

2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of hexachloroethane. 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

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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 or exposure levels below which no adverse effects 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 hexachloroethane are indicated in Table 2-2 and Figure 2-2. Because cancer effects could occur at lower exposure levels, Figure 2-2 also shows a range 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 hexachloroethane. 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 1990e), 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.

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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

Hexachloroethane is a solid that sublimates at ambient air temperatures. At 20°C the saturated vapor concentration is 670-700 ppm (Weeks et al. 1979); thus, there is a limitation on the vapor concentration that can be used in studies using the inhalation route of exposure. In circumstances where the saturation threshold is exceeded, microcrystalline hexachloroethane forms in the atmosphere and is inhaled by the exposed animals along with the volatilized hexachloroethane.

2.2.1.1 Death

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

Rats were exposed to vapor concentrations of either 260 or 5,900 ppm hexachloroethane for 8 hours (Weeks et al. 1979). The 5,900 ppm vapor concentration was generated at 50°C and crystallized as it entered the exposure chamber. At the higher concentration the exposed animals showed signs of distress (staggering gait) during exposure, and 2 of 6 were dead at the end of 8 hours. No animals died at the lower exposure concentration.

Following 6 weeks of inhalation exposure to 15-260 ppm, no deaths in quail were reported; however, 2 of 50 rats, 4 of 10 guinea pigs, and 1 of 4 dogs died at the 260 ppm concentration (Weeks et al. 1979). Based on clinical signs, the dogs seemed to be particularly sensitive to hexachloroethane exposure. The animals developed tremors and ataxia and closed their eyes. The one dog that died experienced convulsions before death. The rats and guinea pigs that succumbed to exposure died during weeks 4 or 5 and, thus, appear to be less sensitive. The quail were the most resistant to death following hexachloroethane exposure.

All identified LOAEL values for lethality in each species and duration category are recorded in Table 2- 1 and plotted in Figure 2- 1. These values indicate that hexachloroethane is lethal to animals exposed intermittently to 260 ppm for 6 weeks; however, no deaths occurred in animals acutely exposed for 8 hours to the same concentration. Concentrations of 48 ppm and lower were not lethal in rats, guinea pigs, dogs, or quail.

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

Key ^a to figure	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
ACUTE EXPOSURE							
Death							
1	Rat (Sprague-Dawley)	8 hr				5900 M (2/6 rats died)	Weeks et al. 1979
Systemic							
2	Rat (Sprague-Dawley)	8 hr	Resp	260 M	5900 M (interstitial pneumonitis in 2/4 survivors)		Weeks et al. 1979
			Cardio	5900 M			
			Gastro	5900 M			
			Musc/skel	5900 M			
			Hepatic	5900 M			
			Renal	5900 M			
			Endocr	5900 M			
			Derm	5900 M			
			Bd Wt	260 M	5900 M (reduced body weight gain, quantitative data not provided)		
Neurological							
3	Rat (Sprague-Dawley)	6 hr		260 M		5900 M (staggered gait in 1/6 rats)	Weeks et al. 1979
4	Rat (Sprague-Dawley)	11 d Gd 6-16 6hr/d		48 ^b F		260 F (tremors)	Weeks et al. 1979

TABLE 2-1. Levels of Significant Exposure to Hexachloroethane - Inhalation (continued)

Key ^a to figure	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
Reproductive							
5	Rat (Sprague- Dawley)	11 d Gd 6-16 6hr/d		260 F			Weeks et al. 1979
Developmental							
6	Rat (Sprague- Dawley)	11 d Gd 6-16 6hr/d		260			Weeks et al. 1979
INTERMEDIATE EXPOSURE							
Death							
7	Gn pig (Hartley)	6 wk 5d/wk 6hr/d				260 M (4/10 guinea pigs died)	Weeks et al. 1979
8	Dog (Beagle)	6 wk 5d/wk 6hr/d				260 M (1/4 dogs died)	Weeks et al. 1979

TABLE 2-1. Levels of Significant Exposure to Hexachloroethane - Inhalation (continued)

