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 of 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.

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

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

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 first by route of exposure - inhalation, oral, and dermal; and then by health effect - death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods - acute (14 days or less), intermediate (15 - 364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into “less serious” or “serious” effects. “Serious” effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). “Less serious” effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point 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 end points. 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.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAEL) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of hexachlorobutadiene are indicated in Table 2-2. Because cancer effects could occur at lower exposure levels, Figures 2-l and 2-2 also show ranges for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for hexachlorobutadiene. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. 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 within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990h), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity

reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

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

2.2.1 Inhalation Exposure

2.2.1.1 Death

No studies were located regarding death in humans after inhalation exposure to hexachlorobutadiene.

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).

2.2.1.2 Systemic Effects

No studies were located regarding cardiovascular, gastrointestinal, musculoskeletal , or dermal/ocular effects in humans or animals after inhalation exposure to hexachlorobutadiene. Limited data are available on hepatic effects in humans and on the respiratory and renal effects of hexachlorobutadiene in animals. These effects are discussed below. The highest NOAEL value and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 2-l and plotted in Figure 2-l.

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

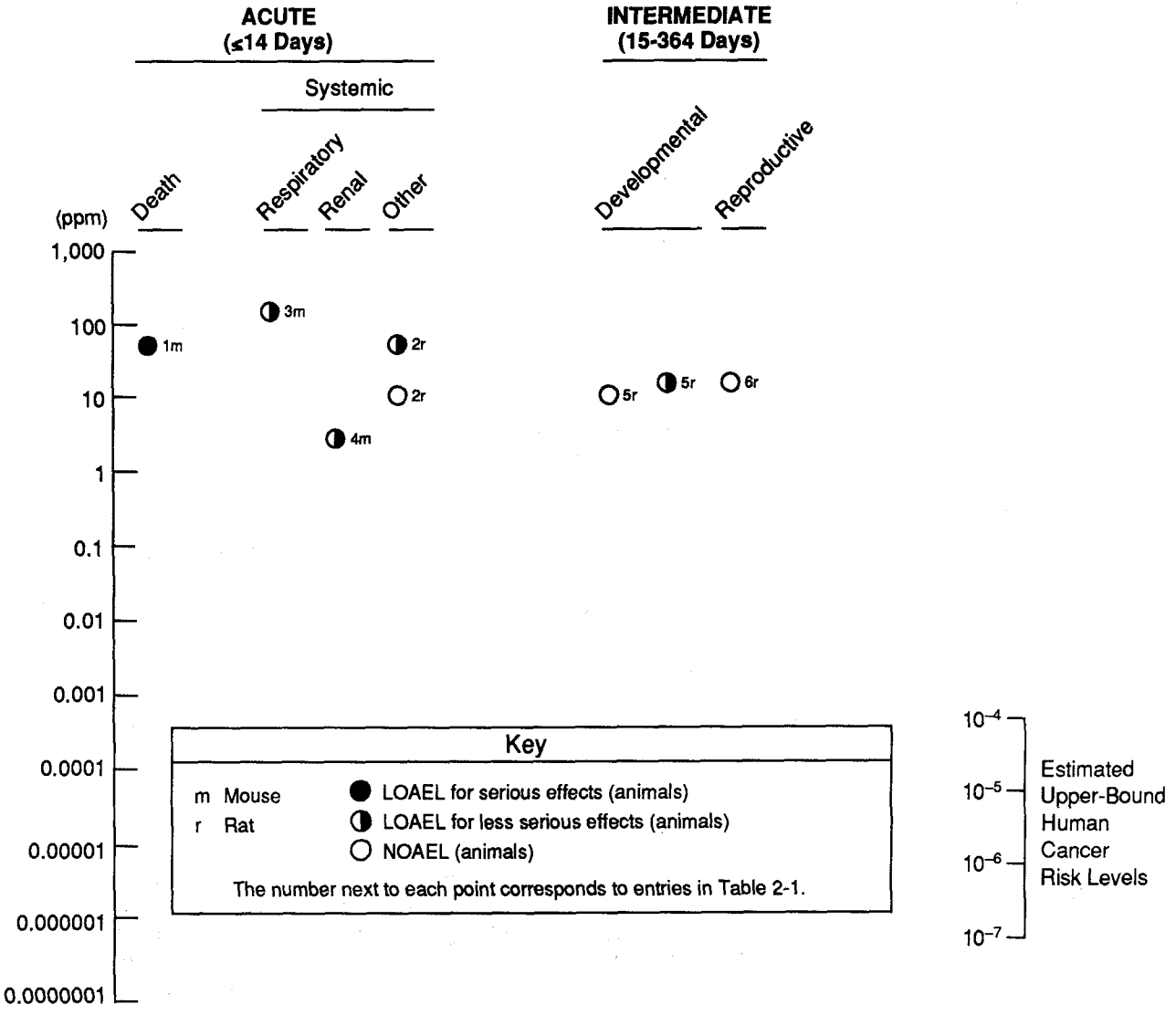
TABLE 2-1. Levels of Significant Exposure to Hexachlorobutadiene - Inhalation

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
ACUTE EXPOSURE								
Death								
1	Mouse		5 d 7hr/d				50 (100% mortality)	NIOSH 1981
Systemic								
2	Rat		5 d 7hr/d	Other	10	50 (body weight reduced 14%)		NIOSH 1981
3	Mouse		15 min	Resp		155 (decreased respiratory rate 36%)		de Ceaurriz et al. 1988
4	Mouse		4 hr	Renal		2.75 (damaged cortical proximal tubules)		de Ceaurriz et al. 1988
INTERMEDIATE EXPOSURE								
Developmental								
5	Rat		Gd 6-20 6hr/d		10	15 (fetal body weight reduced 9.5% in males and 12.5% in females)		Saillenfait et al. 1989
Reproductive								
6	Rat		15 d Gd 6-20 6hr/d		15			Saillenfait et al. 1989

[a]The number corresponds to entries in Figure 2-1.

d = day(s); Gd = gestation day; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; min = minute(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory

FIGURE 2-1 Levels of Significant Exposure to Hexachlorobutadiene – Inhalation



2. HEALTH EFFECTS

Hepatic Effects. 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, glycinedeoxycholic 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 reduced.

Renal Effects. Mice that were exposed to vapors of hexachlorobutadiene (2.75-25 ppm) for 4 hours showed an increase (4-91%) in the number of damaged cortical renal tubules (de Ceaurriz et al. 1988). Degeneration of the tubule midsection resulted from exposures to 250 ppm hexachlorobutadiene for 4 hours on each of 2 consecutive days (Gage 1970). Damage (not specified) to renal proximal tubules was also reported in rats exposed to vapors at a concentration of 25 ppm for 15 days (6 hours/day); the kidneys were pale and enlarged. At a higher concentration (100 ppm), degeneration of renal cortical tubules with epithelial regeneration occurred after 12 days of exposure (Gage 1970). Quantitative data on renal effects were limited.

2.2.1.3 Immunological Effects

No studies were located regarding immunological effects in humans or animals after inhalation exposure to hexachlorobutadiene.

2.2.1.4 Neurological Effects

No studies were located regarding neurological effects in humans or animals after inhalation exposure to hexachlorobutadiene.

2.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to hexachlorobutadiene.

2. HEALTH EFFECTS

In animals, the frequency of abnormal sperm morphology did not increase significantly over controls in mice exposed to concentrations of 10 ppm hexachlorobutadiene (NIOSH 1981). When mice were exposed to 50 ppm (the only other concentration tested), all animals died during the 5 week posttreatment period. Thus, a reliable NOAEL value for reproductive effects cannot be identified for this study. When rat dams were exposed to vapors of hexachlorobutadiene (up to 15 ppm) during gestation (gestation days 6-20), the mean number of implantation sites, total fetal loss, resorptions and number of live fetuses were comparable to unexposed controls (Saillenfait et al. 1989).

Studies evaluating the genotoxic potential of hexachlorobutadiene indicate that hexachlorobutadiene does not affect fertility in male rats. In dominant lethal tests in rats, fertility indices, number of corpora lutea or implantations, or the 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).

2.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans after inhalation exposure to hexachlorobutadiene .

In animals, data are limited to one intermediate-duration study in which rats were exposed to vapors of hexachlorobutadiene at concentrations up to 15 ppm during gestation days 6-20 (Saillenfait et al. 1989). The only effect observed was a reduction ($p < 0.01$) in fetal body weights at concentrations of 15 ppm (highest dose tested). No fetotoxic effects were observed at concentrations of 10 ppm or less. Embryotoxicity was not observed at any dose tested and there were no exposure-related external, visceral, or skeletal anomalies. It should be noted that reduced maternal body weight gain was observed at the 15 ppm vapor concentration.

The highest NOAEL value and a LOAEL value for developmental toxicity in rats are recorded in Table 2-l and plotted in Figure 2-l.

2. HEALTH EFFECTS

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after inhalation exposure to hexachlorobutadiene.

Hexachlorobutadiene did not cause dominant lethal mutations in rats after inhalation of vapors at concentrations of 10 or 50 ppm for up to 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).

Other genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

No studies were located regarding cancer in humans or animals after inhalation exposure to hexachlorobutadiene. However, EPA has derived an inhalation unit risk of 0.022 $(mg/m^3)^{-1}$ (IRIS 1993), based on oral exposure data (see Section 2.2.2.8). Exposure levels corresponding to excess cancer risks of 10^{-4} to 10^{-7} are shown in Figure 2-l.

2.2.2 Oral Exposure

2.2.2.1 Death

No studies were located regarding death in humans after oral exposure to hexachlorobutadiene.

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

Mice exposed to 1,000 and 3,000 ppm hexachlorobutadiene in their diet (19-36 mg/kg/day) died after 3-5 days (NTP 1991; Yang et al. 1989). Animals exposed to 30-300 ppm (3-49 mg/kg/day)

survived the 15 day exposure period. Survival was not reduced in rats exposed to 100 mg/kg/day hexachlorobutadiene for 30 days or at dose levels of 15.6 mg/kg/day for 13 weeks (Harleman and Seinen 1979) and 100 mg/kg/day (Kociba et al. 1971). Mice survived dose levels of up to 19.2 mg/kg/day for 13 weeks (NTP 1991). In lifetime studies, survival was reduced significantly in male rats exposed to hexachlorobutadiene at a dose level of 20 mg/kg/day (Kociba et al. 1977a). Although the cause of death was not reported, renal damage, a major effect manifested by this compound, may have been a contributing factor.

A LOAEL value for lethality in rats after chronic exposure is recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2. Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, or dermal/ocular effects in humans after oral exposure to hexachlorobutadiene for any duration category.

Studies have been conducted in animals to evaluate the respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, and renal effects. These effects are discussed below. No studies were located on dermal/ocular effects. The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

Respiratory Effects. Intermediate-duration (30-148 days) exposure to 20 mg/kg/day (Schwetz et al. 1977) or 100 mg/kg/day (Kociba et al. 1971) and lifetime exposures to 20 mg/kg/day (Kociba et al. 1977a) did not cause treatment-related lesions of the lungs or changes in lung weight in rats exposed to hexachlorobutadiene at dose levels of 20 mg/kg/day.

Cardiovascular Effects. Hexachlorobutadiene did not alter heart weights or cause treatment-related lesions of the heart in rats or mice exposed for intermediate durations (90-148 days) at dose levels of 19.2-20 mg/kg/day (NTP 1991; Schwetz et al. 1977; Yang et al. 1989) and 100 mg/kg/day (Kociba et al. 1971) or after lifetime exposure at dose levels of 20 mg/kg/day (Kociba et al. 1977a).

