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 hexachlorobutadiene. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

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

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 provides an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to hexachlorobutadiene, but may not be inclusive of the entire body of literature.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. Animal inhalation studies are presented in Table 2-1 and Figure 2-2, animal oral studies are presented in Table 2-2 and Figure 2-3, and dermal data are presented in Table 2-3.

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 hexachlorobutadiene 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.

The health effects of hexachlorobutadiene have primarily been evaluated in animal studies. As illustrated in Figure 2-1, data are available following inhalation, oral, or dermal exposure, with about half of the studies involving oral exposure. Animal data are available for each health effect category and exposure duration category. The most examined endpoints were renal (approximately 70% of the animal studies examined this endpoint), body weight (approximately 62%), and hepatic (approximately 42%). Inhalation and dermal exposure studies also examined a range of endpoints. Only two epidemiology studies were identified; these studies examined hepatic and renal endpoints.

The animal studies suggest that the kidney, respiratory tract, and developing organisms are sensitive targets of hexachlorobutadiene toxicity. Liver and hematological effects also have been observed at relatively low doses, but the effects have not been consistently observed across studies.

- **Renal Endpoints:** Experimental animal studies provide strong evidence that the kidney is the most sensitive target of toxicity for hexachlorobutadiene. An epidemiology study also found some evidence of impaired renal function. In rats and mice, exposure to hexachlorobutadiene results in damage to the proximal tubules, particularly in the pars recta region. The observed lesions include epithelial degeneration, regeneration, and necrosis. Increases in urinary protein and glucose levels and increases in blood urea nitrogen levels have also been observed, suggesting impairment of renal function.

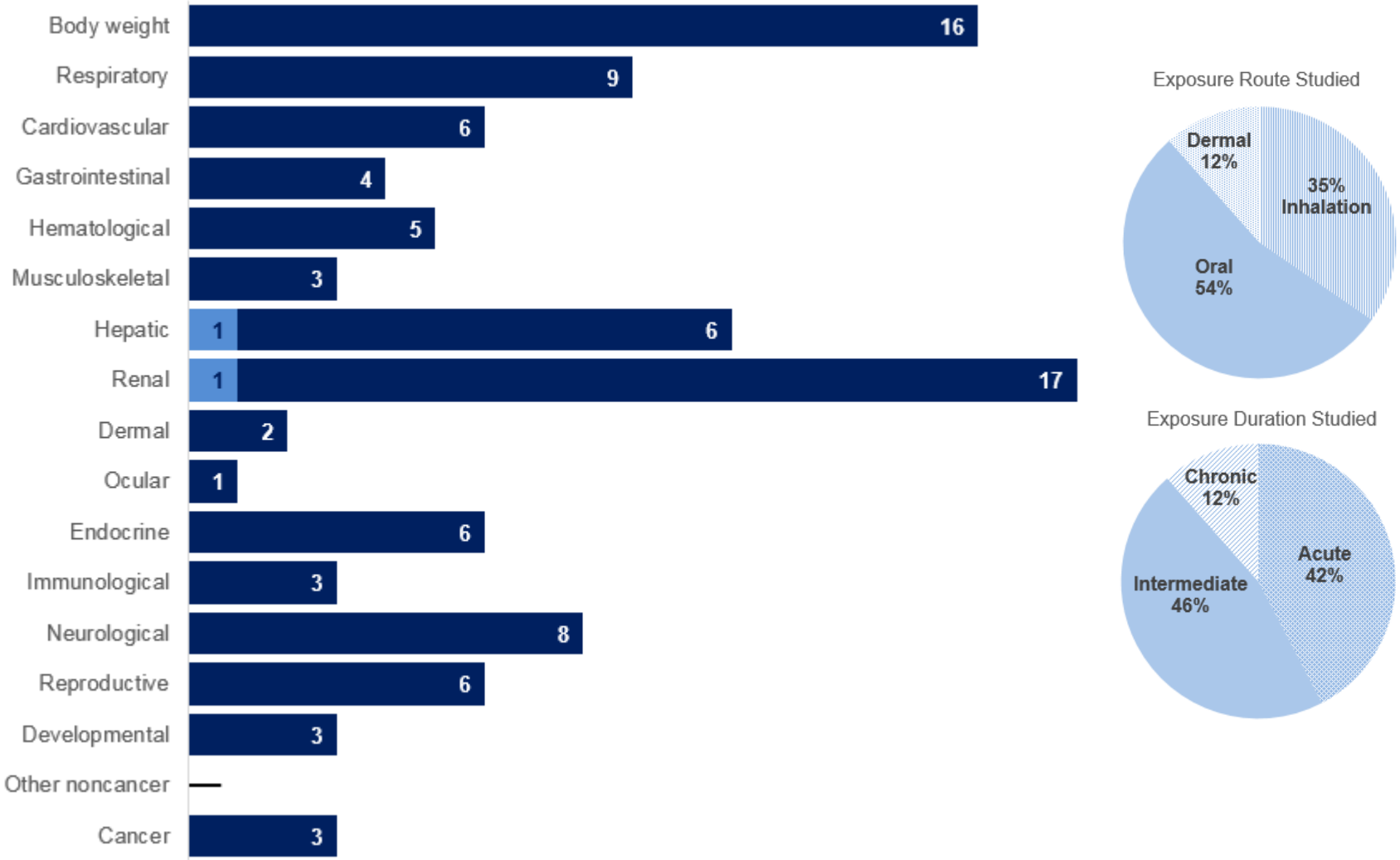
- **Respiratory Endpoints**: Inhalation studies have reported evidence of respiratory irritation in animals following acute- or intermediate-duration exposures. The observed effects include nasal irritation, decreases in respiratory rate, and breathing difficulty.

- **Developmental Endpoints:** Experimental animal studies have consistently found decreases in fetal or pup body weights, but did not find increases in the occurrence of anomalies or malformations. Maternal decreases in body weight was also observed at concentrations or doses resulting in fetal/pup effects.

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

Most studies examined the potential renal, body weight, and hepatic effects of hexachlorobutadiene

More studies evaluated health effects in **animals** than **humans** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 26 studies include those finding no effect. Most studies examined multiple endpoints.

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effect
ACUTE EXPOSURE									
1	Rat (Alderly Park) 4 M, 4 F	2 days 4 hours	250	CS, HP	Resp		250		Nose irritation and respiratory difficulty
					Renal		250		Degeneration of proximal tubules
					Endocr		250		Degeneration of adrenal cortex
					Ocular		250		Eye irritation
Gage 1970									
2	Rat (CD) 10 M	5 days 7 hours/day	0,10, 50	CS, BW, OF	Bd wt			10	57% decrease in body weight gain
					Neuro	10	50		Animals appeared subdued and showed little response to audio stimuli
					Repro	50			No alterations in dominant lethality test
NIOSH 1981									
3	Mouse Swiss 6 M	15 minutes	83, 143, 155, 210, 246	OF	Resp		155		36% decrease in respiratory rate
De Ceaurriz et al. 1988									
4	Mouse Swiss NS M	4 hours	0, 2.75, 5.00, 10.00, 25.00		Renal		2.75		Histochemical evidence of damaged proximal tubules
De Ceaurriz et al. 1988									
5	Mouse (B6C3F1) 10 M	5 days 7 hours/day	0, 10, 50	CS, BW, OF	Death			50	100% mortality
					Bd wt			10	Weight loss
					Repro	10			No alteration in frequency of abnormal sperm
NIOSH 1981									

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

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (ppm)	Parameters monitored	Endpoint	NOAEL (ppm)	Less serious LOAEL (ppm)	Serious LOAEL (ppm)	Effect
INTERMEDIATE EXPOSURE									
6	Rat (Alderly Park) 4 M, 4 F	6 hours/day 5 days/week 12–15 exposures	5, 10, 25, 100	CS, BW, HE, GN, HP	Death			100F	2/4 females died
					Bd wt	5	10		Decreased body weight gain (magnitude not reported)
					Resp	10	25		Respiratory difficulty; nasal irritation was noted at 100 ppm
					Hemato	25	100		Anemia (no additional information provided)
					Renal	10	25		Histological alterations in proximal tubules (no additional information provided); degeneration of cortical tubules and epithelial regeneration were observed at 100 ppm
Gage 1970									
7	Rat Sprague Dawley (19–23 F)	GDs 6–20 6 hours/day	0, 2, 5, 10, 15		Bd wt	2	5	15	Decreased maternal body weight gain (15, 12, and 39% at 5, 10, and 15 ppm)
					Develop	10	15		Fetal body weight reduced by 9.5% in males and 12.5% in females
Saillenfait et al. 1989									

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

Bd wt or BW = body weight; CS = clinical signs; Develop = developmental; Endocr = endocrine; F = female(s); GD = gestation day; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; LOAEL = lowest-observed-adverse-effect level; M = male(s); Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; Repro = reproductive; Resp = respiratory