Key ^a to figure	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
Systemic							
9	Rat (Sprague- Dawley)	6 wk 5d/wk 6hr/d	Resp	48	260	(reduced resistance to endemic mycoplasma infection; mucopurulent exudate in the nasal cavities; lymphoid hyperplasia in the trachea and pneumonitis)	Weeks et al. 1979
			Cardio	260			
			Gastro	260			
			Musc/skel	260			
			Hepatic	260			
			Renal	260			
			Endocr	260			
			Derm	260			
			Ocular	48	260	(irritation)	
			Bd Wt	48	260	M (reduced body weight, quantitative data not provided)	
10	Rat (Sprague- Dawley)	6 wk 5d/wk 6hr/d	Resp	260	M		Weeks et al. 1979
			Hepatic	260	M		
			Renal	260	M		
			Bd Wt	48	M	260 M (mean body weights decreased 11% in older animals)	

TABLE 2-1. Levels of Significant Exposure to Hexachloroethane - Inhalation (continued)

Key ^a to figure	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect)		Reference		
					Less serious (ppm)	Serious (ppm)			
11	Gn pig (Hartley)	6 wk 5d/wk 6hr/d	Resp	260	M			Weeks et al. 1979	
			Cardio	260	M				
			Gastro	260	M				
			Musc/skel	260	M				
			Hepatic	260	M				
			Renal	260	M				
			Derm	260	M				
			Ocular	260	M				
			Bd Wt	48	M	260	M (reduced body weight gain, quantitative data not provided)		
12	Dog (Beagle)	6 wk 5d/wk 6hr/d	Resp	260	M			Weeks et al. 1979	
			Cardio	260	M				
			Hemato	260	M				
			Musc/skel	260	M				
			Hepatic	260	M				
			Renal	260	M				
			Endocr	260	M				
			Derm	260	M				
			Ocular	48	M	260	M (irritation)		
			Bd Wt	260	M				
Neurological									
13	Rat (Sprague-Dawley)	6 wk 5d/wk 6hr/d		260	M			Weeks et al. 1979	

TABLE 2-1. Levels of Significant Exposure to Hexachloroethane - Inhalation (continued)

Key ^a to figure	Species (strain)	Exposure duration/ frequency	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
14	Rat (Sprague- Dawley)	6 wk 5d/wk 6hr/d		48 ^c		260 (tremors)	Weeks et al. 1979
15	Dog (Beagle)	6 wk 5d/wk 6hr/d		48 M		260 M (tremors; ataxia; fasciculations; severe head bobbing)	Weeks et al. 1979

^aThe number corresponds to entries in Figure 2-1.

^bUsed to derive an acute-duration inhalation minimal risk level (MRL) of 6 ppm. The NOAEL was converted to a Human Equivalent Concentration (HEC) of 181 ppm by multiplying by (0.22 m³/day/0.204 kg), the reference value for Sprague-Dawley rats (EPA 1988a), and dividing by (20 m³/day/70kg), the reference value for humans (EPA 1988a). The HEC was divided by an uncertainty factor of 30 (3 to extrapolate from animals to humans, and 10 for human variability).

^cUsed to derive an intermediate-duration inhalation MRL of 6 ppm. The NOAEL was converted to a Human Equivalent Concentration (HEC) of 174 ppm by multiplying by (0.245 m³/day/0.236 kg), the reference values for Sprague-Dawley rats (EPA 1988a), and divided by (20 m³/day/70kg), the reference values for humans (EPA 1988a). The HEC was divided by an uncertainty factor of 30 (3 to extrapolate from animals to humans, and 10 for human variability).

Bd Wt = body weight; Cardio = cardiovascular; d = day(s); Derm = dermal; Endocr = endocrine; F = female; Gastro = gastrointestinal; Gd = gestation day(s); Gn pig = guinea pig; Hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; M = male; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