TABLE 2-2. Levels of Significant Exposure to Hexachlorobutadiene - Oral

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
ACUTE EXPOSURE								
Systemic								
1	Rat	(F)	14 d	Hepatic	35			Harleman and Seinen 1979
				Renal		4.6 (proximal convoluted tubule degeneration)		
				Other		4.4 (body weight reduced 9.5% in females)		
INTERMEDIATE EXPOSURE								
Systemic								
2	Rat	(GO)	13 wk	Hepatic	2.5	6.3 (increased cytoplasmic basophilia)		Harleman and Seinen 1979
				Hemato	15.6			
				Renal	1	2.5 (degeneration proximal tubules)		
				Other	2.5		6.3 (body weight decreased 29% in females and 13% in males)	
3	Rat	(F)	30 d	Resp	100			Kociba et al. 1971
				Cardio	100			
				Gastro	100			
				Hemato	3	10 (increased hemoglobin concentration)		
				Hepatic	10	30 (centrilobular *hepatocellular* swelling)		
				Renal	10	30 (tubular degeneration, necrosis)		
				Other	100	(thyroid, parathyroid, pituitary and adrenal glands)		

TABLE 2-2. Levels of Significant Exposure to Hexachlorobutadiene - Oral *(continued)*

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
4	Rat	(F)	10-18 wk	Renal			15 (tubular degeneration, necrosis)	Harleman and Seinen 1979
				Other		15 (maternal body weight decreased 15%)		
5	Rat	(F)	4 wk	Hepatic	1.25	5 (absolute liver weight decreased 41%)	20 (increased plasma aspartate amino-transferase and bilirubin)	Jonker et al. 1993b
				Renal	1.25	5 (tubular cytomegaly and decreased plasma creatinine)		
				Other	1.25	5 (body weight decreased 10% in males and 15% in females, decreased adrenal weight, 17%)		
6	Rat	(F)	148 d	Resp	20			Schwetz et al. 1977
				Cardio	20			
				Gastro	20			
				Hemato	20			
				Musc/skel	20			
				Hepatic	20			
				Renal	0.2	2 (tubular degeneration)		
				Other	2	20 (body weight gain reduced 8-9% in males and 5-17% in females)		

TABLE 2-2. Levels of Significant Exposure to Hexachlorobutadiene - Oral *(continued)*

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
7	Mouse	(F)	13 wk	Resp	19.2			NTP 1991, Yang et al. 1989
				Cardio	19.2			
				Gastro	19.2			
				Musc/skel	19.2			
				Hepatic	19.2			
				Renal		0.2[b] (tubular degeneration)		
				Derm/oc	19.2			
				Other	1.5	4.9 (body weight gain reduced 49% in males)		
Neurological								
8	Rat	(GO)	13 wk		15.6			Harleman and Seinen 1979
9	Rat	(F)	148 d		20			Schwetz et al. 1977
10	Rat	(F)	10-18 wk		15		150 (ataxia, demyelination and degeneration of femoral nerve fiber)	Harleman and Seinen 1979
11	Rat	(F)	30 d		100			Kociba et al. 1971
12	Mouse	(F)	13 wk		19.2			NTP 1991, Yang et al. 1989
Developmental								
13	Rat	(F)	43 d Gd1-22; Ld1-21		2	20 (neonatal weight decreased 13%)		Schwetz et al. 1977
14	Rat	(F)	6 wk			15 (reduced pup weight, 16-19%)		Harleman and Seinen 1979

TABLE 2-2. Levels of Significant Exposure to Hexachlorobutadiene - Oral *(continued)*

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
Reproductive								
15	Rat	(F)	10-18 wk		15		150 (infertility)	Harleman and Seinen 1979
16	Rat	(F)	148 d		20			Schwetz et al. 1977
17	Mouse	(F)	13 wk		19.2			NTP 1991, Yang et al. 1989
CHRONIC EXPOSURE								
Death								
18	Rat	(F)	2 yr				20 (increased mortality)	Kociba et al. 1977a
Systemic								
19	Rat	(F)	2 yr	Resp	20			Kociba et al. 1977a
				Cardio	20			
				Gastro	20			
				Hemato	20			
				Musc/skel	20			
				Hepatic	20			
				Renal	0.2	2 (tubular hyperplasia)		
				Other	2	20 (reduced mean body weight in males [8-20%] and females [5-12%])		
Neurological								
20	Rat	(F)	2 yr		20			Kociba et al. 1977a

TABLE 2-2. Levels of Significant Exposure to Hexachlorobutadiene - Oral *(continued)*

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference
Cancer								
21	Rat	(F)	2 yr				20 (CEL: kidney tumors)	Kociba et al. 1977a

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an intermediate oral minimal risk level (MRL) of 0.0002 mg/kg/day; dose divided by an uncertainty factor of 1,000 (10 for extrapolation from animals to humans, 10 for use of a LOAEL and 10 for human variability).

Cardio = cardiovascular; d = day(s); (F) = feed; Gastro = gastrointestinal; Gd = gestation day; (GO) = gavage - oil; Hemato = hematological; Ld = lactation day(s); LOAEL = lowest-observed-adverse-effect level; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); yr = year(s)

FIGURE 2-2 Levels of Significant Exposure to Hexachlorobutadiene – Oral

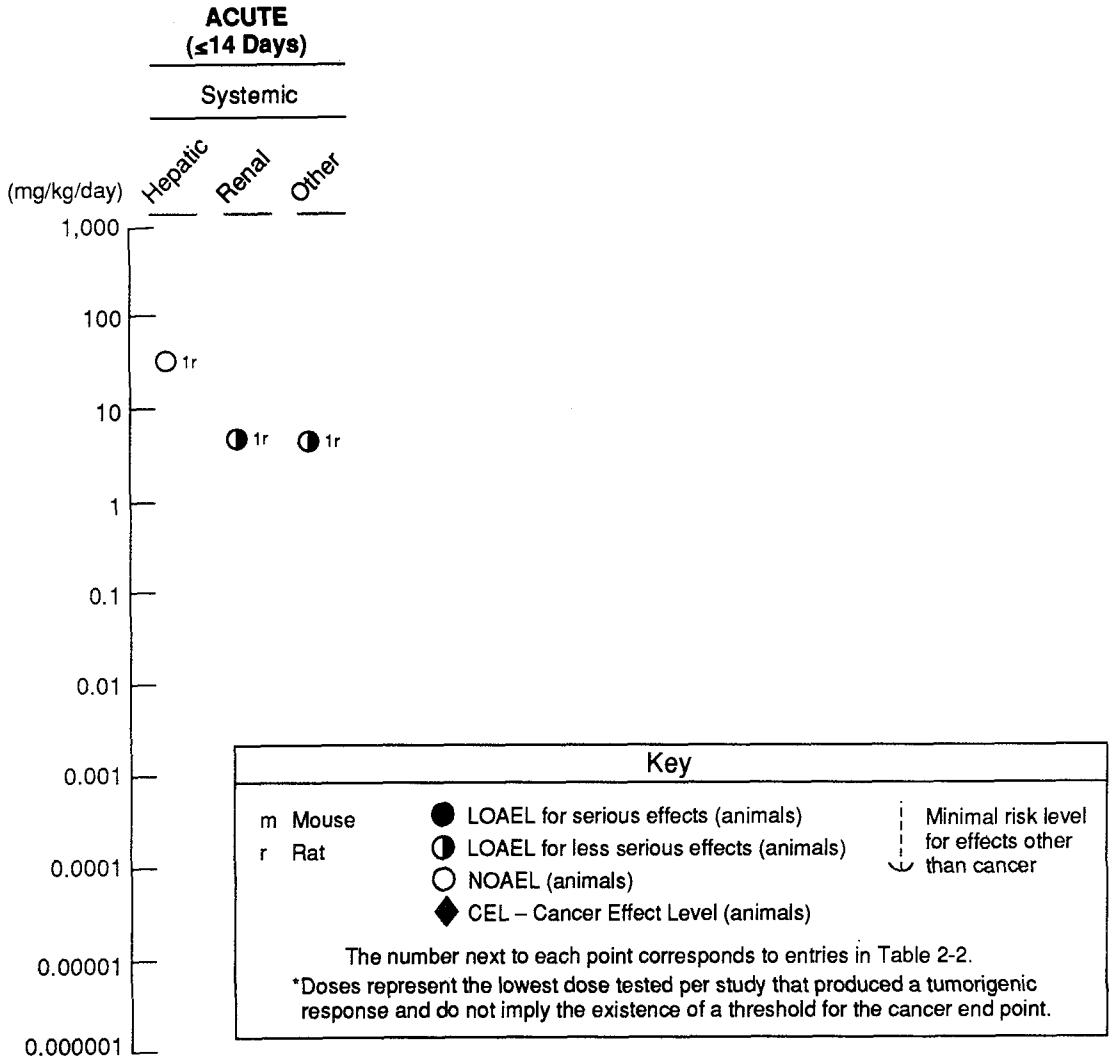


FIGURE 2-2 Levels of Significant Exposure to Hexachlorobutadiene – Oral (Continued)

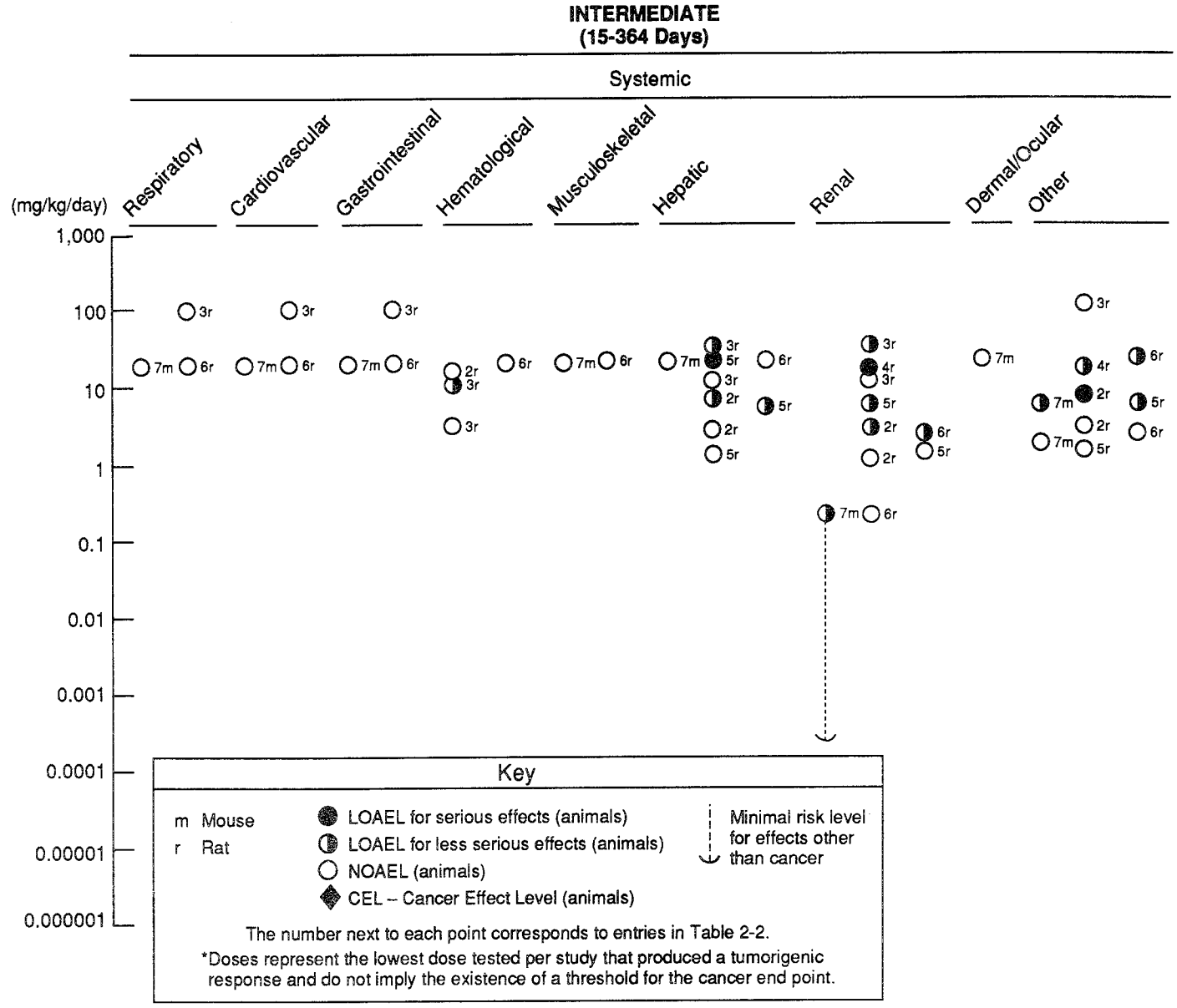


FIGURE 2-2 Levels of Significant Exposure to Hexachlorobutadiene – Oral (Continued)