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

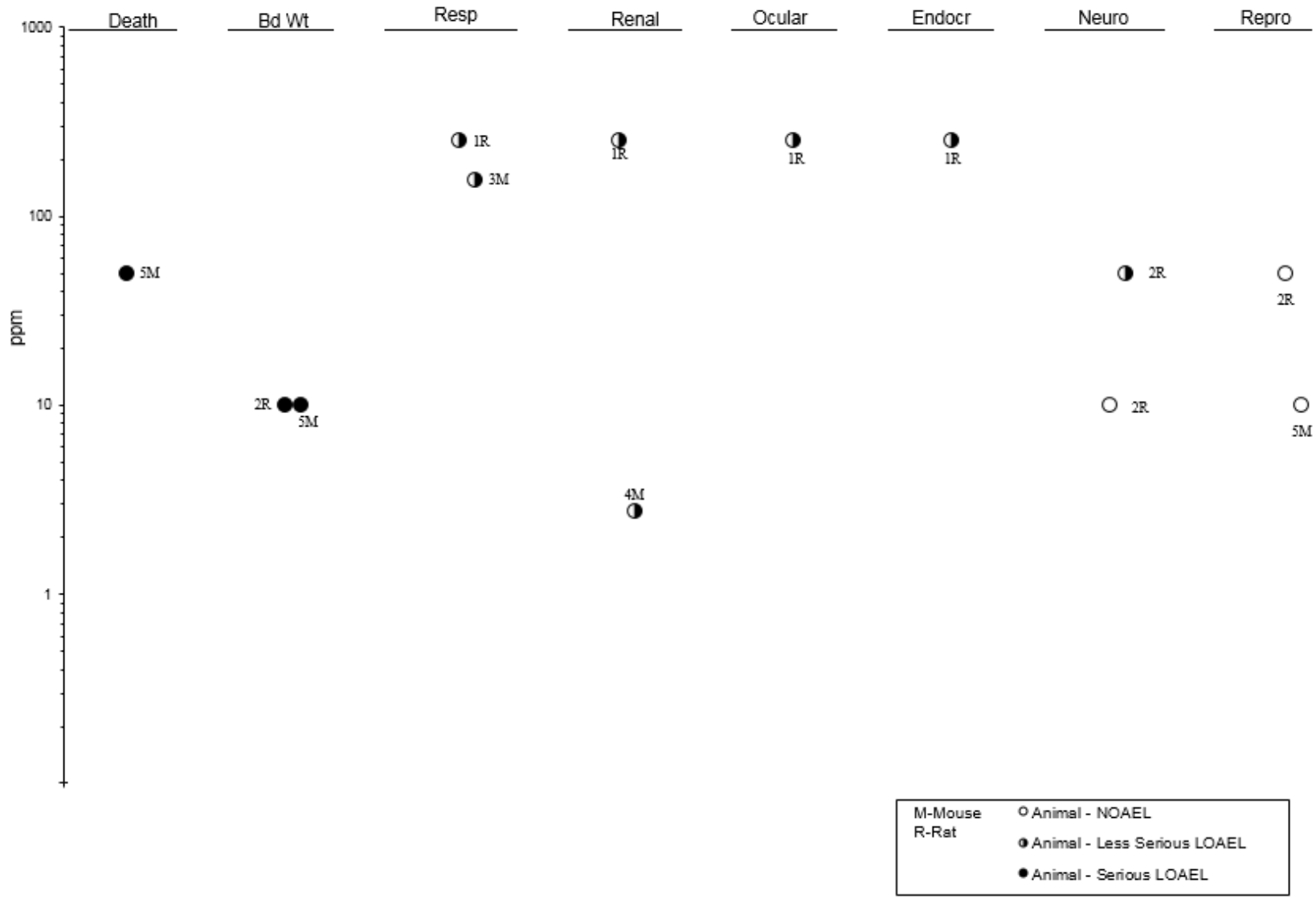


Figure 2-2. Levels of Significant Exposure to Hexachlorobutadiene – Inhalation
Intermediate (15–364 days)

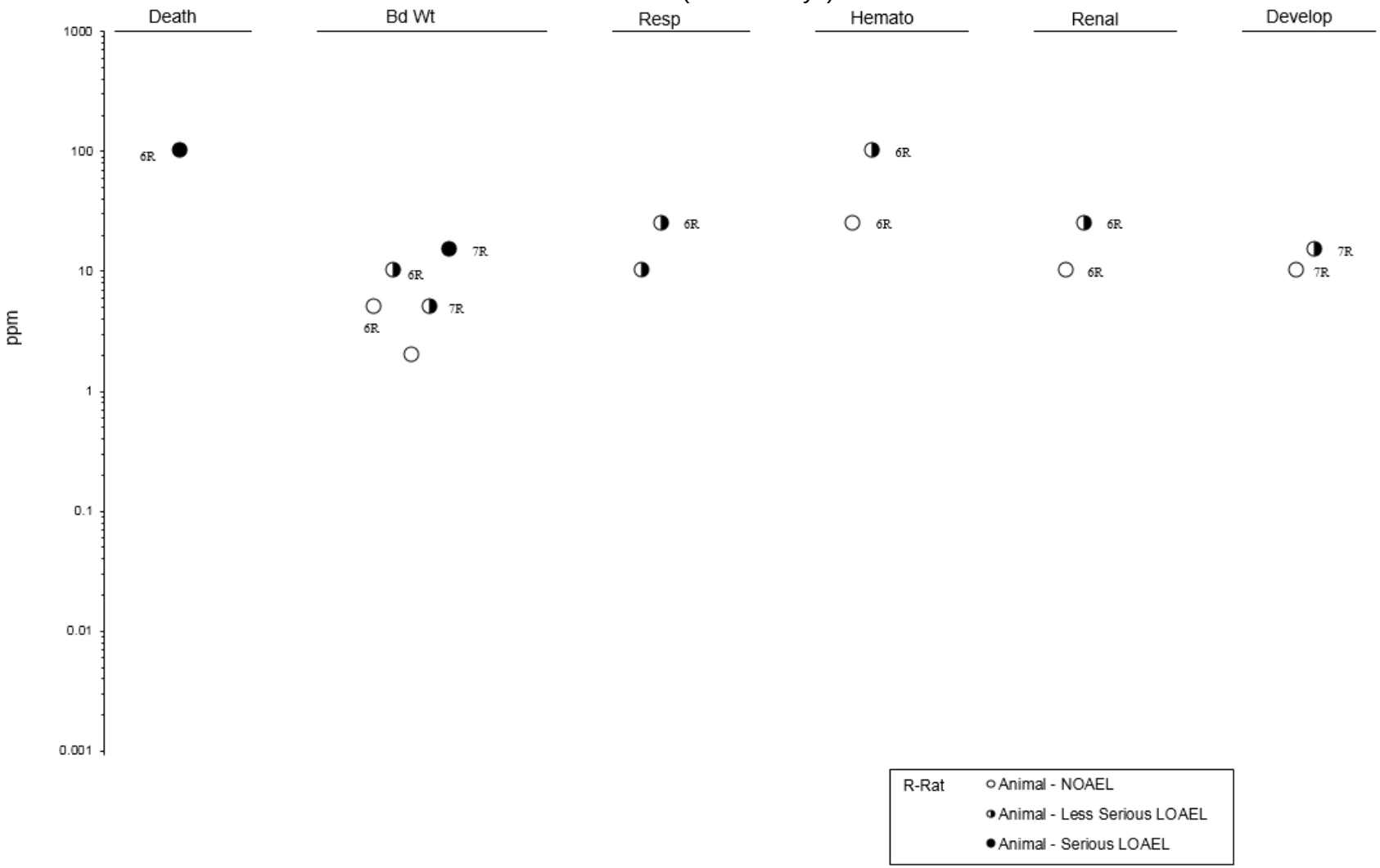


Table 2-2. Levels of Significant Exposure to Hexachlorobutadiene – Oral

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 (Wistar) 4 M, 4 F	Once (GO)	0, 200	HP	Hepatic		200		Cytoplasmic lipid droplets and apoptotic cells in liver
					Renal			200	Extensive epithelial necrosis and degeneration of epithelia in proximal tubules
Birner et al. 1995									
2	Rat (Wistar) 6 M, 6 F	14 days (F)	M: 0, 5.9, 19, 59 F: 0, 6.2, 20, 62	BW, OW, HP	Bd wt	5.9M	19M 6.2F		Body weight was reduced by 9.5% in females
					Hepatic	59M 62F			
					Renal		5.9M[b] 6.2F		Proximal convoluted tubule degeneration
Harleman and Seinen 1979									
3	Rat (Wistar) 5 M	Once (GO)	0, 10, 100, 200	FI, BW, BC, UR, OW, HP	Renal	10	100	200	Increased relative kidney weight, proximal tubular necrosis, increases in serum creatinine; at 200 mg/kg, increases in BUN, and urinary protein, and glucose, increased urine volume and decreased urine density
Jonker et al. 1993a									
INTERMEDIATE EXPOSURE									
4	Rat (Wistar) 10 M, 10 F	13 weeks (GO)	0, 0.4, 1, 2.5, 6.3, 15.6	BW, FI, HE, BC, UR, OW, HP	Bd wt	2.5		6.3	Body weight decreased by 29% in females and by 13% in males
					Resp	15.6			
					Cardio	15.6			
					Gastro	15.6			
					Hemato	15.6			

Table 2-2. Levels of Significant Exposure to Hexachlorobutadiene – 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
					Hepatic	6.3M	15.6M		Increased cytoplasmic basophilia
					Renal	2.5M 1F	6.3M 2.5F		Enlarged hyperchromatic nuclei in the proximal tubules in females at 2.5 mg/kg/day and males at 6.3 mg/kg/day; decreased urine osmolarity in females at 2.5 mg/kg/day
					Endocr	15.6			
					Immuno	15.6			No histological alterations
					Neuro	15.6			No histological alterations
Harleman and Seinen 1979									
5	Rat (Wistar) 6 F	10–18 weeks (F)	0, 15, 150	CS, BW, OW, HP, DX	Bd wt		15		Body weight decreased by 15%
					Resp	150			
					Cardio	150			
					Hepatic	15	150		Slight proliferation of bile duct epithelium
					Renal		15	150	Tubular degeneration and necrosis at 15 mg/kg/day; extensive tubular degeneration at 150 mg/kg/day
					Endocr	150			
					Immuno	150			No histological alterations
					Neuro	15		150	Ataxia, demyelination and fragmentation of femoral nerve fibers
					Repro	15		150	Infertility
					Develop		15		16–19% reduction in pup body weight
Harleman and Seinen 1979									

Table 2-2. Levels of Significant Exposure to Hexachlorobutadiene – 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
6	Rat (Wistar) 5 M, 5 F	4 weeks (F)	0, 2.5, 9.9, 40	BW, HP, OW, UR	Bd wt	2.5	9.9		Reduced body weight in males (9.7%) and females (15%)
					Hepatic	40			
					Renal		2.5F		Decreased BUN at ≥2.5 mg/kg/day in females; diffuse tubular cytomegaly in the inner cortex in females at ≥9.9 mg/kg/day and males at 40 mg/kg/day
Jonker et al. 1993b									
7	Rat (Wistar) 5 F	32 days (GO)	0, 1, 4	BW, HP, OW, UR	Bd wt	4			
					Renal	1	4		Increased GGT in urine (79%), increased relative kidney weight (12.6%), and focal tubular vacuolization
Jonker et al. 1996									
8	Rat (Sprague-Dawley) 4 F	30 days (F)	0, 1, 3, 10, 65, 100	CS, BC, HE, FI, BW, OW, GN, HP	Bd wt	10	30		Decreased body weight gain; 10.5% at 30 mg/kg/day
					Resp	100			
					Cardio	100			
					Gastro	100			
					Hemato	3	10		Increased hemoglobin concentration
					Hepatic	65	100		Centrilobular hepatocellular swelling
					Renal	10	30		Tubular degeneration, necrosis, regeneration
					Endocr	100			
					Immuno	100			No histological alterations
					Neuro	100			No histological alterations
Kociba et al. 1971									

