Toxicological Profile for Hexachlorobutadiene

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U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

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DISCLAIMER

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FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a relevance to public health discussion which would allow a public health professional to make a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to the protection of public health are identified by ATSDR.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance due to associated acute, intermediate, and chronic exposures;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, intermediate, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staffs of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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*Legislative Background

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL) and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the NPL, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

VERSION HISTORY

Date	Description
March 2021	Final toxicological profile released
December 2019	Draft for public comment toxicological profile released
December 2012	Addendum to the toxicological profile released
May 1994	Final toxicological profile released

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ATSDR Office of Community Health and Hazard Assessment; ATSDR, Office of Capacity Development and Applied Prevention Science; ATSDR, Office of Science; NCEH, Division of Laboratory Science; NCEH, Division of Environmental Health Science and Practice; EPA, National Center for Environmental Assessment.

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These experts collectively have knowledge of toxicology, chemistry, and/or health effects. All reviewers were selected in conformity with Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for this compound.

The listing of peer reviewers should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with ATSDR.

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CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

1.1 OVERVIEW AND U.S. EXPOSURES

Hexachlorobutadiene (C_4Cl_6; CAS No. 87-68-3) is a colorless liquid with a turpentine-like odor with an odor threshold of approximately 1 ppm. The main source of hexachlorobutadiene in the United States is its production as a byproduct of chlorinated hydrocarbon synthesis.

Low levels of hexachlorobutadiene can be detected in air, water, and sediment. Atmospheric levels of hexachlorobutadiene in rural and urban air samples typically range from 2 to 11 ppt, with a mean value of 2–3 ppt. Higher levels can be detected at areas near industrial and chemical waste disposal sites and production sites. Hexachlorobutadiene is infrequently detected in ambient waters, but has been detected in drinking water at levels of 2–3 ppt. Sediments contain higher levels of hexachlorobutadiene than the waters from which they were obtained. Foodstuffs generally do not contain detectable levels of hexachlorobutadiene, except for fish in which concentrations of 0.1–4.7 mg/kg have been reported. Thus, exposure can occur through ingestion of contaminated water or food or inhalation of contaminated air.

Hexachlorobutadiene has been detected in human adipose tissue and blood samples, although general population monitoring data are not available.

1.2 SUMMARY OF HEALTH EFFECTS

Information on the toxicity of hexachlorobutadiene primarily comes from studies in laboratory animals; only two epidemiology studies were identified. Both epidemiology studies were limited in scope examining either hepatic or renal endpoints. A wide range of potential endpoints were examined in the 24 experimental animal studies. Although hexachlorobutadiene toxicity has been more well-studied following oral exposure, the results of inhalation and dermal studies suggest similar targets.

As illustrated in Figures 1-1 and 1-2, the kidney is the most sensitive target of toxicity. Other targets include body weight gain, developmental toxicity, respiratory effects, hematological alterations, and hepatic toxicity; additionally, there is some evidence that chronic exposure can result in kidney tumors. Although body weight effects are also observed at lower doses, they are likely secondary to other effects. At higher doses, neurological effects, endocrine, ocular, and dermal effects (following direct contact exposure), and reproductive effects have been observed. The six sensitive targets of toxicity are discussed below.

Figure 1-1. Health Effects Found in Animals Following Inhalation Exposure to Hexachlorobutadiene

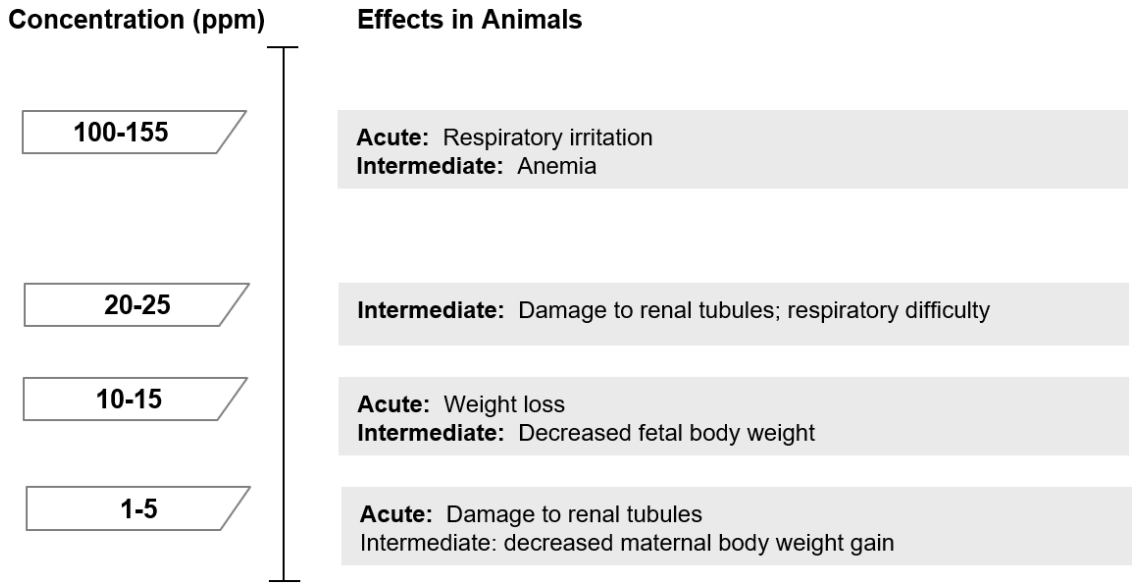
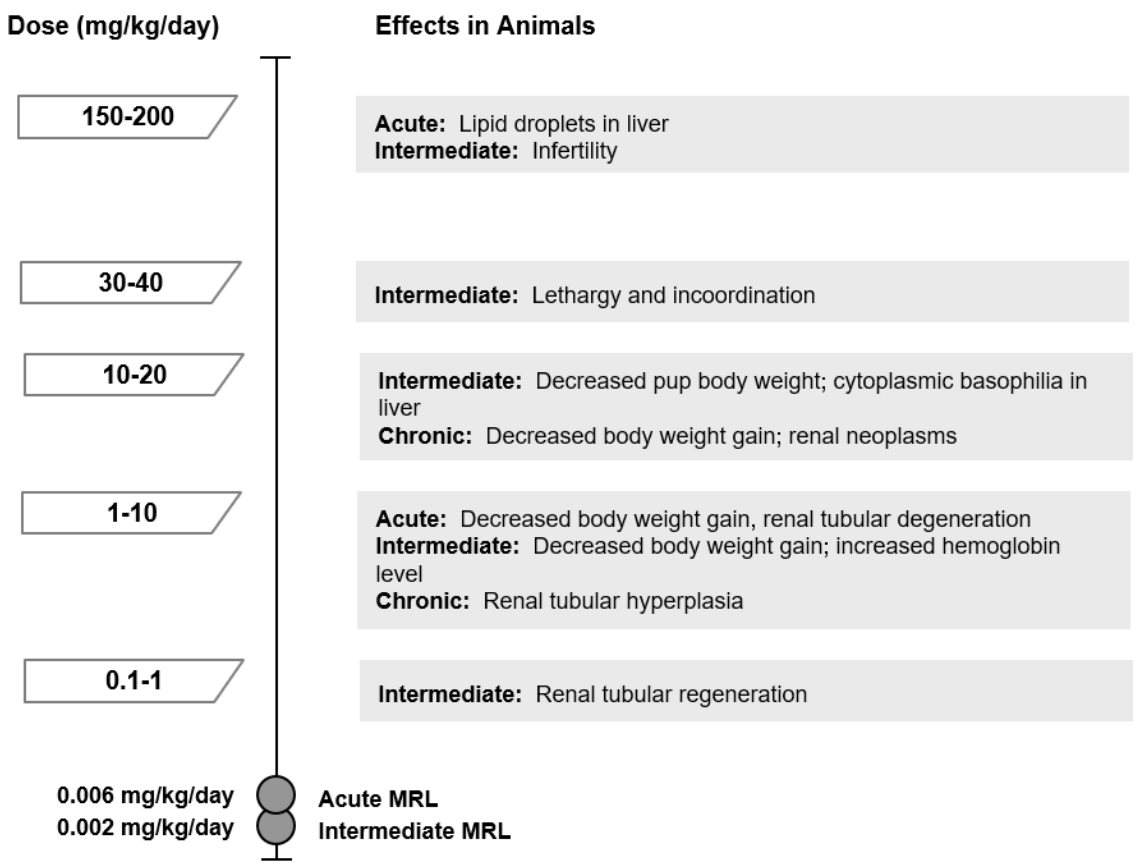


Figure 1-2. Health Effects Found in Animals Following Oral Exposure to Hexachlorobutadiene



1. RELEVANCE TO PUBLIC HEALTH

Renal Effects. An epidemiology study found alterations in several biomarkers of renal toxicity (α-glutathione-S-transferase, γ-glutamyltransferase, leucine aminopeptidase, and π-glutathione-S-transferase) among residents living in homes contaminated with hexachlorobutadiene (Staples et al. 2003). The biomarkers returned to normal levels in most of the subjects 10 months after exposure termination, suggesting that the effects may be reversible. In experimental animals, renal toxicity, characterized as histological damage and alterations in biomarkers of impaired renal function, has been observed following inhalation, oral, dermal, and parenteral exposures and has been observed in single and repeated exposure studies. The histological damage was typically found in the pars recta region of the proximal tubules, although the S1/S2 regions have also been affected. The lesions included epithelial degeneration (Gage 1970; Harleman and Seinen 1979; Kociba et al. 1971; NTP 1991; Schwetz et al. 1977), epithelial regeneration (Schwetz et al. 1977; NTP 1991), hyperplasia (Kociba et al. 1977), and necrosis at higher concentrations/doses (Birner et al. 1995; Harleman and Seinen 1979; Jonker et al. 1993a; Kociba et al. 1971; NTP 1991). Several urinary biomarkers of renal function have been altered by hexachlorobutadiene exposure including increases in N-acetyl-β-glucosaminidase, protein, and glucose levels, increases in volume, and decreases in urine concentrating ability (Harleman and Seinen 1979; Jonker et al. 1993a).

Developmental Effects. Inhalation and oral exposure studies in rats have consistently reported decreases in fetal or pup body weights (Harleman and Seinen 1979; Saillenfait et al. 1989; Schwetz et al. 1977); decreases in maternal body weight gain were typically observed at the same dose level. No other developmental effects including fetal loss, resorptions, pup survival, or occurrence of anomalies or malformations were observed.

Respiratory Effects. Respiratory irritation, characterized as nasal irritation, decreases in respiratory rate, and breathing difficulties have been observed in rats and mice exposed to hexachlorobutadiene vapor (de Ceaurriz et al. 1988; Gage 1970). Histological examinations were not conducted in the inhalation studies, and oral studies have not found evidence of lung damage.

Hematological Effects. Two studies reported altered hematological parameters (Gage 1970; Kociba et al. 1971); however, the results were not consistent with each other or with other studies that found no alterations (Harleman and Seinen 1979; Kociba et al. 1977; Schwetz et al. 1977). An inhalation study reported anemia (Gage 1970) and an oral study reported increases in hemoglobin levels (Kociba et al. 1971). The inconsistency of the results makes it difficult to evaluate the relevance of this effect to humans.

Hepatic Effects. An increase in serum bile acids was reported in a study of workers exposed to hexachlorobutadiene, along with other solvents such as carbon tetrachloride and tetrachloroethylene (Driscoll et al. 1992); the study does not allow for an interpretation of whether these effects were due to hexachlorobutadiene exposure or to the other solvents that are known hepatotoxicants. Some oral exposure animal studies have reported hepatic effects (Birner et al. 1995; Harleman and Seinen 1979; Kociba et al. 1971), whereas other studies have not found histological alterations (Harleman and Seinen 1979; Kociba et al. 1977; NTP 1991; Schwetz et al. 1977). Observed effects included cytoplasmic lipid droplets (Birner et al. 1995), cytoplasmic basophilia (Harleman and Seinen 1979), and hepatocellular swelling (Kociba et al. 1971).

Cancer Effects. Increases in the incidence of renal neoplasms were observed in male and female rats orally exposed to hexachlorobutadiene for 2 years (Kociba et al. 1977). No increases in tumor incidences were observed in mice dermally exposed for 1.2–1.6 years (Van Duuren et al. 1979). Based on the results of the Kociba et al. (1977) study, EPA classified hexachlorobutadiene as a possible human carcinogen (Group C) (IRIS 1993). IARC categorized hexachlorobutadiene as not classifiable as to its carcinogenicity in humans (Group 3) (IARC 1999).

1.3 MINIMAL RISK LEVELS (MRLs)

The inhalation database was not considered adequate for deriving inhalation MRLs. As presented in Figure 1-3, the available inhalation data for hexachlorobutadiene suggest that sensitive targets include the kidney, respiratory tract, and developing organisms (fetal body weight); body weight effects have also been observed at low concentrations.

The oral database was considered adequate for derivation of acute- and intermediate-duration MRLs for hexachlorobutadiene; but was not considered adequate for derivation of a chronic-duration MRL. The kidney is the most sensitive target following acute, intermediate, or chronic duration exposure. As illustrated in Figure 1-4, body weight, hematological, and developmental effects are also observed at low doses. The oral MRLs for hexachlorobutadiene are summarized in Table 1-1 and discussed in greater detail in Appendix A.

Figure 1-3. Summary of Sensitive Targets of Hexachlorobutadiene – Inhalation

The kidney is the most sensitive target of hexachlorobutadiene.
Numbers in circles are the lowest LOAELs (ppm) for all health effects in animals; no reliable concentration-response is available for humans.

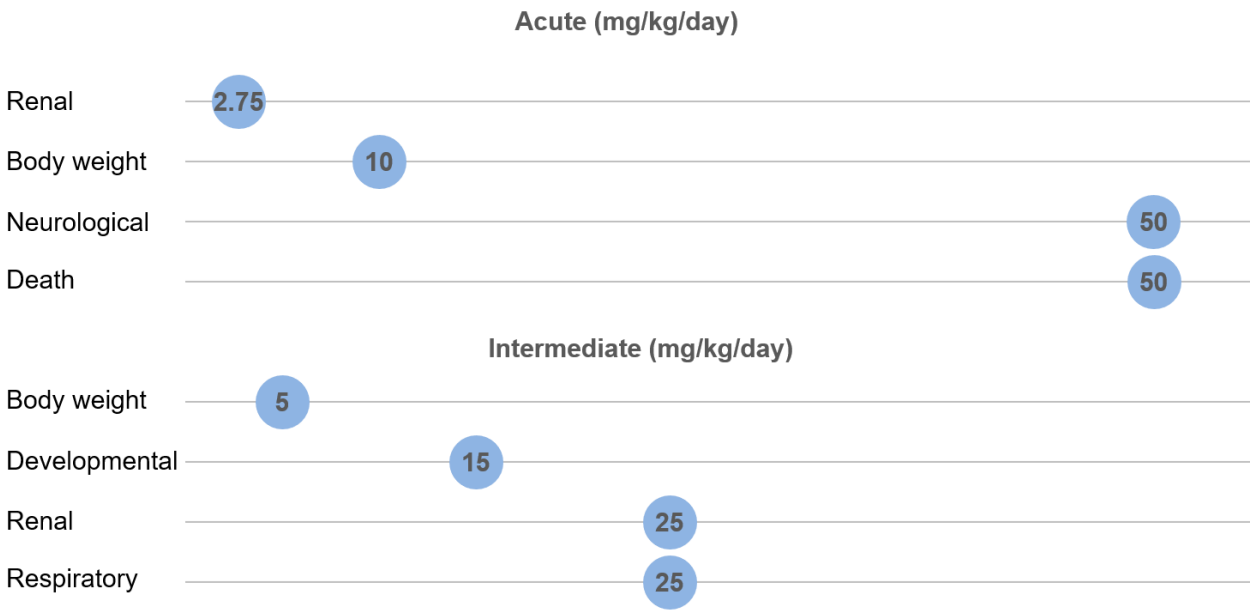


Figure 1-4. Summary of Sensitive Targets of Hexachlorobutadiene – Oral

The kidney is the most sensitive target of hexachlorobutadiene.
Numbers in circles are the lowest LOAELs (mg/kg/day) for all health effects in animals; no human data were identified.

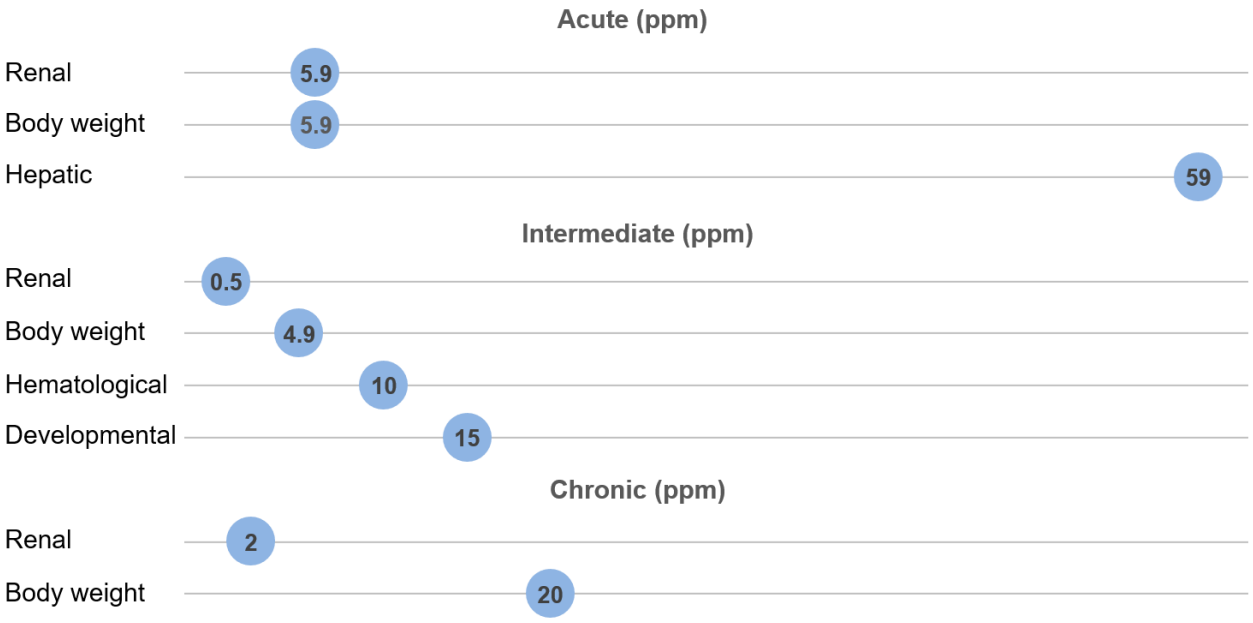


Table 1-1. Minimal Risk Levels (MRLs) for Hexachlorobutadiene[a]

Exposure duration	MRL	Critical effect	Point of departure	Modifying and uncertainty factors	Reference
Inhalation exposure (ppm)					
Acute	Insufficient data for MRL derivation				
Intermediate	Insufficient data for MRL derivation				
Chronic	Insufficient data for MRL derivation				
Oral exposure (mg/kg/day)					
Acute	0.006	Renal proximal tubule degeneration	5.9 (LOAEL)	1,000	Harleman and Seinen 1979
Intermediate	0.002	Renal proximal tubule regeneration	0.2 (NOAEL)	100	NTP 1991
Chronic	Insufficient data for MRL derivation				

[a]See Appendix A for additional information.

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

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.

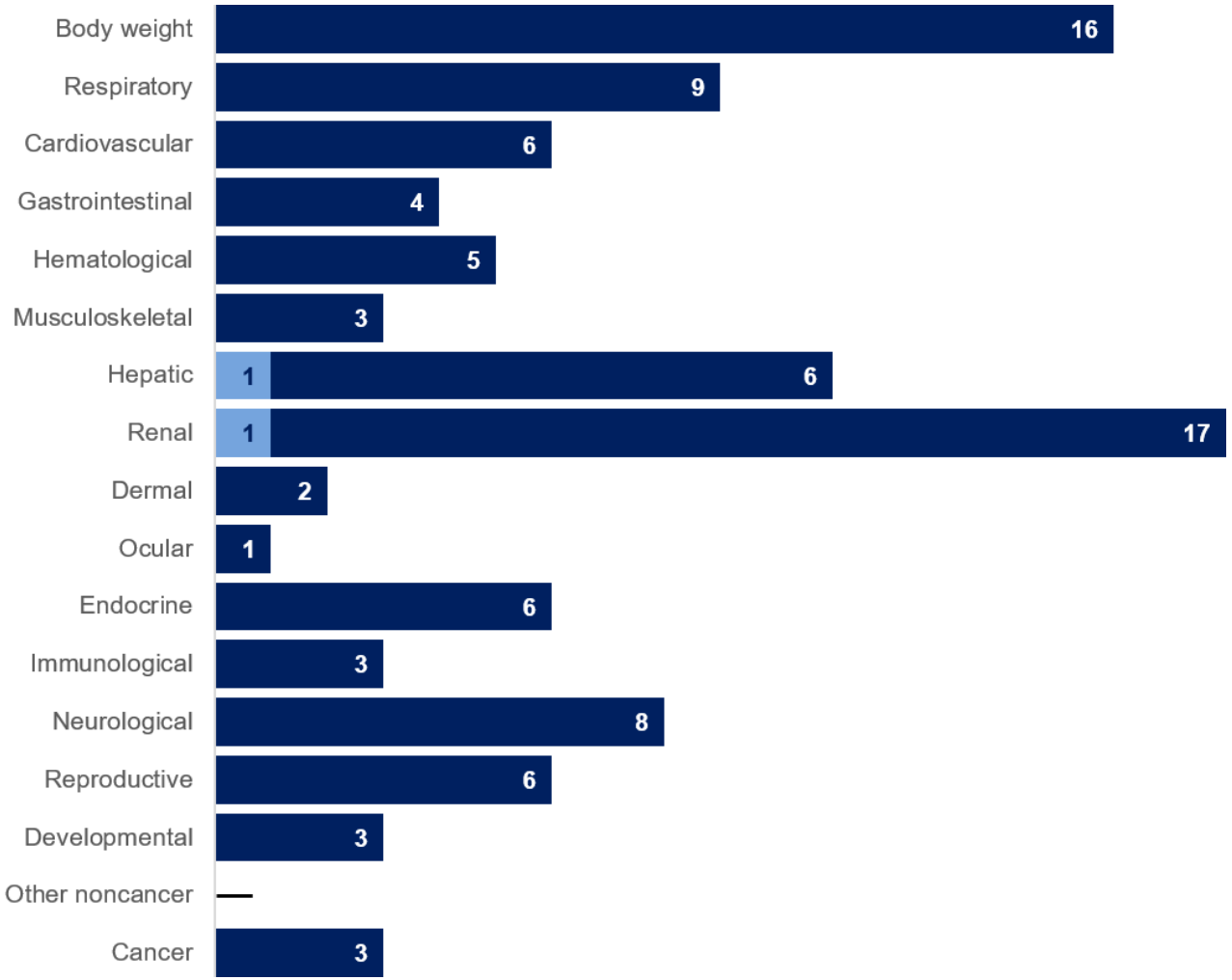
- **Renal Endpoints:** Experimental animal studies provide strong evidence that the kidney is the most sensitive target of toxicity for hexachlorobutadiene. An epidemiology study also found some evidence of impaired renal function. In rats and mice, exposure to hexachlorobutadiene results in damage to 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.