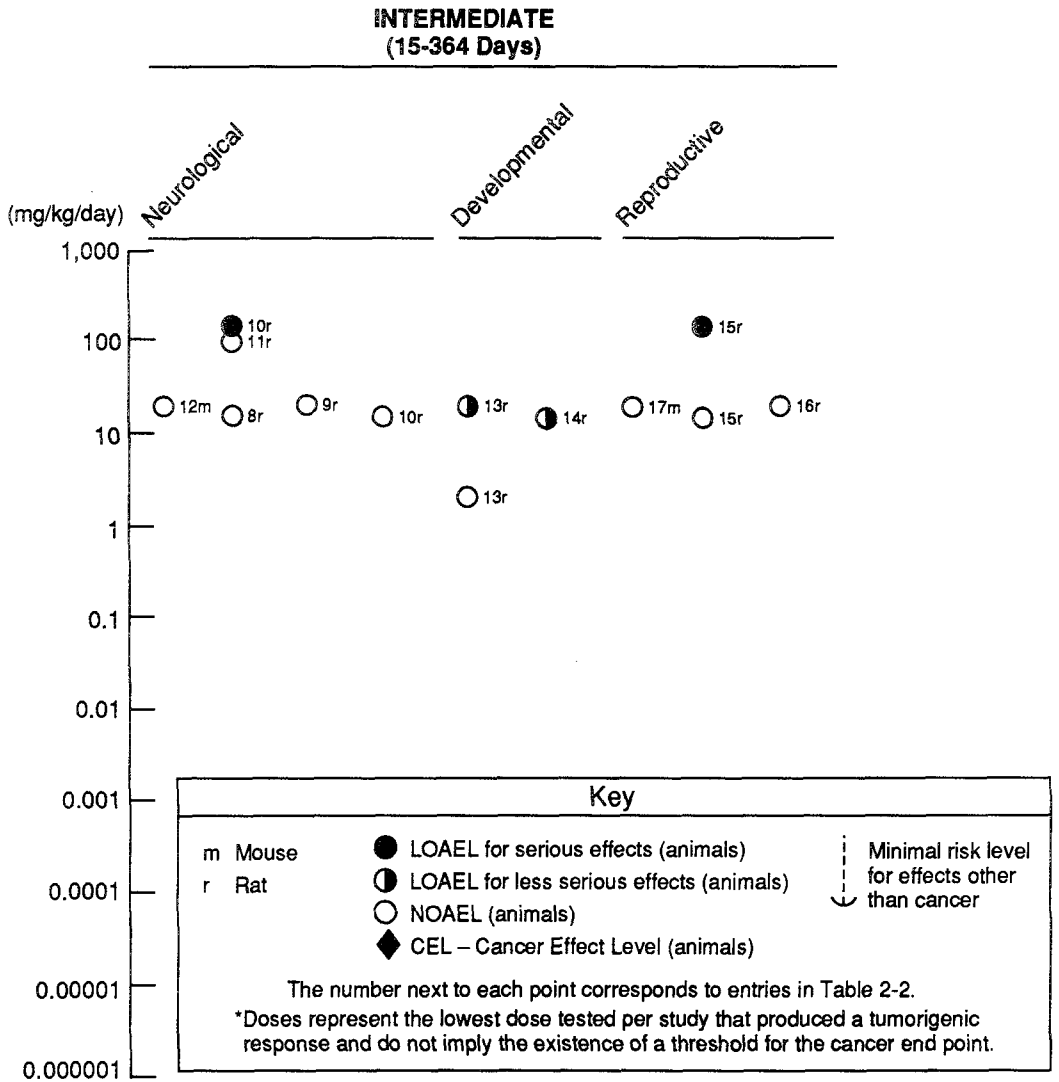
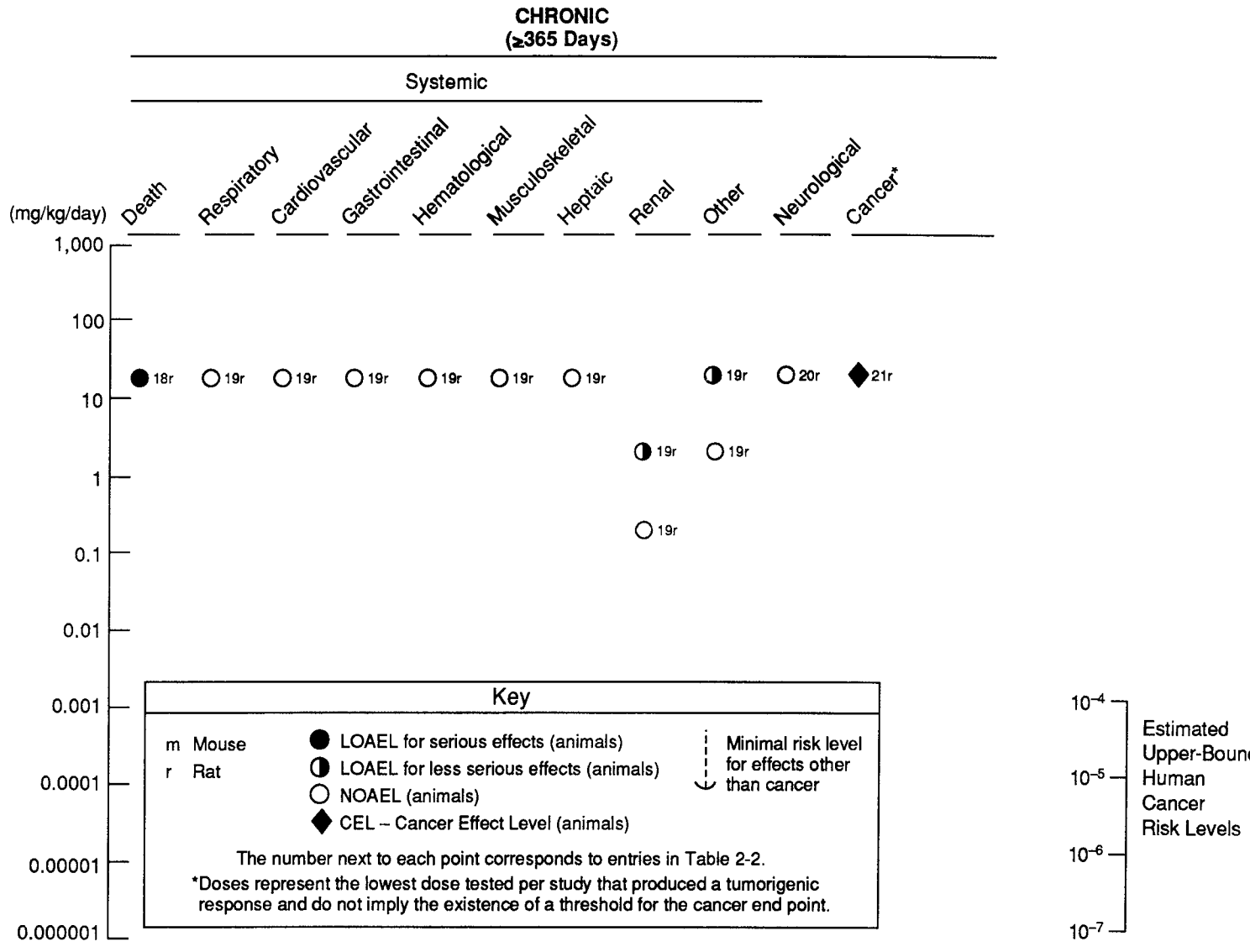


FIGURE 2-2 Levels of Significant Exposure to Hexachlorobutadiene – Oral (Continued)



2. HEALTH EFFECTS

Gastrointestinal Effects. Intermediate-duration (30-148 days) 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 20 mg/kg/day (Schwetz et al. 1977) or levels up to 100 mg/kg/day (Kociba et al. 1971). Lifetime exposure at dose levels of 20 mg/kg/day (Kociba et al. 1977a) did not result in any effect on this system.

Hematological Effects. 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 exposure (90-148 days) (Harleman and Seinen 1979; Schwetz et al. 1977). Similarly, one lifetime oral exposure (20 mg/kg/day) also did not cause hematological effects (Kociba et al. 1977a). However, in another study, hemoglobin concentration increased in rats at dose levels from 10-100 mg/kg/day, but not at 3 mg/kg/day. Other hematologic parameters, as mentioned above, were within normal values (Kociba et al. 1971).

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

Hepatic Effects. An animal study revealed that hexachlorobutadiene can affect the liver. However, the effects were less serious compared to effects in the kidney at the same dose. Liver weights were decreased in female rats fed 5 mg/kg/day or greater hexachlorobutadiene for 4 weeks (Jonker et al. 1993b). Histological examinations were not performed. However, evaluation of serum biochemical parameters in males revealed increased enzyme activity (aspartate aminotransferase, $p < 0.02$) and total bilirubin levels ($p < 0.02$) at doses of 20 mg/kg/day (highest dose tested). Cytoplasmic basophilia and liver weights were increased in male rats exposed to hexachlorobutadiene by gavage at dose levels of 6.3 mg/kg/day or greater for 13 weeks; treatment-related lesions were not observed in females (Harleman and Seinen 1979). In another study, hepatocellular swelling occurred at a dose level of 30 mg/kg/day and liver weights decreased at dose levels of 30-100 mg/kg/day or greater in female rats that were fed diets containing hexachlorobutadiene for 30 days (Kociba et al. 1971). Males were not evaluated concurrently. Although histological lesions were not observed in lifetime studies, urinary excretion of coproporphyrin increased at dose levels of 20 mg/kg/day, suggesting alterations in heme synthesis in the liver (Kociba et al. 1977a).

2. HEALTH EFFECTS

Renal Effects. The kidney appears to be the primary target organ following oral exposure to hexachlorobutadiene. Focal necrosis and increased urinary parameters (lactate dehydrogenase, N-acetyl-β-glucosaminidase) were reported in rats administered hexachlorobutadiene (100 mg/kg) for 24 hours (Jonker et al. 1993a). These effects were not seen at 10 mg/kg. In acute studies in which rats were fed hexachlorobutadiene (4.6 mg/kg/day) in the diet for 14 days, there was degeneration of tubular epithelial cells mainly confined to the straight limbs of the proximal tubules located in the outer zone of the medulla (Harleman and Seinen 1979). Similar effects were seen following intermediate-duration exposure in other species. Cell necrosis and regeneration were found as well as tubular epithelial cell degeneration in rats exposed to dose levels of 30-100 mg/kg/day for 30 days (Kociba et al. 1971). Diffuse tubular cytomegaly was observed in the cortex of the kidneys at dose levels of 5 mg/kg/day or greater in rats following oral exposure to hexachlorobutadiene for 4 weeks (Jonker et al. 1993b). Tubular degeneration also occurred in mice at dose levels from 0.2-19.2 mg/kg/day in females exposed to hexachlorobutadiene for 13 weeks (NTP 1991; Yang et al. 1989). Based on a value of 0.2 mg/kg/day, an intermediate oral MRL was calculated as described in the footnote in Table 2-2.

Kidneys were roughened and had a mottled cortex in males exposed to dose levels of 2 and 20 mg/kg/day for 148 days (Schwetz et al. 1977). Alterations in the kidney were also observed following longer exposures. For the most part, these effects were manifested as renal tubular hyperplasia which occurred at dose levels of 2 and 20 mg/kg/day, but not at a dose level of 0.2 mg/kg/day (Kociba et al. 1977a). Kidney weights were also affected. Intermediate- and chronic-duration oral exposures caused increased relative kidney weights or kidney/body weight ratios at dose levels from 2-20 mg/kg/day (Jonker et al. 1993b; Kociba et al. 1977a; NTP 1991; Schwetz et al. 1977; Yang et al. 1989).

Impaired kidney function accompanied morphological evidence of kidney damage. The ability to concentrate urine was significantly reduced in female rats at dose levels from 2.5-15.6 mg/kg/day for 13 weeks. The same effect was observed in males at 15 mg/kg/day (Harleman and Seinen 1979). On the other hand, alterations in various clinical chemistry indices (e.g., blood urea nitrogen, creatinine, y-glutarnyl transpeptidase, and alanine aminotransferase) were comparable to untreated controls in rats exposed to hexachlorobutadiene at dose levels up to 20 mg/kg/day up to 148 days (Harleman and Seinen 1979; Schwetz et al. 1977). Plasma urea levels decreased ($p < 0.05$) in female rats at dose

levels of 1.25 mg/kg/day or greater and in males at 20 mg/kg/day, while creatinine levels decreased ($p < 0.05$) in females at dose levels of 5 mg/kg/day or greater for 4 weeks (Jonker et al. 1993b).

2.2.2.3 Immunological Effects

No studies were located regarding immunological effects in humans after oral exposure to hexachlorobutadiene.