Table 2-2. Levels of Significant Exposure to Hexachlorobutadiene – 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
9	Rat (Wistar) 3 M	3 weeks (F)	0, 7.1, 37, 190	BW, HP	Bd wt	7.1	37		15% reduction in body weight
					Renal	37	190		Proximal tubules lined with basophilic epithelium
Nakagawa et al. 1998									
10	Rat (Wistar) 21 M	30 weeks (F)	0, 94	BW, OW, HP	Bd wt		94		23% decrease in mean final body weight
					Renal	94			No change in BUN or creatinine levels, no histological alterations
Nakagawa et al. 1998									
11	Rat (Sprague-Dawley) 10–12 M, 20–24 F	90 days premating, 15-day mating, GDs 1–21, LDs 1–21 (F)	0, 0.2, 2, 20	CS, BW, FI, BC, UR, HE, OW, HP, RX, DX	Bd wt	2	20		7–17% decreased body weight gain in females; decreases in food intake also observed.
					Resp	20			
					Cardio	20			
					Gastro	20			
					Hemato	20			
					Musc/Skel	20			
					Hepatic	20			
					Renal	0.2F	2F		Tubular dilatation and hypertrophy with foci of epithelial degeneration and regeneration in females at ≥2 mg/kg/day and males at 20 mg/kg/day
					Neuro	20			
					Repro	20			
					Develop	2	20		13% decrease in neonatal weight
Schwetz et al. 1977									

Table 2-2. Levels of Significant Exposure to Hexachlorobutadiene – 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
12	Mouse (B6C3F1) 5 M, 5 F	15 days (F)	M: 0, 3, 12, 40, 19, 24; F: 0, 5, 16, 49, 30, 36	CS, BW, OW, GN, HP	Death				100% mortality at the two highest doses
					Bd wt	3M 5F		12M 16F	Weight loss
					Neuro	12M 16F		40M 49F	Lethargy, hunched position, incoordination
NTP 1991									
13	Mouse (B6C3F1) 10 M, 10 F	13 weeks (F)	M: 0, 0.1, 0.4, 1.5, 4.9, 16.8 F: 0, 0.2, 0.5, 1.8, 4.5, 19.2	CS, BW, FI, OW, GN, HP	Bd wt	1.5M 4.5F	4.9M 19.2F		Body weight gain reduced by 9.9% in males
					Resp	19.2M 16.8F			
					Cardio	19.2M 16.8F			
					Gastro	19.2M 16.8F			
					Musc/skel	19.2M 16.8F			
					Hepatic	19.2M 16.8F			
					Renal	1.5M 0.2F[c]	4.9M 0.5F		Tubular epithelial regeneration in females at ≥0.5 mg/kg/day and in males at ≥4.9 mg/kg/day
					Endocr	19.2M 16.8F			
					Dermal	19.2M 16.8F			
					Immuno	19.2M 16.8F			No histological alterations
					Neuro	19.2M 16.8F			No histological alterations

Table 2-2. Levels of Significant Exposure to Hexachlorobutadiene – 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
					Repro	19.2M 16.8F			No dose-related decreases in sperm motility at 1.5 mg/kg/day; no alterations in sperm count, incidence of abnormal sperm, estrual cyclicity, average estrous cycle length
NTP 1991; Yang et al. 1989									
CHRONIC EXPOSURE									
14	Rat (Sprague-Dawley) 40 M, 40 F	2 years (F)	0, 0.2, 2, 20	BW, HE, BC, UR, OW, GN, HP	Death			20M	Increased mortality in males
					Bd wt	2	20		Mean body weight reduced by 8–20% in males and 5–12% in females
					Resp	20			
					Cardio	20			
					Gastro	20			
					Hemato	20			
					Musc/skel	20			
					Hepatic	20			
					Renal	0.2	2		Tubular epithelial hyperplasia
					Endocr	20			

Table 2-2. Levels of Significant Exposure to Hexachlorobutadiene – 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
					Neuro	20			
					Repro	20			No histological alterations
					Cancer			20	CEL: kidney tumors
Kociba et al. 1977a									

[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 a provisional acute oral Minimal Risk Level (MRL) of 0.006 mg/kg/day; the LOAEL dose was divided by an uncertainty factor of 1,000 (10 for the use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability)
[c]Used to derive a provisional intermediate oral minimal risk level (MRL) of 0.002 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

BC = blood chemistry; Bd wt or BW = body weight; BUN = blood urea nitrogen; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; G = gavage; Gastro = gastrointestinal; GGT = gamma-glutamyl transferase; GN = gross necropsy; (GO) = gavage in oil vehicle; HE = hematology; Hemato = hematological; HP = histopathological; Immuno = immunological; LOAEL = lowest-observed-adverse-effect level; LD_{50} = lethal dose, 50% kill; M = male(s); Musc/skel = muscular/skeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; UR = urinalysis

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

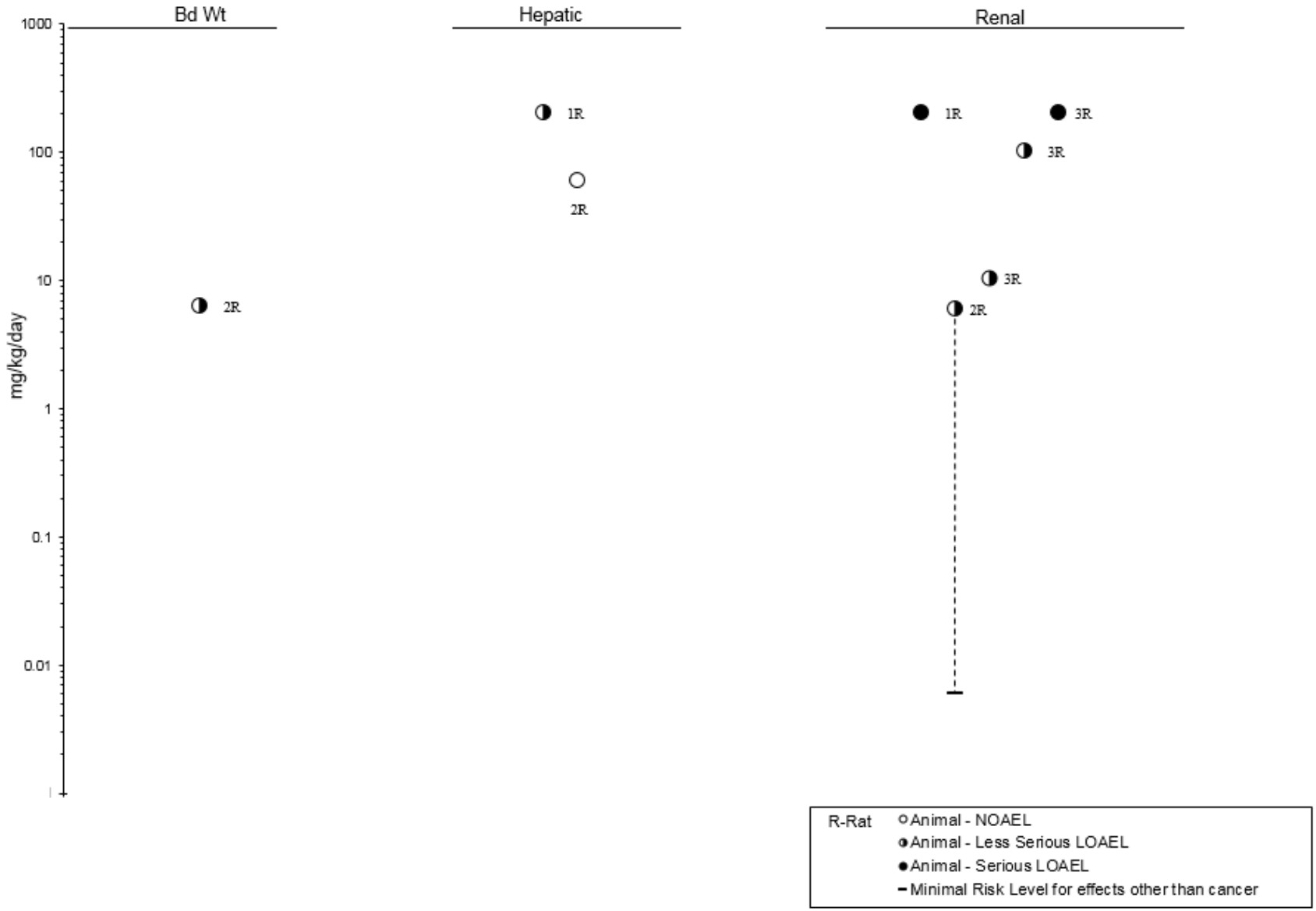


Figure 2-3. Levels of Significant Exposure to Hexachlorobutadiene – Oral

Intermediate (15–364 days)