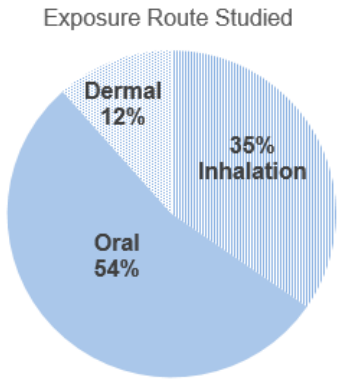
- **Respiratory Endpoints**: Inhalation studies have reported evidence of respiratory irritation in animals following acute- or intermediate-duration exposures. The observed effects include nasal irritation, decreases in respiratory rate, and breathing difficulty.

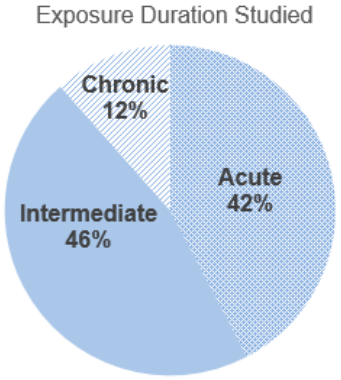
- **Developmental Endpoints:** Experimental animal studies have consistently found decreases in fetal or pup body weights, but did not find increases in the occurrence of anomalies or malformations. Maternal decreases in body weight was also observed at concentrations or doses resulting in fetal/pup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

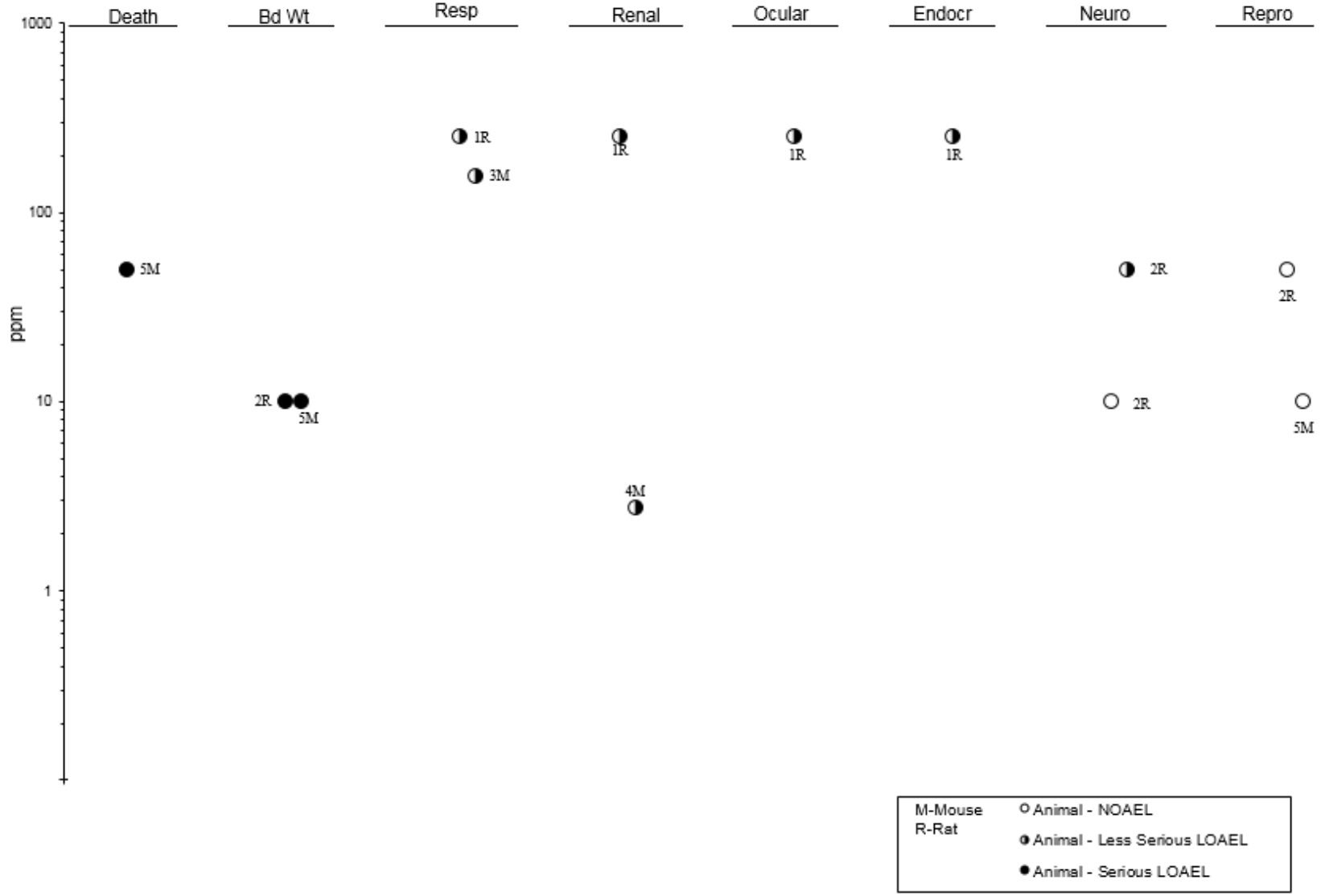


Figure 2-2. Levels of Significant Exposure to Hexachlorobutadiene – Inhalation

Intermediate (15–364 days)

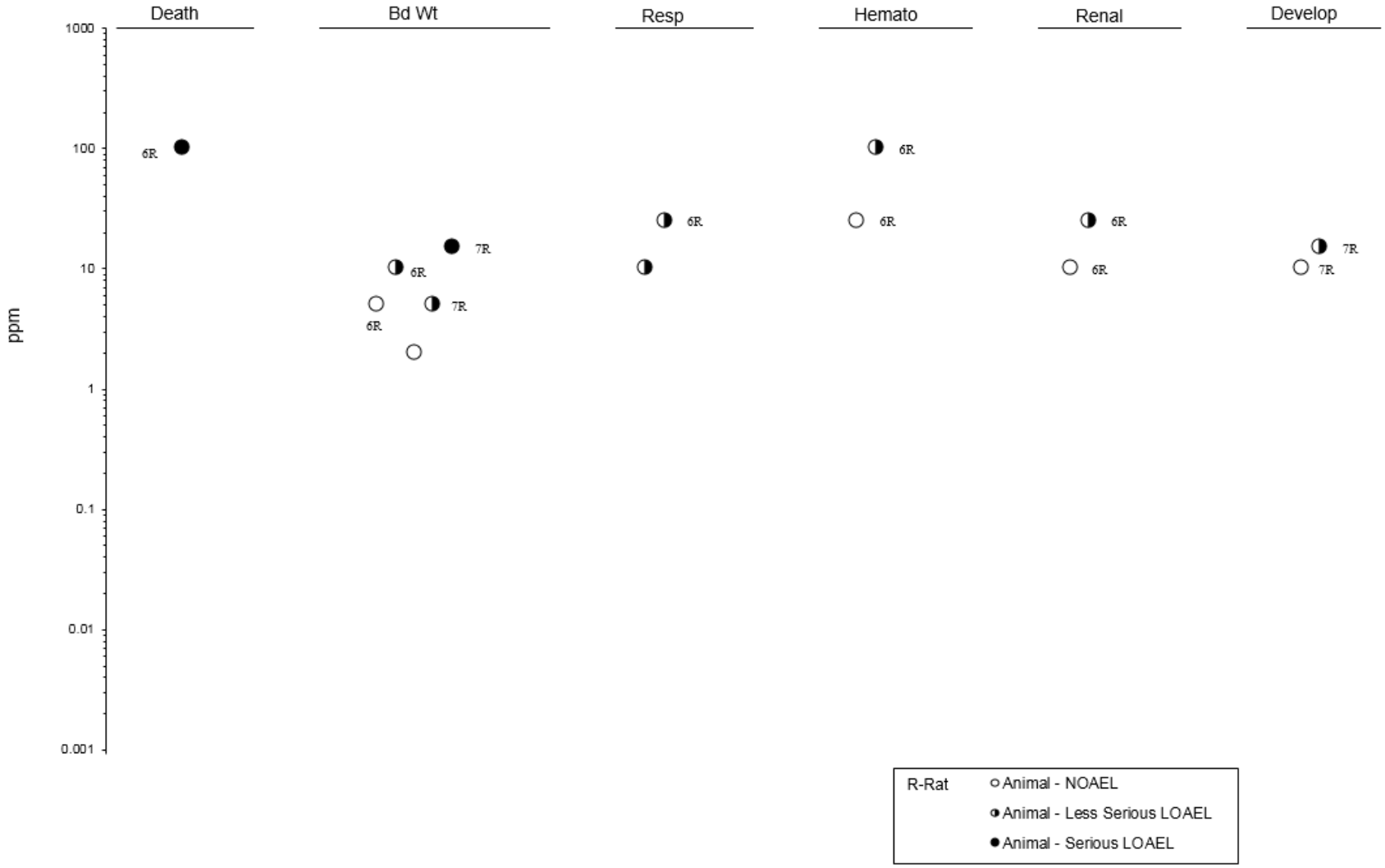


Table 2-2. Levels of Significant Exposure to Hexachlorobutadiene – Oral

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			
					Hepatic	6.3M	15.6M		Increased cytoplasmic basophilia

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
					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									
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%)

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	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. 1977									

[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 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 an intermediate oral 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)

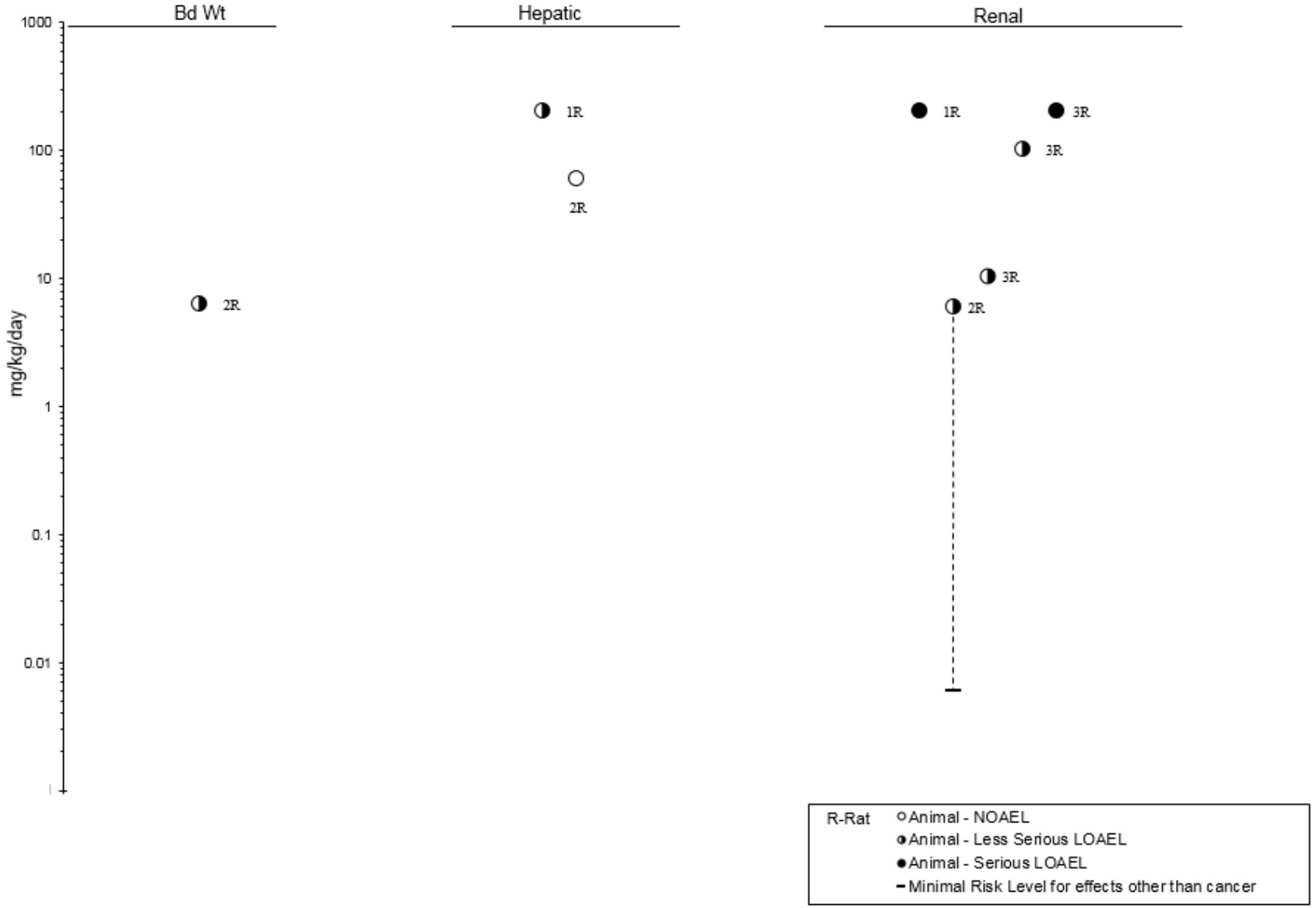


Figure 2-3. Levels of Significant Exposure to Hexachlorobutadiene – Oral

Intermediate (15–364 days)

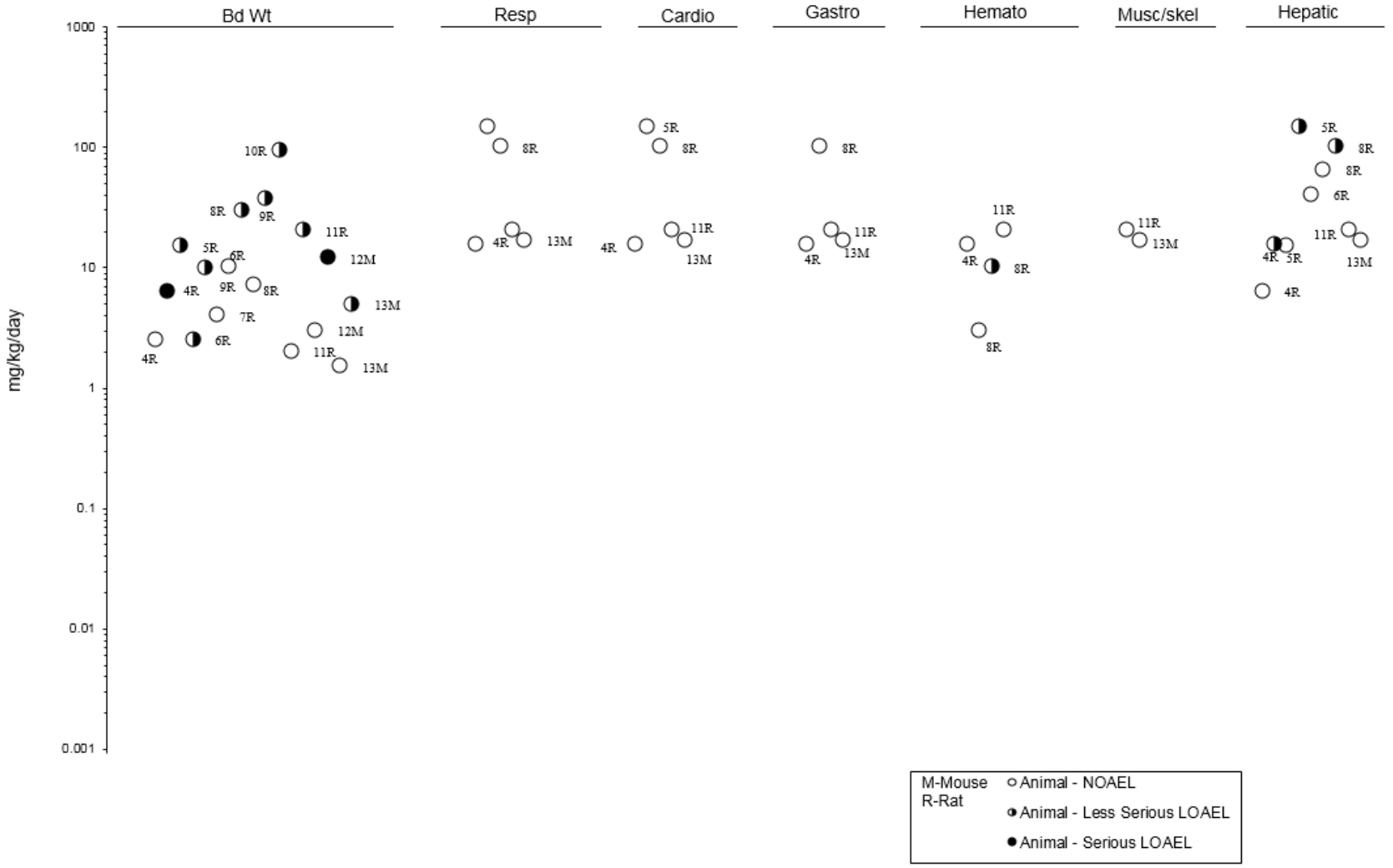


Figure 2-3. Levels of Significant Exposure to Hexachlorobutadiene – Oral

Intermediate (15–364 days)

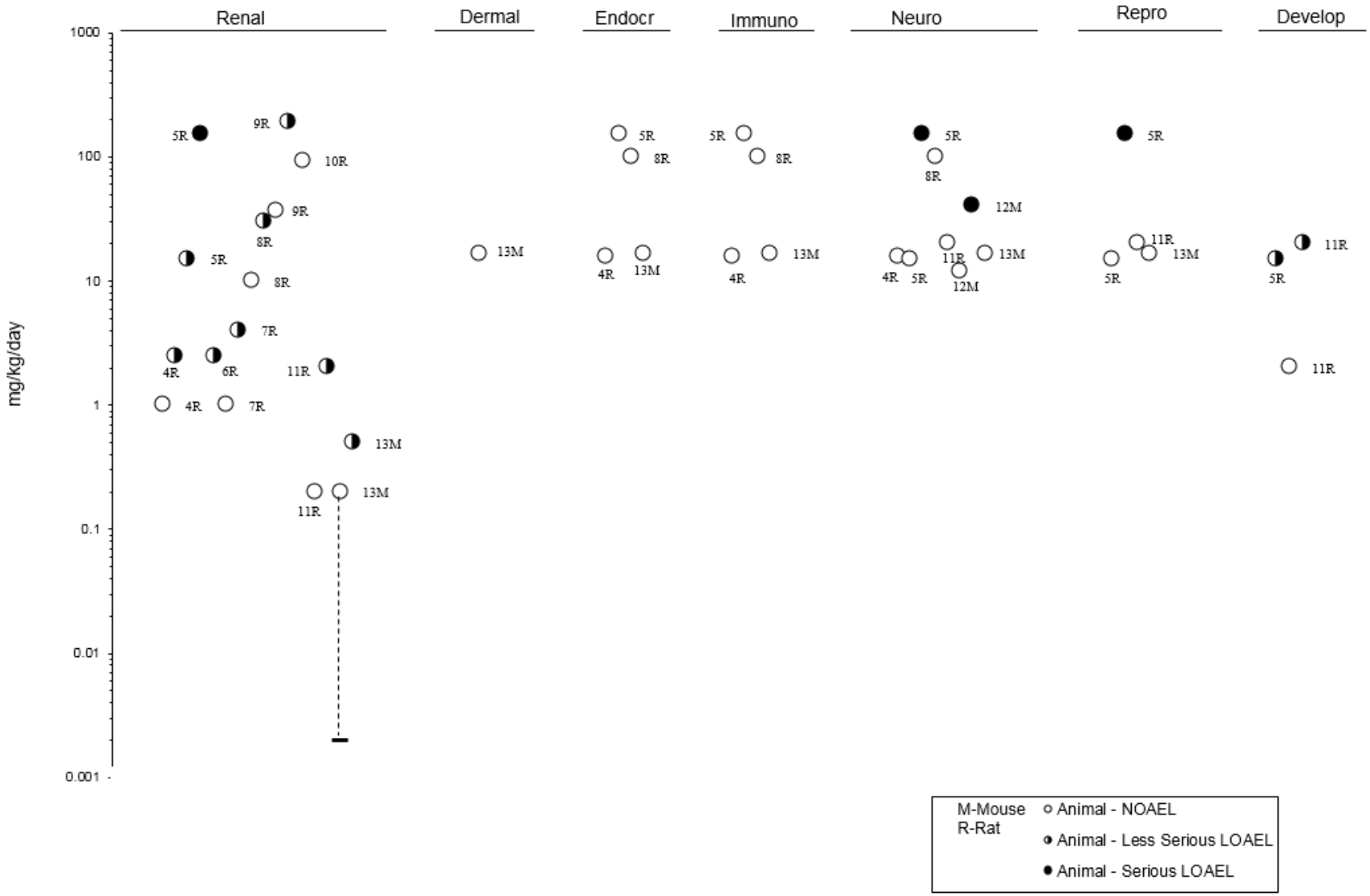


Figure 2-3. Levels of Significant Exposure to Hexachlorobutadiene – Oral

Chronic (≥365 days)