In animals, histological examination of lymphoid organs including the thymus and spleen did not reveal treatment-related lesions at dose levels up to 100 mg/kg/day rats (Harleman and Seinen 1979; Kociba et al. 1971, 1977a). Depletion and necrosis of lymphoid tissue in the lymph nodes, spleen, and thymus were noted in mice exposed to lethal doses of hexachlorobutadiene 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 mg/kg/day (NTP 1991; Yang et al. 1989). Tests on effects of immune function have not been evaluated.

2.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to hexachlorobutadiene.

In animals, ataxia, and demyelination and degeneration 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); however, 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; Yang et al. 1989). 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 of 100 mg/kg/day or less (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, and sciatic nerve in rats exposed to hexachlorobutadiene (20 mg/kg/day) for 2 years (Kociba et al. 1977a). Neurochemical and neurophysiological parameters have not been monitored.

The highest NOAEL values and all LOAEL values from each reliable study for neurotoxicity in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to hexachlorobutadiene.

In animals, fertility was reduced 100% in Wistar-derived rat dams administered 150 mg/kg/day hexachlorobutadiene during a 10-week study. The mean litter size and the resorption rate did not differ significantly from controls in dams fed 15 mg/kg/day during an 18-week study (Harleman and Seinen 1979). The actual total exposure time for this study is not clear; the rats were exposed for at least 10 weeks at the high dose and 12 weeks (of an 18-week study) at the low dose. No determination of a reliable LOAEL or NOAEL value was possible for this study. 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 or incidence of abnormal sperm in mice exposed to hexachlorobutadiene (19 mg/kg/day) for 13 weeks (NTP 1991; Yang et al. 1989). Lifetime exposures up to 20 mg/kg/day did not reveal treatment-related lesions in the reproductive organs (Kociba et al. 1977a).

The highest NOAEL values and all LOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to hexachlorobutadiene.

In animal studies, rat dams were fed hexachlorobutadiene at dose levels of 15 mg/kg/day during gestation (as part of an 18-week study). Rat pup weights were reduced at birth and weaning. However, embryotoxicity and teratogenicity were not observed at this dose (Harleman and Seinen 1979). In another 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 throughout gestation and lactation; body weights were not reduced in pups from dams exposed to 2 mg/kg/day. No other signs of fetotoxicity were evident at doses up to 20 mg/kg/day. Teratogenic effects were not observed nor was hexachlorobutadiene embryotoxic at the doses tested (Schwetz et al. 1977).

The highest NOAEL values and all LOAEL values from each reliable study for developmental effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans after oral exposure to hexachlorobutadiene.

In animals, there is some evidence that hexachlorobutadiene interacts with genetic material. Male rats administered a single gavage dose of hexachlorobutadiene (20 mg/kg/day) showed a 40% increase in renal deoxyribonucleic acid (DNA) repair and 0.78 alkylations per million nucleotides (Stott et al. 1981). On the other hand, when hexachlorobutadiene was administered in the diet, it did not cause chromosomal aberrations in rat bone marrow cells (Schwetz et al. 1977).

Other genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding carcinogenic effects in humans after oral exposure to hexachlorobutadiene.

Studies in rats reported renal tubular adenomas and adenocarcinomas in male and female animals at doses of 20 mg/kg/day (Kociba et al. 1977a). Metastasis to the lungs was observed. Combined incidences of renal tubular neoplasms in males (9/39, 23 %) and in females (6/40, 15 %) increased ($p < 0.05$) over controls (males-l/90, females-0/90, 0%). The tumor incidence was not increased in the 0.2 and 2 mg/kg/day dose groups but there were some indications of hyperplasia in animals exposed to 2 mg/kg/day. The EPA (1990f) evaluated these data and calculated a human potency factor of 7.8×10^{-2} $(mg/kg/day)^{-1}$ (q_1*), representing a 95% upper confidence limit of extra lifetime

human risk. Based on this value, cancer risk levels of 10^{-4}, 10^{-5}, and 10^{-6} correspond to exposures of 0.001, 0.0001, 0.00001 mg/kg/day.

2.2.3 Dermal Exposure

2.2.3.1 Death

No studies were located regarding death in humans after dermal exposure to hexachlorobutadiene.

During the 14 day observation period some rabbits (2-8) died after 8-hour exposure to doses of 775-1,550 mg/kg applied directly to shaved skin (3.2 cm^2), but no deaths occurred in the 388 mg/kg dose group. The author 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.

A LOAEL value for lethality in rabbits after acute-duration exposure is recorded in Table 2-3.

2.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal/ocular, or other effects in humans after dermal exposure to hexachlorobutadiene. Liver, kidney, and dermal/ocular effects were reported in animals. These effects are discussed below. All LOAEL values for systemic effects in rabbits after acute-duration exposure are recorded in Table 2-3.

Hepatic Effects. Hydropic changes, fatty degeneration, and glycogen reduction were observed in rabbits after exposure of the skin to 388 mg/kg or greater hexachlorobutadiene for 8 hours (Duprat and Gradiski 1978). These effects were reversible within 3 weeks.

TABLE 2-3. Levels of Significant Exposure to Hexachlorobutadiene - Dermal

Species	Exposure duration/ frequency	System	NOAEL (mg/kg)	LOAEL (effect) Less serious (mg/kg)	LOAEL (effect) Serious (mg/kg)	Reference
ACUTE EXPOSURE						
Death						
Rabbit	Once 8 hr				116 (LD50)	Duprat and Gradiski 1978
Systemic						
Rabbit	Once 8 hr	Hepatic		388 (fatty degeneration, hydropic changes)		Duprat and Gradiski 1978
		Renal		388 (tubular necrosis)		
		Derm/oc		388 (cutaneous necrosis)		

Derm/oc = dermal/ocular; hr = hour(s); LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; mg/kg = milligrams per kilogram; NOAEL = no-observed-adverse-effect level

Renal Effects. 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.

Dermal/Ocular Effects. Skin necrosis was evident at the site of application in rabbits after exposure of the skin to dose levels of 388 mg/kg hexachlorobutadiene for 8 hours (Duprat and Gradiski 1978). However, most skin lesions had healed within 2 weeks.

2.2.3.3 lmmunological Effects

No studies were located regarding immunological effects in humans or animals after dermal exposure to hexachlorobutadiene.

2.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans after dermal exposure to hexachlorobutadiene.

Rabbits exposed to doses of 388-1550 mg/kg applied to shaved skin exhibited evidence of aneral nervous system depression (stupor) during exposure and in the l-2 hour period after exposure (Duprat and Gradiski 1978).

No studies were located regarding the following health effects in humans or animals after dermal exposure:

2.2.3.5 Developmental Effects

2.2.3.6 Reproductive Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.4.

2. HEALTH EFFECTS

2.2.3.8 Cancer

No studies were located regarding carcinogenic effects in humans after dermal exposure to hexachlorobutadiene.

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). Data from this exploratory study are not sufficient to rule out carcinogenic effects via dermal exposure.

2 . 3 TOXICOKINETICS

In analogy with other unsaturated chlorinated compounds, hexachlorobutadiene absorption presumably occurs across the lipid portion of the intestinal matrix rather than by active or protein-facilitated transport. After absorption, most of the hexachlorobutadiene is carried to the liver where it is conjugated with glutathione and excreted in the bile. Mono- and bis-substituted glutathione conjugates are formed. The bile also contains the cysteinyl/glycinyl and cysteinyl derivatives of the glutathione conjugate. Biliary metabolites are resorbed from the intestines and undergo enterohepatic recirculation.

Hexachlorobutadiene and its metabolites preferentially distribute to the kidney, liver, adipose deposits, and possibly the brain. Some hexachlorobutadiene metabolites inhibit mitochondrial metabolism and react with DNA, resulting in cell death or tumorigenesis. Hexachlorobutadiene metabolites are excreted in the urine and feces. There is limited degradation to carbon dioxide which is exhaled from the lungs.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

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.

2. HEALTH EFFECTS

2.3.1.2 Oral Exposure

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 which 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.

2.3.1.3 Dermal Exposure

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).

2. HEALTH EFFECTS

2.3.2 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 the route of exposure.

2.3.2.1 Inhalation Exposure

No studies were located regarding distribution in humans or animals after inhalation exposure to hexachlorobutadiene.

2.3.2.2 Oral Exposure

No studies were located regarding distribution in humans after oral exposure to hexachlorobutadiene. In animals, 5-14 % of (^{14}C) radiolabeled 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 0.11%, and the plasma 0.013% from the intestinally absorbed material. In the recipient rats, the kidney contained 0.15% of the dose, the liver 0.97%, and the plasma 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.

2.3.2.3 Dermal Exposure

No studies were located regarding distribution in humans or animals after dermal exposure to hexachlorobutadiene.

2.3.2.4 Other Exposure Routes

In a study using doses of 0.1 and 300 mg/kg intraperitoneally-administered 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.

2.3.3 Metabolism

2.3.3.1 Inhalation Exposure

No studies were located regarding metabolism in humans or animals after inhalation exposure to hexachlorobutadiene.

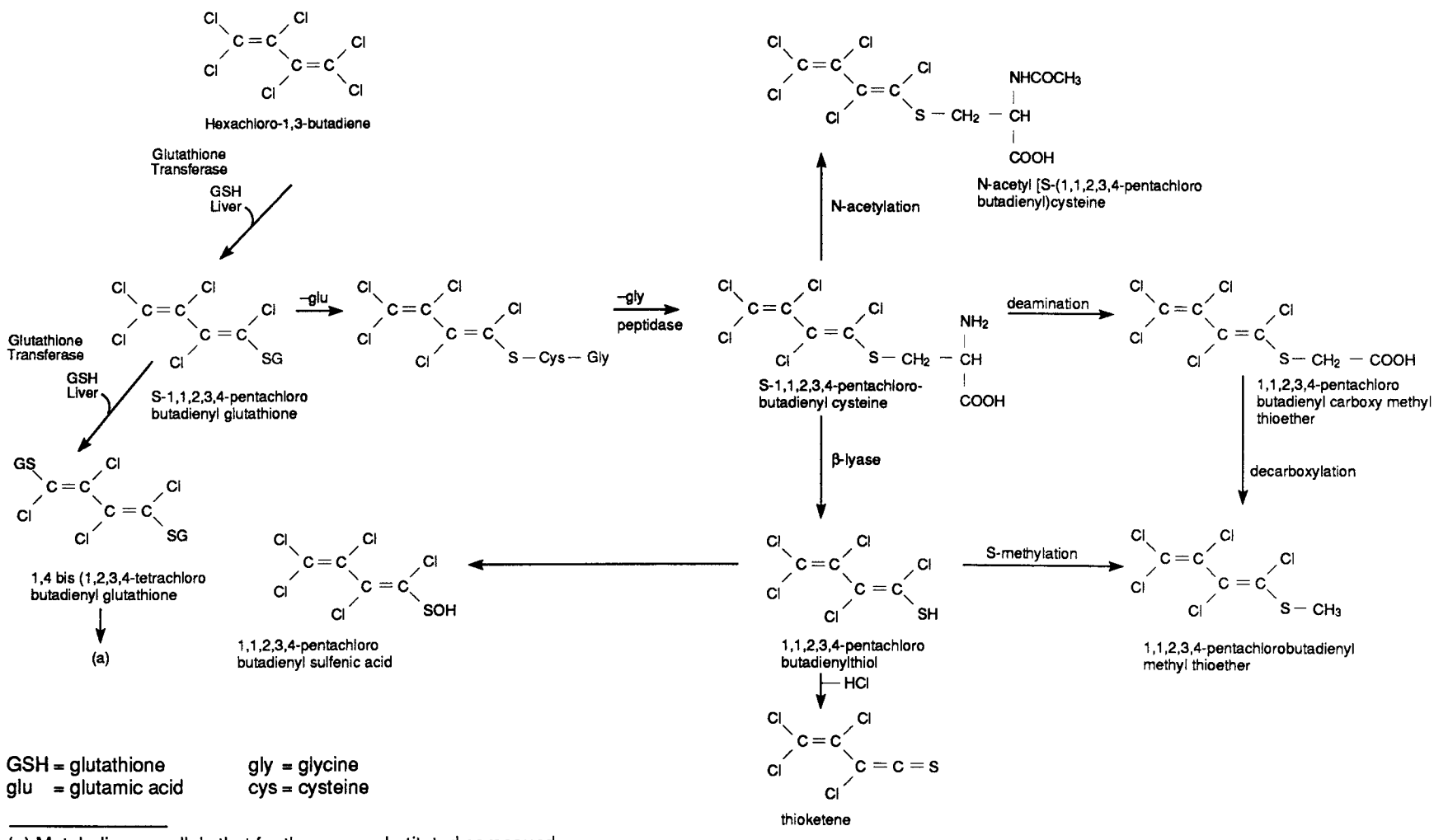
2.3.3.2 Oral Exposure

No studies were located regarding metabolism in humans after oral exposure to hexachlorobutadiene.