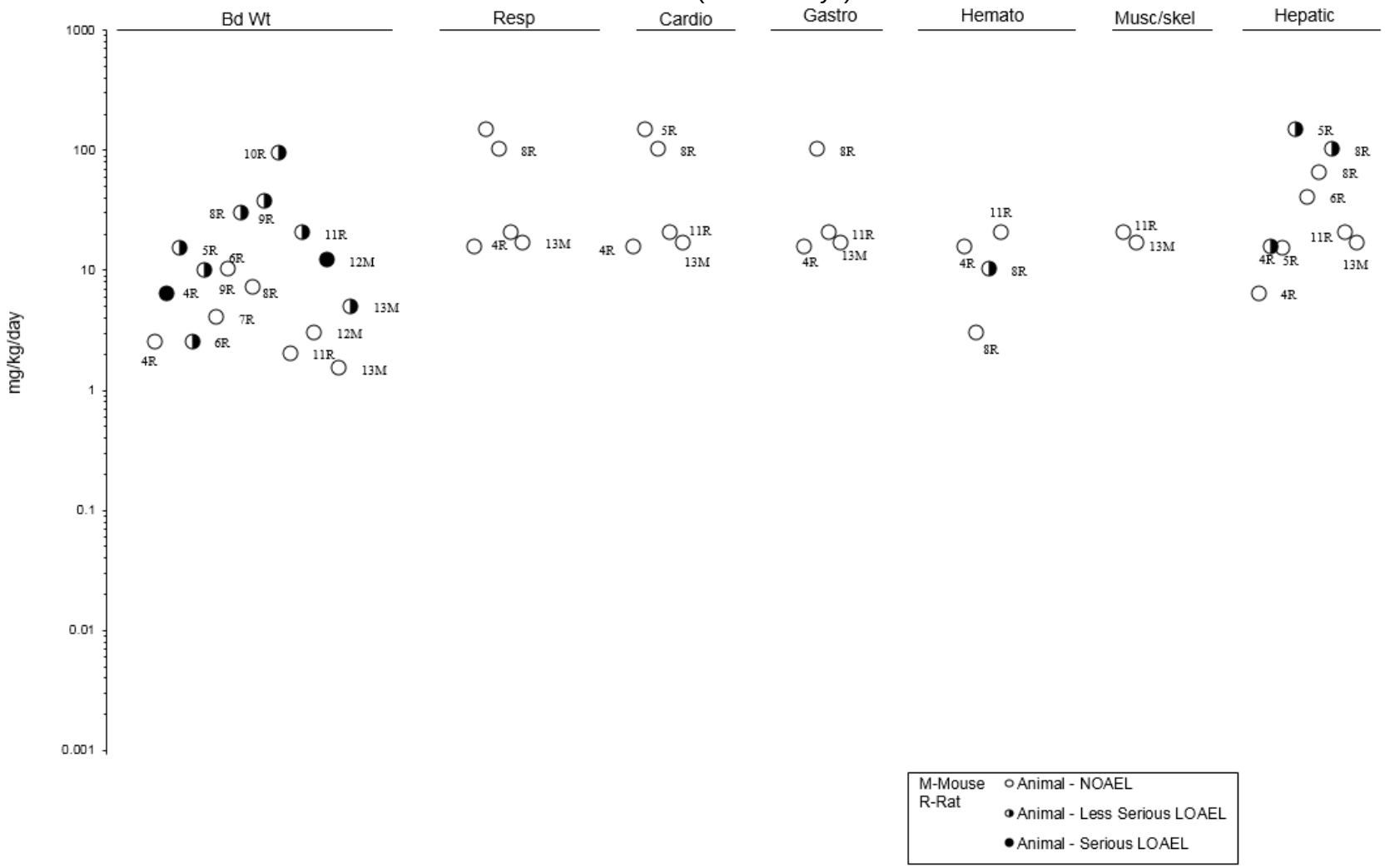


Figure 2-3. Levels of Significant Exposure to Hexachlorobutadiene – Oral
Intermediate (15–364 days)

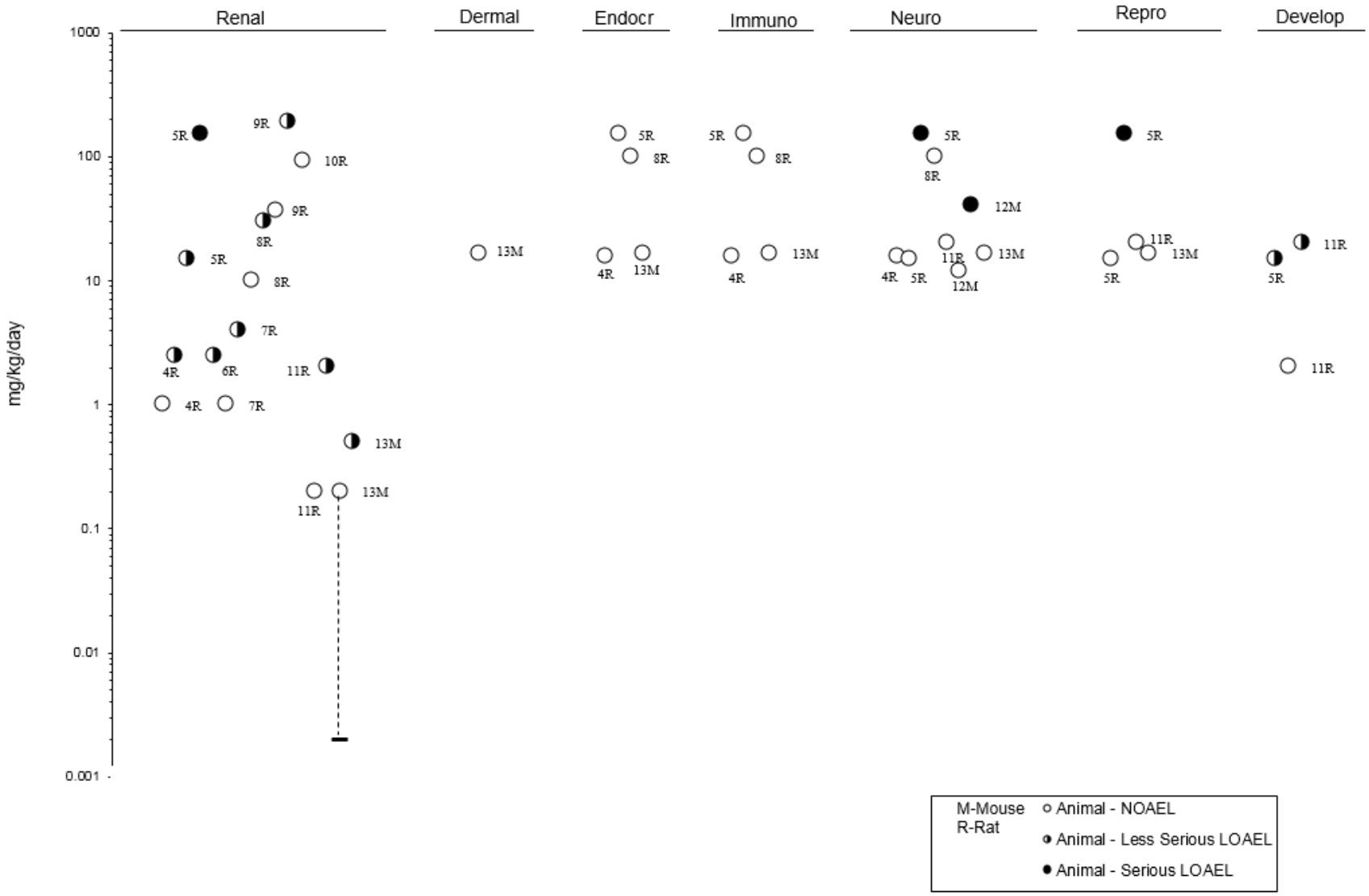


Figure 2-3. Levels of Significant Exposure to Hexachlorobutadiene – Oral
Chronic (≥365 days)