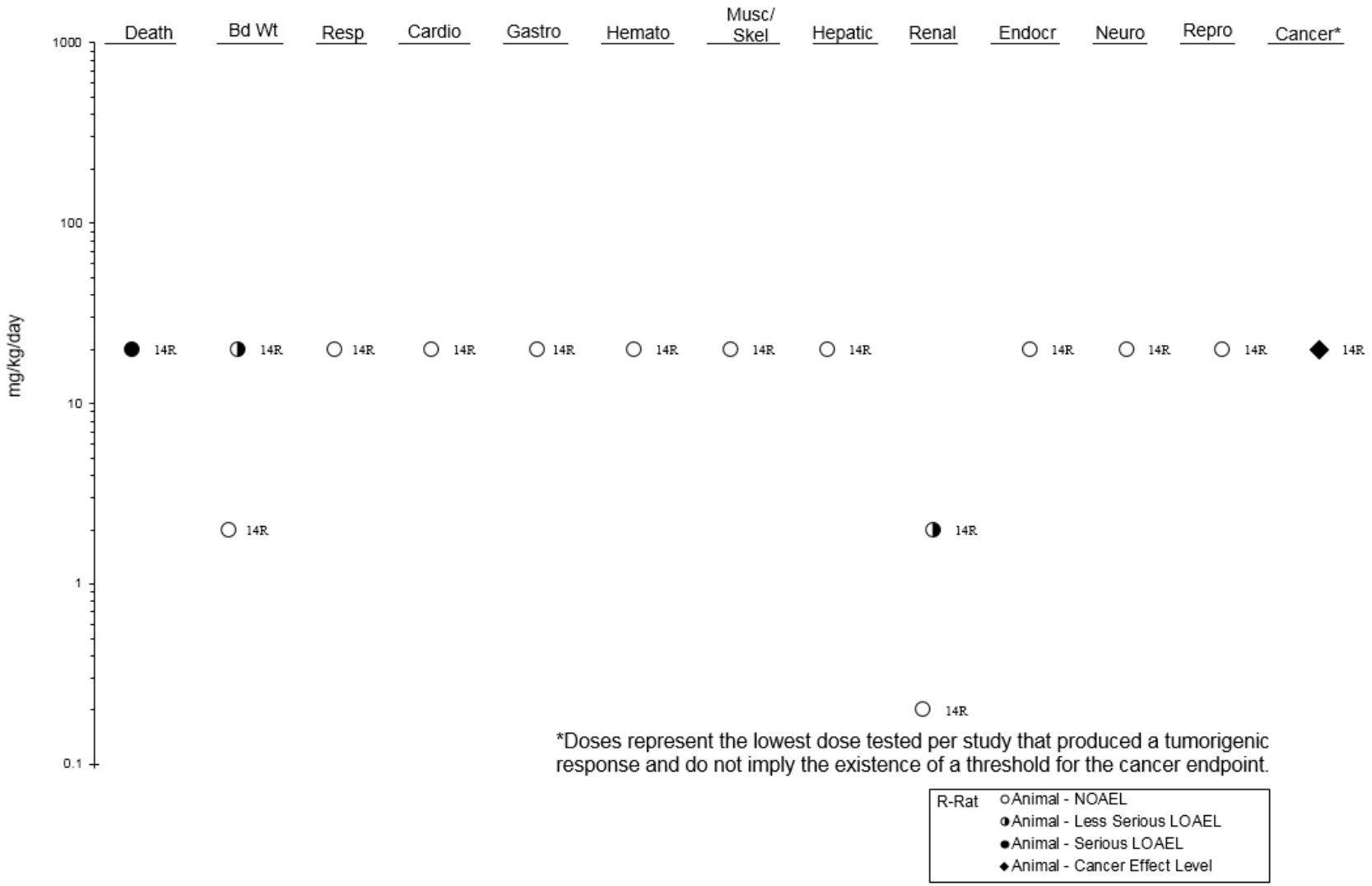


Table 2-3. Levels of Significant Exposure to Hexachlorobutadiene – Dermal

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. 1977). 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. 1977). 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. 1977).

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; NTP 1991; Schwetz et al. 1977) or chronic exposures to 20 mg/kg/day (Kociba et al. 1977) 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. 1977).

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 (NTP 1991). Lifetime exposure at dose levels of 20 mg/kg/day (Kociba et al. 1977) 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. 1977). 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. 1977).

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. 1977).

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, 1977; 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. 1977), 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. 1977). 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. 1977; 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 damaged 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. 1990; 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. 1977), 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, 1977). 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. 1977). 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 hexachloro-butadiene 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. 1977).

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. 1977). 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. 1990; 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. (1977) 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. 1988) 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. 1988). 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. 1988
S. typhimurium (TA98, TA100, TA2638)	Gene mutation	+[c]	+[c]	Vamvakas et al. 1988
S. typhimurium (TA98, TA100, TA2638)	Gene mutation	–[d]	–[d]	Vamvakas et al. 1988
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).

CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

No studies were located regarding the toxicokinetics of hexachlorobutadiene in humans, but there are limited data from studies in animals. These data are summarized below.

- Based on health effect and excretion data, hexachlorobutadiene is absorbed following inhalation, oral, or dermal exposure.
- Absorbed hexachlorobutadiene is distributed throughout the body with the highest concentrations found in the kidney, liver, and fat.
- The predominant pathway for hexachlorobutadiene metabolism is conjugation with glutathione in the liver and subsequent metabolism to form cysteine conjugates. The cysteine conjugates can be further metabolized by β-lyase to form reactive intermediates.
- The major route of excretion of hexachlorobutadiene is expiration of the parent compound or metabolite (carbon dioxide) through the lung, urinary excretion of metabolites and parent compound, and fecal excretion of parent compound and metabolites.

3.1.1 Absorption

No studies were located regarding absorption in humans or animals after inhalation exposure to hexachlorobutadiene. The occurrence of effects after exposure (de Ceaurriz et al. 1988; Gage 1970) indicate that absorption does occur.

No studies were located regarding absorption in humans after oral exposure to hexachlorobutadiene. There have also been no direct studies of absorption in animals although data on excretion and distribution provide information that suggests that absorption does occur from the gastrointestinal tract (Reichert et al. 1985). In animals, absorption is rapid and virtually complete at low doses of hexachlorobutadiene (1 mg/kg). At a higher dose (50 mg/kg), unmetabolized hexachlorobutadiene is found in the fecal matter (Reichert et al. 1985).

When Alderley Park rats were given a single dose of 200 mg/kg of radiolabeled hexachlorobutadiene and sacrificed at 2, 4, 8, and 16 hours, an autoradiogram of longitudinal sections of whole animals sacrificed 4 hours after dosing demonstrated that the label was concentrated in the intestines. The intestinal label was determined to be 85% unmodified, unabsorbed hexachlorobutadiene. At 8 hours, the intestinal

concentration of the label was no longer apparent as hexachlorobutadiene was absorbed and distributed to the tissues (Nash et al. 1984).

Most of the data pertaining to oral administration of hexachlorobutadiene utilized triglycerides (corn oil or tricaprylin) as a gavage dosing medium. Because of its high lipophilicity and low water solubility, it is likely that the absorption of hexachlorobutadiene from an aqueous solution would differ from that from a triglyceride media. When 1 mg/kg hexachlorobutadiene in tricaprylin was administered to female Wistar rats, 30.61% was excreted in the urine over 72 hours (Reichert et al. 1985), while when the same dose in aqueous polyethylene glycol solution was given to male Sprague Dawley rats, only 18% was in the urine (Payan et al. 1991). These data suggest that absorption from the lipid solvent was greater than that with the aqueous solvent.

No studies were located regarding absorption in humans after dermal exposure to hexachlorobutadiene. In animals, pure hexachlorobutadiene (388–1,550 mg/kg) applied to the skin of rabbits was completely absorbed in 8 hours (Duprat and Gradiski 1978).

3.1.2 Distribution

Hexachlorobutadiene has been identified in samples of human adipose tissue (Mes et al. 1985). The tissue samples were obtained from cadavers and, thus, no data were available pertaining to exposure.

No studies were located regarding distribution in humans after oral exposure to hexachlorobutadiene. In animals, 5–14 % of ^{14}C-hexachlorobutadiene was retained in the tissues and carcass 72 hours after compound administration (Dekant et al. 1988a; Reichert et al. 1985). The kidney (outer medulla), liver, and adipose tissue appeared to concentrate hexachlorobutadiene label when single doses of up to 200 mg/kg ^{14}C-hexachlorobutadiene in corn oil were administered by gavage (Dekant et al. 1988a; Nash et al. 1984; Reichert et al. 1985). In one report, the brain was also determined to contain a relatively high concentration of label 72 hours after exposure (Reichert et al. 1985). Label in the kidney 72 hours after exposure was more extensively covalently bound to proteins than that in the liver (Reichert et al. 1985).

Levels of label in the liver, kidney, and plasma were determined for the donor and recipient rats when secretions from bile duct cannulated donor rats given a dose of 100 mg/kg hexachlorobutadiene were infused directly into the bile duct of nonexposed recipient rats, and thereby into their intestines (Payan et al. 1991). In the donor rats, after 30 hours, the kidney contained 0.26% of the dose, the liver contained

0.11%, and the plasma contained 0.013% from the intestinally absorbed material. In the recipient rats, the kidney contained 0.15% of the dose, the liver contained 0.97%, and the plasma contained 0.009% from the resorbed biliary metabolites. For each tissue, the level of label from resorbed metabolites was about two-thirds of that from the original dose. The kidneys contained more of the label than the liver in both instances, clearly identifying the kidneys as a target organ for distribution. This is consistent with studies showing that the kidney is the most sensitive organ of hexachlorobutadiene toxicity.

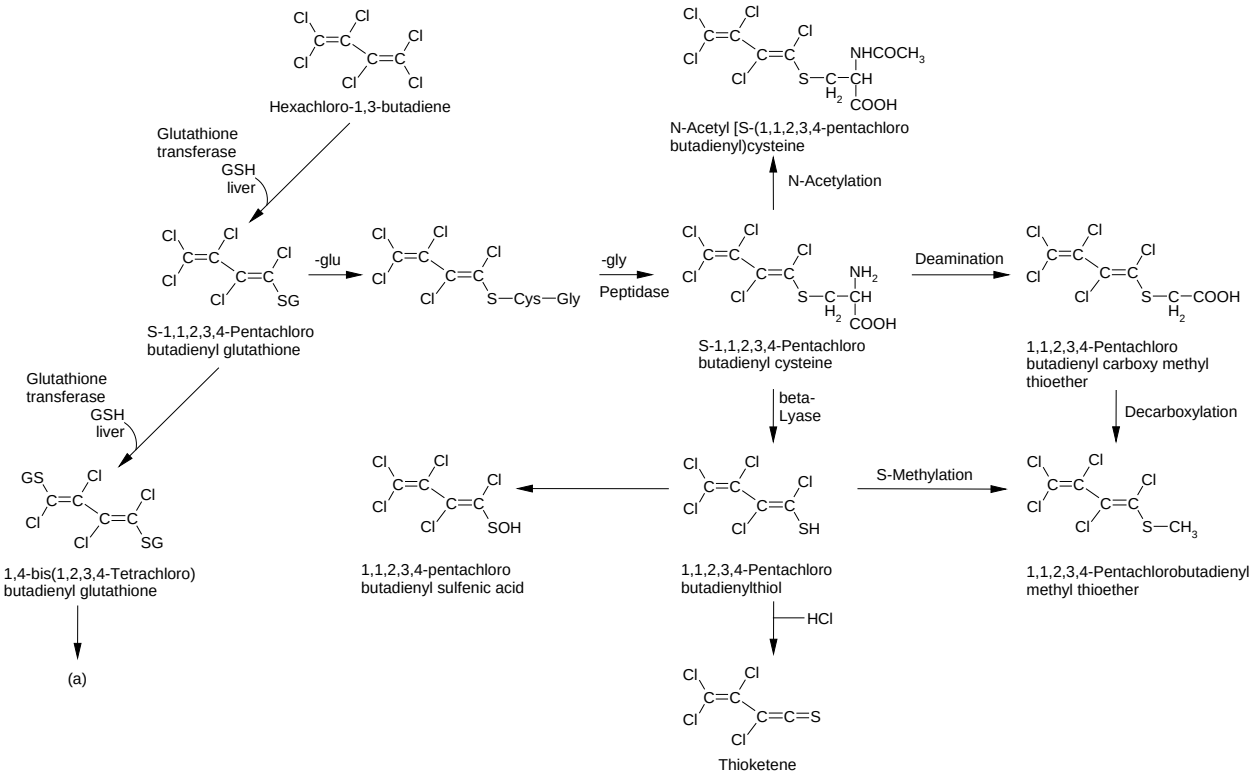
In a study using doses of 0.1 and 300 mg/kg intraperitoneally-administered radiolabeled hexachlorobutadiene, the label was found in the liver, kidney, and adipose tissue. Very little of the label was found in the brain, lung, heart, and muscle tissue at 48 hours after dosing (Davis et al. 1980). The reported levels in the brain in this study differ from those reported at 72 hours following oral administration (Reichert et al. 1985). This may indicate that there is a gradual deposition of labeled hexachlorobutadiene and/or its metabolites in the brain lipids over time.

3.1.3 Metabolism

There is a considerable amount of information available concerning the metabolism of hexachlorobutadiene in animals. Figure 3-1 presents a proposed metabolic pathway for hexachlorobutadiene. This pathway is based on the metabolites identified in urine and bile using chromatographic techniques.

Most of the absorbed hexachlorobutadiene is transported via the portal circulation to the liver where it is conjugated with glutathione (Garle and Fry 1989). In rat livers, both mono- and di-substituted conjugates have been identified (Jones et al. 1985), whereas mice appear to produce only the monosubstituted conjugate (Dekant et al. 1988a). There was a dose-related decrease in hepatic levels of glutathione following exposure to hexachlorobutadiene, and pretreatment of experimental animals with agents that interfere with glutathione synthesis or conjugation reactions decreased the amount of glutathione conjugate that can be synthesized (Gietl and Anders 1991). There appears to be no oxidation of the hexachlorobutadiene by the mixed function oxidase system enzymes prior to conjugation (Garle and Fry 1989).

Figure 3-1. Proposed Pathways for Hexachlorobutadiene Metabolism



(a) = metabolism parallels that for the monosubstituted compound; glu = glutamic acid; gly = glycine; GSH = glutathione

Sources: Dekant et al. 1991; Jaffe et al. 1983; Jones et al. 1985; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985; Wild et al. 1986; Wolf et al. 1984

The glutathione conjugate (S-(1,2,3,4,4-pentachlorobutadienyl)glutathione, PCBG) is excreted with the bile into the intestinal tract. A portion of the material is hydrolyzed with the removal of glutamate or glutamate and glycine from the glutathione tripeptide to form the cysteine derivative [S-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine, PCBC] or the cysteinylglycine derivative (Gietl and Anders 1991; Gietl et al. 1991; Nash et al. 1984). In one study, the glutathione conjugate accounted for 40% of the label in the bile and the cysteine derivative for 15% of the label. Another 45% of the label was present as unidentified compounds (Nash et al. 1984).

The conversion of the glutathione conjugate to its cysteinyl derivative is mediated, at least in part, by enzymes in the intestinal epithelial cells. PCBG and PCBC are partially reabsorbed from the intestines and transported to the liver and subsequently to the body tissues (Gietl et al. 1991). Only a portion of the reabsorbed material is taken up by the liver for additional metabolism. When liver uptake of the glutathione conjugate was measured using perfused rat livers, the maximum uptake observed was 39%

(Koob and Dekant 1992). A portion of this material was re-excreted in bile without any metabolic modification.

The cysteine conjugate, acetylated cysteine conjugate, and six bis-substituted metabolites were synthesized from the glutathione conjugate and excreted in bile. Two of the bis-substituted metabolites were identified as the bis-1,4-glutathione conjugate and the bis-1,4-cysteine conjugate. The cysteine conjugate was taken up by the liver to a greater extent than the glutathione conjugate (Koob and Dekant 1992). Up to 79% of the cysteine conjugate was absorbed, but this metabolite appeared to be toxic to the liver and caused decreased bile flow within 20 minutes. There were only small portions of the cysteine derivative and acetylated cysteine derivative in the bile. Bis-substituted derivatives, including the 1-cysteinyl-4-glutathionyl tetrachlorobutadiene, bis-1,4-cysteinyl tetrachlorobutadiene, and 1-cysteinyl-4-cysteinyl glycine tetrachlorobutadiene, were formed.

Additional processing of the hexachlorobutadiene metabolites produces the compounds identified in the urine (1,1,2,3-tetrachlorobutenoic acid, 1,1,2,3,4-pentachloro-1:3-butadienyl sulfenic acid, N-acetyl-S-(1,1,2,3,4-pentachlorobutadienyl)-L-cysteine, S-1,1,2,3,4-pentachlorobutadienylmercaptoacetic acid, 1,1,2,3,4-pentachlorobutadiene methylthioether, and 1,1,2,3,4-pentachlorobutadiene carboxymethylthio-ether) (Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985). Birner et al. (1995) also demonstrated that N-acetyl-S-1, 1,2,3,4-pentachlorobutadienyl-L-cysteine could be further metabolized to form N-acetyl-S-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine sulfoxide, but that may only occur in male rats; the metabolite was not detected in female rat urine.

A very small portion of the absorbed hexachlorobutadiene is oxidized to carbon dioxide. This pathway can be saturated since an increase in the hexachlorobutadiene dose does not cause a corresponding increase in excretion of labeled carbon dioxide (Dekant et al. 1988b; Payan et al. 1991; Reichert et al. 1985).

Green et al. (2003) compared the *in vitro* metabolism of hexachlorobutadiene in human and rat tissues and found higher metabolic rates and affinity constants in rat tissues than in human tissues (Table 3-1). The ratios (rat:human) of the V_{max} values ranged from 2.4 to 73.6. The investigators developed a physiologically based pharmacokinetic (PBPK) model using these data, as well as other published pharmacokinetic modeling parameters (see Section 3.1.5 for more information on the model); the model predicted that humans would need to be exposed to a 10-fold higher hexachlorobutadiene air concentration than rats to obtain the same β-lyase metabolite levels as found in rats.

Table 3-1. Metabolic Rate (V_{max}) and Affinity Constant (K_m) of Liver and Kidney Enzymes Measured in Human and Rat Tissues (*In Vitro*)

	V_{max} (nmol/minute/mg)			K_m (mM)		
Enzyme	Rat	Human[a]	Ratio	Rat	Human[a]	Ratio
Glutathione S-transferase (liver microsomes)	1.23	0.25	4.9	0.21	0.16	1.3
β-lyase (kidney cytosol)	1.13	<0.05[b]	22.6[c]	0.3	–	–
β-lyase (kidney mitochondria)	4.17	1.76	2.4	0.12	0.25	0.48
Cysteine N-acetyl transferase (kidney microsomes)	144.9	37.6	3.8	0.39	0.19	2.0
Acylase (kidney cytosol)	7.36	<0.10[b]	73.6[c]	1.43	–	–

[a]Mean of results from 3–6 tissue samples.
[b]No measurable rate, limit of detection shown.
[c]Calculated using the limit of detection for humans.

Source: Green et al. 2003

3.1.4 Excretion

In animals, hexachlorobutadiene and its metabolites are excreted in exhaled air, urine, and feces. In studies where radiolabeled (^{14}C) hexachlorobutadiene was administered at doses of 1, 30, 50, or 100 mg/kg, 4–8% of the dose was removed from the body in the exhaled air as unmetabolized hexachlorobutadiene and carbon dioxide within the 72 hours after compound administration (Dekant et al. 1988b; Payan et al. 1991; Reichert et al. 1985). In another study, 1.1 and 2.0% of ^{14}C-hexachlorobutadiene was excreted in exhaled air 48 hours after a single dose of 200 mg/kg in male and female rats, respectively (Birner et al. 1995); 0.02 and 0.03% of the dose was exhaled as carbon dioxide.

With single doses ranging from 1 to 200 mg/kg ^{14}C-hexachlorobutadiene, the percent of the label in the urine ranged from 3.1 to 30.6%, with the highest percentage associated with the lowest dose (Birner et al. 1995; Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985). These data suggest that the absorption or excretion may be saturable. At the higher doses, urinary excretion values of 5–10% were common (Nash et al. 1984; Reichert and Schutz 1986). Some of the hexachlorobutadiene label excreted in the urine originates from the biliary metabolites reabsorbed from the intestinal tract and processed by the kidneys for excretion. A study of bile duct cannulated rats and noncannulated rats estimated the contribution of reabsorbed biliary metabolites to urinary excretion. When a dose of 1 mg/kg hexachlorobutadiene in polyethylene glycol solution was given to bile duct cannulated male rats,

the urine contained 11% of the label after 72 hours; in noncannulated rats given the same dose, it contained 18 % of the label (Payan et al. 1991). When a dose of 100 mg/kg was given, the urine of the cannulated rats contained 7% of the label and the urine of the noncannulated rats contained 9% after 72 hours.