There is a considerable amount of information available concerning the metabolism of hexachlorobutadiene in animals. Figure 2-3 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

FIGURE 2-3 Proposed Pathways for Hexachlorobutadiene Metabolism*



GSH = glutathione gly = glycine
glu = glutamic acid cys = cysteine

(a) Metabolism parallels that for the monosubstituted compound

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

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).

Glutathione conjugate 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 or the cysteineiglycine derivative (Gietl et al. 1991; Gietl and Anders 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. S-(Pentachlorobutadienyl)glutathione and S-(pentachlorobutadienyl)-L-cysteine 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.

2. HEALTH EFFECTS

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-l, 1,2,3,4-pentachlorobutadienyl-L-cysteine, S-l, 1,2,3,4-pentachlorobutadienylmercaptoacetic acid, 1,1,2,3,4-pentachlorobutadiene methylthioether, and 1,1,2,3,4-pentachlorobutadiene carboxymethylthioether) (Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985).

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).

2.3.3.3 Dermal Exposure

No studies were located regarding metabolism in humans or animals after dermal exposure to hexachlorobutadiene.

2.3.4 Excretion

2.3.4.1 Inhalation Exposure

No studies were located regarding excretion in humans or animals after inhalation exposure to hexachlorobutadiene.

2.3.4.2 Oral Exposure

No studies were located regarding excretion in humans after oral exposure to hexachlorobutadiene.

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).

2. HEALTH EFFECTS

With single doses ranging from 1 to 200 mg/kg ^{14}C hexachlorobutadiene, the percent of the label in the urine ranged from 4.5 to 30.6% with the highest percentage associated with the lowest dose (Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985). At the higher doses urinary excretion values of 5 to 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.

The contribution of reabsorbed biliary metabolites to urinary excretion is reflected in the differences in urinary excretion of label from bile duct cannulated rats and noncannulated rats. 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-pentachlorobutadienyl-mercaptoacetic acid; 1,1,2,3,4-pentachlorobutadiene methylthioether and 1,1,2,3,4-pentachlorobutadiene carboxymethylthioether (Dekant et al. 1988b; Nash et al. 1984; Reichert and Schutz 1986; Reichert et al. 1985).

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). The only metabolite that had been identified in the feces is 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.

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 cannrulated 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.

2.3.4.3 Dermal Exposure

No studies were located regarding excretion in humans or animals after dermal exposure to hexachlorobutadiene.

2.3.4.4 Other Exposure Routes

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 less than 0.5 % for both doses. The bile contained 89% of the 1 mg/kg dose and 72% of the 100 mg/kg dose.

2.3.5 Mechanism of Toxicity

Much of the data related to the mechanism of hexachlorobutadiene toxicity indicate that the intermediates produced by modification of the S- 1,1,2,3,4-pentachlorodienyl cysteine derivative are responsible for the observed effects on the proximal tubules of the nephrons. The cysteine derivative is formed from the hexachlorobutadiene conjugate 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.

The cysteine derivative is further metabolized to simpler sulphur derivatives through the action of β-lyase. β-Lyase is present in the rodent liver, intestines, and kidneys (MacFarlane et al. 1989; Jones et al. 1988). In the kidney, the highest concentration of β-lyase is located in the pars recta of the proximal tubule, the same area that is damaged by hexachlorobutadiene. It should be noted that β-lyase has been detected in the entire proximal segment (Jones et al. 1988). It is present in both the cytosol and mitochondria and is pyridoxal phosphate dependent (MacFarlane et al. 1989). It degrades the cysteine conjugate to pyruvate, ammonia, and one or more reactive thiols (Dekant et al. 1990b; Schnellmann et al. 1987). A highly reactive thioketene (Figure 2-3) may form as an intermediate and cause local tissue damage (Dekant et al. 1991; Koob and Dekant 1992).

The effects of the cysteine conjugate 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.

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

2.4 RELEVANCE TO PUBLIC HEALTH

Data regarding the effects of exposure to hexachlorobutadiene on humans are sparse. Serum bile acids were increased in workers exposed to vapor concentrations of 0.005-0.02 ppm. These effects could not be attributed to hexachlorobutadiene alone because the workers were also potentially

exposed to other chemicals (carbon tetrachloride and perchloroethylene) and there were no background data on employee health, smoking habits, alcohol consumption, or other confounding variables. Animals have been studied more extensively. Although there are studies available on the systemic (respiratory) effects following inhalation, data are not sufficient to identify a reliable NOAEL value by this route. Much of the available data involve oral exposures in rats. The primary health effect associated with intermediate-duration and chronic-duration oral exposures to hexachlorobutadiene is kidney damage, which occurred in this organ at doses of 0.2 to 20 mg/kg/day. The liver was affected to a lesser extent and effects occurred at doses of 6.3 or 15.6 mg/kg/day. Acute dermal studies, although limited, confirm the toxic effects of hexachlorobutadiene on these organs.

Hexachlorobutadiene did not adversely affect reproduction in animals except at high doses (150 mg/kg/day for 10 weeks). Although there was some evidence of fetotoxicity in animals after inhalation (10 ppm) or oral (15 mg/kg/day) exposure, embryolethality and teratogenicity were not detected. Oral studies in animals indicate that hexachlorobutadiene may increase the risk of renal cancer at dose levels of 20 mg/kg/day. The effects of hexachlorobutadiene are most pronounced after repeated chronic exposure to low doses, suggesting that effects are cumulative. For this reason, there is greater concern for populations living near hazardous waste sites, where exposure to low levels may occur for long periods of time, than for acute exposure scenarios.

Minimal Risk Levels for Hexachlorobutadiene

Inhalation MRLs

Inhalation MRLs have not been derived for any duration category due to the lack of sufficient data to identify a target organ and reliable NOAEL values.

Oral MRLs

- An MRL of 0.0002 mg/kg/day has been derived for intermediate-duration oral exposure to hexachlorobutadiene.

This MRL was derived using a LOAEL value of 0.2 mg/kg/day, based on the presence of kidney damage in female mice (NTP 1991). Tubular cell degeneration and regeneration in the renal cortex were found in treated animals.

Other intermediate-duration oral studies confirm that the kidney is the primary target organ following oral exposure to hexachlorobutadiene. Renal damage, characterized as tubular hyperplasia, tubular epithelial degeneration, and tubular regeneration, was seen at dose levels of 2 mg/kg/day or greater (Harleman and Seinen 1979; Schwetz et al. 1977). A chronic study in rats reported renal tubular hyperplasia at dose levels of 2 mg/kg/day or greater (Kociba et al. 1977a).

No data were located on the effects of acute-duration oral exposure in humans. Two studies are available on the acute oral toxicity in animals. A LOAEL value of 4 mg/kg/day (based on kidney effects) was reported in one study in rats exposed to hexachlorobutadiene for 14 days (Harleman and Seinen 1979). On the other hand, a NOAEL value of 10 mg/kg was reported for kidney effects in a 24-hour rat study (Jonker et al. 1993a). Neither of these studies was considered suitable for the MRL determination because of the small numbers of animals evaluated.

No data were located on the effects of chronic-duration exposure in humans. A chronic-duration study in animals revealed tubular hyperplasia in rats at dose levels of 2 mg/kg/day or greater (Kociba et al. 1977a), but not at 0.2 mg/kg/day, the LOAEL for kidney effects from the intermediate-duration study in mice. Because the intermediate-duration MRL protects against chronic exposures, a chronic MRL has not been proposed.

Death. No studies were located regarding lethality in humans. Hexachlorobutadiene reduced survival in rats following acute- and chronic-duration exposures. Young rats may be more sensitive than adult rats. Acute oral doses of 580 mg/kg (male) and 200-400 mg/kg (female) were lethal to 50% of adult rats. Death occurred at lower dose levels in weanling females (46 mg/kg) and males (65 mg/kg) (Kociba et al. 1977a). However, in a rat reproduction study in which dams received intermediate-duration oral exposures to hexachlorobutadiene at doses of 20 mg/kg/day during gestation (days 1 to 22) and lactation (days l-21), pup survival was not affected even at doses that were maternally toxic (Schwetz et al. 1977). Acute-duration dermal exposures (775 mg/kg) can also reduce survival (50%) in animals (Duprat and Gradiski 1978). Based on these considerations,

lethality may be of concern in humans following exposure to hexachlorobutadiene. The basis for differential susceptibility between adult and young rats may be due to metabolic differences or differences in disposition of hexachlorobutadiene. As discussed in Section 2.3.2, hexachlorobutadiene distributes to body fat. The smaller fraction of fat in the newborn reduces the amount of sequestered hexachlorobutadiene; therefore, more of the compound may reach target organs (Hook et al. 1983).

Systemic Effects

Respiratory Effects. No studies were located regarding respiratory effects in humans. The only data available indicating respiratory effects were reports of irritation of the nasal cavity in mice after acute (15 minutes) inhalation of vapors of hexachlorobutadiene at concentrations of 155 ppm or greater (de Ceaurriz et al. 1988). The importance of this finding to human health is uncertain.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans. In animals, intermediate-duration or chronic-duration oral exposure to hexachlorobutadiene at dose levels up to 100 mg/kg/day did not cause treatment-related lesions of the heart in rats or mice (Kociba et al. 1971, 1977a; NTP 1991; Schwetz et al. 1977; Yang et al. 1989). On the other hand, heart weights decreased significantly in mice at doses of 16.8 mg/kg/day (NTP 1991; Yang et al. 1989) or 65 mg/kg/day or greater in rats (Kociba et al. 1971). There were no histopathological lesions. Because treatment-related lesions were not observed even at doses higher than those causing other organ toxicity, cardiovascular toxicity may not be an area of concern in humans following exposure to hexachlorobutadiene.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans. Intermediate-duration (up to 100 mg/kg/day) or chronic-duration oral (20 mg/kg/day) exposure to hexachlorobutadiene did not cause treatment-related lesions of the gastrointestinal tract in rats (Kociba et al. 1971, 1977a; Schwetz et al. 1977). Because histological lesions were not observed even at doses higher than those causing other organ toxicity, gastrointestinal toxicity may not be an area of concern in humans following exposure to hexachlorobutadiene.

Hematological Effects. No studies were located regarding hematological effects in humans. Animal studies evaluating the hematological effects of hexachlorobutadiene involved mainly intermediate-duration and chronic-duration oral exposures up to 20 mg/kg/day in rats (Harleman and Seinen 1979;

Kociba et al. 1977a; Schwetz et al. 1977). There was increased hemoglobin concentration in rats after intermediate-duration oral exposure (10 mg/kg/day or greater) (Kociba et al. 1971). However, hematocrit, red blood cell, and differential leucocyte counts were comparable to untreated controls. For this reason, hematological effects may not be an area of major concern in humans following exposure to hexachlorobutadiene.

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans. Intermediate-duration or chronic-duration oral exposure to hexachlorobutadiene at dose levels up to 20 mg/kg/day did not cause treatment-related lesions of the musculoskeletal system in rats (Harleman and Seinen 1979; Kociba et al. 1977a; Schwetz et al. 1977). Because histological lesions were not observed even at doses higher than those causing other organ toxicity, musculoskeletal effects may not be an area of concern in humans following exposure to hexachlorobutadiene.

Hepatic Effects. Data in humans are limited to one study which reported significant dose-related increases in the concentration of serum bile acids in workers after inhalation exposure to hexachlorobutadiene (0.005-0.02 ppm) (Driscoll et al. 1992). The practical importance of this finding is reduced because workers were also potentially exposed to other solvents (carbon tetrachloride and perchloroethylene) and background information on other confounding variables was minimal. No studies were located regarding other hepatic effects in humans.