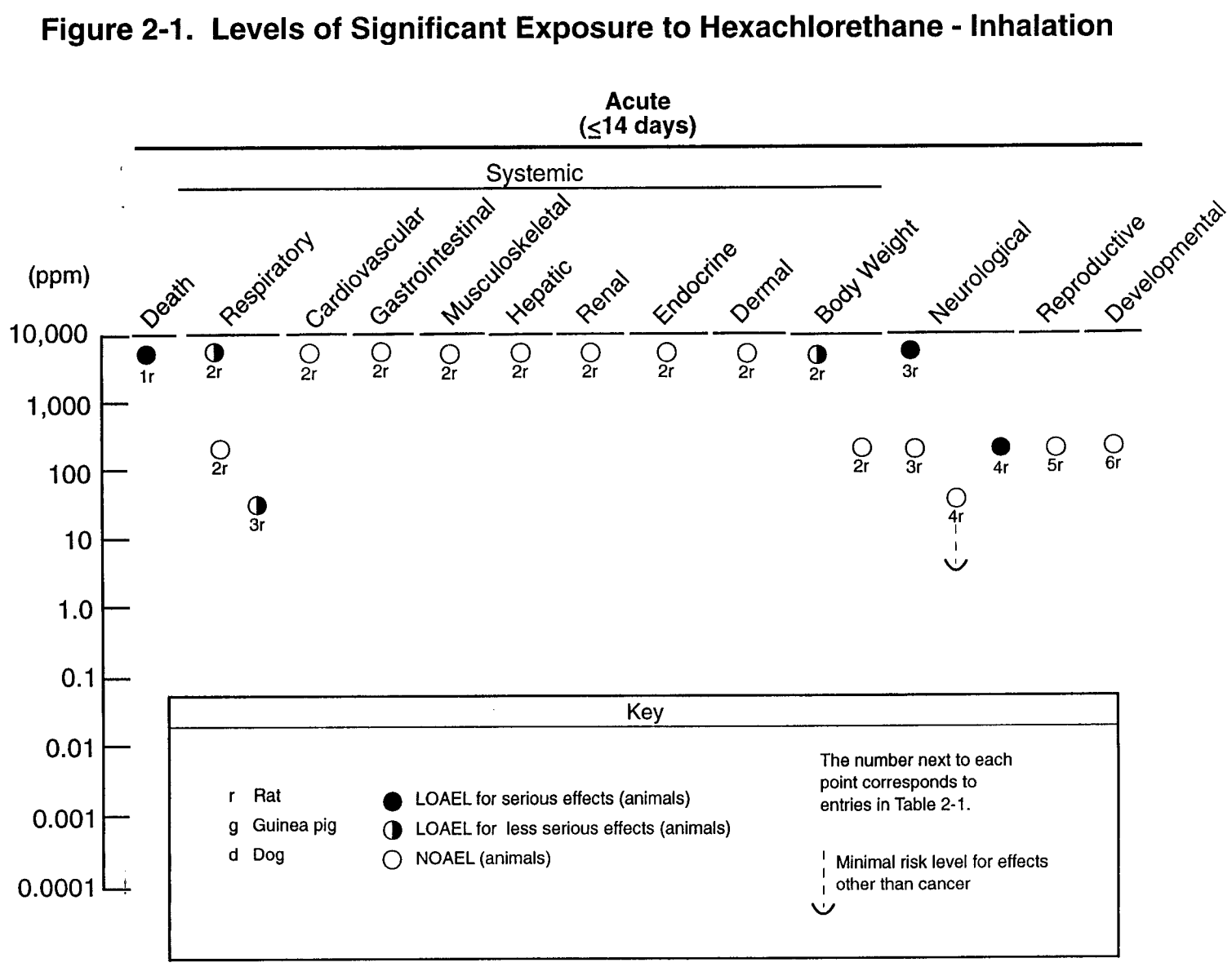
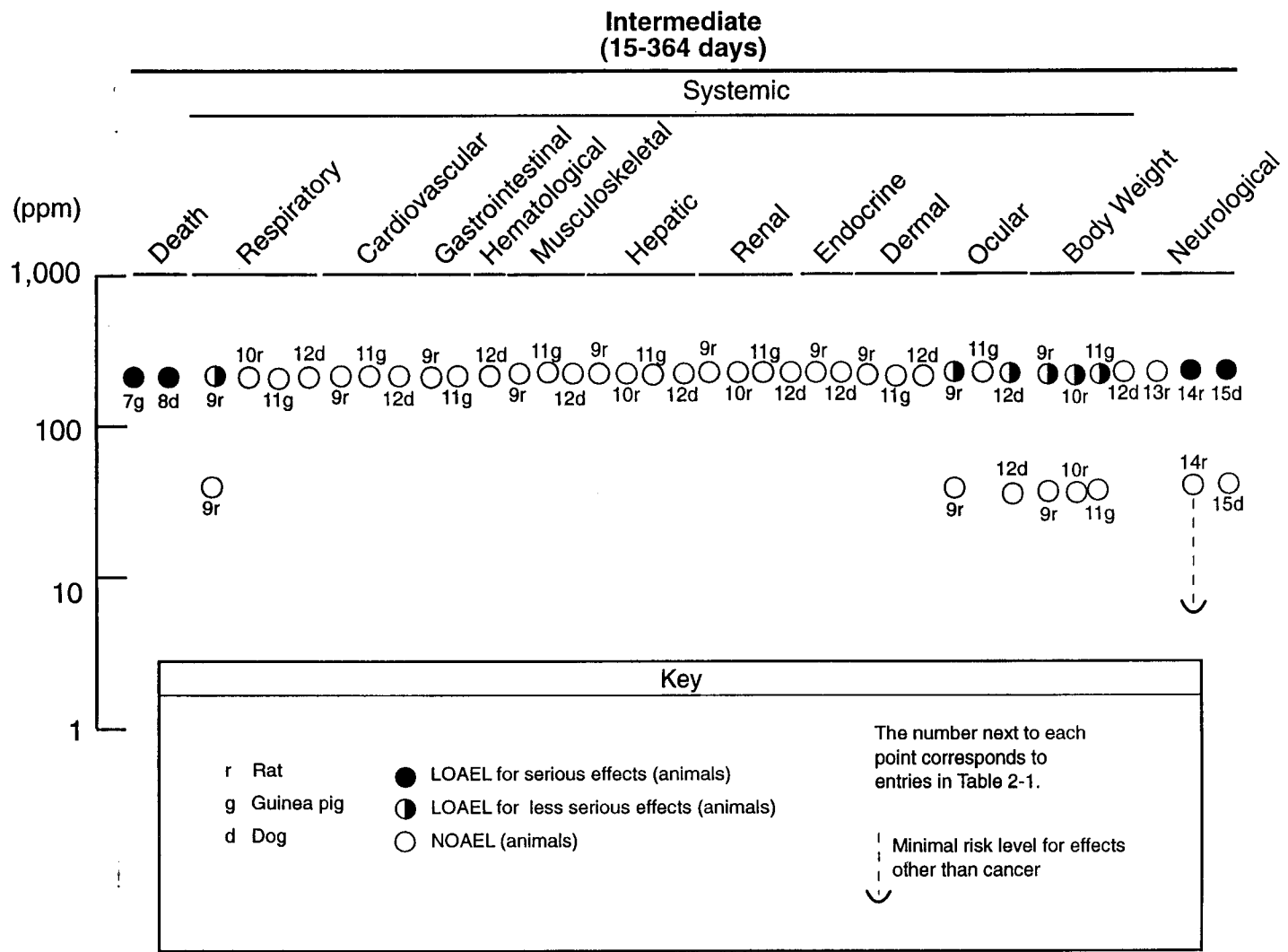