Metabolites identified in the urine include: S-(1,1,2,3,4-pentachlorobutadienyl)glutathione; S-(1,1,2,3,4-pentachlorobutadienyl) cysteine; 1,1,2,3-tetrachlorobutenoic acid; 1,1,2,3,4-pentachloro-1: 3-butadienyl sulfenic acid; N-acetyl-S- 1,1,2,3,4-pentachlorobutadienyl)-L-cysteine; S-pentachloro-butadienylmercaptoacetic acid; 1,1,2,3,4-pentachlorobutadiene methylthioether; and 1,1,2,3,4-penta-chlorobutadiene carboxymethylthioether (Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985). Birner et al. (1995) found sex-related differences in the identification and quantification of urinary metabolites between male and female rats administered a single 200 mg/kg gavage dose of hexachlorobutadiene. In females, the primary urinary metabolite was N-ac-PCBC; radiolabel was also detected as part of the minor urinary metabolite, PCBC. In males, N-ac-PCBC and PCBC were minor metabolites and the radiolabel was primarily detected as unchanged hexachloro-butadiene and as N-acetyl-S-(pentachlorobutadienyl)-L-cysteine sulfoxide.

Fecal excretions contained unmetabolized, unabsorbed hexachlorobutadiene plus a portion of the hepatic metabolites excreted with the bile. At the lower doses, almost all of the label in the feces originated with the biliary metabolites, whereas at the higher doses, there was also some unabsorbed hexachlorobutadiene in the fecal matter (Dekant et al. 1988b). In rats given 200 mg/kg, feces collected during the 5-day period contained a total of 39% of the dose. Only 5% was excreted in the first 2 days after dosing. In another study, the feces and contents of the gastrointestinal tract contained 62% of a 1 mg/kg dose and 72% of a 100 mg/kg dose (Payan et al. 1991). A third study found that 15.6 and 11.1% of ^{14}C-hexachlorobutadiene label was excreted in the feces during the 2-day period following a gavage dose of 200 mg/kg in male and female rats (Birner et al. 1995). The only metabolite that had been identified in the feces was S-(1,1,2,3,4-pentachlorobutadienyl) glutathione (Dekant et al. 1988b), although unidentified metabolites were also present and most likely included the cysteine derivatives. Similarly, Birner et al. (1995) reported the radiolabel in the feces represented both unmetabolized hexachlorobutadiene and S-conjugates.

In one study where a single 200 mg/kg dose was given to rats by gavage, 35% of the label was found in the bile in the first 2 days after dosing. The biliary label was equally distributed over the 2 days of collection. In a different study, 66% of a 1 mg/kg dose was excreted in the bile of bile duct cannulated

rats in 72 hours and 58% of a 100 mg/kg dose (Payan et al. 1991). Secretions from bile duct cannulated rats given a dose of 100 mg/kg hexachlorobutadiene were infused directly into the bile duct of nonexposed rats (Payan et al. 1991). The levels of label in the urine, bile, and feces of both the donor and recipient rats were measured 30 hours after dosing. The label in the urine and bile of the recipient rats represented label that was reabsorbed from the gastrointestinal tract. It was determined that 80% of the biliary metabolites were reabsorbed and only 20% remained in the feces and gastrointestinal tract.

The distribution of radiolabel in excreta was measured in male rats for the 72-hour period after intravenous administration of doses of 1 or 100 mg/kg (Payan et al. 1991). At both doses, about 8% of the radiolabel was exhaled. The amount of label in the urine was 21% of the low dose and 9% of the high dose; the amount in the feces was 59% of the low dose and 72% of the high dose. In a parallel study, the fecal, urinary, and biliary excretions were measured for rats with cannulated bile ducts. The urine contained 6–7 % of the dose and the feces <0.5 % for both doses. The bile contained 89% of the 1 mg/kg dose and 72% of the 100 mg/kg dose.

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

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

Green et al. (2003) developed a PBPK model for hexachlorobutadiene based on an inhalation PBPK model for styrene with an added kidney compartment. The model was designed to estimate an inhalation concentration that would result in the same body burden as an oral dose of 0.2 mg/kg/day (NOAEL for renal toxicity). The renal concentration of β-lyase metabolites was then estimated for the inhalation concentration. Lastly, an inhalation concentration that would result in the same concentration of β-lyase metabolites in humans was estimated. The model used published partition coefficients and physiological parameters, and enzyme metabolic rates and affinity constants were calculated using *in vitro* human and rat liver and kidney samples. The model predicted that the level of β-lyase metabolites associated with an

oral dose of 0.2 mg/kg/day (137.7 mg/L) would be equivalent to an inhalation concentration of 0.07 ppm for rats. In humans, an inhalation concentration of 1.41 ppm would be needed to obtain 137.7 mg/L of β-lyase metabolites. The results of this model should be interpreted cautiously since the model was constructed using limited available data and has not been validated in humans or rats.

3.1.6 Animal-to-Human Extrapolations

The results of an *in vitro* study suggest differences in hexachlorobutadiene metabolism between rats and humans (Green et al. 2003) and predicted that 10 times less β-lyase metabolites are formed in humans than rats at a given air concentration. However, the study did not investigate whether this would result in differences in hexachlorobutadiene toxicity between the species. The limited available toxicity data in humans do not allow for a comparison with experimental animal species. Based on the toxicity studies presented in Table 2-2, rats and mice appear to have similar targets of toxicity, with the kidney being the most sensitive target. Although direct comparisons of dose-response curves in rats and mice are not possible due to differences in exposure durations, subroutes of exposure (gavage versus diet), and range of doses tested, the available data do not appear to suggest large differences in the sensitivity of rats and mice to the renal toxicity of hexachlorobutadiene.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to hexachlorobutadiene are discussed in Section 5.7, Populations with Potentially High Exposures.

Studies in animals revealed that hexachlorobutadiene causes damage to the proximal tubules of the kidney. Accordingly, people with preexisting kidney damage may have compromised organ functions and are expected to be more vulnerable to chemical insult than people with normal kidney function. This is supported by a study that found that compromised glomerular function increased the renal toxicity of hexachlorobutadiene (Kirby and Bach 1995). Pre-treatment with Adriamycin, which induces glomerular damage, resulted in an increase in the severity of the hexachlorobutadiene-induced damage to the proximal tubules.

Several studies have evaluated potential age-related differences in the toxicity of hexachlorobutadiene. Kociba et al. (1977) reported much lower LD_{50} values in weanling rats (65 mg/kg in males) as compared to adult rats (580 mg/kg in males). Another study which examined rats aged 1, 3, 6, 9, and 12 months did not find any differences in the renal response to an intraperitoneal injection of 100 mg/kg hexachlorobutadiene (Zanetti et al. 2010). Studies examining the developmental toxicity of hexachlorobutadiene reported decreases in fetal or pup body weights (Harleman and Seinen 1979; Saillenfait et al. 1989; Schwetz et al. 1977), but did not find increases in anomalies or malformations.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

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

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to hexachlorobutadiene are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for hexachlorobutadiene from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health

impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by hexachlorobutadiene are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Human exposure to hexachlorobutadiene can be determined by measuring the parent compound in blood and adipose tissue (Bristol et al. 1982; Mes et al. 1985). Data in animals are limited, but do suggest that hexachlorobutadiene can be detected in urine and exhaled air. Approximately 4–31% of the administered radioactivity was detected in the urine of mice or rats within 72 hours following the administration of single oral doses of ^{14}C-hexachlorobutadiene (1–200 mg/kg) (Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985). No information was located on how long before it can no longer be detected. Unmetabolized hexachlorobutadiene was detected in exhaled air after animals were given doses of 1–100 mg/kg (Dekant et al. 1988b; Payan et al. 1991; Reichert et al. 1985). Cysteine conjugates of hexachlorobutadiene are converted to thio derivatives (e.g., 1,1,2,3,4-pentachlorobutadiene methylthioether and 1,1,2,3,4-pentachlorobutadiene carboxy methylthioether), which have been detected in urine (Reichert et al. 1985). Accordingly, tests to determine concentrations of these sulfur derivatives in urine may be useful in determining if exposure to hexachlorobutadiene has occurred.

3.3.2 Biomarkers of Effect

Data are sparse regarding biomarkers of the effects of hexachlorobutadiene in humans. Workers chronically exposed to the compound (along with carbon tetrachloride and perchloroethylene) had increased serum bile acids (Driscoll et al. 1992). Because the workers were also exposed to other chemicals, effects reported cannot be attributed to hexachlorobutadiene alone.

As discussed in Chapter 2, renal damage is the primary toxic effect associated with exposure to hexachlorobutadiene in animals (Harleman and Seinen 1979; Kociba et al. 1971, 1977; NTP 1991; Schwetz et al. 1977). Several studies have investigated the reliability of biomarkers to assess hexachlorobutadiene-induced renal toxicity in rats receiving a single intraperitoneal injection of hexachlorobutadiene (Maguire et al. 2013; Swain et al. 2011, 2012). These biomarkers are not specific to hexachlorobutadiene and may be altered due other causes of renal damage. These studies identify urinary α-glutathione S-transferase (α-GST) and albumin as the most sensitive biomarkers of early renal damage. In a study testing multiple dose levels (Swain et al. 2012), the lowest dose causing minimal proximal tubular damage also resulted in significant increases in urinary α-GST levels (approximately 300% higher than controls). The urinary α-GST level increase followed a dose-related pattern; at the highest dose tested (90 mg/kg), which caused very marked degeneration, α-GST levels were approximately 65,000 times higher than in controls. Albumin levels were not increased at the lowest adverse dose level; however, the magnitude of the increase at higher doses was greater than other biomarkers examined. In addition to α-GST and albumin, urinary levels of glucose, kidney molecule-1 (KIM-1), β-hydroxy-butyrate, osteopontin, and clusterin were found to be sensitive biomarkers of renal damage (Maguire et al. 2013; Swain et al. 2012); with the exception of β-hydroxybutyrate, increases in these biomarkers were not observed at doses resulting in minimal proximal tubular damage (Swain et al. 2012). β-Hydroxybutyrate levels were increased at the lowest dose tested, which did not result in histological alterations (Swain et al. 2012). KIM-1 and clusterin levels appeared to be reliable biomarkers of the regeneration and repair that occurred several days after exposure (Maguire et al. 2013; Swain et al. 2011, 2012). In contrast to the urinary biomarkers, plasma biomarkers appeared to be relatively insensitive. Increases in plasma creatinine and urea levels were only observed at doses resulting in marked tubular degeneration (Swain et al. 2012).

3.4 INTERACTIONS WITH OTHER CHEMICALS

Several studies have been conducted to assess factors which influence the toxicity of hexachloro butadiene. Most of these studies have involved effects of mixed function oxidase activity (MFO) on renal toxicity. The administration of MFO inhibitors, including SKF-525A (Lock and Ishmael 1981) and piperonyl butoxide (Davis 1984; Hook et al. 1982), did not alter hexachlorobutadiene-induced renal damage. Similar results were reported in tests evaluating MFO inducers such as phenobarbital (Lock and Ishmael 1981), β-naphthoflavone, isosafrole, and Aroclor 1254 (Hook et al. 1982). Renal toxicity was not exacerbated by prior exposure to ketonic solvents (Hewitt and Brown 1984).

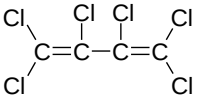
There are reports of interactions of hexachlorobutadiene with other chemicals. Combined administration of minimally toxic doses of hexachlorobutadiene with mercuric chloride and potassium dichromate for 24 hours caused synergistic effects as evident by marked increases in urinary (6–24 hours) alkaline phosphatase, lactate dehydrogenase, and NAG activities, as well as more severe tubular necrosis than caused by treatment with hexachlorobutadiene alone (Jonker et al. 1993a). Antagonistic effects were evident as characterized by smaller increases in urinary γ-glutamyl transferase activity compared to treatment with hexachlorobutadiene alone. Combined administration of the same chemicals did not cause additive interactions regarding biochemical parameters or histopathological changes in the kidney (Jonker et al. 1993a). An additional study revealed that when animals are treated for 4 weeks with minimally toxic doses of hexachlorobutadiene in combination with other chemicals (mercuric chloride, δ-limonene, and lysinoalanine), there is an increase in growth retardation and renal toxicity (renal weight, urine concentrating ability, and renal structure) in male rats, but not in female rats (Jonker et al. 1993b).

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of hexachlorobutadiene is located in Table 4-1.

Table 4-1. Chemical Identity of Hexachlorobutadiene

Characteristic	Information	Reference
Chemical name	Hexachlorobutadiene	Montgomery and Welkom 1990
Synonym(s) and registered trade name(s)	Perchlorobutadiene; HCBD; 1,1,2,3,4,4-Hexachloro-1,3-butadiene; 1,3-Hexachlorobutadiene; Dolen-Pur; GP-40-66:120	Montgomery and Welkom 1990; NLM 2020
Chemical formula	C_4Cl_6	Montgomery and Welkom 1990
Chemical structure		Montgomery and Welkom 1990
CAS Registry Number	87-68-3	Montgomery and Welkom 1990

CAS = Chemical Abstracts Service

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of hexachlorobutadiene is located in Table 4-2.

Table 4-2. Physical and Chemical Properties of Hexachlorobutadiene

Property	Information	Reference
Molecular weight	260.76	Montgomery and Welkom 1990
Color	Colorless	NLM 2020
Physical state	Liquid	Montgomery and Welkom 1990
Melting point	-21°C	Montgomery and Welkom 1990
Boiling point	215°C	Montgomery and Welkom 1990
Density at 20°C	1.55 g/cm^3	NLM 2020
Odor	Mild to pungent	Montgomery and Welkom 1990
Odor threshold:		
Water	No data	
Air	12.0 mg/m^3	Ruth 1986

Table 4-2. Physical and Chemical Properties of Hexachlorobutadiene

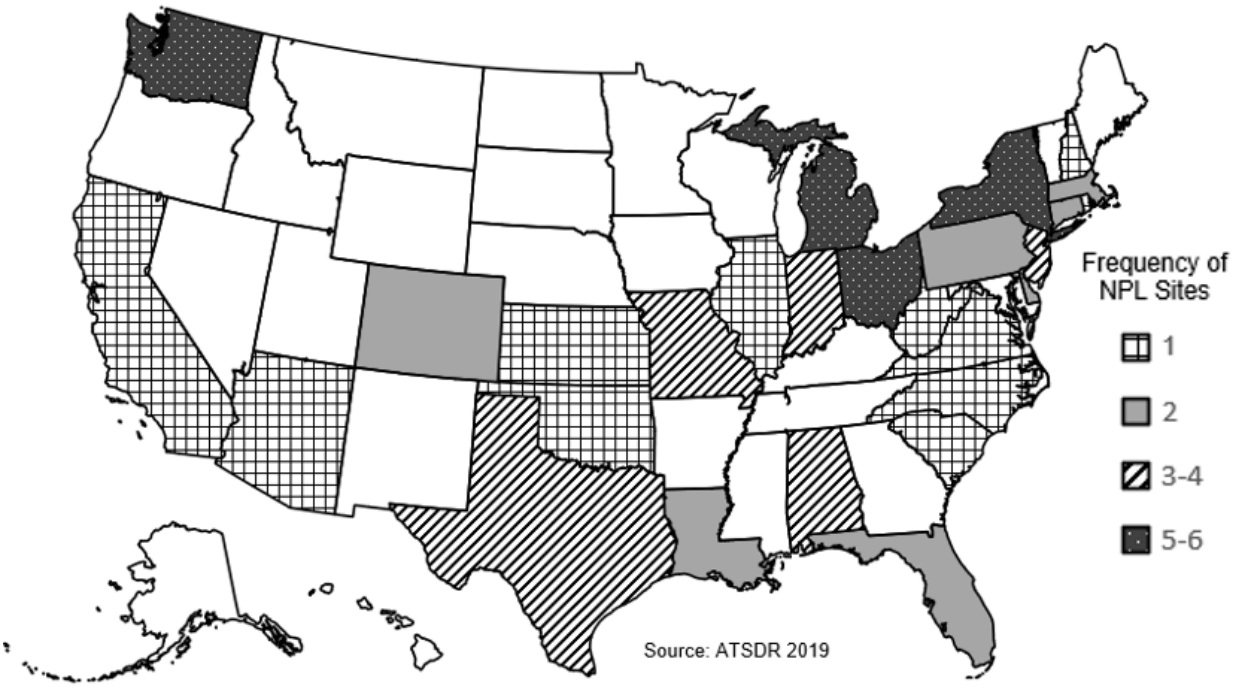
Solubility:		
Water at 20°C	2–2.55 mg/L	Montgomery and Welkom 1990
Organic solvents	Soluble in ethanol and ether	Montgomery and Welkom 1990
Partition coefficients:		
Log K_{ow}	4.78	Montgomery and Welkom 1990
Log K_{oc}	3.67	Montgomery and Welkom 1990
Vapor pressure at 25°C	0.15 mmHg	Montgomery and Welkom 1990
Henry's law constant	0.001–0.026 atm-m^3/mol	Montgomery and Welkom 1990
Autoignition temperature	610°C	NLM 2020
Flashpoint	Noncombustible	Montgomery and Welkom 1990
Flammability limits	Noncombustible	Montgomery and Welkom 1990
Conversion factors	1 ppm=10.5 mg/m^3 1 mg/m^3=0.095 ppm	
Explosive limits	No data	

CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

Hexachlorobutadiene has been identified in at least 62 of the 1,867 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2019). However, the number of sites in which hexachlorobutadiene has been evaluated is not known. The number of sites in each state is shown in Figure 5-1.

Figure 5-1. Number of NPL Sites with Hexachlorobutadiene Contamination



- The most likely route of exposure for the general public to hexachlorobutadiene is through ingestion of contaminated food and water and inhalation.
- There are no natural sources of hexachlorobutadiene that contribute to environmental levels. The main source is its production as a byproduct of chlorinated hydrocarbon synthesis.
- Its principal use is as a chemical intermediate in the manufacture of rubber compounds.
- There are limited data on the fate and transport of hexachlorobutadiene. The available data suggest that it will bind to soil particles and sediments and particulates in air and water. Some volatilization of hexachlorobutadiene from surface waters and soils may also occur.

- In air, it will likely react with reactive oxygen species and have a half-life ranging from 60 days to 1.6 years. The estimated half-life in surface water is estimated to be between 3 and 300 days.

5.2 PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.2.1 Production

Hexachlorobutadiene was first prepared in 1877 by the chlorination of hexyl oxide (IARC 1979). Commercial quantities of hexachlorobutadiene have never been produced in the United States. The primary source of hexachlorobutadiene found in the United States is inadvertent production as a waste byproduct of the manufacture of certain chlorinated hydrocarbons, such as tetrachloroethylene, trichloroethylene, and carbon tetrachloride (EPA 1980; Yang 1988). In 1982, EPA reported an annual volume of about 28 million pounds of hexachlorobutadiene inadvertently produced as a waste byproduct from this source (EPA 1982a; NLM 2020). Table 5-1 summarizes information on U.S. companies that manufactured or used hexachlorobutadiene in 2018 (TRI18 2020).

Table 5-1. Facilities that Produce, Process, or Use Hexachlorobutadiene

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
IN	1	100	999	12
KY	1	100,000	999,999	1, 3, 6
LA	5	10,000	9,999,999	1, 5, 6, 12, 13
OH	1	1,000	9,999	12
TX	4	100	999,999	1, 5, 6, 12, 13, 14

[a]Post office state abbreviations used.
[b]Amounts on site reported by facilities in each state.
[c]Activities/Uses:

1. Produce
2. Import
3. Used Processing
4. Sale/Distribution
5. Byproduct
6. Reactant
7. Formulation Component
8. Article Component
9. Repackaging
10. Chemical Processing Aid
11. Manufacture Aid
12. Ancillary
13. Manufacture Impurity
14. Process Impurity

Source: TRI18 2020 (Data are from 2018)

5.2.2 Import/Export

Since 1974, most hexachlorobutadiene used commercially in the United States has been imported from Germany. Imported quantities remained fairly constant in the late 1970s, averaging about

500,000 pounds annually, but dropped to 145,000 pounds in 1981 (EPA 1980, 1982b). More recent information on the volume of imported or exported hexachlorobutadiene is not available.

5.2.3 Use

Hexachlorobutadiene is used as a chemical intermediate in the manufacture of rubber compounds (EPA 1982b). Lesser quantities of hexachlorobutadiene are used as a solvent, a fluid for gyroscopes, a heat transfer liquid, a hydraulic fluid, and a chemical intermediate in the production of chlorofluorocarbons and lubricants (EPA 1980; IARC 1979; Verschueren 1983). Small quantities are also used as a laboratory reagent (EPA 1982b). In the international market, Russia is reported to be one of the major users of hexachlorobutadiene, where it is used as a fumigant on grape crops. Hexachlorobutadiene is also used as a fumigant in France, Italy, Greece, Spain, and Argentina (IARC 1979; NTP 1991). Prior to 1975, the largest domestic use of hexachlorobutadiene was for the recovery of “snift” (chlorine-containing) gas in chlorine plants (NLM 2020). More recent information from U.S. chlorine producers indicates that hexachlorobutadiene is no longer used for this process (EPA 1982b; IARC 1979).