In animals, liver damage may occur after oral exposure to hexachlorobutadiene; however, the effects are less severe than those associated with renal damage. Intermediate-duration oral exposures caused liver damage in male rats at dose levels of 6.3 mg/kg/day or greater (Harleman and Seinen 1979; Kociba et al. 1971). Histological lesions were not found in female rats. Relative liver weights were increased in female rats, but occurred at higher dose levels (15 mg/kg/day) than in male rats (6.3 mg/kg/day). In another study, liver weights were decreased in female rats at dose levels of 5 mg/kg/day or greater following exposure to hexachlorobutadiene for 4 weeks (Jonker et al. 1993b). In the same study, serum biochemical parameters (aspartate aminotransferase activity and total bilirubin) were increased at doses of 20 mg/kg/day. Urinary excretion of coproporphyrin increased at dose levels of 20 mglkglday in lifetime studies; however, histopathological lesions were not found (Kociba et al. 1977a).

2. HEALTH EFFECTS

Dermal studies in which rabbits received applications of hexachlorobutadiene (775 mg/kg or greater) directly to the skin identified hepatocyte damage and fatty degeneration of the centrilobular area as effects of exposure. Effects were reversible within 3 weeks (Duprat and Gradiski 1978). Studies using intraperitoneal injection support findings of morphological alterations in the liver. A single intraperitoneal dose of hexachlorobutadiene (100 mg/kg or greater) caused disruption of mitochondria in periportal hepatocytes which resulted in an influx of water and ions into the cell without effects on the sodium pump (Lock et al. 1982, 1985). Alterations in serum biochemical parameters have been reported following intraperitoneal injection. Alkaline phosphatase activity increased ($p < 0.05$) at doses of 52 mg/kg/day or greater (Bai et al. 1992). Aspartate aminotransferase activity and total bilirubin levels decreased ($p < 0.05$) at a dose of 104 mg/kg/day (highest dose tested).

Renal Effects. No studies were located regarding renal effects in humans. Acute-, intermediate- and chronic-duration oral studies in rats and mice revealed that the kidney is the primary target organ for hexachlorobutadiene toxicity. Acute exposure (24 hours) to hexachlorobutadiene (100 mg/kg or greater) caused focal necrosis and an increase in plasma creatinine levels (Jonker et al. 1993a). When rats were exposed to hexachlorobutadiene (5 mg/kg/day) for 4 weeks, tubular cytomegaly was reported (Jonker et al. 1993b). Tubular cell degeneration and regeneration in the renal cortex occurred in female mice at dose levels of 0.2 mg/kg/day for 13 weeks (NTP 1991; Yang et al. 1989). Tubular degeneration and cell necrosis occurred in rats after short duration exposures (30-148 days) at dose levels of 20 or 30 mg/kg/day (Harleman and Seinen 1979; Kociba et al. 1971; Schwetz et al. 1977).

Lifetime exposures at dose levels from 2.5 mg/kg/day revealed renal hyperplasia in rats (Kociba et al. 1977a). For the most part, kidney lesions were more pronounced in females and occurred at lower dose levels. Morphological changes were found in female rats in the 2.5 mg/kg/day dose groups, whereas comparable alterations were first seen in male rats at dose levels of 6.3 mg/kg/day. Kidney weights or kidney/body weight ratios were increased at dose levels causing morphological damage (Harleman and Seinen 1979; Kociba et al. 1977a; NTP 1991; Schwetz et al. 1977; Yang et al. 1989). Results of evaluations of impairment in kidney functions were consistent with morphological alterations. The capacity to concentrate urine was reduced in female rats at dose levels of 2.5 mg/kg/day and in males at 15 mg/kg/day (Harleman and Seinen 1979). Although histological lesions were not evident at the low dose in this study, kidney damage has been reported at comparable dose levels following chronic oral exposures (Kociba et al. 1977a).

2. HEALTH EFFECTS

Biochemical indices (blood urea nitrogen, creatinine) were comparable to controls at dose levels up to 20 mg/kg/day in some studies (Harleman and Seinen 1979; Kociba et al. 1977a; Schwetz et al. 1977). However, blood urea levels increased significantly at dose levels of 200 mg/kg (highest dose tested) and creatinine levels increased at 100 mg/kg in an acute study (24 hours) (Jonker et al. 1993a). Similarly, urinary lactate dehydrogenase and N-acetyl-β−glucosaminidase increased significantly in the 24 hours after exposure to a single dose of 100 mg/kg or greater. In a different study, blood creatinine levels decreased in females at dose levels of 5 mg/kg/day or greater, while blood urea levels decreased at doses of 1.25 mg/kg/day or greater hexachlorobutadiene for 4 weeks (Jonker et al. 1993b). Although statistically significant differences in biochemical parameters were found, the importance of these effects is reduced because the effects were not completely dose-related. Accordingly, these parameters may not be reliable indicators of renal damage following hexachlorobutadiene exposure.

Kidney damage was also seen following an 8 hour dermal exposure in rabbits that received applications of hexachlorobutadiene (775 mg/kg or greater). The effects were reversible within 3-5 weeks (Duprat and Gradiski 1978). Intraperitoneal studies revealed patterns of damage similar to those for other routes and duration categories. Species differences were reported. Renal tubular necrosis was evident in rats and was confined mainly to the straight limb of the proximal tubules involving the medulla. Effects were evident within 24 hours at dose levels of 100 mg/kg (Lock and Ishmael 1979). At higher (200 mg/kg) doses, necrosis was evident by 8 hours (Ishmael et al. 1982). Studies in mice also revealed that hexachlorobutadiene causes damage to the proximal tubules of the kidney; effects were observed at lower dose levels (50 mg/kg) than in rats and were observed in both the cortex and the medulla *(*Ishmael et al. 1984). It was also noted that active regeneration of the tubular epithelium was evident by 5 days after treatment, and by 14 days, tubular morphology had returned to normal (Ishmael et al. 1984).

Metabolites of hexachlorobutadiene (glutathione conjugate, cysteine conjugate, and its N-acetyl cysteine conjugate) produced effects at lower doses than the parent compound after intraperitoneal injection and there was differential susceptibility between sexes (Ishmael and Lock 1986). A single intraperitoneal dose of 25 mg/kg of the conjugates caused minimal to moderate necrosis in males and severe necrosis in females. On the other hand, a comparable dose caused no effect in males and females after exposure to the parent compound (Ishmael and Lock 1986).

2. HEALTH EFFECTS

One or two hexachlorobutadiene metabolites appear to cause some of the compound-induced renal damage (see Section 2.3.5) and are more toxic than the parent compound, causing comparable lesions in the kidneys at lower doses. These active thioacylating metabolites are capable of modifying DNA, as indicated by the isolation of sulfur-containing nucleides from hydrolyzed DNA from renal cells exposed to the hexachlorobutadiene cysteine derivative (Vamvakas et al. 1988b).

Overall, the kidney is highly susceptible to the toxicity of hexachlorobutadiene, in contrast to other organs, due to the activity of β-lyase and other mercapturic acid processing enzymes (Vamvakas et al. 1988b). The greater sensitivity of females may be due to differences in renal enzymes responsible for the tissue levels of the active metabolites (Hook et al. 1983). Based on data in animals, renal toxicity is a major concern in humans who may be chronically exposed to this material from hazardous waste sites or other sources.

Dermal/Ocular Effects. No studies were located regarding dermal/ocular effects in humans. Acute-duration dermal exposure caused skin necrosis in rabbits; however, effects were reversible within 2 weeks (Duprat and Gradiski 1978). Nasal irritation resulted from 15 minute exposure to vapor concentrations of 155 ppm (de Ceaurriz et al. 1988). No dermal/ocular effects were seen following intermediate- or chronic-duration dermal exposure in rabbits. Based on acute effects in rabbits, hexachlorobutadiene may pose some risk to humans following skin contact with the chemical depending on the area exposed. Inhalation of vapors may cause irritation of the nasal mucosa.

Immunological Effects. No studies were located regarding immunological effects in humans. Oral intermediate or chronic exposures to hexachlorobutadiene did not produce treatment-related histopathological lesions in lymphoid tissue (spleen or thymus) in mice (NTP 1991; Yang et al. 1989) or rats (Harleman and Seinen 1979; Kociba et al. 1971, 1977a) after 13 weeks of exposure. Necrosis of lymphoid tissue did occur in the spleen, lymphoids, and thymus of mice exposed to lethal doses (NTP 1991; Yang et al. 1989). No data are available on immunological effects following acute-duration oral exposure. In the absence of tests that evaluate impairment of immune functions, firm conclusions cannot be made about the potential for hexachlorobutadiene to affect immune processes in humans.

Neurological Effects. No studies were located regarding neurological effects in humans. Intermediate oral exposure to hexachlorobutadiene caused damage to the nervous system in rats.

2. HEALTH EFFECTS

Ataxia associated with demyelination and fragmentation of femoral nerve fibers was reported in adult female rats that received 150 mg/kg hexachlorobutadiene. Effects were not seen at lower dose levels (15 mg/kg/day) (Harleman and Seinen 1979). No neurological effects were reported following chronic oral exposures (Kociba et al. 1977a). Dermal application of 388-1550 mg/kg induced stupor in rabbits during the 8-hour exposure period and for the 2-hour period immediately after exposure (Duprat and Gradiski 1978). Although neurological symptoms were not present in all studies, these data, and the fact that hexachlorobutadiene has been found in brain tissue, suggest neurological effects may occur in humans following hexachlorobutadiene exposure.

Reproductive Effects. No studies were located regarding reproductive effects in humans. Acute-duration inhalation exposure to hexachlorobutadiene (10 ppm) did not adversely affect sperm morphology in mice (NIOSH 1981). In a developmental toxicity study, the mean number of implantation sites, total fetal loss, and live fetuses per litter in rat dams exposed to 15 ppm hexachlorobutadiene during gestation were comparable to unexposed controls (Saillenfait et al. 1989). No data were found on intermediate- or chronic-duration inhalation exposure in mice. Intermediate-duration oral exposure did not adversely affect fertility, gestation, viability, and lactation indices in rats at dose levels of 20 mg/kg/day (Schwetz et al. 1977). Similarly, hexachlorobutadiene did not adversely affect mean litter size and resorption rate in rats fed 15 mg/kg/day hexachlorobutadiene (Harleman and Seinen 1979). There were no histological lesions in the gonads or accessory sex organs after intermediate- or chronic-duration exposures (Kociba et al. 1977a; Schwetz et al. 1977). Based on these data and the fact that the compound has not been detected in reproductive tissue, hexachlorobutadiene does not appear to pose a significant risk to human reproduction.

Developmental Effects. No studies were located regarding developmental effects in humans. In intermediate-duration inhalation animal studies, fetal body weight was reduced in rat pups following exposure of dams to hexachlorobutadiene vapors at concentrations of 15 ppm for 15 days (Saillenfait et al. 1989). After intermediate oral exposure in rat dams administered hexachlorobutadiene (during gestation and lactation) at dose levels of 20 mg/kg/day, body weights decreased on lactation day 21 (Schwetz et al. 1977) and pup weights were reduced at dose levels of 15 mg/kg/day for 6 weeks (during gestation and lactation) during an 18 week study (Harleman and Seinen 1979). In both cases, no other fetotoxic effects were reported. Similar results were found in rat pups of dams administered a single dose of hexachlorobutadiene by intraperitoneal injection at dose levels of 10 mg/kg (Hardin et al. 1981). Because the fetotoxic effects occurred at concentrations that were also maternally toxic

and the fact that embryolethality and major malformations were not observed, it is not likely that low levels of hexachlorobutadiene will pose any significant risk to renal development and function in humans.

Genotoxic Effects. No studies were located regarding genotoxic effects in humans. For the most part, acute inhalation studies evaluating chromosomal damage in rats and gene mutation in Drosophila revealed that hexachlorobutadiene is not mutagenic (NIOSH 1981). On the other hand, results of oral studies in rats do not agree suggesting differences in activation and detoxification. Hexachlorobutadiene can affect genetic material as evident by the induction of DNA repair and alkylation (Stott et al. 1981) (Table 2-4). The compound did not cause chromosomal aberrations in rat bone marrow cells (Schwetz et al. 1977).

