterranean library room. The greatest prevalence of symptoms occurred in those directly involved in the cleanup, where the potential for exposure to the highest concentrations was greatest. Concentrations in air, taken ≈4.5 months after the spill, ranged from 0.1 to 0.3 $\mu g/m^3$. Because air concentration data were not available for the first 4 days of exposure, concentrations associated with the observed effects

cannot be estimated. No neurological effects, however, were found in epidemiological studies of workers in chlordane manufacture (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951). In a retrospective mortality study of 1,403 men employed for ≥3 months at two plants, a significantly increased risk of death from cerebrovascular disease was found, but the authors could not definitively attribute this excess to chlordane exposure (Wang and MacMahon 1979a).

By accident, part of the public water system of Chattanooga, Tennessee, became contaminated with chlordane, and the tap water of 42 houses had concentrations ranging from 0.1 to 92,500 ppb (Harrington et al. 1978). When the affected residents were surveyed, 18% reported neurological symptoms consistent with acute chlordane toxicity. Serum levels of *trans*-nonachlor and oxychlordane, however, were approximately twice as high in asymptomatic as in symptomatic individuals, raising serious questions about the validity of the association between exposure to chlordane and the reported effects. Most of the information on acute human oral exposure comes from cases of accidental or suicidal ingestion; therefore, doses of ingested chlordane are not readily quantifiable. Determination of a dose-effect response is further complicated because vomiting or lavage reduced the amount of ingested chlordane actually available for systemic absorption. In one such case, ingestion of 32 mg/kg of chlordane by a girl resulted initially in diplopia, blurred vision, and twitching of the extremities, followed by vomiting and eventually muscle tremors and generalized convulsions (Dadey and Kammer 1953). The investigators also estimated that after vomiting, only about 10 mg/kg was available for absorption. In another case, a man who ingested 3,041 mg/kg chlordane developed seizures and became comatose (Olanoff et al. 1983), but he also vomited, invalidating this dose. Clonic convulsions also developed in a 4-year-old girl who ingested chlordane (Aldrich and Holmes 1969). A dose of 0.15 mg/kg was estimated after gastric lavage. In a 15-month-old child who ingested 11.1 mg/kg chlordane, tremors and convulsions began about 3 hours after ingestion, which was prior to gastric lavage (Lensky and Evans 1952). These subsided by the second day, followed by moderate ataxia and irritability. Convulsions were also observed in patients who ingested unknown quantities of chlordane (Curley and Garrettson 1969; Kutz et al. 1983).

Kilburn and Thornton (1995) assessed measures of neurobehavioral function in a group of 216 adults who had been exposed to chlordane at an apartment complex in April of 1987 following application to external wood surfaces and soil. Later in 1987 and in 1988, the group was exposed to additional chlordane and chlorpyrifos applications. Tests for chlordane residue in 1990 and 1991 revealed concentrations ≥0.5 μg/929 cm^2 on 85% of 81 samples from external wood surfaces. Indoor concentrations as high as 13.6 μg/929 cm^2 were obtained on wipe samples, and 8-hour air samples taken from some of the apartments revealed chlorinated insecticide levels >0.5 $\mu g/m^3$. Eight subjects occupying the apartments

had elevated blood levels of heptachlor (range 110–186 ppb), oxychlordane (70–150 ppm), and *trans*-nonachlor (76–200 ppm). During June–September 1994, each of the 216 subjects underwent a battery of neurophysiological and neuropsychological testing and provided information regarding the frequency of 35 respiratory, neurologic, and vegetative complaints. A referent group of 174 adults of similar age, educational level, weight, height, and sex ratio was likewise assessed. Compared to the referents, exposed subjects exhibited significantly impaired performance of balance, reaction times, Culture Fair (measure of nonverbal nonarithmetical intelligence), digit symbol, verbal recall, and trail-making (visual attention and task switching); significantly elevated mood-state scores (tension, depression, anger, vigor, fatigue, confusion); and elevated frequencies of respiratory, neurobehavioral, and rheumatic symptoms.

Abnormal respiratory movements, excess salivation, and convulsions occurred in rats intermittently exposed by inhalation to chlordane technical for 11 exposures at 154 mg/m^3 or 3 exposures at 413 mg/m^3 (EPA 1987; Khasawinah et al. 1989). Female rats intermittently exposed by inhalation for 28 days at 28.2 mg/m^3 showed hypersensitivity to touch from day 16 onward (EPA 1987f; Khasawinah et al. 1989). There were no overt signs of neurotoxicity or histopathologic brain lesions in rats or monkeys intermittently exposed by inhalation for 90 days at 10 mg/m^3.