5.2.4 Disposal

Waste streams resulting from the inadvertent production of hexachlorobutadiene as a byproduct of certain chlorinated hydrocarbons typically contain 33–80% hexachlorobutadiene. These wastes are disposed of by various methods. Disposal practices have shifted from landfilling to incineration. Incineration, which is considered the preferred method of disposal, reportedly achieves >99.9% destruction efficiency (EPA 1982b). In 1982, approximately 68% of an estimated 27 million pounds of hexachlorobutadiene wastes were disposed of by incineration, 32% by deep well injection, and <0.2% by hazardous waste landfill operations (EPA 1982b).

The generation, treatment, storage, and disposal of hexachlorobutadiene-containing wastes are subject to regulation under the Resource Conservation and Recovery Act (RCRA). Underground injection of hexachlorobutadiene is subject to permits issued under an Underground Injection Control program promulgated under the Safe Drinking Water Act (EPA 1982b).

5.3 RELEASES TO THE ENVIRONMENT

The Toxics Release Inventory (TRI) data should be used with caution because only certain types of facilities are required to report (EPA 2005). This is not an exhaustive list. Manufacturing and processing

facilities are required to report information to the TRI only if they employ ≥10 full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20–39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005).

5.3.1 Air

Estimated releases of 2,192 pounds (~0.99 metric tons) of hexachlorobutadiene to the atmosphere from 12 domestic manufacturing and processing facilities in 2018, accounted for about 99.4% of the estimated total environmental releases from facilities required to report to the TRI (TRI18 2020). These releases are summarized in Table 5-2.

Table 5-2. Releases to the Environment from Facilities that Produce, Process, or Use Hexachlorobutadiene[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
IN	1	0	0	0	0	0	0	0	0
KY	1	19	0	0	0	0	19	0	19
LA	5	2,157	0	0	13	0	2,157	13	2,170
OH	1	0	0	0	0	0	0	0	0

Table 5-2. Releases to the Environment from Facilities that Produce, Process, or Use Hexachlorobutadiene[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
TX	4	15	0	0	0	0	15	0	15
Total	12	2,192	0	0	13	0	2,192	13	2,205

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, wastewater treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other onsite landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

Source: TRI18 2020 (Data are from 2018)

There are no known natural sources of hexachlorobutadiene that contribute to environmental levels. The predominant source of hexachlorobutadiene is inadvertent production from the synthesis of certain chlorinated hydrocarbons (EPA 1982a). In 1975, the production of hexachlorobutadiene in the United States was estimated to be 8 million pounds, with 0.1 million pounds released to the environment (NSF 1975). Sixty-eight percent of the 27 million pounds of hexachlorobutadiene waste generated in the United States in 1982 was disposed of by incineration. This process typically obtains a 99.99% destruction efficiency, indicating that approximately 1,900 pounds were released to the atmosphere.

5.3.2 Water

There were no releases of hexachlorobutadiene to surface water or publicly owned treatment works (POTWs) from 12 domestic manufacturing and processing facilities in 2018 required to report to the TRI (TRI18 2020). These releases are summarized in Table 5-2.

Hexachlorobutadiene may be released to underground and surface waters through discharge from industrial facilities, by leaching from industrial discharges, by leaching from landfills or soils, or by urban runoff. Hexachlorobutadiene was detectable in 1.6% of 1,190 industrial effluent samples reported in the EPA Storage and Retrieval (STORET) database (Staples et al. 1985). The median concentration for all samples, including nondetects was <6 ppb. This chemical was also detected in leachate from an industrial landfill at a concentration of 0.109 ppm (Brown and Donnelly 1988) and from a hazardous waste site (Hauser and Bromberg 1982). In 1982, of the 27 million pounds of hexachlorobutadiene waste produced in the United States as a byproduct of chlorinated hydrocarbon production, 9 million pounds were disposed of by deep well injection (EPA 1982a).

5.3.3 Soil

Estimated releases of 13 pounds (~0.006 metric tons) of hexachlorobutadiene to soils from 12 domestic manufacturing and processing facilities in 2018, accounted for ~0.6% of the estimated total environmental releases from facilities required to report to the TRI (TRI18 2020). No hexachlorobutadiene was released via underground injection (TRI18 2020). These releases are summarized in Table 5-2.

Hexachlorobutadiene may be released to soil by disposal of wastes in landfill operations. In 1982, only 0.2% of the 27 million pounds of hexachlorobutadiene waste produced as a byproduct of chlorinated hydrocarbon-synthesis was disposed of in landfill operations (EPA 1982a). These data indicate that the release to soil was approximately 54,000 pounds.

5.4 ENVIRONMENTAL FATE

5.4.1 Transport and Partitioning

Air. Hexachlorobutadiene can exist in the atmosphere as a vapor or adsorbed to airborne particulate matter. The atmospheric burden of hexachlorobutadiene has been estimated to be 3.2 and 1.3 million kg/year for the northern and southern hemispheres, respectively (Class and Ballschmiter 1987). Significant dispersion of hexachlorobutadiene has been confirmed by the detection of hexachlorobutadiene at areas that are far removed from release sources (Class and Ballschmiter 1987). A high partition coefficient (log K_{oc}) value of 3.67 (Montgomery and Welkom 1990) for hexachlorobutadiene indicates that adsorption to soils with high organic carbon content can occur. Wind erosion of contaminated surface soils can then lead to airborne hexachlorobutadiene-containing particulate matter.

Levels of hexachlorobutadiene have been detected in fly ash from the incineration of hexachlorobutadiene-containing hazardous waste (Junk and Ford 1980). The transport of particulate matter is a function of particle size and wind speed; however, no data were located regarding the transport of hexachlorobutadiene-containing particles in air.

Water. Transport and partitioning of hexachlorobutadiene in water involves volatilization to the atmosphere and sorption to soil and sediments particulates. The high partition coefficient (log K_{ow}) of 4.78 (Montgomery and Welkom 1990) for hexachlorobutadiene leads to preferential partitioning to sediments and biota over water. Environmental surveys generally report higher levels of hexachlorobutadiene in sediments than in the waters that contain them (Elder et al. 1981; EPA 1976a; Oliver and Charlton 1984). Hexachlorobutadiene has a vapor pressure of 0.15 mmHg (25°C) (Montgomery and Welkom 1990), indicating that volatilization from water occurs. Volatilization is reduced by adsorption to organic material in the water.

Sediment and Soil. The transport and partitioning of hexachlorobutadiene in soils involve volatilization and adsorption. An estimated high partition coefficient (log K_{oc}) of 3.67 (Montgomery and Welkom 1990) for hexachlorobutadiene in soil indicates that soil adsorption can occur, particularly in soils with a high organic carbon content. Sorption was the predominant fate process for hexachlorobutadiene during anaerobic digestion of sludges (Govind et al. 1991). Data indicate that hexachlorobutadiene is mobile in sandy soils, which have relatively low organic-carbon contents (Piet and Zoeteman 1980). Volatilization from surface soils is relatively low; binding to the organic carbon content of the soil further reduces hexachlorobutadiene release.

The desorption of hexachlorobutadiene from field samples of contaminated soil to water was shown to occur in two stages: a loosely bound stage and a tightly bound stage, exhibiting a hysteresis effect on desorption. The initial partitioning from desorption of loosely bound hexachlorobutadiene in soil is quicker than the more tightly bound hexachlorobutadiene desorption characterized as a long-term resistant phase (Chen et al. 1999; Kommalapati et al. 2002).

Other Media. In rainbow trout, the bioconcentration factor (BCF) was dependent on water concentration (Oliver and Niimi 1983). At low concentrations of 0.10 ng/L, a BCF of 5,800 was obtained, compared to a value of 17,000 obtained with higher water concentrations of 3.4 ng/L. Hexachlorobutadiene preferentially accumulates in the liver of fish (Pearson and McConnell 1975). In mussels, the BCF was determined to be between 900 and 2,000 (Pearson and McConnell 1975).

However, lower values were obtained for algae, crayfish, and bass (160, 60, and 29, respectively) (EPA 1976a). The EPA reviewed new BCF data and recommended a value of 392 (EPA 1989).

Bioaccumulation factors based on freely dissolved lipid-normalized concentrations in water for hexachlorobutadiene range from 6,761 to 575,440 in various aquatic species including *Callinectes sapidus*, *Fundulus heteroclitus*, *Micropoganias undulates*, and *Brevoortia patronus* (Burkhard et al. 1997).

The mean concentration of hexachlorobutadiene found in fish in a contaminated swamp in the lower Mississippi River in Baton Rouge, Louisiana was more than 300 times greater than the mean sediment concentrations suggesting high bioaccumulation (Bart et al. 1998).

5.4.2 Transformation and Degradation

Air. No data were located regarding the transformation and degradation of hexachlorobutadiene in air. Based on the monitoring data, the tropospheric half-life of hexachlorobutadiene was estimated by one author to be 1.6 years in the northern hemisphere (Class and Ballschmiter 1987). However, analogy to structurally similar compounds such as tetrachloroethylene indicates that the half-life of hexachlorobutadiene may be as short as 60 days, predominantly due to reactions with photochemically produced hydroxyl radicals and ozone (Atkinson 1987; Atkinson and Carter 1984). Oxidation constants of $<10^3$ and 6 $(m \bullet hr)^{-1}$ were estimated for reactions with singlet oxygen and peroxy radicals, respectively (EPA 1982c).

Water. Data concerning the transformation and degradation of hexachlorobutadiene in waters are limited. Under aerobic conditions, hexachlorobutadiene underwent complete biodegradation after 7 days in water inoculated with domestic sewage (Tabak et al. 1981). Biodegradation of hexachlorobutadiene also occurred during anaerobic digestion of wastewater sludges, although sorption was the predominant fate process (Govind et al. 1991). However, biodegradation did not occur in anaerobic waters (Johnson and Young 1983). Based on monitoring data, the half-life of hexachlorobutadiene in rivers and lakes was estimated to be 3–30 and 30–300 days, respectively (Zoeteman et al. 1980). Data regarding the hydrolysis or photolysis of hexachlorobutadiene in water were not located.

Sediment and Soil. Data regarding the transformation and degradation of hexachlorobutadiene in soil were not located. However, based on the observation that hexachlorobutadiene was completely

biodegraded in water under aerobic conditions (Tabak et al. 1981), biodegradation probably occurs in nonarid soils as well.

5.5 LEVELS IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to hexachlorobutadiene depends, in part, on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of hexachlorobutadiene in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on hexachlorobutadiene levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable.

Table 5-3 shows the lowest limit of detections that are achieved by analytical analysis in environmental media. An overview summary of the range of concentrations detected in environmental media is presented in Table 5-4.

Table 5-3. Lowest Limit of Detection Based on Standards[a]

Media	Detection limit	Reference
Air	0.02 µg/sample	NIOSH 1994
Water	0.11 µg/L	EPA 1992
Soil/solid waste	Laboratory specific	EPA 2006
Whole blood	18 ng/L	Kastl and Hermann 1983

[a]Detection limits based on using appropriate preparation and analytics. These limits may not be possible in all situations.

Table 5-4. Summary of Environmental Levels of Hexachlorobutadiene

Media	Low	High	For more information
Outdoor air (ppt)	2	37	Section 5.5.1
Indoor air (ppt)	–	38	Section 5.5.1
Surface water (ppb)	0.82	22	Section 5.5.2
Drinking water (ppt)	1.6	2.7	Section 5.5.2
Sediment (µg/kg)	2.9	11	Section 5.5.3
Food (mg/kg)	0.06	0.3	Section 5.5.4

Detections of hexachlorobutadiene in air, water, and soil at NPL sites are summarized in Table 5-5.

Table 5-5. Hexachlorobutadiene Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Medium	Median[a]	Geometric mean[a]	Geometric standard deviation[a]	Number of quantitative measurements	NPL sites
Water (ppb)	120	560	5,600	8	6
Soil (ppb)	230,000	23,000	220	10	6
Air (ppbv)	7.2	6.8	420	15	7

[a]Concentrations found in ATSDR site documents from 1981 to 2019 for 1,867 NPL sites (ATSDR 2019). Maximum concentrations were abstracted for types of environmental media for which exposure is likely. Pathways do not necessarily involve exposure or levels of concern.

5.5.1 Air

In the United States, the reported average concentration of hexachlorobutadiene, based on 72 samples from urban and source dominated areas, was 36 ppt (0.38 µg/m^3) (EPA 1988a; Shah and Singh 1988). Hexachlorobutadiene levels ranging from 2 to 11 ppt were reported in a number of cities (EPA 1978; Singh et al. 1980, 1982). Higher levels of hexachlorobutadiene were reported in Niagara Falls, with concentrations of up to 37 ppt detected in ambient air levels and up to 38 ppt detected in the basement air of homes near industrial and chemical waste disposal sites (Pellizzari 1982). Hexachlorobutadiene was not detected in 31 ambient air samples from two superfund sites (Intermountain Waste Oil Refinery; Ogden Railyard) (WQP 2020).

Occupational exposures can be significantly higher for individuals who work at plants that produce chlorinated hydrocarbons. Maximum air levels off plant property, at a plant boundary, and within a plant were reported to be 22, 938, and 43,000 ppt, respectively (EPA 1976b).

5.5.2 Water

Hexachlorobutadiene has been detected in some surface waters, but the incidence of detection is low. It was detected in about 4% of 2,518 ambient water samples compiled from the STOrage and RETrieval (STORET) and National Water Information System (NWIS) databases, but levels were below the lower reporting limit (WQP 2020). Historically, hexachlorobutadiene was detected in 1 of 204 surface water sites sampled across the United States with a concentration of 22 ppb (EPA 1977). Low levels of

hexachlorobutadiene were detected in the Niagara River at 0.82 ppt (Oliver and Charlton 1984). Hexachlorobutadiene was not detected in rainwater (Pankow et al. 1984) or urban storm water runoff (Cole et al. 1984; WQP 2020) in a number of U.S. cities. It has not been detected in open ocean waters; however, the coastal waters of the Gulf of Mexico were reported to contain 3–15 ppt (Sauer 1981).

Low levels of hexachlorobutadiene (<1 ppb) may be found in drinking water (EPA 1989). Finished drinking water samples from two U.S. cities were found to contain 1.6 and 2.7 ppt, respectively (EPA 1984). Hexachlorobutadiene was detected in about 31% of 4,329 groundwater samples, with only about 10% of samples testing above the reporting limit with a maximum concentration of 6.38 ppm (WQP 2020).

5.5.3 Sediment and Soil

Hexachlorobutadiene adsorbs to sediments in contaminated water. Sediments from the Niagara River were found to contain 2.9–11 µg/kg (Oliver and Charlton 1984). Hexachlorobutadiene was analyzed for, but not detected, above the quantitation limit in >2,500 sediment samples reported from the Great Lakes National Program (WQP 2020). The median quantitation limit was <300 ppb. Hexachlorobutadiene was not detected in 84 sediment samples from the city and county of Honolulu with lower reporting levels of 26–42 ppb, nor was it detected in 43 ocean site sediment samples.

Abdelghani et al. (1995) conducted field and laboratory studies to determine the levels of hexachlorobutadiene in various samples collected from a swamp area in Louisiana. Hexachlorobutadiene levels ranged from <0.05 to 0.40 ppb in sediment samples.

Hexachlorobutadiene was not detected in 333 soil samples tested in response to Hurricane Katrina, in 133 soil samples from superfund site Ogden Railyard or in 325 soil samples reported from U.S. Geological Survey sites in Alabama, Florida, Hawaii, Indiana, New Mexico, and Texas (WQP 2020).

5.5.4 Other Media

Hexachlorobutadiene was detected in several foodstuffs in the United Kingdom (McConnell et al. 1975) and Germany (Kotzias et al. 1975), but it was not detected in the United States in milk, eggs, or vegetables even when the samples were obtained from within a 25-mile radius of facilities producing chlorinated hydrocarbons (Yip 1976; Yurawecz et al. 1976). Fish from the Great Lakes generally did not contain detectable levels of hexachlorobutadiene (Camanzo et al. 1987; DeVault 1985), with the

exception of trout from Lake Ontario, which were reported to contain 0.06–0.3 mg/kg (Oliver and Niimi 1983). Hexachlorobutadiene was not detectable in any of 993 fish tissue samples collected from 2000 to 2020 reported on the STORET database including data from 39 U.S. states in 60 species of fish (WQP 2020).

Macgregor et al. (2010) investigated concentrations of persistent organic pollutants (POPs), including hexachlorobutadiene, in eels from 30 sites across Scotland. While several of the other POPs were detected in all samples, hexachlorobutadiene was detected in only one sample. Eels were used as an ideal biomonitor because of their high lipid content.

Hexachlorobutadiene was not detected in sewage influents (EPA 1979), in sewage samples (EPA 1990), or landfill leachate (WQP 2020).

In a study of black tattoo ink, hexachlorobutadiene was detected in 6 of 14 samples; the levels ranged from 0.08 to 4.52 µg/g (Lehner et al. 2011).

5.6 GENERAL POPULATION EXPOSURE

The general population can be exposed to low levels of hexachlorobutadiene in air, food, and water. Estimates of source or route-specific exposures to humans were not located. Hexachlorobutadiene has been detected in human adipose tissue with a concentration ranging from 0.8 to 8 µg/kg wet weight (McConnell et al. 1975; Mes et al. 1982). Higher concentrations were reported in human liver samples, with values ranging from 5.7 to 13.7 µg/kg wet weight (McConnell et al. 1975). These data indicate that exposure to hexachlorobutadiene occurs in humans, but do not identify sources or routes of exposure. Although exposure from foods is probably a minor route of exposure, people who consume large amounts of fish obtained from contaminated waters may be exposed to significant quantities of hexachloro-butadiene. Similarly, persons who live in source-dominated areas or work in plants that produce chlorinated hydrocarbons may be exposed to significant levels of hexachlorobutadiene in the air. No information was found on the number of workers potentially exposed to hexachlorobutadiene.

Fat tissue samples from 50 children living in farm areas in the Murcia region of Spain were found to have mean concentrations of hexachlorobutadiene of 0.70 µg/g (from 13 positive samples) ranging from 0.23 to 2.43 µg/g (Olea et al. 1999).

5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

People who live in source-dominated areas (at or near hazardous waste sites or chlorinated hydrocarbon production plants) and workers in these areas are potentially exposed to high levels of hexachlorobutadiene. Individuals who consume large amounts of fish from contaminated waters may also be exposed to above-average levels of hexachlorobutadiene.

CHAPTER 6. ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of hexachlorobutadiene is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the adverse health effects (and techniques for developing methods to determine such health effects) of hexachlorobutadiene.

Data needs are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

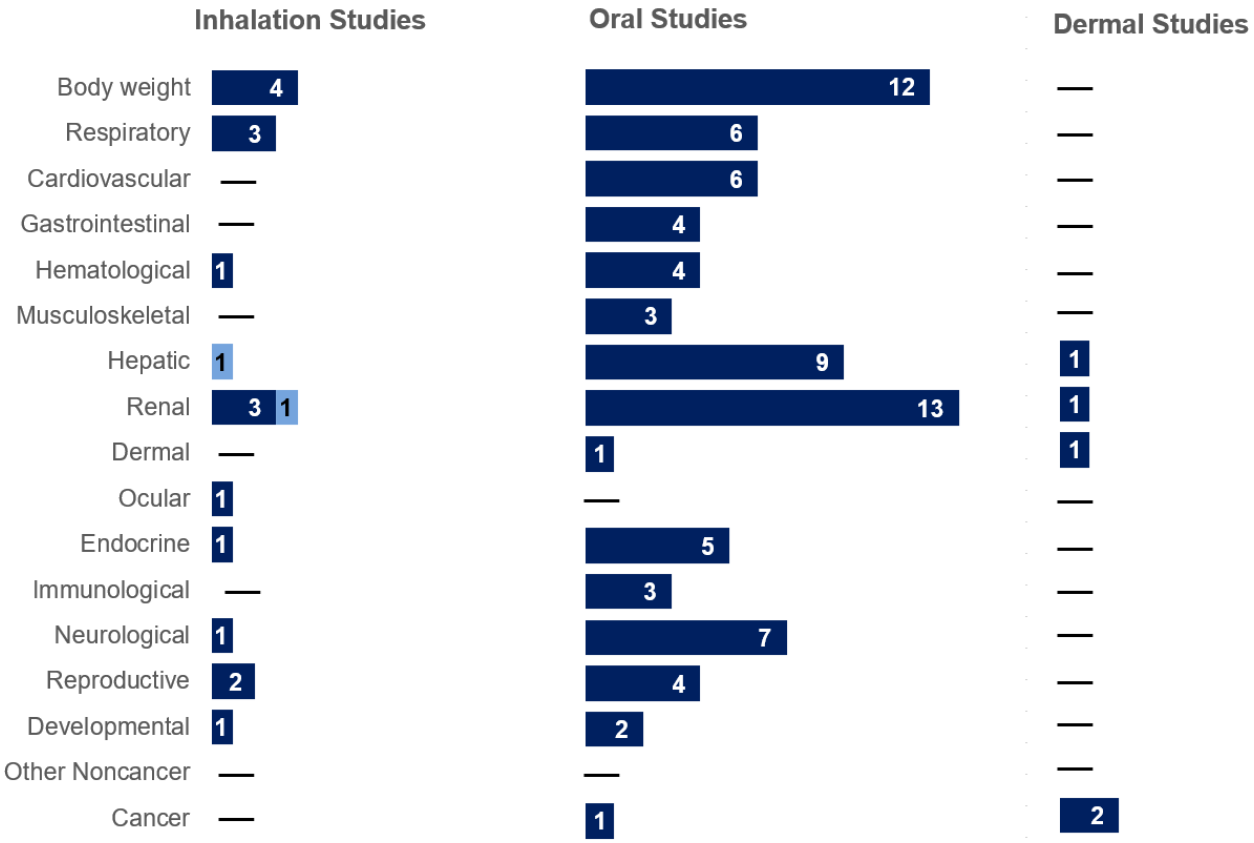
6.1 INFORMATION ON HEALTH EFFECTS

Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to hexachlorobutadiene that are discussed in Chapter 2 are summarized in Figure 6-1. The purpose of this figure is to illustrate the information concerning the health effects of hexachlorobutadiene. The number of human and animal studies examining each endpoint is indicated regardless of whether an effect was found and the quality of the study or studies.