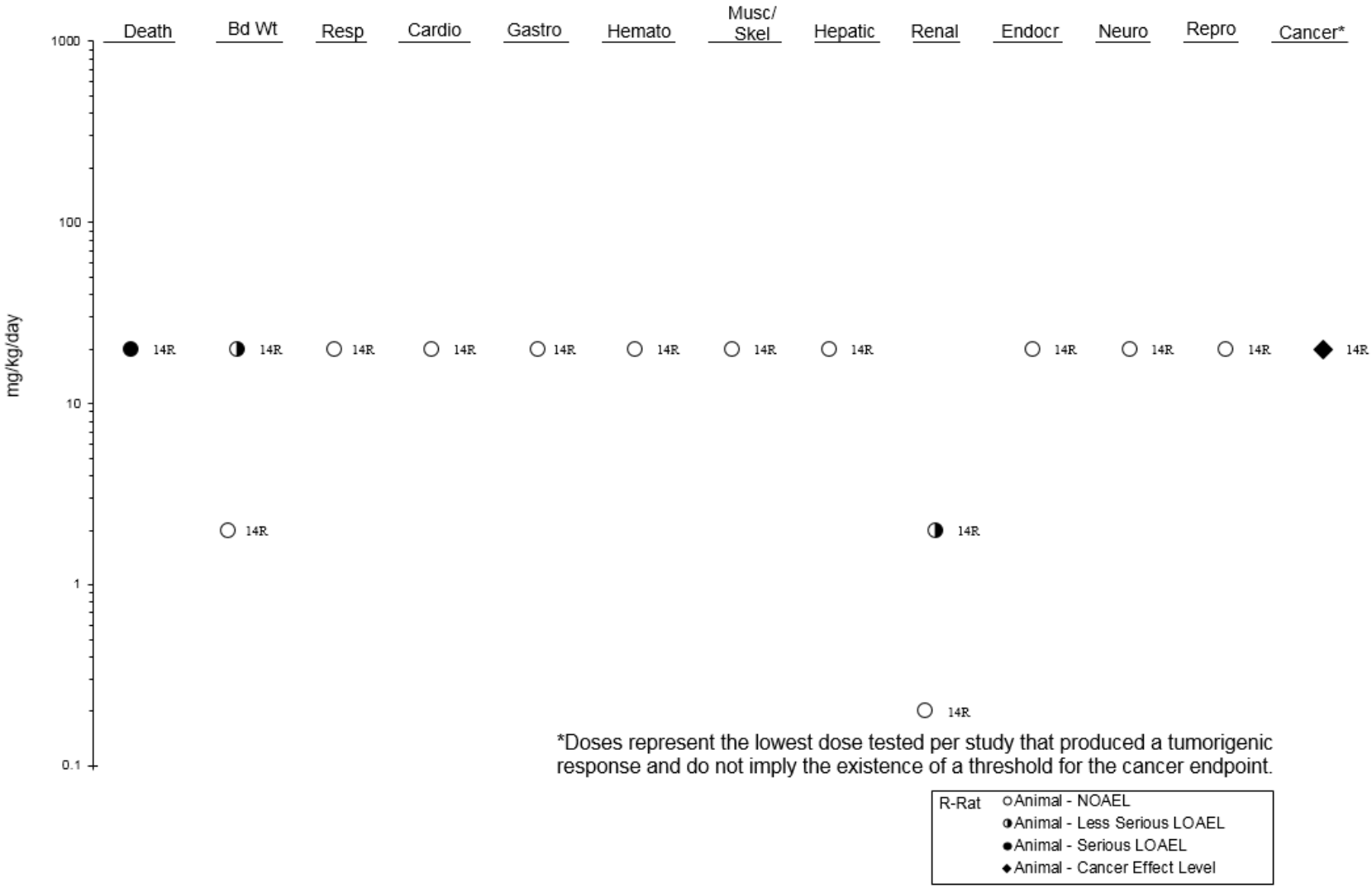


Table 2-3. Levels of Significant Exposure to Hexachlorobutadiene – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effect
ACUTE EXPOSURE								
Rabbit (New Zealand) 10 F	Once 8 hours	0, 388, 775, 1,160, 1,550 mg/kg	LE, CS, OW, HP	Death			1,116	LD_{50}
				Hepatic		388		Hydropic changes at ≥388 mg/kg; fatty degeneration at ≥775 mg/kg
				Renal		388	1,550	Epithelial regeneration at ≥388 mg/kg; necrotizing nephritis at 1,550 mg/kg
				Dermal		388		Cutaneous necrosis
Duprat and Gradiski 1978								
Mouse (Swiss) 30 F	Once	15 mg/mouse	GN, HP	Cancer				No increases in incidence of papillomas in initiation/promotion assay
Van Duuren et al. 1979								
CHRONIC EXPOSURE								
Mouse (Swiss) 30 F	3 times/week 440–594 days	6.0 mg/mouse	GN, HP	Cancer				No increases in tumor incidences
Van Duuren et al. 1979								

CS = clinical signs; F = female(s); GN = gross necropsy; HP = histopathology; LD_{50} = lethal dose, 50 % kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect-level; NS = not specified; OW = organ weight

2.2 DEATH

In animals, all mice that were exposed to vapors of 50 ppm hexachlorobutadiene for 5 days died, but no deaths occurred at 10 ppm (NIOSH 1981).

In an acute oral exposure study, young rats were more sensitive to hexachlorobutadiene than adult rats. LD_{50} values for adult rats were 580 mg/kg (males) and 200-400 mg/kg (females). The LD_{50} values for weanling male and female rats were 65 and 46 mg/kg, respectively (Kociba et al. 1977a). Important experimental details of this study were not available for review.

In an intermediate-duration study, mice exposed to high concentrations of hexachlorobutadiene in their diet (1,000 or 3,000 mg/kg diet) died after 3–5 days of exposure (NTP 1991; Yang et al. 1989); no deaths were observed in mice exposed to lower dietary concentrations (30–300 mg/kg diet, equivalent to doses of 5–49 mg/kg/day) for 15 days. Survival was not reduced in rats exposed to 100 mg/kg/day hexachlorobutadiene for 30 days (Kociba et al. 1971) or 15.6 mg/kg/day for 13 weeks (Harleman and Seinen 1979). Male and female mice survived dose levels of up to 16.8 or 19.2 mg/kg/day for 13 weeks (NTP 1991). In lifetime studies, survival was reduced significantly in male rats exposed to 20 mg/kg/day hexachlorobutadiene in the diet for 2 years (Kociba et al. 1977a). Although the cause of death was not reported, renal damage, a major effect manifested by this compound, may have been a contributing factor.

In an acute dermal lethality study, 2–8 rabbits died during the 14-day observation period after 8-hour exposure to doses of 775–1,550 mg/kg applied directly to shaved skin, but no deaths occurred in the 388 mg/kg dose group. The authors calculated an LD_{50} of 1,116 mg/kg from these data (Duprat and Gradiski 1978). Central nervous system depression was evident, as manifested by stupor. Some animals were weak and anorexic, while others showed signs of dyspnea and cyanosis. The lungs, liver, and kidneys were congested in animals that died. Death was reportedly due to respiratory or cardiac failure.

2.3 BODY WEIGHT

Decreases in body weight gain have been observed in rats and mice following inhalation and/or oral exposure to hexachlorobutadiene. Inhalation exposure to ≥10 ppm 6 hours/day, 5 days/week for 12–15 exposures resulted in decreases in body weight gain, but the magnitude of the effect was not reported (Gage 1970). In an acute oral study, a 9.5% decrease in body weight gain was observed in rats (Harleman and Seinen 1979). Dietary exposure to 9.9–94 mg/kg/day for 3–18 weeks resulted in 10–23% decreases in body weight gain in rats (Harleman and Seinen 1979; Jonker et al. 1993b; Kociba et al. 1971;

Nakagawa et al. 1998). A 29% decrease in terminal weights was observed in female rats administered via gavage hexachlorobutadiene in oil for 13 weeks (Harleman and Seinen 1979). In a companion study, 18 weeks of dietary exposure to 15 mg/kg/day resulted in a 15% decrease in terminal body weights in female rats (Harleman and Seinen 1979), suggesting that gavage administration may have a greater impact on body weight gain than dietary exposure. An approximate 10% decrease in body weight gain was observed in mice exposed to 4.9 mg/kg/day for 13 weeks (NTP 1991); weight loss was observed following a 15-day exposure to 12 mg/kg/day (NTP 1991). Chronic duration exposure to 20 mg/kg/day in the diet for 2 years resulted in 5–20% decreases in body weight gain in rats (Kociba et al. 1977a).

Inhalation and oral studies also reported decreases in maternal body weight gain. In an inhalation study, decreases in maternal weight gain were observed in rat dams exposed to 5 ppm on gestation days (GDs) 6–20 (Saillenfait et al. 1989); at 15 ppm, a 39% decrease in body weight gain was observed. In rats exposed for 90 days prior to mating and throughout the mating, gestation, and lactation periods, 7–17% decreases in body weight gain were observed at 20 mg/kg/day (Schwetz et al. 1977); the study also reported a decrease in food intake at this dose level.

2.4 RESPIRATORY

Respiratory rates were decreased in mice exposed to vapors of hexachlorobutadiene at concentrations of ≥155 ppm for 15 minutes. The authors characterized the responses as a reaction to nasal irritation (de Ceaurriz et al. 1988). Nasal irritation and respiratory difficulty were also reported in rats exposed to vapors at a concentration of 250 ppm for 2 days (4 hours/day) or 100 ppm for 12 exposures (6 hours/day, 5 days/week) (Gage 1970). Breathing difficulty was also observed in rats exposed to 25 ppm 6 hours/day, 5 days/week for 15 exposures.

Intermediate-duration exposure to doses as high as 150 mg/kg/day (Harleman and Seinen 1979; Kociba et al. 1971; NCI 1991; Schwetz et al. 1977) or chronic exposures to 20 mg/kg/day (Kociba et al. 1977a) did not cause treatment-related lesions of the lungs or changes in lung weight in rats or mice.

2.5 CARDIOVASCULAR

Hexachlorobutadiene did not alter heart weights or cause treatment-related lesions of the heart in rats or mice exposed for intermediate durations at dose levels of 15.6–150 mg/kg/day (Harleman and Seinen 1979; Kociba et al. 1971; NTP 1991; Schwetz et al. 1977; Yang et al. 1989) or after chronic exposure at dose levels of 20 mg/kg/day (Kociba et al. 1977a).

2.6 GASTROINTESTINAL

Intermediate-duration exposure did not cause treatment-related histopathological lesions in the esophagus, stomach, small intestines, or large intestines in rats exposed to hexachlorobutadiene at dose levels up to 100 mg/kg/day (Harleman and Seinen 1979; Kociba et al. 1971; Schwetz et al. 1977) or in mice exposed to doses as high as 19.2 mg/kg/day (NCI 1991). Lifetime exposure at dose levels of 20 mg/kg/day (Kociba et al. 1977a) did not result in any effect on this system.

2.7 HEMATOLOGICAL

Evaluations of hematological parameters in rats revealed no treatment-related alterations in packed cell volume, red blood cell count, hemoglobin concentration, total white blood cell count, or differential white blood cell count in animals exposed to a dose level of 15.6 or 20 mg/kg/day after intermediate-duration oral exposure (Harleman and Seinen 1979; Schwetz et al. 1977). Similarly, chronic oral exposure (20 mg/kg/day) did not cause hematological effects (Kociba et al. 1977a). However, inhalation and oral studies reported hematological alterations. Anemia was reported in rats exposed to 100 ppm airborne hexachlorobutadiene for 12 exposures (6 hours/day, 5 days/week) (Gage 1970); no additional information was provided. In the oral study, hemoglobin concentration increased in rats at dose levels from 10 to 100 mg/kg/day, but not at 3 mg/kg/day, for 30 days (Kociba et al. 1971). Other hematologic parameters were within normal values.

2.8 MUSCULOSKELETAL

No treatment-related lesions of the musculoskeletal system were observed in rats exposed to dose levels of 20 mg/kg/day hexachlorobutadiene for up to 148 days (Harleman and Seinen 1979; Schwetz et al. 1977) or 2 years (Kociba et al. 1977a).

2.9 HEPATIC

Although the liver is not a major target of hexachlorobutadiene toxicity, there is some indication that it may be adversely affected following exposure in humans. Serum bile acids (deoxycholic acid, glycine deoxycholic acid, taurine-chenodeoxycholic acid, and total deoxycholate) increased following chronic exposure in workers to estimated exposure levels of 0.005–0.02 ppm (Driscoll et al. 1992). It should be noted that the workers were also potentially exposed to other solvents (carbon tetrachloride and

perchloroethylene). For this reason, and the fact that data are absent on morphological changes as well as other effects on liver function, the practical importance of this finding is questionable.