Central nervous system effects consisting of tremors, convulsions, and paralysis of the hindlimbs occurred in rats following single gavage doses of *cis*-chlordane ≥200 mg/kg; hypothermia was noted at (Hrdina et al. 1974). Histological examination of the brains of rats and mice given a single oral dose of chlordane technical at 200 mg/kg and hamsters given a single dose of 1,200 mg/kg revealed congestion in the brain (Truhaut et al. 1975). In rats administered chlordane technical by gavage once at ≥200 mg/kg/day or for 9–12 days at 50 mg/kg/day, convulsions preceded death (Ambrose et al. 1953a). No ataxia or change in the level of cerebral amino acids were observed in mice treated by gavage with 25 mg/kg/day for 45 consecutive days (Matin et al. 1977). In a 12-week dietary study, a dose of 5 mg/kg/day caused convulsions in rats (Drummond et al. 1983). In an 80-week study, analytical-grade chlordane induced tremors in female rats at 22.15 mg/kg/day, but not at 11.08 mg/kg/day and only during week 44 (NCI 1977). Similar signs were not observed in male rats, which were tested at up to 32.13 mg/kg/day. No brain lesions were found in rats of either sex. Similarly-treated male and female mice exhibited tremors at 9.64 and 11.02 mg/kg/day, respectively, in the absence of histopathologic brain lesions (NCI 1977). Neither clinical signs nor histopathological lesions of the nervous system were observed in a 30-month study in which rats received chlordane technical from the diet at up to 1.18 and 1.41 mg/kg/day, respectively (EPA 1985a; Khasawinah and Grutsch 1989a) or a similarly-designed

24-month dietary study of mice at doses up to 1.65 mg/kg/day (EPA 1985a; Khasawinah and Grutsch 1989b).

2.16 REPRODUCTIVE

Chronic exposure in homes treated with chlordane for termites was associated with an increased incidence of unspecified ovarian and uterine disease, compared with a reference population (Menconi et al. 1988). Because aldrin and heptachlor were included in the analysis for chlordane in indoor air, these effects cannot be unequivocally attributed to chlordane.

There was no histopathological evidence of treatment-related effects on the reproductive organs of rats intermittently exposed to chlordane technical by inhalation for 28 days at 28.2 mg/m^3, or other rats or monkeys similarly exposed for 90 days at 10 mg/m^3 (EPA 1987; Khasawinah et al. 1989). However, reproductive function was not assessed. Histological examination of the testes of rats and mice given a single oral dose of 200 mg/kg or hamsters given a single oral dose of 1,200 mg/kg revealed no lesions (Truhaut et al. 1975), but reproductive function was not assessed. In the only evaluation of fertility with oral exposure, Ambrose et al. (1953a) reported reduced fertility, reflected as a reduction in the number of mated females that delivered litters, when male and female rats were fed a diet that provided chlordane technical at 16 mg/kg/day. Treatment began at weaning of the parental generation and continued through lactation. None of the litters survived to weaning. Treatment of male mice by gavage at 100 or 300 mg/kg/day for 30 days resulted in reduced size of seminiferous tubules and degeneration of spermatogenic epithelium (Balash et al. 1987). Consumption of chlordane from the diet at 19.5 mg/kg/day by male rats for 90 days increased androgen receptor sites in the ventral prostate (Shain et al. 1977). There were no effects on ventral prostate or testicular weight, or on plasma testosterone level, and the toxicological significance of this observation to humans is unclear.

No treatment-related histopathological lesions were observed in the reproductive tracts of male or female rats consuming chlordane technical for up to 30 months at doses up to 1.18 and 1.41 mg/kg/day, respectively (EPA 1985a; Khasawinah and Grutsch 1989a), or in male and female mice similarly treated for up to 24 months at doses up to 1.65 mg/kg/day (EPA 1985a; Khasawinah and Grutsch 1989b). In male and female rats given diets providing up to 16 mg/kg/day chlordane technical for 407 days, no histopathological lesions were found in reproductive organs (Ambrose et al. 1953a). However, only five rats/sex/group were used. No treatment-related histopathological lesions were observed in the reproductive tracts of male or female rats consuming analytical-grade chlordane for up to 80 weeks at

doses up to 32.13 and 22.15 mg/kg/day, respectively, or in similarly treated male and female mice at doses up to 9.64 and 11.02 mg/kg/day, respectively (NCI 1977).

2.17 DEVELOPMENTAL

Trabert et al. (2012) assessed the association between *in utero* exposure to chlordane and cryptorchidism (absence of one or both testes in scrotum) and hypospadias (abnormal positioning of urethra opening). Levels of *trans*-chlordane and oxychlordane were measured among pregnant women enrolled in the Collaborative Perinatal Project (CPP) from 1959 to 1965 who delivered sons with cryptorchidism or hypospadias and sons without either condition. Results did not support an association between chlordane levels and cryptorchidism and hypospadias.

Gladen et al. (2003) assessed whether weight at birth is associated with prenatal exposure to persistent organochlorine compounds, including *trans*-nonachlor and oxychlordane. From 1993 to 1994, organochlorine compounds were measured in breast milk 4–5 days after birth and were used as the index for prenatal exposure for 197 singleton infants selected from the at-large population in two Ukrainian cities. Infants within the upper tertile of oxychlordane (82 ng/g milk fat) and *trans*-nonachlor (73 ng/g milk fat) had slightly smaller, though statistically insignificant, mean birth weights compared with the lower tertile. Seven of eight preterm infants were in the upper oxychlordane tertile, but the small number of preterm births in this study prevented the authors from drawing any conclusions. The authors reported that prenatal exposure to the concentrations of chemicals studied did not affect weight at birth.

Fenster et al. (2006) found no association between selected birth outcomes (length of gestation, birth weight, and crown-heel length) and maternal serum levels of oxychlordane (a chlordane metabolite), *trans*-nonachlor (a major component of technical chlordane), or heptachlor epoxide (a component of technical chlordane and metabolite of heptachlor) in a birth cohort of 385 low-income Latinas living in the agricultural community of Salinas Valley, California.