As illustrated in Figure 6-1, most of the data on the toxicity of hexachlorobutadiene come from studies in experimental animals. The most commonly examined endpoints were the kidney, body weight, and liver. Two human studies were identified examining potential liver and kidney effects; the exposure route for these studies is presumed to be inhalation. Hexachlorobutadiene toxicity has been evaluated in 24 inhalation, oral, or dermal exposure studies; approximately half of the animal studies were conducted by the oral exposure route.

Figure 6-1. Summary of Existing Health Effects Studies on Hexachlorobutadiene By Route and Endpoint*

Potential kidney, body weight, and liver effects were the most studied endpoints
The majority of the studies examined oral exposure in **animals** (versus **humans**)



*Includes studies discussed in Chapter 2; the number of studies include those finding no effect. Many studies examined multiple endpoints.

6.2 IDENTIFICATION OF DATA NEEDS

Missing information in Figure 6-1 should not be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Acute-Duration MRLs. Several studies have evaluated the acute toxicity of hexachlorobutadiene following inhalation exposure; however, the database was not considered adequate for derivation on an inhalation MRL due to the lack of repeated exposure studies examining the kidney. The overall database for hexachlorobutadiene suggests that the kidney is one of the most sensitive targets of toxicity. Two studies have examined the kidney, but neither study involved exposure for more than 2 days and the study identifying the lowest LOAEL was only for 4 hours. Additionally, repeated exposure studies examining a wide range of potential targets is needed to establish that the kidney is the most sensitive target and for derivation of an MRL. Three studies evaluated acute oral toxicity and were considered adequate for derivation of an oral MRL with the support of intermediate- and chronic-duration studies. Additional studies are needed to verify that the kidney is the most sensitive target of toxicity following acute-duration exposure.

Intermediate-Duration MRLs. The intermediate-duration inhalation database consists of a general toxicity study and a developmental toxicity study. Given the limited number of endpoints examined in both studies and the lack of study details provided for the general toxicity study, neither study was considered adequate for derivation of an MRL. Additional studies involving exposure durations of at least 13 weeks examining a wide variety of potential endpoints over a range of exposure concentrations are needed to identify the most sensitive targets of toxicity and establish concentration-response relationships that could be used to derive an MRL. A number of comprehensive intermediate-duration oral studies support the derivation of an intermediate-duration MRL. The principal study (NTP 1991) demonstrated a very steep dose-response curve; the incidence of renal lesions went from 1/10 at 0.2 mg/kg/day to 9/10 at 0.5 mg/kg/day. Thus, benchmark dose modeling could not be used to derive the MRL. Studies testing doses between 0.2 and 0.5 mg/kg/day would be useful for establishing dose-response relationships and decreasing the uncertainty in the MRL.

Chronic-Duration MRLs. No chronic-duration inhalation studies were identified, and studies are needed for derivation of a chronic-duration inhalation MRL. One chronic-duration oral study was identified (Kociba et al. 1977). Although the study examined a wide range of effects at three dose levels, the insufficient reporting of the results, including lack of a description of the renal lesions and incidence data, precluded an independent assessment of the results and the use of the study as the basis of an MRL. An additional chronic oral study is needed for derivation of a chronic-duration oral MRL.

Health Effects.

Respiratory. Acute- and intermediate-duration inhalation studies suggest that hexachlorobutadiene is a respiratory irritant. However, none of the available inhalation studies included histological examination of the respiratory tract; these studies are needed to determine if the respiratory tract is a sensitive target and for comparison of the effect levels for respiratory and renal effects.

Renal. A number of studies have evaluated the renal toxicity of hexachlorobutadiene, particularly following intermediate-duration oral exposure. Available studies have examined potential effects on kidney weight, histology, and renal function. However, very few studies included both histological examination and measurement of renal function parameters; two studies reported histological and function alterations at the same dose level (Harleman and Seinen 1979; Jonker et al. 1993a) and a third study found histological alterations, but no alterations in urinary parameters (Kociba et al. 1977). Additional studies evaluating histology and urinary renal function parameters would be useful to understand the toxicity of hexachlorobutadiene and to assess whether renal function is affected at lower doses than those resulting in histological alterations.

Dermal. There are limited data on the dermal toxicity of hexachlorobutadiene. In a lethality study, hepatic, renal, and dermal effects were observed at the lowest dose tested. Additional studies are needed to establish the most sensitive endpoint following repeated exposure to lower doses.

Immunotoxicity. The potential of hexachlorobutadiene to impair immune function has not been evaluated following inhalation, oral, or dermal exposure, although studies have conducted histological examinations of immune tissues. An *in vitro* study did find inhibition of B or

T lymphocyte mitogenesis. Whether similar effects would occur following *in vivo* exposure is not known and future studies should include examination of immune function.

Cancer. Information on the carcinogenic potential of hexachlorobutadiene is limited to a chronic oral and dermal studies and a dermal initiation/promotion study. The oral rat study (Kociba et al. 1977) reported increases in the total number of renal neoplasms. Additional studies in another species is needed to confirm these results and to decrease the uncertainty in the carcinogenicity assessment.

Epidemiology and Human Dosimetry Studies. There are limited epidemiology data available for hexachlorobutadiene. An occupational exposure study reported alterations in serum bile acids in chronically exposed workers (Driscoll et al. 1992), although interpretation of the results is limited by potential exposure to other hepatotoxic chemicals. A second study found some indications of altered renal function in residents living in homes with elevated hexachlorobutadiene levels (Staples et al. 2003). Experimental animal studies consistently demonstrated that the kidney is the most sensitive target of toxicity. Well-conducted epidemiological studies are needed to determine if similar patterns of damage occur in humans.

Biomarkers of Exposure and Effect. There is no single biological indicator of exposure to hexachlorobutadiene. Various tests of renal function and biochemical changes associated with renal damage may be measured to detect effects resulting from short-term, intermediate, and long-term exposure. Because similar effects can also occur following exposure to other substances, these tests are not specific for hexachlorobutadiene exposure. Although hexachlorobutadiene and its metabolites are excreted in urine, the metabolism of the compound has not been characterized in humans. Additional tests addressing the dose-response relationship between hexachlorobutadiene excretion in breath and the excretion of sulfur-containing metabolites in urine would prove valuable.

Absorption, Distribution, Metabolism, and Excretion. Data are available on the toxicokinetics of hexachlorobutadiene in animals by the oral route, but not in humans. There are no data in humans or animals on exposures to hexachlorobutadiene by the inhalation or dermal routes. Because of the key role of the liver in producing the metabolites that are responsible for the nephrotoxicity of this compound, knowledge of the toxicokinetics of inhalation and dermal exposures would be valuable. Oral studies reported the presence of the enzymes responsible for the glutathione conjugation reaction and the subsequent formation of derivatives in the liver, intestines, and kidney. It is not known at this time how

hexachlorobutadiene is distributed and metabolized by inhalation and dermal routes. It is postulated that distribution and metabolism by these routes would be similar to that for the oral route.

Comparative Toxicokinetics. There are no data on metabolism of hexachlorobutadiene in humans. On the other hand, toxic metabolites and proposed mechanisms of renal toxicity have been evaluated in animals employing both *in vivo* and *in vitro* test systems (Dekant et al. 1990; Schnellmann et al. 1987). It is not known if similar metabolic pathways and metabolites occur in humans.

Children's Susceptibility. A study in rats found that weanlings were much more sensitive to the lethality of orally administered hexachlorobutadiene than adults (Kociba et al. 1977). However, a study examining renal toxicity did not find differences in the response to injected hexachlorobutadiene in rats aged 1–12 months (Zanetti et al. 2010). More information is needed to determine if children would be more susceptible to hexachlorobutadiene toxicity than adults or if there would be differences in target tissues.

Physical and Chemical Properties. The physical and chemical properties of hexachlorobutadiene are sufficient to make estimations on its fate in the environment. No data regarding the odor threshold of hexachlorobutadiene in water were located.

Production, Import/Export, Use, Release, and Disposal. Hexachlorobutadiene is not produced for commercial purposes in the United States; however, small amounts are imported from Germany. Hexachlorobutadiene is mainly produced as a byproduct of chlorinated hydrocarbon synthesis and is a primary component of "hex-wastes" (EPA 1982a). Its uses as a pesticide and fumigant have been discontinued. Hexachlorobutadiene is disposed chiefly by incineration, and to a lesser extent by deep well injection and landfill operations (EPA 1982a). More recent production and release data would be helpful in estimating human exposure to hexachlorobutadiene.

Environmental Fate. Much of the environmental fate information on hexachlorobutadiene consists of modeling based on its physical and chemical properties and its similarity to related compounds. Further studies that determine the extent to which hexachlorobutadiene volatilizes from surface waters and soils, and the effects of organic-carbon content on this process would be helpful. Studies that experimentally determine the specific reactions and rates that drive the degradation of hexachlorobutadiene in air, water, and soil would be valuable. Data are lacking on hexachlorobutadiene adsorption to soil or its

biodegradation in this medium. More information on the fate of the compound in soil would be useful since this medium may be a pathway of exposure for populations living near emission sources.

Bioavailability from Environmental Media. Toxicity studies in animals indicate that absorption of hexachlorobutadiene through the gastrointestinal tract, respiratory tract, and skin can occur. Studies that identify the relationship between absorption and the matrix of soils, sediments, and foods would be useful in establishing whether or not absorption is significantly affected by such factors.

Food Chain Bioaccumulation. BCFs have been determined for algae, shellfish, and fish and exhibit a wide range (29–17,000) (EPA 1976a; Oliver and Niimi 1983; Pearson and McConnell 1975). This wide range may be explained, in part, by species differences in metabolism or differences in concentrations tested. Studies also indicate that hexachlorobutadiene preferentially accumulates in the livers of fish. Further studies that might explain the wide range of BCF values would be helpful. No information was located regarding the bioaccumulation of hexachlorobutadiene in plants or aquatic organisms. More information is needed to determine the importance of terrestrial/aquatic food chain bioaccumulation as a potential human exposure pathway.

Exposure Levels in Environmental Media. Data are available on the occurrence of hexachlorobutadiene in air, water, and foodstuff. The majority of the monitoring data on hexachlorobutadiene are outdated, and therefore, more recent information on the levels typically found in the environment would allow for more accurate estimation of human exposures, and could also serve to indicate time-dependent trends when compared with older data. No data were located regarding the occurrence of hexachlorobutadiene in groundwater or soil. Reliable monitoring data for the levels of hexachlorobutadiene in contaminated media at hazardous waste sites are also needed to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Exposure Levels in Humans. Hexachlorobutadiene has been detected in human adipose tissues and blood (Bristol et al. 1982; Mes et al. 1985). However, biomonitoring data are limited and hexachlorobutadiene has not been included in the National Report on Human Exposure to Environmental Chemicals (CDC 2018). Studies that establish a correlation between exposure levels in environmental media and the resulting levels in human tissues and excreta would be valuable in predicting exposures and corresponding health risks in humans who live at or near hazardous waste sites and who are likely to be exposed to hexachlorobutadiene.

Exposures of Children. No data specifically measuring exposure levels in children were located. General population monitoring studies should include an assessment on whether children may be exposed to higher levels than adults.

Analytical Methods. Hexachlorobutadiene can be measured in human blood and adipose tissue. No hexachlorobutadiene was detected in blood from controls or residents near a hazardous waste site (Bristol et al. 1982), indicating that the method was not sensitive enough to measure background levels of hexachlorobutadiene in the general population. It is likely that this method would be sensitive enough to measure levels at which biological effects occur. Hexachlorobutadiene was detected in adipose tissue of victims of accidental and nonaccidental deaths, with about twice as much in accident than nonaccident victims (Mes et al. 1985). This indicates that the gas chromatography/electron capture detection (GC/ECD) and GC/mass spectrometry (MS) method is sensitive enough to measure background levels of hexachlorobutadiene in the general population as well as levels at which biological effects occur.

Existing methods for analysis of air and water appear to be sufficiently sensitive, specific, and reliable to measure background levels in the environment. Matrix interference and contamination by co-eluting chemicals may limit the sensitivity and specificity of methods for analysis of hexachlorobutadiene in soil and solid waste. Development of analytical methods specific for hexachlorobutadiene could provide a rapid, inexpensive, and sensitive method for detecting hexachlorobutadiene in environmental samples.

6.3 ONGOING STUDIES

No ongoing studies were identified in the National Institute of Health (NIH) RePORTER (2020) database.

CHAPTER 7. REGULATIONS AND GUIDELINES

Pertinent international and national regulations, advisories, and guidelines regarding hexachlorobutadiene in air, water, and other media are summarized in Table 7-1. This table is not an exhaustive list, and current regulations should be verified by the appropriate regulatory agency.

ATSDR develops MRLs, which are substance-specific guidelines intended to serve as screening levels by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites. See Section 1.3 and Appendix A for detailed information on the MRLs for hexachlorobutadiene.

Table 7-1. Regulations and Guidelines Applicable to Hexachlorobutadiene

Agency	Description	Information	Reference
	Air		
EPA	RfC	Not evaluated	IRIS 1993
WHO	Air quality guidelines	Not listed	WHO 2010
	Water & Food		
EPA	Drinking water standards and health advisories		EPA 2018a
	1-Day health advisory	0.3 mg/L	
	10-Day health advisory	0.3 mg/L	
	DWEL	0.01 mg/L	
	Lifetime health advisory	No data	
	10^{-4} Cancer risk	0.09 mg/L	
	National primary drinking water regulations	Not listed	EPA 2009
	RfD	Withdrawn	IRIS 1993
	Provisional chronic and subchronic RfD	0.001 mg/kg/day	EPA 2007
WHO	Drinking water quality guidelines		WHO 2017
	Guideline value	0.0006 mg/L (0.6 µg/L)	
	TDI	0.2 µg/kg body weight	
FDA	Substances added to food[a]	No data	FDA 2020
	Cancer		
HHS	Carcinogenicity classification	No data	NTP 2016
EPA	Carcinogenicity classification	C[b]	IRIS 1993
IARC	Carcinogenicity classification	Group 3[c]	IARC 1999
	Occupational		
OSHA	PEL (8-hour TWA) for general industry, construction, and shipyards	No data	OSHA 2019a, 2019b, 2019c
NIOSH	REL (up to 10-hour TWA)	0.02 ppm (0.24 mg/m^3)[d,e]	NIOSH 2016
	IDLH	No data[e]	

Table 7-1. Regulations and Guidelines Applicable to Hexachlorobutadiene

Agency	Description	Information	Reference
	Emergency Criteria		
EPA	AEGLs-air	Not listed	EPA 2018b
DOE	PACs-air		DOE 2018a
	PAC-1[f]	1 ppm	
	PAC-2[f]	3 ppm	
	PAC-3[f]	10 ppm	

[a]The Substances Added to Food inventory replaces EAFUS and contains the following types of ingredients: food and color additives listed in FDA regulations, flavoring substances evaluated by FEMA or JECFA, GRAS substances listed in FDA regulations, substances approved for specific uses in food prior to September 6, 1958, substances that are listed in FDA regulations as prohibited in food, delisted color additives, and some substances "no longer FEMA GRAS."
[b]C: possible human carcinogen.
[c]Group 3: not classifiable as to its carcinogenicity to humans.
[d]Skin notation.
[e]Potential occupational carcinogen.
[f]Definitions of PAC terminology are available from U.S. Department of Energy (DOE 2018b).

AEGL = acute exposure guideline levels; DOE = Department of Energy; DWEL = drinking water equivalent level; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; FEMA = Flavor and Extract Manufacturers Association of the United States; GRAS = generally recognized as safe; HHS = Department of Health and Human Services; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health concentrations; IRIS = Integrated Risk Information System; JECFA = Joint FAO/WHO Expert Committee on Food Additives; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PAC = protective action criteria; PEL = permissible exposure limit; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; TDI = tolerable daily intake; TWA = time-weighted average; WHO = World Health Organization

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APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are based on noncancer health effects only; cancer effects are not considered. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the NOAEL/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

APPENDIX A

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Office of Innovation and Analytics, Toxicology Section, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published MRLs. For additional information regarding MRLs, please contact the Office of Innovation and Analytics, Toxicology Section, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop S102-1, Atlanta, Georgia 30329-4027.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Hexachlorobutadiene
CAS Numbers: 87-68-3
Date: March 2021
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for hexachlorobutadiene.

Rationale for Not Deriving an MRL: The acute-duration inhalation database was not considered suitable for derivation of an MRL due to the lack of a repeated exposure study which examined the kidney and serious body weight effects at the lowest concentration (10 ppm) tested in a 5-day study.

Five studies have evaluated the acute toxicity of inhaled hexachlorobutadiene in rats or mice and reported respiratory tract, kidney, body weight, eye, adrenal gland, and nervous system effects; the results of these studies are summarized in Table A-1. In addition to these effects, NIOSH (1981) reported 100% mortality in mice exposed to 50 ppm hexachlorobutadiene for 5 days (7 hours/day).

Table A-1. Summary of Effects Resulting from Acute-Duration Inhalation Exposure to Hexachlorobutadiene

Species	Exposure	NOAEL (ppm)	LOAEL (ppm)	Effect	Reference
Respiratory effects					
Mouse 6 M	15 minutes		155	Decreased respiratory rate	de Ceaurriz et al. 1988
Rat 4 M, 4 F	4 hours/day 2 days		250	Nose irritation and respiratory difficulty	Gage 1970
Renal effects					
Mouse NS M	4 hours		2.75	Histochemical evidence of damaged proximal tubules	de Ceaurriz et al. 1988
Rat 4 M, 4 F	4 hours/day 2 days		250	Degeneration of proximal tubules	Gage 1970
Body weight effects					
Rat 10 M	7 hours/day 5 days		10*	57% decrease body weight gain	NIOSH 1981
Mouse 10 M	7 hours/day 5 days		10*	Weight loss (4.6% loss between days 1 and 2)	NIOSH 1981
Ocular effects					
Rat 4 M, 4 F	4 hours/day 2 days		250	Eye irritation	Gage 1970
Endocrine effects					
Rat 4 M, 4 F	4 hours/day 2 days		250	Degeneration in adrenal cortex	Gage 1970

Table A-1. Summary of Effects Resulting from Acute-Duration Inhalation Exposure to Hexachlorobutadiene

Species	Exposure	NOAEL (ppm)	LOAEL (ppm)	Effect	Reference
Neurological effects					
Rat 10 M	7 hours/day 5 days	10	50	Animals appeared subdued and showed little response to audio stimuli	NIOSH 1981
Death					
Mouse 10 M	7 hours/day 5 days		50 (serious LOAEL)	100% mortality	NIOSH 1981

F = female(s); LOAEL = lowest-observed-adverse-effect level; M = males(s); NOAEL = no-observed-adverse-effect level

The lowest LOAEL identified in the available studies is 2.75 ppm for histochemical evidence of proximal tubule damage in mice (de Ceaurriz et al. 1988). This study is not suitable as the basis of an MRL because it involved a single 4-hour exposure to hexachlorobutadiene and there is considerable uncertainty that the value would be protective of continuous exposure for 14 days. The lowest LOAEL in repeated exposure studies is 10 ppm in rats and mice exposed 7 hours/day for 5 days (NIOSH 1981). However, 10 ppm was categorized as a serious LOAEL for large decreases in body weight gain in rats and weight loss in mice. Additionally, this study did not evaluate the kidney, which is the presumed most sensitive target of toxicity. Given the uncertainty in using a single exposure study and the lack of a repeated exposure study that examined the kidney and identified a NOAEL or less serious LOAEL, the acute-inhalation database was not considered suitable for derivation of an MRL.

Agency Contacts (Chemical Managers): Carolyn Harper, Ph.D.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Hexachlorobutadiene
CAS Numbers: 87-68-3
Date: March 2021
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for hexachlorobutadiene.

Rationale for Not Deriving an MRL: The intermediate-duration inhalation database was not considered suitable for derivation of an MRL due to the limited number of endpoints examined in the two available studies and the lack of study details reported in the general toxicity study.

Two studies have examined the toxicity of hexachlorobutadiene following intermediate-duration inhalation exposure. In rats exposed to hexachlorobutadiene 6 hours/day, 5 days/week for 15 exposures, Gage (1970) reported decreases in body weight gain at 10 ppm and respiratory difficulty and histological alterations in the renal proximal tubules at 25 ppm. At 100 ppm (animals only exposed 12 times), 50% of the female rats died and anemia and degeneration of the renal cortical tubules were observed. No adverse effects were observed at 5 ppm. The second study is a developmental toxicity study that found decreases in maternal weight gain at ≥5 ppm and decreases in fetal body weight at 15 ppm; no other developmental effects were observed (Saillenfait et al. 1989). The intermediate-duration database was not considered suitable for derivation of an MRL because the two available studies examined a limited number of endpoints; additionally, the general toxicity study (Gage 1970) did not provide information on the magnitude of the body weight changes, description of the renal lesions observed at 25 ppm, incidence data, or statistical analyses.

Agency Contacts (Chemical Managers): Carolyn Harper, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Hexachlorobutadiene
CAS Numbers: 87-68-3
Date: March 2021
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for hexachlorobutadiene.

Rationale for Not Deriving an MRL: No chronic duration inhalation studies were identified for hexachlorobutadiene.