Oral studies in experimental animals provide some evidence that hexachlorobutadiene can affect the liver, but the results are inconsistent across studies. Cytoplasmic lipid droplets and apoptotic cells were observed in the livers of rats receiving a single gavage dose of 200 mg/kg hexachlorobutadiene (Birner et al. 1995). No liver effects were observed at the highest dose tested (59 mg/kg/day in males and 62 mg/kg/day in females) in another acute oral study (Harleman and Seinen 1979). Absolute liver weights were decreased in female rats fed ≥9.9 mg/kg/day and in males at 40 mg/kg/day hexachlorobutadiene for 4 weeks (Jonker et al. 1993b); these decreases may be secondary to the decreases in body weight also observed at these doses. Relatively small increases in aspartate aminotransferase levels (22–43%) and increases in total bilirubin levels (145– 577%) were observed in males and females at 40 mg/kg/day; no histological examination of the liver was conducted. This study (Jonker et al. 1993b) does not provide conclusive evidence of hepatotoxicity, and the highest dose was categorized as a NOAEL. Cytoplasmic basophilia and increased liver weights were observed in male rats administered hexachlorobutadiene by gavage at dose levels of 15.6 mg/kg/day for 13 weeks; treatment-related lesions were not observed in females (Harleman and Seinen 1979). In a second study by this group, slight proliferation of the bile duct epithelium was observed in female rats exposed to 150 mg/kg/day hexachlorobutadiene in the diet for 18 weeks (Harleman and Seinen 1979). Hepatocellular swelling and decreases in absolute and relative liver weights at 100 mg/kg/day and decreases in absolute liver weight at 65 mg/kg/day were observed in female rats fed diets containing hexachlorobutadiene for 30 days (Kociba et al. 1971). Two other intermediate-duration dietary studies did not report adverse hepatic histological alterations in rats exposed to 20 mg/kg/day for approximately 150 days (Schwetz et al. 1977) or male and female rats exposed to 19.2 or 16.8 mg/kg/day, respectively, for 13 weeks (NTP 1991; Yang et al. 1989). Although histological lesions were not observed in a chronic study, urinary excretion of coproporphyrin increased at dose levels of 20 mg/kg/day, suggesting alterations in heme synthesis in the liver (Kociba et al. 1977a).

Hydropic changes at 388 mg/kg and fatty degeneration at ≥775 mg/kg were observed in rabbits after dermal exposure to hexachlorobutadiene for 8 hours (Duprat and Gradiski 1978). These effects were reversible within 5 weeks.

2.10 RENAL

Data on the renal toxicity of hexachlorobutadiene in humans are limited to a study of residents living near a contaminated site containing hexachlorobutadiene (Staples et al. 2003). Indoor air monitoring in 20 homes revealed hexachlorobutadiene concentrations as high as 6.8 ppm. Urinary biomarkers of renal toxicity were measured in 47 adults and children living in homes with hexachlorobutadiene levels of at least 0.0006 ppm; urine samples were collected 2 months after the subjects vacated their homes. Abnormal levels of several biomarkers of proximal tubular damage (α-glutathione-S-transferase, γ-glutamyltransferase, leucine aminopeptidase) and a biomarker of distal tubular damage (π-glutathione-S-transferase) were observed in 19–22% of the subjects; no increases in biomarkers of glomerular damage (urinary albumin and transferrin levels) were found. Urinary biomarkers were re-evaluated 10 months after the subjects left their homes; proximal and distal tubular biomarkers significantly decreased, although 14, 8, and 8% of the subjects still had abnormal γ-glutamyltransferase, leucine aminopeptidase, and π-glutathione-S-transferase levels, respectively.

Acute-, intermediate-, and chronic-duration studies in experimental animals provide strong evidence that the kidney is the primary target organ following inhalation, oral, dermal, or parenteral exposure to hexachlorobutadiene; the toxicity does not appear to be route-specific. The renal effects are characterized as alterations in organ weight (increases or decreases), histological damage to the proximal convoluted tubules, and alterations in serum and urinary parameters indicative of damage or decreased renal function.

Inhalation exposure of mice to 2.75–25 ppm for 4 hours showed an increase in the number of damaged cortical renal tubules (de Ceaurriz et al. 1988). The percentage of damaged tubules increased with exposure concentration; ranging from 4% at 2.75 ppm to 91% at 25 ppm (percentage of damaged tubules were 0.18–1.51% in controls). Degeneration in the proximal tubule was observed in rats following exposure to 250 ppm hexachlorobutadiene for 4 hours on each of 2 consecutive days (Gage 1970). In the only intermediate-duration inhalation study examining the kidneys (Gage 1970), unspecified damage was observed in the proximal tubules of rats exposed to 25 ppm for 15 days (6 hours/day, 5 days/week); at 100 ppm (6 hours/day, 5 days/week), degeneration of renal cortical tubules with epithelial regeneration occurred after 12 days of exposure. No renal lesions were observed in rats exposed to 10 ppm for 15 days.

Oral studies have reported alterations in kidney weights; most studies reported increases in relative kidney weight (Harleman and Seinen 1979; Jonker et al. 1993a, 1996; Kociba et al. 1971, 1977a; Schwetz et al.

1977) and one study in mice reported a decrease in relative kidney weight (NTP 1991). However, the alterations in kidney weight were sometimes observed at doses higher than those associated with histological alterations (Harleman and Seinen 1979; Jonker et al. 1993b; Kociba et al. 1977a), suggesting that kidney weight may not be a sensitive biomarker of hexachlorobutadiene-induced renal toxicity.

Proximal tubule lesions have been observed after acute-, intermediate-, and chronic-duration exposures to hexachlorobutadiene in the diet or administered via gavage. Single gavage doses of 100 or 200 mg/kg have resulted in proximal tubular necrosis in rats (Birner et al. 1995; Jonker et al. 1993a); at 200 mg/kg, the necrosis was characterized as extensive (Birner et al. 1995). No renal lesions were observed at 10 mg/kg (Jonker et al. 1993a). In acute studies in which rats were fed hexachlorobutadiene (5.9 and 6.2 mg/kg/day in males and females) in the diet for 14 days, degeneration of tubular epithelial cells mainly confined to the straight limbs of the proximal tubules located in the outer zone of the medulla were observed (Harleman and Seinen 1979). Intermediate-duration exposure of rats to hexachlorobutadiene resulted in tubular dilatation with hypertrophy and foci of epithelial degeneration and regeneration at ≥2 mg/kg/day (Schwetz et al. 1977), enlarged hyperchromatic nuclei (Harleman and Seinen 1979) or diffuse tubular cytomegaly (Jonker et al. 1993b) at ≥2.5 mg/kg/day, focal tubular vacuolization at 4 mg/kg/day (Jonker et al. 1996), and tubular degeneration, necrosis, and regeneration at ≥15 mg/kg/day (Harleman and Seinen 1979; Kociba et al. 1971). In mice, necrosis and regeneration were observed in males and females exposed to 3 and 5 mg/kg/day for 15 days (NTP 1991) and degeneration was observed in males and females at ≥4.9 and 0.5 mg/kg/day for 13 weeks (NTP 1991). In the only available chronic-duration study, hyperplasia was observed in the proximal tubules of rats exposed to 20 mg/kg/day for 2 years; the investigators noted that similar lesions were observed in a smaller number of animals in the 2 mg/kg/day group, but did not provide incidence data (Kociba et al. 1977a). It is noted that increases in renal neoplasms were also observed at 20 mg/kg/day. No effects were observed at 0.2 mg/kg/day.

Oral studies in rats have found alterations in serum and urinary parameters of renal function. Increases in urinary N-acetyl-β-glucosaminidase (NAG) were observed in rats receiving a single gavage dose of 100 mg/kg hexachlorobutadiene (Jonker et al. 1993a). At 200 mg/kg, increases in urinary protein and glucose excretion, increases in urinary volume, and decreases in urine density were also observed. In a 13-week gavage study, the ability to concentrate urine (as measured by urine osmolarity) was significantly reduced in female rats at dose levels of 2.5–15.6 mg/kg/day and in male rats at 15.6 mg/kg/day (Harleman and Seinen 1979); a decrease in urine production was observed in females at ≥6.3 mg/kg/day. High-dose exposures (≥100 mg/kg) have been associated with increases in serum

creatinine and urea nitrogen levels (Jonker et al. 1993a). At lower doses, increases in these serum parameters have not been observed (Harleman and Seinen 1979; Kociba et al. 1977a; Schwetz et al. 1977). Jonker et al. (1993b) reported decreases in serum urea nitrogen levels in female rats exposed to ≥2.5 mg/kg/day; however, decreases in urea nitrogen are not typically associated with impaired renal function.

Acute-duration dermal exposure in rabbits caused tubular necrosis 24 hours after exposure at dose levels 388 mg/kg or greater (Duprat and Gradiski 1978). The effects were partly reversible, as evident by epithelial regeneration 2 and 5 weeks after exposure.

Oral exposure studies provide evidence of sex-related differences in the renal toxicity of hexachlorobutadiene. Although similar effects were observed in males and females, effects occurred at lower doses in females. In most studies testing males and females, the LOAELs in females were NOAELs for males (Harleman and Seinen 1979; NTP 1991; Schwetz et al. 1977); see Table 2-4. Possible sex-related differences in renal toxicity may be due to metabolic differences between male and female rats (Birner et al. 1995). In females, cysteine conjugates were the primary urinary metabolites which contrasts with the finding in males that cysteine conjugates were minor metabolites and high levels of parent compound was found in the urine (see Section 3.1.3 for additional details).

Table 2-4. Comparison of LOAELs for Renal Effects in Males and Females

Species	Exposure	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Effect	Reference
Rat	14 days		6.2 F[a] 5.9 M[a]	Tubular degeneration	Harleman and Seinen 1979
Rat	13 weeks (GO)	1F 2.5 M	2.5 F 6.3 M	Enlarged hyperchromatic nuclei in the proximal tubules decreased urine osmolarity in females	Harleman and Seinen 1979
Mouse	13 weeks (F)	0.2 F 1.5 M	0.5 F[a] 4.9 M	Tubular epithelial regeneration	NTP 1991
Rat	147 days (F)	0.2 F 2 M	2 F 20 M	Tubular dilation and hypertrophy with foci of degeneration and regeneration	Schwetz et al. 1977

[a]Lowest dose tested.