There was no effect on the incidence of malformations and no evidence of fetal toxicity, including retarded skeletal development, in the fetuses of rats administered chlordane technical by gavage during gestation at up to 80 mg/kg/day, although 4/8 high-dose maternal rats died (Usami et al. 1986). No effects on viability and postnatal growth were observed in the offspring of mice treated with an undescribed sample of chlordane at 50 mg/kg/day during gestation days (GDs) 8–12 (Chernoff and Kavlock 1982). The offspring of mice treated at 1 and 2.5 mg/kg/day during the third trimester exhibited

depressed acquisition of avoidance response, increased seizure threshold, and increased exploratory activity in a study that assessed neurobehavioral effects after in utero exposure (Al-Hachim and Al-Baker 1973). The authors concluded that chlordane affected the fetal brain. Exposure could also have occurred via nursing, because the pups were allowed to nurse the treated dams. The study identified a LOAEL of 1 mg/kg/day, which was used to derive an acute-duration oral MRL as described in the footnote in Table 2-2 and Appendix A.

Cranmer et al. (1984) administered analytical-grade chlordane in peanut homogenate to maternal mice throughout gestation at up to at 8 mg/kg/day to measure endocrinological performance of adult offspring. Although mice in treated groups gave birth to approximately equal numbers of viable offspring of "average" body weight that were grossly normal in appearance, 55% of the offspring of the high-dose dams died within the first week of the nursing period. The authors stated only that the cause of death was not apparent from gross necropsy; however, it is possible that exposure to high levels of chlordane and/or metabolites in the dam's milk may have been responsible for these deaths. Postweaning survival was not affected by treatment. Plasma corticosterone in the offspring measured at 400 days of age was elevated in females at 0.16 mg/kg/day, but not at 8.0 mg/kg/day, and in males at both dose levels. These effects were not apparent at 800 days of age in either sex, although not enough high-dose males survived for evaluation. The investigators hypothesized that elevated plasma levels of corticosterone may reflect the diminished ability of the liver to metabolically reduce corticosterone. The effects on plasma corticosterone levels in females did not occur in a dose-related fashion and the toxicological significance of this effect is unclear. Therefore, this effect is not considered in estimating levels of significant exposure.

In other studies, pregnant mice were treated with chlordane technical, and the effects on the immune system of the offspring were assessed (Barnett et al. 1985a, 1985b; Menna et al. 1985; Spyker-Cranmer et al. 1982). These studies suggested to the investigators that *in utero* and/or neonatal exposure to chlordane suppressed cell-mediated immunity, as manifested by depressed delayed-type hypersensitivity reactions in the offspring of treated mice. There was no effect on humoral-mediated immunity. It is likely that the nursing pups continued to be exposed to chlordane because chlordane is excreted in milk. Subsequent studies by these investigators indicate that prenatal treatment of mice depressed granulocyte-macrophage and spleen-forming stem cells in the bone marrow (Barnett et al. 1990a) and the liver (Barnett et al. 1990b), but had no effect on cytotoxic T-lymphocyte activity (Blaylock et al. 1990). Further mechanistic studies demonstrated that prenatal exposure of mice to chlordane (dams treated with 8 mg/kg/day during GDs 1–18) altered the macrophage in such a manner that it exhibited phenotypic characteristics of a cell

that achieved inflammatory status (Theus et al. 1992). The significance of this effect is not well understood.

Blyler et al. (1994) administered analytical-grade chlordane (in peanut butter) to groups of pregnant BALB/c mice (number per group not specified) on GDs 1–18 at 8 mg/kg/day. Myeloid hemopoietic activity of bone marrow cells from 6-week-old offspring was evaluated for *in vitro* colony-forming units-in-culture in response to exogenously added recombinant forms of the cytokines granulocyte/macrophage-colony stimulating factor (CSF), macrophage-CSF, and interleukin 3 (IL-3). Female, but not male, offspring exhibited a significant depression of the numbers of bone marrow colony forming units-granulocyte/macrophage (CFU-GM), CFU-IL-3, and CFU-M. Chlordane treatment did not significantly affect the number of recoverable, viable bone marrow cells in male or female offspring.

Narotsky and Kavlock (1995) administered technical chlordane to groups of timed-pregnant Fischer 344 rats (19–23/group) by gavage on GDs 6–19 at doses of 0, 21, or 28 mg/kg/day and assessed maternal and developmental effects. Both chlordane groups exhibited initial maternal weight loss (GDs 6–8) and significantly depressed gestational weight gain (GDs 6–20; approximately 30 and 55%, respectively, less than controls). No clinical signs of maternal toxicity were reported. No significant, treatment-related effects appeared in numbers of implants, resorptions, or live litters at birth. Both chlordane groups exhibited significantly decreased mean numbers of live pups at 6 days postpartum (5.2 and 1.5 pups/litter for the 21 and 28 mg/kg/day groups, respectively, compared with 7.9 pups/litter in controls). No gross signs of treatment-related malformations were observed.