Several *in vitro* assays have been evaluated; 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; Reichert et al. 1983; Stott et al. 1981; Vamvakas et al. 1988a) or in the presence of activation (Roldan-Arjona et al. 1991). On the other hand, results were positive in other bacterial assays employing S. typhimurium (Reichert et al. 1984; Roldan-Arjona et al. 1991; Vamvakas et al. 1988a). Certain metabolites of hexachlorobutadiene have also been evaluated. Monooxidation products of hexachlorobutadiene were mutagenic in *Salmonella* with and without metabolic activation (Reichert et al. 1984). Similarly, monooxidation products induced unscheduled DNA synthesis as well as morphological transformations in cultured Syrian hamster embryo fibroblasts (Schiffmann et al. 1984). However, results did not agree for hexachlorobutadiene in an *in vitro* unscheduled DNA synthesis assay employing rat hepatocytes (Stott et al. 1981). 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. On the other hand, some influence of genetic mechanisms cannot be ruled out since there was limited evidence of renal DNA repair and alkylation (Stott et al. 1981).

TABLE 2-4. Genotoxicity of Hexachlorobutadiene *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium (TA98, TA100, TA1535, TA1537)	Gene mutation	-	-	Haworth et al. 1983
S. typhimurium (TA98, TA100)	Gene mutation	-	-	Reichert et al. 1983
S. typhimurium (TA98, TA100, TA2638)	Gene mutation	+	-	Vamvakas et al. 1988a
S. typhimurium (TA98, TA100, TA2638)	Gene mutation	+[a]	+[a]	Vamvakas et al. 1988a
S. typhimurium (TA98, TA100, TA2638)	Gene mutation	-[b]	-[b]	Vamvakas et al. 1988a
S. typhimurium (TA100)	Gene mutation	+	-	Reichert et al. 1984
S. typhimurium (TA100)	Gene mutation	+[c]	+[c]	Reichert *et al.* 1984
S. typhimurium (TA100)	Gene mutation	+[d]	+[d]	Wild et al. 1986
S. typhimurium (TA100)	Gene mutation	-[e]	-[e]	Wild et al. 1986
S. typhimurium (TA98, TA100, TA1530, TA1535, TA1538)	Gene mutation	-	-	DeMeester et al. 1980
S. typhimurium (TA98, TA100, TA2638)	Gene mutation	+[f]	Not tested	Dekant et al. 1986
S. typhimurium	-	-	-	Stott et al. 1981
S. typhimurium	Gene mutation	-	+	Roldan-Arjona et al. 1991
Mammalian cells:				
Syrian hamster embryo fibroblast	Unscheduled DNA synthesis	+	+	Schiffmann et al. 1984

TABLE 2-4. Genotoxicity of Hexachlorobutadiene *In Vitro (continued)*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Syrian hamster embryo fibroblast	Morphological transformation	+	+	Schiffmann et al. 1984

- = negative result; + = positive result; DNA = deoxyribonucleic acid

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

TABLE 2-5. Genotoxicity of Hexachlorobutadiene *In Vivo*

Species (test system)	End point	Results	Reference
Mammalian cells:			
Rat	Dominant lethality	-	NIOSH 1981
Rat (bone marrow cells)	Chromosomal aberration	-	NIOSH 1981
Rat (kidney cells)	DNA alkylation	+	Stott et al. 1981
	DNA repair	+	Stott et al. 1981
Rat (bone marrow cells)	Chromosomal aberration	-	Schwetz et al. 1977
Eukaryotic organism:			
Insect:			
Drosophila	Gene mutation (sex-linked recessive lethal)	-	NIOSH 1981

- = negative result; + = positive result; DNA = deoxyribonucleic acid

Cancer. No studies were located regarding the carcinogenic potential of hexachlorobutadiene in humans. It is carcinogenic in rats after lifetime oral exposures. The incidence of adenomas and adenocarcinomas in the kidney increased over control levels at doses of 20 mg/kg/day. Two neoplasms metastasized to the lungs (Kociba et al. 1977a). The IARC (1979) evaluated the carcinogenic potential of hexachlorobutadiene and concluded there was limited evidence that hexachlorobutadiene is carcinogenic in rats. EPA considers hexachlorobutadiene to be a possible human carcinogen (Group C) (IRIS 1993).

Several studies have assessed the mechanism of hexachlorobutadiene-induced renal tumorigenesis (see Section 2.3.5). The carcinogenic properties of hexachlorobutadiene may result from binding of intermediary metabolites to cellular DNA (Dekant et al. 1990b; Henschler and Dekant 1990). In addition, the occurrence of renal tubular regeneration suggests that cell repair processes which induce the replication of cells with altered DNA may be a factor in the tumorigenesis process. Based on carcinogenic effects in rats, exposure to hexachlorobutadiene may pose some risk for development of kidney tumors in humans.

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers 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 or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that

are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to hexachlorobutadiene are discussed in Section 2.5.1.

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 effects caused by hexachlorobutadiene are discussed in Section 2.5.2.

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 2.7, Populations That Are Unusually Susceptible.

2.5.1 Biomarkers Used to Identify or Quantify Exposure to Hexachlorobutadiene

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 (l-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 l-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.

2.5.2 Biomarkers Used to Characterize Effects Caused by Hexachlorobutadiene

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 Section 2.2, renal damage is the primary toxic effect associated with exposure to hexachlorobutadiene in animals (Harleman and Seinen 1979; Kociba et al. 1971, 1977a; NTP 1991; Schwetz et al. 1977). Because hexachlorobutadiene-induced renal damage is mostly to the proximal convoluted tubules, tests to determine increases in urine glucose creatinine and alkaline phosphatase activity, as well as molecular weight pattern of proteins excreted in the urine, may be useful indicators of kidney damage. Urine volume and specific gravity may be evaluated as part of the overall assessment of kidney damage after exposure to hexachlorobutadiene. Excretion of urinary coproporphyrin was increased in animals at dose levels that did not induce renal tumors. This parameter may be useful in the overall assessment of potential exposure to hexachlorobutadiene. The characteristics renal damage associated with hexachlorobutadiene exposure may also occur with other compounds (e.g., S-C 1,2-dichlorovinyl cysteine and mercuric chloride). Therefore, these parameters are not specific for hexachlorobutadiene exposure. Additional information concerning biomarkers for effects on the immune, renal, and hepatic systems can be found in the CDC/ATSDR Subcommittee Report on Biological Indicators of Organ Damage (CDC/ATSDR 1990), and on the neurological system in the Office of Technology Assessment Report on Identifying and Controlling Poisons of the Nervous System (OTA 1990).

2.6 INTERACTIONS WITH OTHER CHEMICALS

Several studies have been conducted to assess factors which influence the toxicity of hexachlorobutadiene. 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 hour) alkaline phosphatase, lactate dehydrogenase and N-acetyl-β-glucosaminidase 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 females (Jonker et al. 1993b).

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to hexachlorobutadiene than will most persons exposed to the same level of hexachlorobutadiene in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the pre-existing compromised function of target organs (including effects or clearance rates and any resulting endproduct metabolites). For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, Populations With Potentially High Exposure.

2. HEALTH EFFECTS

Studies in animals revealed that hexachlorobutadiene causes damage to the proximal tubules of the kidney and, to a lesser extent, to the liver. Accordingly, people with preexisting kidney and liver damage may have compromised organ functions and are expected to be more vulnerable to chemical insult than people with normal kidney and liver functions. Infants are more likely to be affected following exposure to hexachlorobutadiene than adults. Studies in animals showed that young rats were more sensitive to the acute lethal effects of hexachlorobutadiene than adults. This greater susceptibility in newborns can be attributed to immature organ systems.

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to hexachlorobutadiene. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to hexachlorobutadiene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.8.1 Reducing Peak Absorption Following Exposure

Exposure to hexachlorobutadiene can occur by inhalation of vapors, ingestion, and dermal contact. The compound can cause kidney damage and, to a lesser extent, liver damage after ingestion or if it comes in contact with skin.

Information regarding methods for reducing absorption following exposure to hexachlorobutadiene was obtained primarily from the HSDB. No other sources were available. If inhalation of hexachlorobutadiene has occurred, movement of the patient to fresh air is recommended. No specific treatment is available; however, patients are usually monitored for respiratory distress, respiratory tract irritation, bronchitis and pneumonia. If there has been substantial ingestion of the compound, syrup of ipecac is administered within 30 minutes of ingestion to induce vomiting. Syrup of ipecac is usually not given in cases of severe central nervous system depression or coma because there is risk of pulmonary aspiration. The absorption of hexachlorobutadiene may be reduced following oral exposure by binding the compound in the gastrointestinal tract. Activated charcoal in the form of aqueous suspension or sorbitol slurry may be administered for this purpose. However, if syrup of ipecac is given within 1 hour of ingestion of activated charcoal, it is not effective. Another suggested

treatment following oral ingestion of hexachlorobutadiene may be a cathartic, such as magnesium or sodium sulfate to speed fecal excretion. Lipids are not usually given by mouth because this may increase absorption. In cases where hexachlorobutadiene has been splashed into the eyes, irrigation with copious amounts of water for 15 minutes has been recommended. In order to minimize absorption through the skin, areas of skin that have come in contact with the compound should be washed with soap and water.

2.8.2 Reducing Body Burden

No information was located on the retention of hexachlorobutadiene or its metabolites in humans. In animals, the compound and its metabolites were detected in urine and areas extent in breath within 3 days after exposure. Adverse effects were seen within 24 hours, suggesting that the compound and its metabolites are toxic while retained in the body.

There are no specific treatments for reducing the body burden following absorption of hexachlorobutadiene. As discussed in Section 2.3, there is extensive reabsorption and enterohepatic recirculation of biliary metabolites, which are thought to play a major role in the nephrotoxicity of the compound. One approach to reducing body burden may involve the administration of compounds that would decrease reabsorption of biliary metabolites. Activated charcoal may be used for this purpose.

2.8.3 Interfering with the Mechanism of Action for Toxic Effects

No information is available on treatment methods that employ substances that interfere with the mechanism of toxicity of hexachlorobutadiene. Studies in animals indicate that hexachlorobutadiene exerts its effects on the proximal tubules of the kidney. The major portion of the toxicity of hexachlorobutadiene results from initial formation of glutathione conjugates of the compound and the subsequent uptake of the glutathione-derived conjugates by renal tissues through an organic transport mechanism. Thus, prevention of transport of conjugate anions may reduce the toxicity associated with exposure to hexachlorobutadiene. Use of a uricosuric agent such as probenecid may be an effective treatment. In animals, probenecid blocked the accumulation of a mercapturic acid derivative of hexachlorobutadiene, in renal tissue, the extent of covalent binding of radioactivity to renal protein, and the nephrotoxicity (Lock and Ishmael 1985).

2. HEALTH EFFECTS

2.9 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 the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of hexachlorobutadiene.

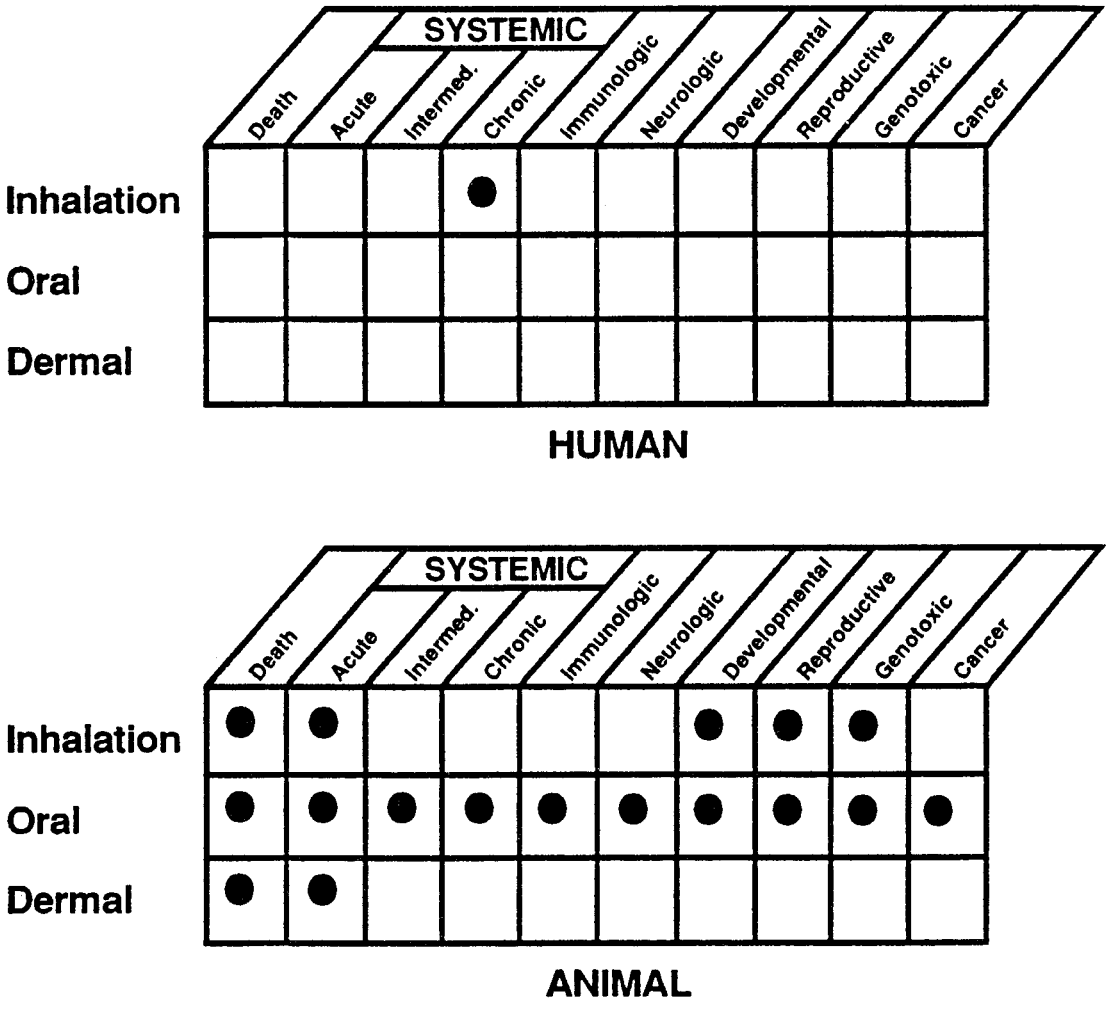
The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean 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.