(F) = feed; F = female(s); (GO) = gavage in oil; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level

Parenteral exposure studies have evaluated the time course of renal damage and possible age-related differences in toxicity. Single intraperitoneal injection studies demonstrate that histological damage to the pars recta portion of the proximal tubules occurs shortly after exposure. In male rats exposed to 100 mg/kg, minimal necrosis was observed 6 hours post-exposure (Cristofori et al. 2013). The severity of the necrosis continued to increase over time and was graded as marked 24 hours post-exposure; 48–72 hours post-exposure, regeneration was observed and marked regeneration was observed 96 hours post-exposure. In male rats administered 45 mg/kg, degeneration was observed 1–3 days post-exposure, followed by regeneration 2–5 days post-exposure; the kidneys appeared normal 28 days after exposure (Maguire et al. 2013). A similar pattern was observed for changes in urinary parameters. Administration of 120 or 170 mg/kg hexachlorobutadiene resulted in increases in urine volume and urinary protein, glucose, and albumin levels within 24 hours of exposure (Kirby and Bach 1995; Swain et al. 2011). Urinary protein levels remained elevated until 7–8 days post-exposure and glucose levels remained elevated until 4 or 7 days post-exposure depending on the administered dose level. At a lower dose level (45 mg/kg), urine volume was not decreased until post-exposure day 5 and urine glucose, protein, and albumin levels were increased on post-exposure days 1–5 (Maguire et al. 2013).

Three studies examining possible age-related differences in renal toxicity in male rats aged 1–12 months and receiving single intraperitoneal injections of 100 mg/kg hexachlorobutadiene did not find age-related differences in damage to the pars recta portion of the proximal tubules (Chiusolo et al. 2008; Cristofori et al. 2013; Zanetti et al. 2010). One study (Zanetti et al. 2010) did find a greater magnitude of increases in urinary protein and NAG levels in 1-month-old rats as compared to the change observed in rats 2, 6, 9, or 12 months of age.

Repeated exposure of young male rats to 25 mg/kg administered via intraperitoneal injection with a corn oil vehicle demonstrated recovery of tissue damage and resistance to future damage (Boroushaki 2003). Extensive renal tubular necrosis of the proximal tubules was observed in the pars recta of rats exposed for 2 or 3 days; marked increases in blood urea nitrogen (BUN) were also observed. In rats exposed for 4 or 7 days, proximal tubule regeneration was observed in the corticomedullary junction; BUN levels were higher than controls, but were not significantly different from rats exposed for 2 days. The investigators suggested that the less severe tissue damage was due to the replacement of the damage cells with cells containing lower levels of cysteine-conjugate β-lyase and were thus resistant to hexachlorobutadiene toxicity. This is supported by the finding in rats exposed to 25 mg/kg/day for 2 days, allowed to recover for 14 or 21 days, and then administered 25 mg/kg/day for 2 days; only minor damage and regenerating tubules were observed after the re-dosing. A similar finding was observed when the animals were re-

dosed with a single dose of 100 mg/kg after a 14-day recovery. But in animals allowed to recover for 21 days, a subsequent single dose of 100 mg/kg resulted in extensive damage in the pars recta.

Much of the data related to the mechanism of hexachlorobutadiene toxicity indicate that the intermediates produced by modification of the cysteine derivative, S-(1,2,3,4,4-pentachlorobuta-1,3-dienyl)-L-cysteine (PCBC), are responsible for the observed effects on the proximal tubules (Cristofori et al. 2015). PCBC is formed from the hexachlorobutadiene conjugate S-1,2,3,4,4-pentachlorobuta-1,3-dienyl)-L-glutathione in the liver, intestines, and/or kidney through the action of γ-glutamyl transferase, which removes the glutamate from the glutathione tripeptide followed by the action of a peptidase that removes the glycine from the carboxy terminus (Cristofori et al. 2015).

PCBC is further metabolized to simpler sulfur derivatives through the action of β-lyase. β-Lyase is present in the rodent liver, intestines, and kidneys (Birner et al. 1998; Jones et al. 1988; MacFarlane et al. 1989). In the kidney, the highest concentration of β-lyase is located in the pars recta of the proximal tubule, the same area that is damaged by hexachlorobutadiene. It should be noted that β-lyase has been detected in the entire proximal segment (Jones et al. 1988). It is present in both the cytosol and mitochondria and is pyridoxal phosphate dependent (MacFarlane et al. 1989). It degrades the cysteine conjugate to pyruvate, ammonia, and one or more reactive thiols (Dekant et al. 1990b; Schnellmann et al. 1987). A highly reactive thioketene may form as an intermediate and cause local tissue damage (Dekant et al. 1991; Koob and Dekant 1992). In male rats, PCBC can also be acetylated in the kidney to form N-acetyl-S-(1,2,3,4,4-pentachlorobuta-1,3-dienyl)-L-cysteine (N-AcPCBC); sulfoxidation of N-AcPCBC results in the formation of N-acetyl-S-(1,2,3,4,4-pentachlorobuta-1,3-dienyl)-L-cyteine sulfoxide (N-AcPCBC-SO) (Birner et al. 1998). N-AcPCBC-SO has also been shown to be nephrotoxic.

The effects of PCBC on the activity of the cells of the proximal tubules was evaluated in cells from New Zealand White rabbits (Schnellmann et al. 1987). These studies indicate that the metabolites of the cysteine conjugate alter the action of the mitochondria in a two-phase process. The first phase apparently causes an uncoupling of oxidative phosphorylation thereby preventing the generation of ATP. The deficiency of ATP in turn limits ATP-dependent active transport in the tubules, inhibiting reabsorption processes. In the second phase, inhibition of cytochrome c-cytochrome oxidase activity and electron transport occur (Schnellmann et al. 1987). These changes result in cell damage, as reflected in a decrease in the cellular retention of lactate dehydrogenase approximately 1 hour after exposure.

2.11 DERMAL

Upper dermis fibrosis and epidermal acanthosis were observed in rabbits exposed to 388 mg/kg hexachlorobutadiene for 8 hours (Duprat and Gradiski 1978); at 1,160 mg/kg, epidermal degeneration, and edema in the dermis and subcutaneous tissues were observed within 12 hours of exposure termination. Effects at 388 mg/kg were not observed until at least 5 days post-exposure.

2.12 OCULAR

There are limited data on the ocular toxicity of hexachlorobutadiene. Eye irritation was reported in rats exposed to 250 ppm hexachlorobutadiene vapor for 4 hours/day for 2 days (Gage 1970).

2.13 ENDOCRINE

The endocrine system does not appear to be a target of hexachlorobutadiene toxicity. Although an acute inhalation study found degeneration of the adrenal cortex in rats exposed to 250 mg/kg/day (Gage 1970); other studies have not found effects. No histological alterations were observed in the adrenal or thyroid glands of rats administered 15.6 mg/kg/day via gavage for 13 weeks (Harleman and Seinen 1979), rats exposed to 20 mg/kg/day in the diet for 2 years (Kociba et al. 1977a), rats exposed to 100 mg/kg/day in the diet for 30 days (Kociba et al. 1971), rats exposed to 150 mg/kg/day hexachlorobutadiene in the diet for 18 weeks (Harleman and Seinen 1979), or male and female mice exposed via the diet to 19.2/16.8 mg/kg/day for 13 weeks (NTP 1991).

2.14 IMMUNOLOGICAL

In animals, histological examination of lymphoid organs including the thymus and spleen did not reveal treatment-related lesions at dose levels up to 150 mg/kg/day rats (Harleman and Seinen 1979; Kociba et al. 1971, 1977a). Depletion and necrosis of lymphoid tissue in the lymph nodes, spleen, and thymus were noted in mice exposed to lethal doses of hexachlorobutadiene (1,000 and 3,000 mg/kg diet) in the 2-week component of the NTP (1991) study. However, no abnormalities in these tissues were seen after 13-week exposures to doses of up to 19.2 or 16.8 mg/kg/day in male and female mice (NTP 1991; Yang et al. 1989). Tests on effects of immune function have not been evaluated in *in vivo* studies. In an *in vitro* study in mouse splenic lymphocytes, dose-related inhibition of B lymphocyte mitogenesis and weak inhibition of T lymphocyte mitogenesis were reported (Sakazaki et al. 2001); the concentration resulting in 50% cell growth inhibition IC50 was 1.0×10^{-5} mol/L.

2.15 NEUROLOGICAL

In animals, ataxia and demyelination and fragmentation of femoral nerve fibers were observed in rat dams exposed to dose levels of 150 mg/kg/day for up to 10 weeks (Harleman and Seinen 1979). Lethargy, hunched position, and incoordination were noted in male and female mice exposed to 40 or 49 mg/kg/day in the diet for 15 days (NTP 1991). No treatment-related brain lesions were seen following exposure to hexachlorobutadiene (Harleman and Seinen 1979; Kociba et al. 1971; NTP 1991; Schwetz et al. 1977). On the other hand, the mean brain/body weight ratio increased at dose levels of 10–100 mg/kg/day, but histopathological lesions were not seen at dose levels ≤100 mg/kg/day (Kociba et al. 1971). Exposure to hexachlorobutadiene did not alter brain weights and there were no treatment-related histopathological lesions of the brain, spinal cord, or sciatic nerve in rats exposed to hexachlorobutadiene (20 mg/kg/day) for 2 years (Kociba et al. 1977a). Neurochemical and neurophysiological parameters have not been monitored.

Rabbits exposed to doses of 388–1,550 mg/kg applied to shaved skin exhibited evidence of nervous system depression (stupor) during exposure and in the 1–2-hour period after exposure (Duprat and Gradiski 1978).

2.16 REPRODUCTIVE

The frequency of abnormal sperm morphology did not increase significantly over controls in mice exposed to airborne concentrations of 10 ppm hexachlorobutadiene for 5 days (7 hours/day) (NIOSH 1981). Studies evaluating the genotoxic potential of hexachlorobutadiene indicate that hexachlorobutadiene does not affect fertility in male rats. In dominant lethal tests in rats, fertility indices, number of corpora lutea or implantations, or frequency of early death did not differ between animals that inhaled vapors of hexachlorobutadiene at concentrations up to 50 ppm and their unexposed controls (NIOSH 1981).

In an oral study, fertility was reduced 100% in Wistar-derived rat dams administered 150 mg/kg/day hexachlorobutadiene during a 10-week study (Harleman and Seinen 1979); this dose level also resulted in weight loss and ataxia. At a lower dose (15 mg/kg/day), the mean litter size and the resorption rate did not differ significantly from controls (Harleman and Seinen 1979). The actual total exposure time for this study is not clear; the rats were exposed for at least 4 weeks prior to mating and it is assumed that they were also exposed for the remaining 6 or 14 weeks of the study for rats in the 150 or 15 mg/kg/day

groups, respectively. In another study, fertility, gestation, viability, and lactation indices were comparable in treated and control groups of Sprague-Dawley rats at dose levels of 20 mg/kg/day for 148 days (Schwetz et al. 1977). No significant changes were seen in sperm count, incidence of abnormal sperm, estrual cyclicity, or average estrous cycle length in mice exposed to hexachlorobutadiene in the diet (19.2 or 16.8 mg/kg/day in males and females) for 13 weeks (NTP 1991; Yang et al. 1989). Lifetime exposures up to 20 mg/kg/day did not reveal treatment-related lesions in the reproductive organs (Kociba et al. 1977a).