Cassidy et al. (1994) assessed the pre- and postnatal effects of technical chlordane in Sprague-Dawley rats. The study included oral administration of chlordane to groups of pregnant rats (5/group) from GD 4 throughout gestation, parturition, and lactation at doses of 0, 0.1, 0.5, or 5 mg/kg/day and oral dosing of the offspring on postnatal days (PNDs) 22–80. In tests conducted between PND 77 and 85, the chlordane-exposed offspring exhibited sex- and dose-related effects on testosterone levels, selected behavioral tests of spatial abilities, and body weight. Female, but not male, offspring exhibited significant increases in body weight, decreases in testosterone levels, improved spatial abilities, and increases in auditory startle-evoked responses. Chlordane-exposed male rats exhibited significant increases in male-typical mating behaviors and decreases in Cl^- uptake in brain microsacs. Male rats did not show a significant decrease in testosterone levels at any dose, though a 10% decrease in testosterone was observed in male rats dosed at 5 mg/kg/day. The authors interpreted these results as indicative of

chlordane-induced masculinization of sexually dimorphic functions and behaviors by mimicking sex steroids, limiting their levels, or both.

2.18 OTHER NONCANCER

Everett and Matheson (2010) used the National Health and Nutrition Examination Survey (NHANES), 1999–2004 to evaluate the associations of total diabetes and prediabetes (glycohemoglobin 5.7–6.4%) with eight pesticide and pesticide metabolites, including oxychlordane, *trans*-nonachlor, and heptachlor epoxide. In separate adjusted logistic regressions, oxychlordane, *trans*-nonachlor, and heptachlor epoxide were positively associated with total diabetes. In a combined logistic regression, oxychlordane and heptachlor epoxide were positively associated with total diabetes. Heptachlor epoxide was positively associated with prediabetes in both the separate and combined models.

Lee et al. (2010) reported a nonlinear association between type 2 diabetes and serum levels of both *trans*-nonachlor and oxychlordane in a nested case-control study that included 90 cases and 90 controls enrolled in a Coronary Artery Risk Development in Young Adults (CARDIA) cohort. Montgomery et al. (2008) reported an increased risk of diabetes among chlordane-using licensed pesticide applicators (372 diabetics and 7,365 nondiabetics) enrolled in an Agricultural Health Study between 1993 and 2003. In a study that included 1,303 Mexican Americans 20–74 years of age from the Hispanic Health and Nutrition Examination Survey, 1982–1984, Cox et al. (2007) reported significant positive associations between self-reported diabetes and serum levels of both *trans*-nonachlor and oxychlordane.

2.19 CANCER

Studies that evaluated possible associations between exposure to chlordane and risk of cancers are largely limited due to unquantified exposure frequency, duration, and concentration, exposure to other compounds, and other confounding factors.

Retrospective mortality studies of workers involved in the manufacture of chlordane (Shindell and Ulrich 1986; Wang and MacMahon 1979a) reported no increased incidence of total deaths due to cancer or to a specific type of cancer. In a prospective study of pesticide applicators, Wang and MacMahon (1979b) reported an increased SMR for death due to bladder cancer that was “on the borderline of statistical significance,” but did not attribute this observation to exposure to chlordane because a similar effect was not observed in the manufacturing study (Wang and MacMahon 1979a). A follow-up study on a larger cohort of pesticide applicators found no association of exposure to chlordane with total deaths due to

cancer or to a specific type of cancer (MacMahon et al. 1988). In another retrospective mortality study of four cohorts (305–1,155 workers/plant exposed for ≥6 months) from four manufacturing plants, there was a significantly increased risk of death from noncancer respiratory disease, and a slight excess risk of cancer of the esophagus, rectum, liver, and hematopoietic system at plant 3, and a slightly greater risk of stomach cancer at plant 1 (Ditraglia et al. 1981). However, chlordane was the only pesticide manufactured at plant 1, while aldrin, dieldrin, endrin, and dichlorodiphenyltrichloroethane were manufactured at plant 3. The statistical power did not allow for a conclusion that no association existed between cause-specific mortality and employment at the plants. However, in a follow-up to the study by Ditraglia et al. (1981), the carcinogenic risk among workers exposed to organochlorines was assessed (Brown 1992). This study added 11 years to the previous follow-up study, thus providing 40 years of observation for the cohort. As 23 years was the minimum time elapsed since each cohort member was first employed at the study plants, this allowed more time for diseases with long latency periods to develop. The investigator concluded that the mortality for all causes and all malignant neoplasms was lower than expected.

A small but insignificant positive association between reported chlordane use and risk of non-Hodgkin's lymphoma was reported among farmers exposed to chlordane in a case-control study (Woods and Polissar 1989). Several cases of leukemia and neuroblastoma were reported in persons exposed to chlordane or heptachlor in their home or garden, or as a result of their profession as pest control operators (Epstein and Ozonoff 1987; Infante et al. 1978). A small case-control study found that levels of chlordane residues (heptachlor epoxide, oxychlordane, *trans*-nonachlor) in the breast fat from 20 women with malignant breast disease were not significantly different from 20 women with benign breast disease (largely nonproliferative fibrocystic changes) (Falck et al. 1992).

A number of case-control studies examined possible associations between risk of selected cancer endpoints and levels of chlordane or chlordane-related substances such as *cis*- and *trans*-nonachlor (major components of technical chlordane) or oxychlordane (a metabolite of chlordane) in plasma or serum samples or adipose tissue. Most case-control studies found no significant associations between levels of chlordane or chlordane-related compounds and risk of breast cancer (Demers et al. 2000; Gammon et al. 2002; Ward et al. 2000; Wolff et al. 2000; Zheng et al. 2000), endometrial cancer (Weiderpass et al. 2000), prostate cancer (Aronson et al. 2010; Ritchie et al. 2003; Sawada et al. 2010), or non-Hodgkin's lymphoma (Cantor et al. 2003).