Agency Contacts (Chemical Managers): Carolyn Harper, Ph.D.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Hexachlorobutadiene
CAS Numbers: 87-68-3
Date: March 2021
Profile Status: Final
Route: Oral
Duration: Acute
MRL: 0.006 mg/kg/day
Critical Effect: Renal proximal tubular degeneration
Reference: Harleman and Seinen 1979
Point of Departure: LOAEL of 5.9 mg/kg/day
Uncertainty Factor: 1,000
LSE Graph Key: 2
Species: Rat

MRL Summary: An acute-duration oral MRL of 0.006 mg/kg/day was derived for hexachlorobutadiene based on an increased incidence of renal proximal tubule degeneration in rats exposed to hexachlorobutadiene in the diet for 14 days (Harleman and Seinen 1979). The MRL is based on a LOAEL of 5.9 mg/kg/day and a total uncertainty factor of 1,000 (10 for the use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

Selection of the Critical Effect: Three studies have evaluated the acute oral toxicity of hexachlorobutadiene. Two studies involved a single gavage dose of hexachlorobutadiene, which resulted in necrosis in the renal proximal tubules in rats exposed to ≥100 mg/kg (Birner et al. 1995; Jonker et al. 1993a); at 200 mg/kg, there was evidence of impaired renal function (increases in blood urea nitrogen, urine volume, urinary protein, and urinary glucose levels) (Jonker et al. 1983a). The third study was a range-finding study that found renal proximal tubule degeneration in male and female rats exposed to 5.9 or 6.2 mg/kg/day, respectively, hexachlorobutadiene in the diet for 14 days (Harleman and Seinen 1979). Decreases in body weight gain (9.5%) were also observed in the female rats exposed to 6.2 mg/kg/day and the male rats exposed to 19 mg/kg/day (21%). The study did not find any histological alterations in the liver of rats exposed to doses as high as 59 or 62 mg/kg/day.

The available acute oral studies identify the kidney as the most sensitive target of hexachlorobutadiene toxicity. Although the studies examined a limited number of potential endpoints, more extensive intermediate-duration studies confirm that the kidney is the most sensitive target of toxicity (Harleman and Seinen 1979; Kociba et al. 1971; NTP 1991; Schwetz et al. 1977).

Selection of the Principal Study: The Harleman and Seinen (1979) study identified the lowest LOAEL for renal effects and was selected as the principal study for the MRL.

Summary of the Principal Study:

Harleman JH, Seinen W. 1979. Short-term toxicity and reproduction studies in rats with hexachloro-(1,3)-butadiene. Toxicol Appl Pharmacol 47:1-14.

Groups of six male and six female Wistar rats were exposed to 0, 50, 150, or 450 ppm hexachlorobutadiene in the diet for 2 weeks. Doses of 0, 5.9, 19, and 59 mg/kg/day for males and 0, 6.2, 20, and 62 mg/kg/day for females were estimated using reported body weights and EPA's allometric equation to calculate food intake. Parameters used to assess toxicity included body weight, food consumption, liver and kidney weights, and histopathological examination of the liver and kidney.

Significant decreases in body weight gain were observed in males at 19 and 62 mg/kg/day (21 and 31% of controls, respectively) and in females at 6.2, 20, and 62 mg/kg/day (9.5, 26, and 33% of controls, respectively). Decreases in food intake were also observed; however, decreases in food efficiency (growth/food intake) were only observed at the highest dose. Significant increases in relative kidney weight were observed in the males and females at ≥19 and 20 mg/kg/day; no alterations in relative liver weight were observed. Degeneration of the proximal tubular epithelial cells was observed at all hexachlorobutadiene exposure levels; no alterations were observed in the liver. Although incidence data were not provided, the investigators noted that histological changes were observed in the kidneys of all animals exposed to hexachlorobutadiene.

Selection of the Point of Departure: The lowest LOAEL value of 5.9 mg/kg/day identified for renal effects in males was selected as the point of departure for the MRL; the study did not identify a NOAEL value. The lack of incidence data precluded using benchmark dose modeling to calculate a point of departure.

Uncertainty Factor: The LOAEL of 5.9 mg/kg/day is divided by a total uncertainty factor (UF) of 1,000:

- 10 for the use of a LOAEL
- 10 for extrapolation from animals to humans
- 10 for human variability

MRL = LOAEL ÷ UFs
5.9 mg/kg/day ÷ (10 x 10 x 10) = 0.006 mg/kg/day

Other Additional Studies or Pertinent Information: No human studies examining the acute oral toxicity of hexachlorobutadiene were identified. In addition to the previously discussed acute studies, a number of intermediate- and chronic-duration oral studies (Harleman and Seinen 1979; Kociba et al. 1971, 1977; NTP 1991; Schwetz et al. 1977) and acute-duration parenteral studies (Boroushaki 2003; Chiusolo et al. 2008; Cristofori et al. 2013; Kirby and Bach 1995; Maguire et al. 2013; Swain et al. 2011; Zanetti et al. 2010) support the identification of the kidney as the most sensitive target of toxicity for hexachlorobutadiene.

Agency Contacts (Chemical Managers): Carolyn Harper, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Hexachlorobutadiene
CAS Numbers: 87-68-3
Date: March 2021
Profile Status: Final
Route: Oral
Duration: Intermediate
MRL: 0.002 mg/kg/day
Critical Effect: Renal proximal tubular regeneration
Reference: NTP 1991
Point of Departure: NOAEL of 0.2 mg/kg/day
Uncertainty Factor: 100
LSE Graph Key: 13
Species: Mouse

MRL Summary: An intermediate-duration oral MRL of 0.002 mg/kg/day was derived for hexachlorobutadiene based on an increased incidence of renal proximal tubule regeneration in mice exposed to hexachlorobutadiene in the diet for 13 weeks (NTP 1991). The MRL is based on a NOAEL of 0.2 mg/kg/day and a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Selection of the Critical Effect: A number of studies have evaluated the toxicity of hexachlorobutadiene toxicity following intermediate-duration oral exposure. These studies have identified several targets of toxicity including body weight, liver, kidney, nervous system, hematological system, reproductive system, and developing organism. A summary of the lowest LOAEL values for these endpoints is presented in Table A-2. A comparison of the LOAEL values across endpoints supports the identification of the kidney as the most sensitive target of toxicity.

Table A-2. Lowest LOAELs Identified in Intermediate-Duration Oral Studies of Hexachlorobutadiene

Endpoint	Effect	NOAEL[a] (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Body weight	9.9% decrease in body weight gain in mice exposed for 13 weeks	1.5	4.9	NTP 1991
Hematological	Increased hemoglobin concentration in rats exposed for 30 days	3	10	Kociba et al. 1971
Hepatic	Increased cytoplasmic basophilia in rats exposed for 13 weeks	6.3	15.6	Harleman and Seinen 1979
Renal	Proximal tubular epithelial regeneration in mice exposed for 13 weeks	0.2	0.5	NTP 1991
Neurological	Lethargy, hunched posture, incoordination in mice exposed to 40 mg/kg/day for 15 days	12	40	NTP 1991
Reproductive	Infertility in female rats exposed for 15 weeks	15	150	Harleman and Seinen 1979

Table A-2. Lowest LOAELs Identified in Intermediate-Duration Oral Studies of Hexachlorobutadiene

Endpoint	Effect	NOAEL[a] (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Developmental	16–19% decrease in pup body weight (rat dams exposed for 18 weeks)		15	Harleman and Seinen 1979

[a]NOAEL identified in the same study as the lowest LOAEL.

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

Selection of the Principal Study: A summary of the NOAEL and LOAEL values for renal effects is presented in Table A-3.

Table A-3. Summary of Renal Effects Observed in Intermediate-Duration Oral Studies of Hexachlorobutadiene

Species	Exposure	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Effect	Reference
Mouse	13 weeks (F)	0.2 F 1.5 M	0.5 F 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
Rat	13 weeks (GO)	1 F 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
Rat	4 weeks (F)		2.5 F	Decreased BUN in females	Jonker et al. 1993b
Rat	32 days (GO)	1 F	4 F	Focal tubular vacuolization and increased relative kidney weight	Jonker et al. 1996
Rat	18 weeks (F)		15 F	Proximal tubular degeneration and necrosis	Harleman and Seinen 1979
Rat	30 days (F)	10 F	30 F	Tubular degeneration, necrosis, and regeneration	Kociba et al. 1971
Rat	3 weeks (F)	37 M	190 M	Proximal tubules lined with basophilic epithelium	Nakagawa et al. 1998
Rat	30 weeks (F)	94 M			Nakagawa et al. 1998

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

In all studies involving exposure of male and female rats or mice, the lower LOAEL values were identified in the females. The lowest LOAEL for renal effects was 0.5 mg/kg/day identified in female mice exposed to hexachlorobutadiene in the diet for 13 weeks (NTP 1991); no effects were observed at 0.2 mg/kg/day. The Schwetz et al. (1977) reproductive/developmental toxicity study also identified a

NOAEL of 0.2 mg/kg/day for kidney effects with a LOAEL of 2 mg/kg/day. The NTP (1991) study was selected as the principal study because it identified the lowest LOAEL for renal effects.

Summary of the Principal Study:

NTP. 1991. National Toxicology Program. Toxicity studies of hexachloro-1,3-butadiene in B6C3F1 mice (feed studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. NIH publication no. 91-3120.

Groups of 10 male and 10 female B6C3F1 mice were exposed to 0, 1, 10, 30, or 100 ppm hexachlorobutadiene in the diet for 13 weeks; the investigators estimated the doses to be 0, 0.1, 0.4, 1.5, 4.9, and 16.8 mg/kg/day in males and 0, 0.2, 0.5, 1.8, 4.5, and 19.2 mg/kg/day in the females. The following parameters were used to assess toxicity: body weight, food intake (measured weekly), organ weight (brain, heart, kidney, liver, spleen, testis), gross necropsy, histopathological examination of major tissues and organs in the controls and high-dose animals and all animals dying early; histopathology of kidneys in all groups; sperm morphology; and vaginal cytology.

One male in the 0.1 mg/kg/day group died early. No overt clinical signs were observed in exposed mice. Decreases in body weight gain were observed in males at 4.9 and 16.8 mg/kg/day (9.9 and 15.8%, respectively) and in females at 19.2 mg/kg/day (15%); no alterations in feed intake were noted. Significant decreases in absolute and relative kidney weights were observed at ≥4.9 mg/kg/day in males; relative kidney weight was also decreased in males at 1.5 mg/kg/day. In females, the only significant alteration in kidney weight was a decrease in absolute weight at 19.2 mg/kg/day. The investigators noted that a decrease in absolute heart weight in males at 16.8 mg/kg/day may be clinically relevant; however, no histological alterations were observed in the heart. Renal tubular epithelial regeneration, prominent in the outer stripe of the outer medullary rays (pars recta) was observed in 0/10, 0/10, 0/10, 0/9, 10/10, and 10/10 males at 0, 0.1, 0.4, 1.5, 4.9, and 16.8 mg/kg/day, respectively, and in 0/10, 1/10, 9/10, 10/10, 10/10, and 10/10 females at 0, 0.2, 0.5, 1.8, 4.5, and 19.2 mg/kg/day, respectively. A significant decrease in sperm motility was observed at 1.5, 4.9, and 16.8 mg/kg/day, but the magnitude of the decrease was not dose-related. No significant alterations in sperm count, incidence of abnormal sperm, estrual cyclicity, or average estrous cycle length were observed.

Selection of the Point of Departure: The NOAEL of 0.2 mg/kg/day identified in female mice was selected as the point of departure for the MRL. The incidence data for renal tubular regeneration was not considered suitable for benchmark dose modeling due to the lack of dose-response data between the extremes in the incidence in the control and lowest dose groups and the incidences in higher dose groups.

Uncertainty Factor: The NOAEL of 0.2 mg/kg/day is divided by a total uncertainty factor (UF) of 100:

- 10 for extrapolation from animals to humans
- 10 for human variability

MRL = LOAEL ÷ UFs
0.2 mg/kg/day ÷ (10 x 10) = 0.002 mg/kg/day

Other Additional Studies or Pertinent Information: No human studies examining the toxicity of hexachlorobutadiene following intermediate-duration oral exposure were identified. The identification of the kidney as the most sensitive target of toxicity is supported by a number of intermediate-duration oral studies and a chronic-duration oral study.

Agency Contacts (Chemical Managers): Carolyn Harper, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Hexachlorobutadiene
CAS Numbers: 87-68-3
Date: March 2021
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for hexachlorobutadiene.

Rationale for Not Deriving an MRL: One study has evaluated the chronic oral toxicity of hexachlorobutadiene (Kociba et al. 1977). In this study, an increase in the incidence of tubular epithelial hyperplasia was observed at 20 mg/kg/day. The investigators also noted that there was a possible increase in incidence of hyperplasia at 2 mg/kg/day; however, no incidence data were provided. An increase in the incidence of total number of renal tubular neoplasms were observed at 20 mg/kg/day. The Kociba et al. (1977) study was not considered suitable for derivation of an MRL because incidence data were not provided to allow for an independent assessment of whether there was a significant increase in the incidence of hyperplasia at 2 mg/kg/day.

Agency Contacts (Chemical Managers): Carolyn Harper, Ph.D.

APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR HEXACHLOROBUTADIENE

The objective of the toxicological profile is to evaluate the potential for human exposure and the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to hexachlorobutadiene.

B.1 LITERATURE SEARCH AND SCREEN

A literature search and screen was conducted to identify studies examining health effects, toxicokinetics, mechanisms of action, susceptible populations, biomarkers, chemical interactions, physical and chemical properties, production, use, environmental fate, environmental releases, and environmental and biological monitoring data for hexachlorobutadiene. ATSDR primarily focused on peer-reviewed articles without publication date or language restrictions. Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of hexachlorobutadiene have undergone peer review by at least three ATSDR-selected experts who have been screened for conflict of interest. The inclusion criteria used to identify relevant studies examining the health effects of hexachlorobutadiene are presented in Table B-1.

Table B-1. Inclusion Criteria for the Literature Search and Screen

Health Effects
Species
Human
Laboratory mammals
Route of exposure
Inhalation
Oral
Dermal (or ocular)
Parenteral (these studies will be considered supporting data)
Health outcome
Death
Systemic effects
Body weight effects
Respiratory effects
Cardiovascular effects
Gastrointestinal effects
Hematological effects
Musculoskeletal effects
Hepatic effects
Renal effects
Dermal effects
Ocular effects
Endocrine effects
Immunological effects
Neurological effects
Reproductive effects
Developmental effects

Table B-1. Inclusion Criteria for the Literature Search and Screen

Other noncancer effects
Cancer
Toxicokinetics
Absorption
Distribution
Metabolism
Excretion
PBPK models
Biomarkers
Biomarkers of exposure
Biomarkers of effect
Interactions with other chemicals
Potential for human exposure
Releases to the environment
Air
Water
Soil
Environmental fate
Transport and partitioning
Transformation and degradation
Environmental monitoring
Air
Water
Sediment and soil
Other media
Biomonitoring
General populations
Occupation populations

A.1.1 Literature Search

The current literature search was intended to update the draft toxicological profile for hexachlorobutadiene released for public comment in 2019; thus, the literature search was restricted to studies published between March 2016 and May 2020. The following main databases were searched in May 2020:

- PubMed
- National Technical Reports Library (NTRL)
- Scientific and Technical Information Network's TOXCENTER

The search strategy used the chemical names, Chemical Abstracts Service (CAS) numbers, synonyms, Medical Subject Headings (MeSH) headings, and keywords for hexachlorobutadiene. The query strings used for the literature search are presented in Table B-2.

The search was augmented by searching the Toxic Substances Control Act Test Submissions (TSCATS), NTP website, and National Institute of Health Research Portfolio Online Reporting Tools Expenditures and Results (NIH RePORTER) databases using the queries presented in Table B-3. Additional databases were searched in the creation of various tables and figures, such as the TRI Explorer, the Substance Priority List (SPL) resource page, and other items as needed. Regulations applicable to hexachlorobutadiene were identified by searching international and U.S. agency websites and documents.

Review articles were identified and used for the purpose of providing background information and identifying additional references. ATSDR also identified reports from the grey literature, which included unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations.

Table B-2. Database Query Strings

Database search date	Query string
PubMed	
05/2020	(87-68-3[rn] OR "1,1,2,3,4,4-Hexachloro-1,3-butadiene"[tw] OR "1,3-Hexachlorobutadiene"[tw] OR "Dolen-pur"[tw] OR "HCBD"[tw] OR "Hexachloro-1,3-butadiene"[tw] OR "Hexachlorobuta-1,3-diene"[tw] OR "Hexachlorobutadiene"[tw] OR "Perchlorobutadiene"[tw] OR "1,3-Butadiene, 1,1,2,3,4,4-hexachloro-"[tw] OR "1,3-Butadiene, hexachloro-"[tw] OR "BUTADIENE, HEXACHLORO-"[tw] OR "D033"[tw] OR "GP-40-66:120"[tw] OR "Perchloro-1,3-butadiene"[tw]) AND (2017/03/01 : 3000[mhda] OR 2017/03/01 : 3000[crdt] OR 2017/03/01 : 3000[edat] OR 2016/03/01 : 3000[dp])
NTRL	
05/2020	"87-68-3" OR "1,1,2,3,4,4-Hexachloro-1,3-butadiene" OR "1,3-Hexachlorobutadiene" OR "Dolen-pur" OR "HCBD" OR "Hexachloro-1,3-butadiene" OR "Hexachlorobuta-1,3-diene" OR "Hexachlorobutadiene" OR "Perchlorobutadiene" OR "1,3-Butadiene, 1,1,2,3,4,4-hexachloro-" OR "1,3-Butadiene, hexachloro-" OR "BUTADIENE, HEXACHLORO-" OR "D033" OR "GP-40-66 120" OR "Perchloro-1,3-butadiene"
Toxcenter	
05/2020	FILE 'TOXCENTER' ENTERED AT 15:05:56 ON 22 MAY 2020 CHARGED TO COST=EH038.06.01.LB.02 L1 2478 SEA FILE=TOXCENTER 87-68-3 L2 2356 SEA FILE=TOXCENTER L1 NOT PATENT/DT L3 110 SEA FILE=TOXCENTER L2 AND ED>=20170301 L4 147 SEA FILE=TOXCENTER L2 AND PY>2015 L5 158 SEA FILE=TOXCENTER L3 OR L4 ACT TOXQUERY/Q --------- L6 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L7 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT) L8 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50) L9 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L10 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L11 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?) L12 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR

Table B-2. Database Query Strings

Database search date	Query string
	DIETARY OR DRINKING(W)WATER?)
	L13 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE))
	L14 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?)
	L15 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR
	OVUM?)
	L16 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?)
	L17 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR
	TERATOGEN?)
	L18 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR
	SPERMATOB? OR SPERMATOC? OR SPERMATOG?)
	L19 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR
	SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?)
	L20 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?)
	L21 QUE (ENDOCRIN? AND DISRUPT?)
	L22 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?)
	L23 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)
	L24 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)
	L25 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR
	NEOPLAS?)
	L26 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L27 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L28 QUE (NEPHROTOX? OR HEPATOTOX?)
	L29 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L30 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L31 QUE L6 OR L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29 OR L30
	L32 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE
	OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE
	OR PORCINE OR MONKEY? OR MACAQUE?)
	L33 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA
	OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L34 QUE L31 OR L32 OR L33
	L35 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR
	PRIMATES OR PRIMATE?)
	L36 QUE L34 OR L35

Table B-2. Database Query Strings

Database search date	Query string

	L38 71 SEA FILE=TOXCENTER L5 AND L36
	L39 6 SEA FILE=TOXCENTER L38 AND MEDLINE/FS
	L40 65 SEA FILE=TOXCENTER L38 NOT L39
	L41 65 DUP REM L39 L40 (6 DUPLICATES REMOVED)
	ANSWERS '1-65' FROM FILE TOXCENTER
	L*** DEL 6 S L38 AND MEDLINE/FS
	L*** DEL 6 S L38 AND MEDLINE/FS
	L42 6 SEA FILE=TOXCENTER L41
	L*** DEL 65 S L38 NOT L39
	L*** DEL 65 S L38 NOT L39
	L43 59 SEA FILE=TOXCENTER L41
	L44 59 SEA FILE=TOXCENTER (L42 OR L43) NOT MEDLINE/FS
	D SCAN L44

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
TSCATS via ChemView	
05/2020	Compounds searched: 87-68-3
NTP	
05/2020	87-68-3 "1,1,2,3,4,4-Hexachloro-1,3-butadiene" "1,3-Hexachlorobutadiene" "Dolen-pur" "HCBD" "Hexachloro-1,3-butadiene" "Hexachlorobuta-1,3-diene" "Hexachlorobutadiene" "Perchlorobutadiene" "1,3-Butadiene, 1,1,2,3,4,4-hexachloro-" "1,3-Butadiene, hexachloro-" "BUTADIENE, HEXACHLORO-" "D033" "GP-40-66 120" "Perchloro-1,3-butadiene"
Regulations.gov	
05/2020	Compounds searched: 87-68-3
NIH RePORTER	
08/2020	Text Search: "1,1,2,3,4,4-Hexachloro-1,3-butadiene" OR "1,3-Hexachlorobutadiene" OR "Dolen-pur" OR "HCBD" OR "Hexachloro-1,3-butadiene" OR "Hexachlorobuta-1,3-diene" OR "Hexachlorobutadiene" OR "Perchlorobutadiene" OR "1,3-Butadiene, 1,1,2,3,4,4-hexachloro-" OR "1,3-Butadiene, hexachloro-" OR "BUTADIENE, HEXACHLORO-" OR "D033" OR "GP-40-66:120" OR "Perchloro-1,3-butadiene" (Advanced), Search in: Projects Admin IC: All, Fiscal Year: Active Projects
Other	Identified throughout the assessment process