Figure 2-1. Levels of Significant Exposure to Hexachloroethane - Inhalation (continued)



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2.2.1.2 Systemic 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- 1 and plotted in Figure 2-1.

Respiratory Effects. Pulmonary function tests (vital capacity, forced expiratory volume at 1 second) were in the normal range in 11 workers occupationally exposed to hexachloroethane at 0.5-2.1 ppm while wearing protective equipment including compressed-air-fed visors or full-facepiece masks with combination filters (Selden et al. 1994). The testing was completed 5 weeks after production at a smoke munitions plant resumed following a 5-week break. Plasma hexachloroethane levels were 0.08 ± 0.14 $\mu\text{g/L}$ before production resumed and 7.3 ± 6.04 $\mu\text{g/L}$ 5 weeks later indicating that despite protective equipment, low-level exposure occurred (Selden et al 1993). Mild skin and mucous membrane irritation were reported in the exposed group, suggesting that exposure may have been through either the inhalation or dermal routes of exposure.

Acute exposure of rats to 5,900 ppm hexachloroethane for 8 hours caused interstitial pulmonary pneumonitis (Weeks et al. 1979). At this exposure concentration there were hexachloroethane particles present in the exposure chamber that were inhaled and probably contributed to the lung irritation. Changes in lung histopathology were noted when the animals were sacrificed after a 14-day recovery period. There were no changes in relative lung weights or tissue histopathology in animals that were exposed to 260 ppm for the same time period. When pregnant female rats were exposed to 0, 15, 48, or 260 ppm hexachloroethane on gestation days 6-16, 85% of the animals in the 48-ppm dose group displayed nasal exudate, and all animals in the 260-ppm dose group were affected. There was an endemic mycoplasma infection in the colony of rats used in the Weeks et al. (1979) study. Therefore, it is not clear if the increase in nasal exudate observed at 48 ppm in the teratology study, but not at 48 ppm in the 6-week study (discussed below), was truly related to exposure.