McGlynn et al. (2008) reported significant positive associations between risk of testicular germ cell tumors and serum levels of *cis*-nonachlor, *trans*-nonachlor, and total chlordanes. McGlynn et al. (2008) also reported significant associations for seminoma (i.e., testicular tumors arising from sperm-forming tissues) and *cis*-nonachlor, *trans*-nonachlor, and oxychlordane. Cook et al. (2011) reported significant positive associations between exposure to *cis*-nonachlor, and *trans*-nonachlor with testicular germ-cell tumors. Hardell et al. (2003) reported a significant association between risk of testicular cancer and lipid-adjusted plasma *cis*-nonachlor levels. Hardell et al. (2003) reported significant positive associations between risk of testicular cancer and plasma levels of *trans*-nonachlor and *cis*-nonachlor in the serum of mothers of the testicular cancer cases. Assessment according to testicular tumor type resulted in significant associations between risk of seminoma and maternal plasma *cis*-chlordane levels and between risk of nonseminoma and maternal plasma *trans*- and *cis*-nonachlor levels. In a subsequent analysis of testicular cancer cases and age-matched controls (Hardell et al. 2006b), no significant association was found between the sum of chlordanes in maternal blood and risk of testicular cancer.

Spinelli et al. (2007) reported significant positive associations between risk of non-Hodgkin's lymphoma and lipid-adjusted plasma levels of oxychlordane and *trans*-nonachlor in a population-based, case-control study involving 422 non-Hodgkin's lymphoma cases and 460 control subjects.

Quintana et al. (2004) reported a significant positive association between risk of non-Hodgkin's lymphoma and levels of oxychlordane in adipose tissue samples collected from cadavers and surgical patients within the EPA National Human Adipose Tissue Survey. Hardell et al. (2006a) reported a significant association between risk of prostate cancer and *trans*-chlordane in adipose tissue. Hardell and colleagues also reported a significant positive association for those prostate cancer cases with prostate-specific antigen (PSA) levels >10 ng/mL. Hardell et al. (2007) reported significant positive associations between risk of pancreatic cancer and adipose tissue levels of *trans*-chlordane, oxychlordane, *trans*-nonachlor, *cis*-nonachlor, and their sum. Hardell et al. (1996) reported a significant positive association between risk of non-Hodgkin's lymphoma and *trans*-nonachlor level in adipose tissue. This study also found significantly increased mean concentrations of *trans*-nonachlor, *cis*-nonachlor, and oxychlordane in adipose tissues from non-Hodgkin's lymphoma patients versus controls.

Possible associations between chlordane and selected cancer endpoints were evaluated in population-based case-control studies. In one study, the risk of non-Hodgkin's lymphoma among farmers was significantly elevated for personal handling, mixing, or application of chlordane as an animal insecticide or as a crop insecticide (Cantor et al. 1992). The odds ratio for non-Hodgkin's lymphoma was also

greater among farmers who first used chlordane before 1965 (15–18 years before diagnosis) or those farmers who did not use protective equipment.

Colt et al. (2006) examined non-Hodgkin's lymphoma risk and use of insecticides in the home and garden. The study included 1,321 non-Hodgkin's lymphoma cases and 1,057 controls from four areas of the country (Iowa, Los Angeles county, Detroit, and Seattle). Study subjects were given a questionnaire regarding the use of insecticides for eight specific types of pests including termites. Termite treatment was associated with a "modest," but not significant elevated risk for non-Hodgkin's lymphoma in all areas except Seattle; and only if treatments were before 1988, when the use of chlordane for termite treatment was banned. Insecticide levels were measured in dust taken from used vacuum cleaner bags (682 cases and 513 controls). A significant positive trend for non-Hodgkin's lymphoma and α-chlordane residue concentrations in dust was observed, and a marginally significant trend was observed for increased levels of γ-chlordane.

Mills and Yang (2005) performed a registry-based, case-control study of breast cancer in farm labor-union members in California. Using available records of pesticide applications between 1970 and 1999, exposures to selected pesticides—including chlordane—were estimated as no exposure, low exposure, or high exposure. Among breast cancer cases diagnosed between 1988 and 1994, a significant positive association was observed between reported "high" use of chlordane and risk of breast cancer. No significant association was observed for breast cancer cases diagnosed between 1995 and 2001. These results may reflect patterns of chlordane use, given that the pesticide was phased out in the 1980s.

Purdue et al. (2006) investigated relationships between cancer incidence and organochlorine insecticide use among pesticide applicators enrolled in the Agricultural Health Study of licensed applicators in Iowa and North Carolina between 1993 and 1997. Information on "ever use" (having ever used) of selected organochlorine pesticides—including chlordane—was collected from self-administered questionnaires at the time of enrollment. A total of 51,011 of the enrolled pesticide applicators reported "ever use" of the selected organochlorine pesticides; 7,244 of these pesticide applicators reported "ever use" of chlordane. Through December 2002, among the chlordane-exposed subjects, 33 cases of rectal cancer had been diagnosed. Among the pesticide applicators with no reported chlordane use (n=43,767), 42 rectal cancer cases had been diagnosed. The study authors reported a significant positive association between "ever use" of chlordane and risk of rectal cancer. However, they found no significant associations between "ever use" of chlordane and other cancers (prostate, lung, colon, bladder, non-Hodgkin's lymphoma, leukemia, and melanoma) or all cancers combined.