2.17 DEVELOPMENTAL

Three studies have evaluated the developmental toxicity of hexachlorobutadiene in laboratory animals. In an inhalation study, no alterations in the mean number of implantation sites, total fetal loss, resorptions, or number of live fetuses were observed in rats exposed to 15 ppm on GDs 6–20 (6 hours/day) (Saillenfait et al. 1989). However, a reduction in fetal body weights was observed at this concentration. No exposure-related external, visceral, or skeletal anomalies were observed.

Dietary exposure of rat dams to 15 mg/kg/day resulted in decreases in pup body weight at birth and weaning (Harleman and Seinen 1979); no gross abnormalities were observed in the pups. In another oral developmental toxicity study, body weight was decreased ($p<0.05$) on day 21 of lactation in rat pups from dams exposed to hexachlorobutadiene at dose levels of 20 mg/kg/day prior to mating and throughout gestation and lactation; body weights were not reduced in pups from dams exposed to 2 mg/kg/day (Schwetz et al. 1977). No other developmental alterations were observed.

2.18 OTHER NONCANCER

No human or animal studies examining other noncancer endpoints were identified.

2.19 CANCER

In the only available oral cancer study, increases in the total incidence of renal neoplasms (tubular adenomas and adenocarcinomas) were observed in male and female animals exposed to 20 mg/kg/day hexachlorobutadiene in the diet for 2 years (Kociba et al. 1977a). Metastasis to the lungs was observed. Combined incidences of renal tubular neoplasms in males (9/39, 23%) and females (6/40, 15%) increased over controls (males-1/90, females-0/90, 0%). The tumor incidence was not increased in the 0.2 or

2 mg/kg/day dose groups, but there were some indications of hyperplasia in animals exposed to 2 mg/kg/day.

Hexachlorobutadiene did not produce skin papillomas, carcinomas, or tumors at distant sites in mice after application of dose levels of 2–6 mg/mouse for 440–594 days (Van Duuren et al. 1979). Similarly, no increases in the incidence of papillomas were observed in a single exposure initiation/promotion assay (Van Duuren et al. 1979).

The carcinogenic properties of hexachlorobutadiene are proposed to result from binding of the sulfenic acid degradation product or a thioketene intermediate to cellular DNA (Dekant et al. 1990b; Henschler and Dekant 1990). Cell necrosis is thought to stimulate replication of cells with altered DNA, enhancing tumorigenesis.

EPA has classified hexachlorobutadiene as a possible human carcinogen (Group C) based on renal neoplasms observed in male and female rats in the Kociba et al. (1977a) study (IRIS 1993). IARC categorized it as not classifiable as to its carcinogenicity to humans (Group 3) (IARC 1999).

2.20 GENOTOXICITY

A number of studies have evaluated the *in vitro* and *in vivo* genotoxicity of hexachlorobutadiene; these data are summarized in Tables 2-5 and 2-6. Several *in vitro* assays evaluated genotoxic potential; however, results were mixed, suggesting differences in activation and detoxification mechanisms (Table 2-5). In bacterial assay systems employing *Salmonella typhimurium*, hexachlorobutadiene was not mutagenic either in the presence or absence of metabolic activation (DeMeester et al. 1980; Haworth et al. 1983; Kirkland et al. 2005; Kubo et al. 2002; Reichert et al. 1983; Stott et al. 1981; Vamvakas et al. 1988a) or in the presence of activation (Roldan-Arjona et al. 1991). On the other hand, results were positive in other bacterial assays employing *S. typhimurium* (Reichert et al. 1984; Roldan-Arjona et al. 1991; Vamvakas et al. 1988a). Certain metabolites of hexachlorobutadiene have also been evaluated. Monooxidation products of hexachlorobutadiene were mutagenic in *Salmonella* with and without metabolic activation (Reichert et al. 1984). Similarly, monooxidation products induced unscheduled DNA synthesis as well as morphological transformations in cultured Syrian hamster embryo fibroblasts (Schiffmann et al. 1984). However, results did not agree for hexachlorobutadiene in an *in vitro* unscheduled DNA synthesis assay employing rat hepatocytes (Stott et al. 1981). Hexachlorobutadiene

significantly increased the number of structural and numerical aberrations in Chinese hamster lung (CHL/IU) cells in the absence of S9 (Matsushima et al. 1999).

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

Species (test system)	Endpoint	Results: Activation: With	Results: Activation: Without	Reference
Prokaryotic organisms				
Salmonella typhimurium (TA98, TA100, TA2638)	Gene mutation	+[a]	ND	DeKant et al. 1986
*S. typhimurium (*TA98, TA100, TA1530, TA1535, TA1538)	Gene mutation	–	–	DeMeester et al. 1980
S. typhimurium (TA98, TA100, TA1535, TA1537)	Gene mutation	–	–	Haworth et al. 1983
S. typhimurium (strains not reported)	Gene mutation	–	–	Kirkland et al. 2005
S. typhimurium (TA98, TA100)	Gene mutation	–	–	Kubo et al. 2002
S. typhimurium (TA98, TA100)	Gene mutation	–	–	Reichert et al. 1983
S. typhimurium (TA100)	Gene mutation	+	–	Reichert et al. 1984
S. typhimurium (TA100)	Gene mutation	+[b]	+[b]	Reichert et al. 1984
S. typhimurium (strains not reported)	Gene mutation	–	+	Roldan-Arjona et al. 1991
S. typhimurium (strains not reported)	Gene mutation	–	–	Stott et al. 1981
S. typhimurium (TA98, TA100, TA2638)	Gene mutation	+	–	Vamvakas et al. 1988a
S. typhimurium (TA98, TA100, TA2638)	Gene mutation	+[c]	+[c]	Vamvakas et al. 1988a
S. typhimurium (TA98, TA100, TA2638)	Gene mutation	–[d]	–[d]	Vamvakas et al. 1988a
S. typhimurium (TA100)	Gene mutation	+[e]	+[e]	Wild et al. 1986
S. typhimurium (TA100)	Gene mutation	–[f]	–[f]	Wild et al. 1986
Mammalian cells				
Syrian hamster (embryo fibroblast cells)	Unscheduled DNA synthesis	+	+	Schiffmann et al. 1984
Rat (hepatocytes)	Unscheduled DNA synthesis	–	–	Stott et al. 1981

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

Species (test system)	Endpoint	Results: Activation With	Results: Activation Without	Reference
Syrian hamster (embryo fibroblast cells)	Morphological transformations	+	+	Schiffmann et al. 1984
Chinese hamster (lung CHL/IU cells)	Chromosome aberrations	ND	+	Matsushima et al. 1999

[a]Conjugate of hexachlorobutadiene: S-1,2,3,4,4-pentachlorobutadiene-1,3-dienylcysteine.
[b]Monooxidation product: perchloro-3-butenoic acid and perchloro-3-butenoic acid chloride.
[c]Conjugates of hexachlorobutadiene: 1-(glutathion-S-yL)-1,2,3,4,4-pentachloro-1,3-butadiene.
[d]Conjugates of hexachlorobutadiene: 1,4-(bis-glutathion-S-yL)-1,2,3,4-tetrachloro-1,3-butadiene and 1,4-(bis-cystein-S-yL)-1,2,3,4-tetrachloro-1,3-butadiene.
[e]Conjugates of hexachlorobutadiene: mercapturic acid and methyl-N-acetyl-S-pentachlorobutadienyl-D-L-homocysteinate.
[f]Conjugates of hexachlorobutadiene: S-pentachlorobutadienyl-mercapto acetic acid and pentachlorobutadienyl-methylthioether.

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

Table 2-6. Genotoxicity of Hexachlorobutadiene *In Vivo*

Species (exposure route)	Endpoint	Results	Reference
Mammalian systems			
Rat (inhalation)	Dominant lethal assay	–	NIOSH 1981
Rat kidney cells (gavage)	DNA alkylation	+	Stott et al. 1981
Rat kidney cells (gavage)	DNA repair	+	Stott et al. 1981
Rat bone marrow cells (inhalation)	Chromosome aberrations	–	NIOSH 1981
Rat bone marrow cell (diet)	Chromosome aberrations	–	Schwetz et al. 1977
Invertebrate systems			
Drosophila melanogaster	Gene mutation (sex-linked recessive lethal)	–	NIOSH 1981

+ = positive results; – = negative results; DNA = deoxyribonucleic acid

Studies of cysteine conjugates of hexachlorobutadiene reported that N-acetyl-S-pentachlorobutadienyl-L-cysteine (mercapturic acid) and D,L-homocysteinate derivatives were mutagenic in *S. typhimurium*, while mercaptoacetic acid and methylthioether derivatives were inactive (Wild et al. 1986). In other tests employing *S. typhimurium*, one cysteine conjugate was mutagenic both with and without activation (Dekant et al. 1986). Overall, results suggest that genotoxicity may not be a major factor in the toxicity of hexachlorobutadiene in humans.

Inhalation and oral studies have evaluated the genotoxic potential of hexachlorobutadiene *in vivo*. Inhalation exposure of *Drosophila* did not result in increases in gene mutations (NIOSH 1981). Hexachlorobutadiene did not cause dominant lethal mutations in rats after inhalation of vapors at concentrations of 10 or 50 ppm 7 hours/day for 5 days (NIOSH 1981). Similarly, there were no increases in the frequency of chromosomal aberrations in bone marrow cells of rats exposed to 10 ppm for up to 5 days (NIOSH 1981). Male rats administered a single gavage dose of hexachlorobutadiene (20 mg/kg/day) showed a 40% increase in renal DNA repair and 0.78 alkylations per million nucleotides (Stott et al. 1981). In another study, dietary exposure of up to 20 mg/kg/day hexachlorobutadiene did not cause chromosomal aberrations in rat bone marrow cells (Schwetz et al. 1977).