2.9.1 Existing Information on Health Effects of Hexachlorobutadiene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to hexachlorobutadiene are summarized in Figure 2-4. The purpose of this figure is to illustrate the existing information concerning the health effects of hexachlorobutadiene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as “data needs. ” 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.

As shown in Figure 2-4, information was located regarding health effects of hexachlorobutadiene in humans after inhalation exposure but not after oral or dermal exposure.

FIGURE 2-4. Existing Information on Health Effects of Hexachlorobutadiene



● Existing Studies

2. HEALTH EFFECTS

In animals, information exists on lethality, acute systemic effects (respiratory), reproductive and developmental effects, and cancer following inhalation exposure, but none was found on other systemic effects after acute, intermediate, or chronic exposure. Much of the information in animals focused on oral exposure. Lethality, systemic effects after acute, intermediate and chronic exposures, immunotoxicity, developmental effects, reproductive effects, genotoxicity, and cancer have been evaluated. Reports on dermal exposure after direct application to the skin involved lethality, acute systemic effects (kidney, liver, dermal/ocular), and cancer.

2.9.2 Identification of Data Needs

Acute-Duration Exposure. No data are available on the effects of hexachlorobutadiene in humans after acute exposure by inhalation, oral, and dermal routes. Hexachlorobutadiene (50 ppm) was lethal in mice after acute (5 days) inhalation exposure and caused irritation of the nasal cavities following 15 minute exposures to concentrations of 15 ppm or greater (de Ceaurriz et al. 1988; NIOSH 1981). Because sufficient data are not available to determine target organs or determine critical effect levels, an acute inhalation MRL cannot be determined.

In one acute-duration (24-hour) oral study in rats, hexachlorobutadiene caused focal necrosis of the kidneys and increased urinary biochemical parameters at doses of 100 mg/kg (Jonker et al. 1993a). Another acute-duration (14 days) oral exposure study revealed that hexachlorobutadiene caused renal tubular epithelial degeneration in rats at dose levels of 4.6 mg/kg/day or greater but no effects were seen in the liver up to doses of 35 mg/kg/day (Harleman and Seinen 1979). The number of animals in both of these studies were small and, thus, the data were not suitable for derivation of an MRL.

Acute-duration dermal exposure to hexachlorobutadiene (388 mg/kg) caused liver and kidney damage in rabbits. For the most part, these effects were reversible within 2-5 weeks (Duprat and Gradiski 1978). In the same dermal study, some rabbits died within 24 hours after exposure to 775 mg/kg hexachlorobutadiene applied directly to the skin for 8 hours, but no deaths occurred at dose levels of 388 mL/kg (Duprat and Gradiski 1978). However, due to the lack of an appropriate methodology for the development of dermal MRLs, no dermal MRLs were derived. Although the vapor pressure of hexachlorobutadiene limits vapor concentration in the air, short-term inhalation exposures are possible and worthy of investigation. There is potential for oral exposures in populations living near

hazardous waste sites; therefore, additional short-term animal studies by oral routes may be useful to more thoroughly assess the potential human health risk.

Intermediate-Duration Exposure. No data are available on the effects of hexachlorobutadiene in humans after intermediate-duration inhalation, oral, or dermal exposures. In animals, data on inhalation exposure are limited to one developmental toxicity study in rats in which maternal body weights were reduced at a concentration (15 ppm) that was also fetotoxic (Saillenfait et al. 1989). Oral studies revealed kidney damage in female mice at dose levels of 0.2 mg/kg/day (NTP 1991; Yang et al. 1989). This LOAEL was used to derive an intermediate-duration oral MRL of 0.0002 mg/kg/day .

Liver damage was evident in male rats at dose levels of 6.3 mg/kg/day but not at dose levels of 2.5 mg/kg/day for 13 weeks (Harleman and Seinen 1979). Treatment-related histopathological hepatic lesions were not seen in females. Some serum biochemical parameters (aspartate aminotransferase and total bilirubin), were increased at doses of 20 mg/kg/day for 4 weeks (Jonker et al. 1993b). No data are available on the effects of hexachlorobutadiene in animals after intermediate-duration dermal exposure. Inhalation exposure to vaporous hexachlorobutadiene can occur when this material is exposed to the environment. Studies of toxicity from material absorbed through the lungs are justified.

Chronic-Duration Exposure and Cancer. Data in humans are limited to one study that reported increases in serum bile acids in workers chronically exposed to vapors of hexachlorobutadiene (0.005-0.02 ppm). Because workers were also potentially exposed to other chemicals (carbon tetrachloride and perchloroethylene), these effects cannot be attributed to hexachlorobutadiene exposure alone. No studies are available on the effects of hexachlorobutadiene in humans after oral or dermal exposure.

In animals, a chronic-duration oral rat study showed that the kidney was the target organ following chronic exposure to hexachlorobutadiene. Kidney damage as well as evidence of impaired kidney function were evident in female rats at dose levels of 2 mg/kg/day, but not at 0.2 mg/kg/day (Kociba et al. 1977a). Since the intermediate-duration oral MRL is protective against affects on the kidney following lifetime exposure, a chronic MRL was not derived. Data are not available to derive a

chronic inhalation MRL. This data need can be evaluated after the results of the suggested acute- and intermediate-duration research becomes available.

No epidemiological studies of hexachlorobutadiene are available. The occurrence of renal tumors after chronic oral exposure in rats suggests carcinogenicity may be an area of concern following occupational exposure to hexachlorobutadiene and long-term exposures from waste sites.

Genotoxicity. No information is available on the genotoxic effects of hexachlorobutadiene in humans. Following exposure to hexachlorobutadiene, results were negative in *in vivo* rat and *Drosophila* tests that evaluated gene mutation and chromosomal damage (NIOSH 1981). The results were negative in *in vitro* tests evaluating gene mutation (De Meester et al. 1980; Haworth et al. 1983; Reichert et al. 1983; Vamvakas et al. 1988a) and DNA repair in *Salmonella* tests (with and without metabolic activation), and positive in mammalian assay systems using Syrian hamster cells (Schiffmann et al. 1984); the overall results were not consistent. Studies of hexachlorobutadiene metabolites have indicated that some of the cysteine derived metabolites are mutagenic while others are not (Wild et al. 1986). Additional studies of the genotoxicity of intermediary metabolites are needed.

Reproductive Toxicity. No data are available on the reproductive toxicity of hexachlorobutadiene in humans. Hexachlorobutadiene did not cause adverse reproductive effects in mice or rats after inhalation or oral exposures, even at dose levels causing kidney and liver damage (Harleman and Seinen 1979; Kociba et al. 1977a; NIOSH 1981; NTP 1991; Saillenfait et al. 1989; Schwetz et al. 1977). No data are available on the reproductive toxicity of hexachlorobutadiene after dermal exposure. Based on existing data in animals, it does not appear that exposure to the compound would pose any significant risk to human reproduction.

Developmental Toxicity. No studies were located regarding developmental effects in humans. Inhalation and oral studies in rat pups revealed that hexachlorobutadiene is fetotoxic, but not embryotoxic or teratogenic, at dose levels that are also maternally toxic (Harleman and Seinen 1979; Saillenfait et al. 1989; Schwetz et al. 1977). Additional oral studies in another species would be useful in clarifying the apparent lack of significant effects of hexachlorobutadiene on development. Systemic toxicity studies in rabbits suggest there is potential for dermal absorption to

hexachlorobutadiene (Duprat and Gradiski 1978). However, the toxicokinetics of this compound by this route has not been evaluated.

Immunotoxicity. No data are available on the immunotoxicity of hexachlorobutadiene in humans following inhalation, oral, or dermal exposure. Data in animals are limited to intermediate and chronic oral studies which examined histological lesions of lymphoid tissue (spleen or thymus) in mice and rats. These studies did not reveal treatment-related lesions except at lethal doses (Harleman and Seinen 1979; Kociba et al. 1971, 1977a; NTP 1991; Schwetz et al. 1977; Yang et al. 1989). Additional studies to evaluate immune function via the oral route would be useful to determine whether this system is susceptible to hexachlorobutadiene toxicity.

Neurotoxicity. No data are available on the neurotoxicity of hexachlorobutadiene in humans after inhalation, oral, or dermal exposure. Histological lesions were not found in the brain in rats (Harleman and Seinen 1979; Kociba et al. 1971; Schwetz et al. 1977) or mice (NTP 1991; Yang et al. 1989). Brain weights were increased after intermediate-duration oral exposure (Kociba et al. 1971). However, such increases were attributed to decreases in body weight gain. Neurological effects were not seen after chronic-duration oral exposure in rats (Kociba et al. 1977a). A comprehensive battery of neurophysiological and neurochemical tests has not been performed and is needed to provide a more thorough assessment of the potential for hexachlorobutadiene to affect the nervous system in humans.

Epidemiological and Human Dosimetry Studies. Information is not available on the adverse health effects of hexachlorobutadiene in humans. Data on rats and mice identified the kidney as the target organ following oral exposure (Harleman and Seinen 1979; Kociba et al. 1971, 1977a; NTP 1991; Yang et al. 1989). Other studies involving inhalation or dermal exposures confirm this finding (de Ceaurriz et al. 1988; Duprat and Gradiski 1978). Well-conducted epidemiological studies are needed to determine if similar patterns of damage occur in humans. More importantly, evidence of cancer in animals is sufficient to cause concern for populations which may be exposed to low levels of hexachlorobutadiene for long periods of time.

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 pharmacokinetics 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 which are responsible for the nephrotoxicity of this compound, knowledge of the pharmacokinetics 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 mechanism of renal toxicity have been evaluated in animals employing both *in vivo* and *in vitro* test systems (Dekant et al. 1990b; Schneumann et al. 1987). It is not known if similar metabolic pathways and metabolites occur in humans.

Methods for Reducing Toxic Effects. Sufficient methods and treatments are available for reducing peak absorption of hexachlorobutadiene following oral exposure and for limiting the concentrations in the body tissues if absorption has occurred (HSDB 1993). However, antidotal methods have not been established that would be effective in treating overdoses of hexachlorobutadiene, based on interference with the mechanism of action of the compound. A key factor in the overall toxicity of hexachlorobutadiene is the accumulation of glutathione derived conjugates in renal tissue due to hexachlorobutadiene-induced impairment of organic ion transport and secretion. Further studies to identify ways to prevent or reduce accumulation in the target tissue are warranted.

2.9.3 On-going Studies

A study is being conducted by R.G. Schnellmann (University of Georgia) for the National Institute of Environmental Health Sciences to evaluate the mechanism of nephrotoxicity of halocarbons, including hexachlorobutadiene. The mechanism of how metabolites alter proximal tubular cellular physiology to produce toxicity is being investigated, with particular emphasis on the effects of metabolites on mitochondria (CRISP 1993).