Xu et al. (2010) examined possible associations between serum concentrations of organochlorine pesticides and selected metabolites (including the chlordane metabolites oxychlordane and *trans*-nonachlor) among 4,237 participants of the 1999–2004 NHANES (4,109 individuals without cancer, 63 breast cancer cases, and 65 prostate cancer cases). No significant association was found between serum oxychlordane and risk of breast cancer. However, a significant positive association was reported for serum *trans*-nonachlor and risk of prostate cancer.

Chronic exposure of humans in homes treated with chlordane for termites was associated with greater incidence of unspecified skin neoplasms, compared with a reference population (Menconi et al. 1988).

Studies using three different strains of mice (one strain had an historically low incidence of spontaneous liver tumors) have demonstrated that dietary administration of chlordane is associated with the development of hepatocellular carcinomas. An unpublished study by IRDC (1973), which was available only in reviews by EPA (1986c, 1987e), Epstein (1976), IRIS (1992), and Reuber (1978), found significant increases in the incidence of hepatocellular carcinomas in male and female CD-1 mice fed analytical-grade technical chlordane in the diet at doses of 3.25 and 6.5 mg/kg/day for 18 months. In an NCI (1977) chronic dietary study with a mixture of analytical-grade chlordane (72% *cis* and 23% *trans* isomers), there was a dose-related increase in the incidence of hepatocellular carcinomas in male and female B6C3Fl mice that was statistically significant in both treated groups of males (doses of 5.13 and 9.64 mg/kg/day) and in the high-dose females (11.02 mg/kg/day). An increased incidence of hepatocellular adenomas and hemangiomas developed in male mice, but not female mice, maintained on a diet providing ≈1.65 mg chlordane/kg/day for 2 years (EPA 1985a; Khasawinah and Grutsch 1989b).

Barrass et al. (1993) reported approximately 50% incidence of hepatocellular tumors among male C57B1/10J mice that had received chlordane in the diet at a concentration of 50 ppm for 2 years (estimated intake of 8.6 mg chlordane/kg/day); hepatic tumor incidence among 400 control mice at the same research facility was approximately 2%. Malarkey et al. (1995) reported 100% incidences of hepatocellular adenomas in groups of male B6C3F1 mice (10 or 20 per group) administered technical chlordane at 55 ppm in the diet for periods of 513–568 days (estimated intake of 9.4 mg chlordane/kg/day). Hepatocellular carcinomas during the same time period were noted in 80–100% of the chlordane-treated mice. Incidences of hepatocellular adenomas and carcinomas in a group of untreated controls at 759 days were 7/43 (16%) and 3/43 (7%), respectively. Results from similar exposure of male B6D2F1 mice indicated that male B6C3F1 mice are more sensitive than are male B6D2F1 mice.

Although chlordane clearly induces liver cancer in mice (EPA 1985a; IRDC 1973; Khasawinah and Grutsch 1989b; NCI 1977), epidemiological data provide no convincing evidence that it induces cancer in humans (Ditraglia et al. 1981; MacMahon et al. 1988; Shindell and Ulrich 1986; Wang and MacMahon 1979a, 1979b). Most genotoxicity tests with chlordane yielded negative results (see Section 2.20), suggesting an epigenetic mechanism of carcinogenicity. Chlordane inhibited gap junction intercellular communication in the rat hepatocyte/liver epithelial system metabolic cooperation assay (Tong and Williams 1988) and in the Lucifer yellow CH dye-coupling test in rat and mouse hepatocytes (Ruth et al. 1990). These results suggest that chlordane acts as a tumor promoter, depressing intercellular communication that checks uncontrolled proliferation of transformed or neoplastic cells (Tong and Williams 1988). Rought et al. (1999) demonstrated the ability of chlordane to reduce retinoblastoma tumor-suppressor gene expression in CEM x 174 cells (a hybrid of human T and B lymphocytes). These results suggest that chlordane is capable of down-regulating retinoblastoma expression at the post-transcriptional level. The authors indicated that such a mechanism could be involved in chlordane's immune-modulatory and tumor-promoting effects. Ruth et al. (1990) suggested that inhibition of intercellular communication may involve alteration of cAMP-dependent protein kinase phosphorylation of hepatocellular gap junction proteins, which would increase permeability at the gap junctions. Moser and Smart (1989), who noted that chlordane stimulated protein kinase C activity in several tissues of mice *in vitro*, provide support for this theory. Nonetheless, Suzaki et al. (1988) did not observe a chlordane-induced increase in protein kinase C activity *in vitro* in the rat brain.

The U.S. Department of Health and Human Services has not classified chlordane as to its carcinogenicity (NTP 2016). EPA categorized it as a probable human carcinogen (Group B2) (IRIS 2002). The International Agency for Research on Cancer categorized it as possibly carcinogenic to humans (Group 2B) (IARC 2001, 2017). The cancer classifications are based on sufficient evidence of carcinogenicity in animal studies and inadequate evidence in humans.