The 2020 results were:

- Number of records identified from PubMed, TOXLINE, and TOXCENTER (after duplicate removal): 85
- Number of records identified from other strategies: 55
- Total number of records to undergo literature screening: 140

A.1.2 Literature Screening

A two-step process was used to screen the literature search to identify relevant studies on hexachlorobutadiene:

- Title and abstract screen
- Full text screen

Title and Abstract Screen. Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

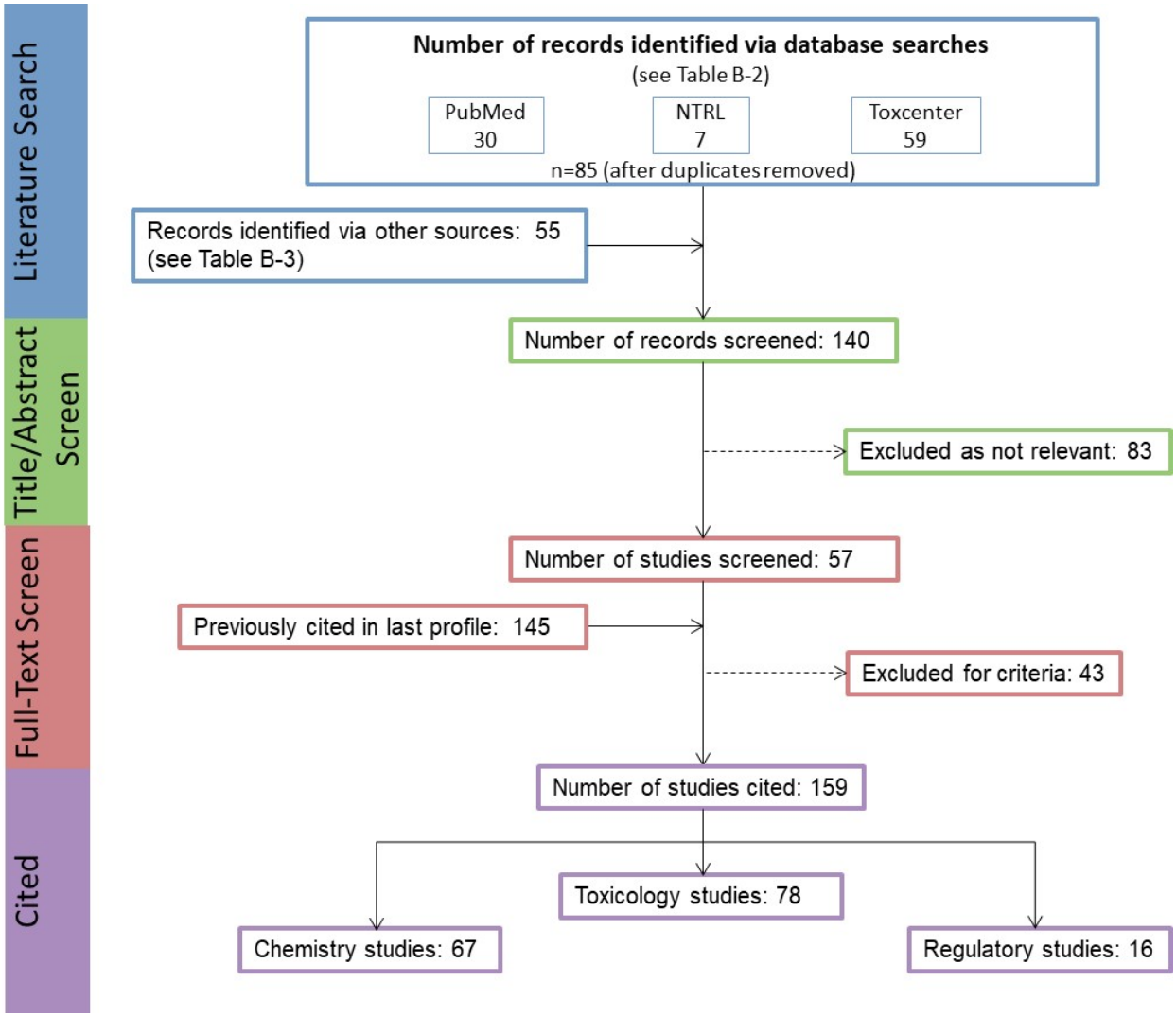
- Number of titles and abstracts screened: 140
- Number of studies considered relevant and moved to the next step: 57

Full Text Screen. The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 57
- Number of studies cited in the pre-public draft of the toxicological profile: 145
- Total number of studies cited in the profile: 159

A summary of the results of the literature search and screening is presented in Figure B-1.

Figure B-1. May 2020 Literature Search Results and Screen for Hexachlorobutadiene



APPENDIX C. USER'S GUIDE

Chapter 1. Relevance to Public Health

This chapter provides an overview of U.S. exposures, a summary of health effects based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information, and an overview of the minimal risk levels. This is designed to present interpretive, weight-of-evidence discussions for human health endpoints by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

Minimal Risk Levels (MRLs)

Where sufficient toxicologic information is available, ATSDR derives MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Section 1.2, Summary of Health Effects, contains basic information known about the substance. Other sections, such as Section 3.2 Children and Other Populations that are Unusually Susceptible and Section 3.4 Interactions with Other Substances, provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a

substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables that are provided in Chapter 2. Detailed discussions of the MRLs are presented in Appendix A.

Chapter 2. Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species and MRLs to humans for noncancer endpoints. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE tables and figures follow. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

TABLE LEGEND

See Sample LSE Table (page C-5)

(1) Route of exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically, when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure (i.e., inhalation, oral, and dermal). LSE figures are limited to the inhalation and oral routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures. Profiles with more than one chemical may have more LSE tables and figures.

(2) Exposure period. Three exposure periods—acute (<15 days), intermediate (15–364 days), and chronic (≥365 days)—are presented within each relevant route of exposure. In this example, two oral studies of chronic-duration exposure are reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Figure key. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 51 identified NOAELs and less serious LOAELs (also see the three "51R" data points in sample LSE Figure 2-X).

(4) Species (strain) No./group. The test species (and strain), whether animal or human, are identified in this column. The column also contains information on the number of subjects and sex per group. Chapter 1, Relevance to Public Health, covers the relevance of animal data to human toxicity and Section 3.1, Toxicokinetics, contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(5) Exposure parameters/doses. The duration of the study and exposure regimens are provided in these columns. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 51), rats were orally exposed to “Chemical X” via feed for 2 years. For a

more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Aida et al. 1992).

(6) Parameters monitored. This column lists the parameters used to assess health effects. Parameters monitored could include serum (blood) chemistry (BC), behavioral (BH), biochemical changes (BI), body weight (BW), clinical signs (CS), developmental toxicity (DX), enzyme activity (EA), food intake (FI), fetal toxicity (FX), gross necropsy (GN), hematology (HE), histopathology (HP), lethality (LE), maternal toxicity (MX), organ function (OF), ophthalmology (OP), organ weight (OW), teratogenicity (TG), urinalysis (UR), and water intake (WI).

(7) Endpoint. This column lists the endpoint examined. The major categories of health endpoints included in LSE tables and figures are death, body weight, respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, endocrine, immunological, neurological, reproductive, developmental, other noncancer, and cancer. "Other noncancer" refers to any effect (e.g., alterations in blood glucose levels) not covered in these systems. In the example of key number 51, three endpoints (body weight, hematological, and hepatic) were investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no adverse effects were seen in the organ system studied. The body weight effect reported in key number 51 is a NOAEL at 25.5 mg/kg/day. NOAELs are not reported for cancer and death; with the exception of these two endpoints, this field is left blank if no NOAEL was identified in the study.

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused an adverse health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. Key number 51 reports a less serious LOAEL of 6.1 mg/kg/day for the hepatic system, which was used to derive a chronic exposure, oral MRL of 0.008 mg/kg/day (see footnote "c"). MRLs are not derived from serious LOAELs. A cancer effect level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases. If no LOAEL/CEL values were identified in the study, this field is left blank.

(10) Reference. The complete reference citation is provided in Chapter 8 of the profile.

(11) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. For example, footnote "c" indicates that the LOAEL of 6.1 mg/kg/day in key number 51 was used to derive an oral MRL of 0.008 mg/kg/day.

FIGURE LEGEND

See Sample LSE Figure (page C-6)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the chronic exposure period are illustrated.

(14) Endpoint. These are the categories of health effects for which reliable quantitative data exist. The same health effect endpoints appear in the LSE table.

(15) Levels of exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) LOAEL. In this example, the half-shaded circle that is designated 51R identifies a LOAEL critical endpoint in the rat upon which a chronic oral exposure MRL is based. The key number 51 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 6.1 mg/kg/day (see entry 51 in the sample LSE table) to the MRL of 0.008 mg/kg/day (see footnote "c" in the sample LSE table).

(17) CEL. Key number 59R is one of studies for which CELs were derived. The diamond symbol refers to a CEL for the test species (rat). The number 59 corresponds to the entry in the LSE table.

(18) Key to LSE figure. The key provides the abbreviations and symbols used in the figure.

APPENDIX C

Table 2-X. Levels of Significant Exposure to [Chemical X] – Oral (1)

Figure key[a] (3)	Species (strain) No./group (4)	Exposure parameters (5)	Doses (mg/kg/day) (5)	Parameters monitored (6)	Endpoint (7)	NOAEL (mg/kg/day) (8)	Less serious LOAEL (mg/kg/day) (9)	Serious LOAEL (mg/kg/day) (9)	Effect
CHRONIC EXPOSURE (2)									
51	Rat (Wistar) 40 M, 40 F	2 years (F)	M: 0, 6.1, 25.5, 138.0 F: 0, 8.0, 31.7, 168.4	CS, WI, BW, OW, HE, BC, HP	Bd wt	25.5	138.0		Decreased body weight gain in males (23–25%) and females (31–39%)
					Hemato	138.0			
					Hepatic		6.1[c]		Increases in absolute and relative weights at ≥6.1/8.0 mg/kg/day after 12 months of exposure; fatty generation at ≥6.1 mg/kg/day in males and at ≥31.7 mg/kg/day in females, and granulomas in females at 31.7 and 168.4 mg/kg/day after 12, 18, or 24 months of exposure and in males at ≥6.1 mg/kg/day only after 24 months of exposure
Aida et al. 1992 (10)									
52	Rat (F344) 78 M	104 weeks (W)	0, 3.9, 20.6, 36.3	CS, BW, FI, BC, OW, HP	Hepatic	36.3			
					Renal	20.6	36.3		Increased incidence of renal tubular cell hyperplasia
					Endocr	36.3			
George et al. 2002									
59	Rat (Wistar) 58M, 58F	Lifetime (W)	M: 0, 90 F: 0, 190	BW, HP	Cancer		190 F		Increased incidence of hepatic neoplastic nodules in females only; no additional description of the tumors was provided
Tumasonis et al. 1985									

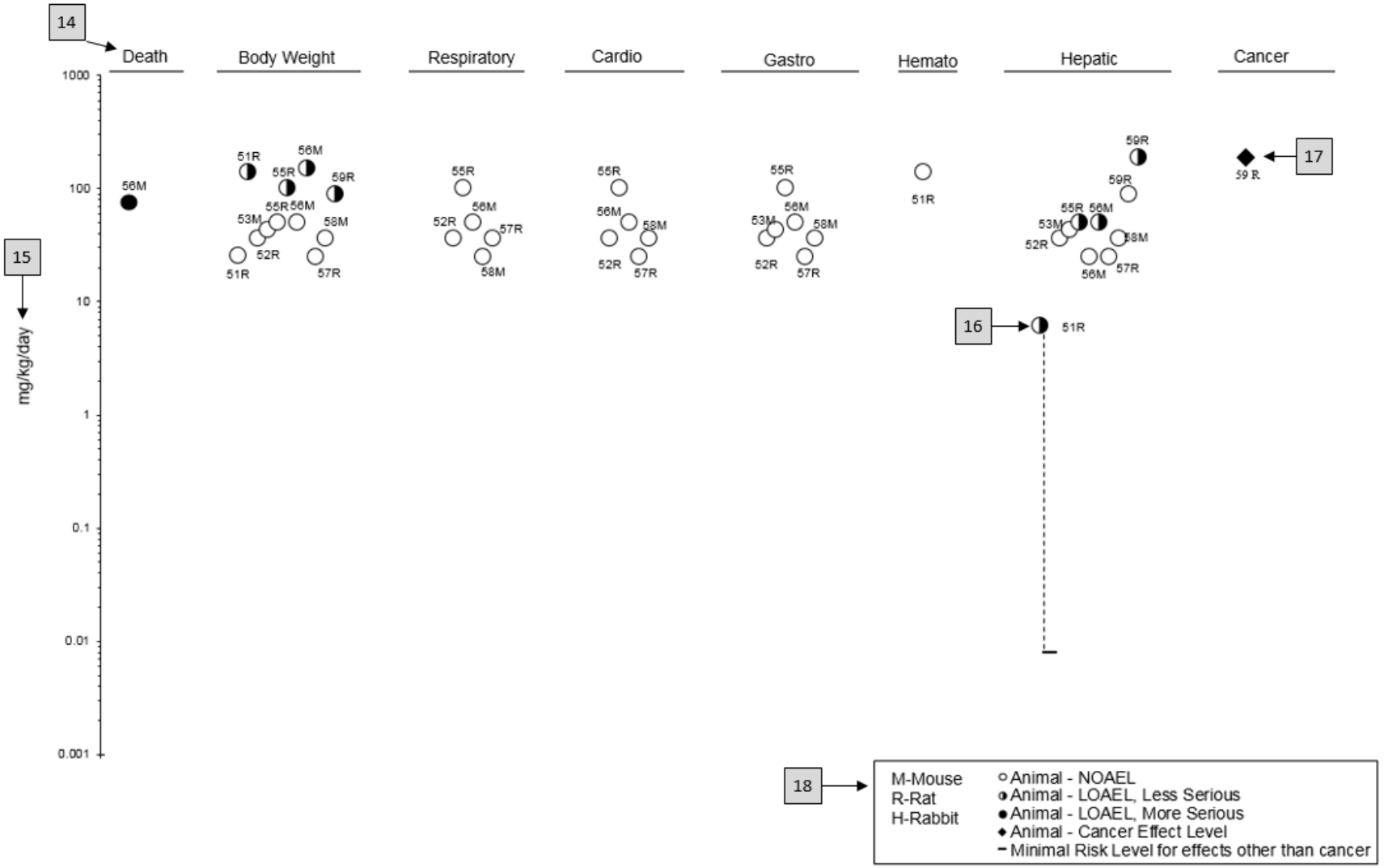
[a]The number corresponds to entries in Figure 2-x.

(11) [b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on the $BMDL_{05}$ of 10 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

[c]Used to derive a chronic-duration oral MRL of 0.008 mg/kg/day based on the $BMDL_{10}$ of 0.78 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral

13 → Chronic (≥365 days)



APPENDIX D. QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances may find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Relevance to Public Health: The Relevance to Public Health Section provides an overview of exposure and health effects and evaluates, interprets, and assesses the significance of toxicity data to human health. A table listing minimal risk levels (MRLs) is also included in this chapter.

Chapter 2: Health Effects: Specific health effects identified in both human and animal studies are reported by type of health effect (e.g., death, hepatic, renal, immune, reproductive), route of exposure (e.g., inhalation, oral, dermal), and length of exposure (e.g., acute, intermediate, and chronic).
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting.

Pediatrics:

Section 3.2 Children and Other Populations that are Unusually Susceptible
Section 3.3 Biomarkers of Exposure and Effect

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
Internet: http://www.atsdr.cdc.gov

The following additional materials are available online:

Case Studies in Environmental Medicine are self-instructional publications designed to increase primary health care providers' knowledge of a hazardous substance in the environment and to aid in the evaluation of potentially exposed patients (see https://www.atsdr.cdc.gov/csem/csem.html).

Managing Hazardous Materials Incidents is a set of recommendations for on-scene (pre-hospital and hospital medical management of patients exposed during a hazardous materials incident (see https://www.atsdr.cdc.gov/MHMI/index.asp).

Fact Sheets (ToxFAQs™) provide answers to frequently asked questions about toxic substances (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015 • Web Page: https://www.cdc.gov/nceh/.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 395 E Street, S.W., Suite 9200, Patriots Plaza Building, Washington, DC 20201 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/niosh/.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212 • Web Page: https://www.niehs.nih.gov/.

Clinical Resources (Publicly Available Information)

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266 • Web Page: http://www.acoem.org/.

The American College of Medical Toxicology (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard, Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

The Pediatric Environmental Health Specialty Units (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

The American Association of Poison Control Centers (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

APPENDIX E. GLOSSARY

Absorption—The process by which a substance crosses biological membranes and enters systemic circulation. Absorption can also refer to the taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of ≤14 days, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD) or Benchmark Concentration (BMC)—is the dose/concentration corresponding to a specific response level estimate using a statistical dose-response model applied to either experimental toxicology or epidemiology data. For example, a BMD_{10} would be the dose corresponding to a 10% benchmark response (BMR). The BMD is determined by modeling the dose-response curve in the region of the dose-response relationship where biologically observable data are feasible. The BMDL or BMCL is the 95% lower confidence limit on the BMD or BMC.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Indicators signaling events in biologic systems or samples, typically classified as markers of exposure, effect, and susceptibility.

Cancer Effect Level (CEL)—The lowest dose of a chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the outcome.

Case Report—A report that describes a single individual with a particular disease or exposure. These reports may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Reports that describe the experience of a small number of individuals with the same disease or exposure. These reports may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration that must not be exceeded.

Chronic Exposure—Exposure to a chemical for ≥365 days, as specified in the Toxicological Profiles.

Clastogen—A substance that causes breaks in chromosomes resulting in addition, deletion, or rearrangement of parts of the chromosome.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome, and who are disease-free at start of follow-up. Often, at least one exposed group is compared to one unexposed group, while in other cohorts, exposure is a continuous variable and analyses are directed towards analyzing an exposure-response coefficient.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at a specific point in time.

Data Needs—Substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the response or amount of the response.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the effect occurs. Effects include malformations and variations, altered growth, and *in utero* death.

Epidemiology—The investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Excretion—The process by which metabolic waste products are removed from the body.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one-half of a quantity of a chemical from the body or environmental media.

Health Advisory—An estimate of acceptable drinking water levels for a chemical substance derived by EPA and based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH)—A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

Immunotoxicity—Adverse effect on the functioning of the immune system that may result from exposure to chemical substances.

Incidence—The ratio of new cases of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Metabolism—Process in which chemical substances are biotransformed in the body that could result in less toxic and/or readily excreted compounds or produce a biologically active intermediate.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—The state of being diseased; the morbidity rate is the incidence or prevalence of a disease in a specific population.

Mortality—Death; the mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations, which are changes in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a hazardous substance.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Although effects may be produced at this dose, they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An odds ratio that is greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests (insects or other organisms harmful to cultivated plants or animals).

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic endpoints. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—A type of physiologically based dose-response model that is comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information, including tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as blood:air partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which a group is followed over time and the pertinent observations are made on events occurring after the start of the study.

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation RfC is expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily oral exposure of the human population to a potential hazard that is likely to be without risk of deleterious noncancer health effects during a lifetime. The oral RfD is expressed in units of mg/kg/day.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). RQs are (1) ≥1 pound or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a hazardous substance. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a hazardous substance.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio/Relative Risk—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio that is greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time-Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

Time-Weighted Average (TWA)—An average exposure within a given time period.

Toxicokinetic—The absorption, distribution, metabolism, and elimination of toxic compounds in the living organism.

Toxics Release Inventory (TRI)—The TRI is an EPA program that tracks toxic chemical releases and pollution prevention activities reported by industrial and federal facilities.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL), Reference Dose (RfD), or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis (3 being the approximate logarithmic average of 10 and 1).

Xenobiotic—Any substance that is foreign to the biological system.

APPENDIX F. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AAPCC	American Association of Poison Control Centers
ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ACMT	American College of Medical Toxicology
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AEGL	Acute Exposure Guideline Level
AIC	Akaike's information criterion
AIHA	American Industrial Hygiene Association
ALT	alanine aminotransferase
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BCF	bioconcentration factor
BMD/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
C	centigrade
CAA	Clean Air Act
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
cm	centimeter
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DWEL	drinking water exposure level
EAFUS	Everything Added to Food in the United States
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
ERPG	emergency response planning guidelines
F	Fahrenheit
F1	first-filial generation
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FR	Federal Register

FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GGT	γ-glutamyl transferase
GRAS	generally recognized as safe
HEC	human equivalent concentration
HED	human equivalent dose
HHS	Department of Health and Human Services
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Level of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
MRL	Minimal Risk Level
MS	mass spectrometry
MSHA	Mine Safety and Health Administration
Mt	metric ton
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NCEH	National Center for Environmental Health
ND	not detected
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences

NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PAC	Protective Action Criteria
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PEL	permissible exposure limit
PEL-C	permissible exposure limit-ceiling value
pg	picogram
PEHSU	Pediatric Environmental Health Specialty Unit
PND	postnatal day
POD	point of departure
ppb	parts per billion
ppbv	parts per billion by volume
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure level/limit
REL-C	recommended exposure level-ceiling value
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase (same as aspartate aminotransferase or AST)
SGPT	serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT)
SIC	standard industrial classification
SMR	standardized mortality ratio
sRBC	sheep red blood cell
STEL	short term exposure limit
TLV	threshold limit value
TLV-C	threshold limit value-ceiling value
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
USNRC	U.S. Nuclear Regulatory Commission

VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization
>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result