2.20 GENOTOXICITY

Chlordane has been evaluated for genotoxicity in a limited number of *in vivo* tests (Table 2-4). Chlordane induced DNA damage in liver cells of orally-exposed rats (Bagchi et al. 1995; Hassoun et al. 1993) and chromosomal aberrations in bone marrow cells of orally-exposed mice (Sarkar et al. 1993). A weakly positive result was obtained for micronuclei in bone marrow of dermally-treated mice (Schop et al. 1990). Chlordane did not induce dominant lethality in male mice treated orally or intraperitoneally (Arnold et al.

1977; Epstein et al. 1972) or DNA adduct formation in liver cells of orally-exposed mice (Whysner et al. 1998).

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

Species (exposure route)	Endpoint	Results	Reference
Rat liver cells (oral)	DNA damage	+	Hassoun et al. 1993
Rat liver cells (oral)	DNA damage	+	Bagchi et al. 1995
Mouse (oral, intraperitoneal)	Dominant lethal	–	Arnold et al. 1977
Mouse (oral, intraperitoneal)	Dominant lethal	–	Epstein et al. 1972
Mouse bone marrow (dermal)	Micronuclei	(+)	Schop et al. 1990
Mouse bone marrow (oral)	Chromosomal aberrations	+	Sarkar et al. 1993
Mouse liver (oral)	DNA adducts	–	Whysner et al. 1998

+ = positive result; (+) = weakly positive result; – = negative result

Chlordane has been evaluated for genotoxicity in a variety of *in vitro* tests (Table 2-5). Chlordane was not mutagenic in *Salmonella typhimurium* or *Escherichia coli* (Gentile et al. 1982; Mortelmans et al. 1986; Probst and Hill 1981; Simmon et al. 1977), rat liver cells (Telang et al. 1981), or Chinese hamster lung V79 diphtheria toxin resistant cells (Tsushimoto et al. 1983). However, in the absence of exogenous metabolic activation, chlordane was mutagenic in assays of human fibroblasts (Tong et al. 1981), mouse lymphoma L5178Y cells (McGregor et al. 1988), and Chinese hamster lung V79 cells (Ahmed et al. 1977b). Negative results were obtained from assays of unscheduled DNA synthesis in a variety of prokaryotic and mammalian test systems (Brandt et al. 1972; Maslansky and Williams 1981; Probst and Hill 1981; Rashid and Mumma 1986; Williams 1980); the only exception was a positive result in human SV-40 fibroblasts when an exogenous metabolic activation system was added (Ahmed et al. 1977a). Chlordane was positive for prophage induction in *E. coli* with and without exogenous metabolic activation (Houk and DeMarini 1987). In *E. coli*, chlordane was also positive for SOS repair induction (Venkat et al. 1995) and DNA strand breaks (Griffin and Hill, 1978) without exogenous metabolic activation. Chlordane induced mitotic gene conversion in *Saccharomyces cerevisiae* (Gentile et al. 1982) with exogenous metabolic activation and sister chromatid exchange in human lymphoid cells (Sobti et al. 1983) with and without exogenous metabolic activation. When exposed to chlordane in the absence of exogenous metabolic activation, Syrian hamster embryo cells were positive for cell transformation but negative for the formation of DNA adducts (Bessi et al. 1995).

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

Species (test system)	Endpoint	Results		Reference
		Activation		
		With	Without	
Prokaryotic organisms				
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Gene mutation	ND	–	Simmon et al. 1977
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Gene mutation	–	–	Gentile et al. 1982
S. typhimurium TA98, TA100, TA1535, TA1537	Gene mutation	–	–	Mortelmanns et al. 1986
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538, G46, C3076, D3052	Gene mutation	–	–	Probst and Hill 1981
Escherichia coli WP2, WP2 *uvrA*	Gene mutation	–	–	Probst and Hill 1981
E. coli $WP2_s$	Prophage induction	+	+	Houk and DeMarini 1987
E. coli PQ37	SOS repair induction	ND	+	Venkat et al. 1995
E. coli K12ColE1	DNA strand breaks	ND	+	Griffin and Hill 1978
S. typhimurium TA1538/TA1978	Unscheduled DNA synthesis	–	–	Rashid and Mumma 1986
E. coli K-12	Unscheduled DNA synthesis	–	–	Rashid and Mumma 1986
Eukaryotic fungal and bacterial cells				
S. cerevisiae D4	Mitotic gene conversion	+	–	Gentile et al. 1982
Mammalian cells				
Human fibroblasts	Gene mutation	–	+	Tong et al. 1981
Rat liver epithelial ARL cells	Gene mutation	ND	-	Telang et al. 1981;
Mouse lymphoma L5178Y cells	Gene mutation	ND	+	McGregor et al. 1988
Chinese hamster lung V79 cells, ouabain resistant	Gene mutation	ND	+	Ahmed et al. 1977b
Chinese hamster lung V79 cells, diphtheria toxin resistant	Gene mutation	ND	–	Tsushimoto et al. 1983
Chinese hamster lung V79 cells, *hprt* locus	Gene mutation	ND	–	Tsushimoto et al. 1983
Human VA-4 fibroblasts	Unscheduled DNA synthesis	–	+	Ahmed et al. 1977a
Human HeLa cells	Unscheduled DNA synthesis	ND	–	Brandt et al. 1972

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

Species (test system)	Endpoint	Results: Activation With	Results: Activation Without	Reference
Rat, mouse, hamster hepatocytes	Unscheduled DNA synthesis	ND	–	Maslansky and Williams 1981
Rat hepatocytes	Unscheduled DNA synthesis	ND	–	Probst and Hill 1981; Williams 1980
Human LAZ-007 lymphocytes	Sister chromatid exchange	+	+	Sobti et al. 1983
Syrian hamster embryo cells	Cell transformation	ND	+	Bessi et al. 1995
Syrian hamster embryo cells	DNA adducts	ND	-	Bessi et al. 1995

– = negative result; + = positive result; +/– = inconclusive results; DNA = deoxyribonucleic acid; ND = no data

2.21 MECHANISMS OF ACTION

2.21.1 Pharmacokinetic Mechanisms

Although the data suggest that chlordane is readily absorbed from the respiratory (Nye and Dorough 1976) and gastrointestinal tracts (Ohno et al. 1986), and that dermal absorption is sufficient to cause toxicity in humans and animals (Derbes et al. 1955; Gaines 1960), data regarding the mechanisms of absorption were not located. Generally, highly lipophilic organic compounds cross membranes largely by passive diffusion. Since chlordane is highly lipophilic, it is expected that absorption of chlordane by all routes of exposure would involve primarily passive diffusion. This is consistent with the observation by Ohno et al. (1986) that little difference in the extent of gastrointestinal absorption occurred over a 10-fold difference in dose.

The metabolism of the components of chlordane and the lipophilicity of the components and metabolites influence their distribution. Initial distribution to the liver and kidneys is more rapid than to fat (Ohno et al. 1986), probably reflecting differences in vascularity of these sites. Subsequently, redistribution results in higher levels in the fat than other tissues. Low levels of *cis*- and *trans*-chlordane in fat and relatively higher levels of oxychlordane and trans-nonachlor reflect the relative lability of the chlordane isomers and stability of the latter two compounds (Hirasawa and Takizawa 1989; Sasaki et al. 1991a, 1992).

Metabolism of the *cis* and *trans* isomers of chlordane by humans and laboratory animals appears to be qualitatively similar (Kutz et al. 1976, 1979), although monkeys may be less efficient than rats

(Khasawinah 1989), and rats may metabolize *trans*-nonachlor more efficiently than humans (Tashiro and Matsumura 1978). Metabolism appears to be largely oxidative, involving hepatic microsomal cytochrome P-450 (Kawano et al. 1989). Epoxide hydrolase is probably the predominant enzyme involved in further degradation of oxychlordane, but the process appears to be slow in animals and humans. In addition, reductive dehalogenation, probably resulting in the formation of reactive free radical intermediates, may be important in the toxicity of chlordane (Brimfield and Street 1981; Kawano et al. 1989).

The strong lipophilicity and relatively weak hydrophilicity of chlordane and its metabolites suggest that excretion would be largely by passive diffusion. This is supported by the observation that fecal (biliary) excretion exceeds urinary excretion in humans and rats (Aldrich and Holmes 1969; Ohno et al. 1986), indicating that renal tubular excretion is probably not a major factor in excretion. Passive tubular resorption probably accounts for the lesser role that renal excretion plays in the fate of chlordane, compared with most organic chemicals, for which biotransformation results in the formation of more polar (hydrophilic) products.

Cis- and *trans*-chlordane and their metabolites bind irreversibly with cellular macromolecules such as protein, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) (Brimfield and Street 1981). Binding to these macromolecules may lead to cell death or altered cellular function. In addition, *cis*- and *trans*-chlordane, heptachlor, and heptachlor epoxide increase the generation of superoxide in cultures of guinea pig polymorphonuclear leukocytes (Suzaki et al. 1988). This was probably an indirect effect of activation of phospholipase C, or of increasing the intracellular concentration of free ionized calcium, rather than a direct effect on protein kinase C.

2.21.2 Mechanisms of Toxicity

Potential mechanisms of chlordane-induced liver effects, immunotoxicity, and cancer are discussed in Sections 2.9, 2.14, and 2.19, respectively. Other potential mechanisms of toxicity are discussed here.

Gauthier and Girard (2001) found that chlordane-induced neutrophil superoxide production in human neutrophils in a concentration-related manner occurred similarly to that induced by the known neutrophil-agonist, phorbol 12-myristate 13-acetate. Chlordane was further shown to enhance neutrophil phagocytosis of sheep red blood cells without altering chemotaxis and apoptosis. Evidence that chlordane-induced superoxide production might involve protein kinase C-dependent mechanisms

included findings that staurosporine and calphostin C (transduction signal inhibitors) inhibited the effect of chlordane on superoxide production.

Bagchi and coworkers (Bagchi et al. 1995; Stohs et al. 1997) examined the effects of chlordane on the production of hepatic and brain lipid peroxidation and DNA single-strand breaks (indices of oxidative stress and oxidative tissue damage) in female rats administered two ¼LD_{50} gavage doses of chlordane 21 hours apart and sacrificed 3 hours later. Chlordane treatment resulted in approximately 3- and 2-fold increases in lipid peroxidation in liver and brain, respectively, and approximately 4- and 1.8-fold increases in single-strand breaks in liver and brain, respectively. The investigators also assessed chlordane-induced changes in the release of lactate dehydrogenase (a measure of cellular damage and cytotoxicity) and DNA single-strand breaks from cultured neuroactive PC-12 cultures. Increases in both releases of lactate dehydrogenase and DNA single-strand breaks were observed. The *in vivo* and *in vitro* results support the notion of chlordane-induced generation of reactive oxygen species.