In rats exposed to 260 ppm hexachloroethane for 6 weeks, there was a significant decrease in oxygen consumption as compared to the controls (Weeks et al. 1979). Oxygen consumption was determined by measuring carbon dioxide exhaled by rats placed in a water-sealed chamber for 15 minutes. The authors hypothesized that the decrease in oxygen consumption could have been a normal response to inhalation of a respiratory tract irritant. There were no significant changes in lung weights, but there was an increase in

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mycoplasma lesions of the nasal turbinates, trachea, and lungs; lymphoid hyperplasia of the trachea; and pneumonitis of the bronchi when the animals were sacrificed after the 6-week exposure period. These changes were not seen in the animals exposed to 15 or 48 ppm or in rats exposed to 260 ppm and sacrificed after a 12-week recovery period. The authors hypothesized that the respiratory tract lesions were the result of hexachloroethane potentiation of an endemic mycoplasma infection rather than systemic effects from inhalation exposure to hexachloroethane. The infection could be the result of lowered host resistance due to either compromised immune defenses or a weakened mucosal barrier along the respiratory epithelium.

In older rats (12-14 weeks), there was a significant increase in relative lung weights as compared to controls following 6 weeks of exposure to 260 ppm hexachloroethane. Oxygen consumption was not measured in these animals, and it is not clear if the tissues were examined histologically (Weeks et al. 1979).

In dogs, there were no significant changes in pulmonary function with exposure to 15-260 ppm hexachloroethane for 6 weeks (Weeks et al. 1979). Intrapleural pressure, transpulmonary pressure, air flow, and tidal volume were measured to obtain scores for compliance and resistance. When the animals were sacrificed at either 6 weeks or after a 12-week recovery period, there were no histopathological changes observed in the lungs. There were also no apparent effects on the respiratory system in guinea pigs from exposure to 15-260 ppm hexachloroethane. Exposure of quail to 260 ppm was associated with increased mucus in the nasal turbinates in 2 of the 10 animals, but this increased mucus did not appear to be associated with a respiratory infection. These changes were considered to be the direct effect of hexachloroethane on the epithelium of the nasal cavity and are discussed in Section 2.2.3.2.

Exposure to hexachloroethane vapors can cause irritation to the respiratory system. Acute exposure to 260 ppm hexachloroethane had no apparent effect on the lungs and air passages in rats, but acute exposure to a concentration where particulate hexachloroethane was present in the atmosphere caused lung irritation (Weeks et al. 1979). On the other hand, intermediate-duration exposure to 260 ppm hexachloroethane appeared to cause some irritation of the respiratory epithelium, which may have increased susceptibility to respiratory infection. When exposure ceased, the animals recovered, so there were no histopathological indications of tissue damage after a 12week recovery period. Lesions of the nasal passages, trachea, and bronchi; increased mycoplasma infections; mucus in the nasal cavities; and decreased oxygen

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consumption were indicators of respiratory tract irritation from repeated episodes of hexachloroethane exposure.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after inhalation exposure to hexachloroethane.

There were no histopathological changes in the heart for rats, guinea pigs, dogs, or quail that were exposed to concentrations of 0, 260, or 5,900 ppm hexachloroethane for 8 hours or to 0, 1548, or 260 ppm hexachloroethane 6 hours/day, 5 days/week for 6 weeks (Weeks et al. 1979).

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after inhalation exposure to hexachloroethane.

There were no histopathological changes in the stomach, small intestines, or large intestines for rats, guinea pigs, dogs, or quail that were exposed to concentrations of 0,260, or 5,900 ppm hexachloroethane for 8 hours or to 0, 15,48, or 260 ppm hexachloroethane 6 hours/day, 5 days/week for 6 weeks (Weeks et al. 1979).

Hematological Effects. Routine blood parameters (hemoglobin, erythrocyte, leukocyte and thrombocyte levels) measured in 11 hexachloroethane workers did not differ from those of the controls (Selden et al. 1994). Plasma hexachloroethane levels in these workers, who wore protective equipment, were 7.3 ± 6.04 $\mu\text{g/L}$ at the time of the hematological analysis and 0.08 ± 0.14 $\mu\text{g/L}$ before production resumed (Selden et al. 1993). Mild skin and mucous membrane irritation were reported in the exposed group, suggesting that exposure may have been through either the inhalation or dermal routes of exposure.

There were no effects on the red blood cell counts of dogs exposed to 0, 15,48, or 260 ppm hexachloroethane for 6 weeks (Weeks et al. 1979). Although other hematological parameters were apparently determined, the red cell count was the only parameter that was specified. Accordingly, it is not possible to speculate whether inhalation exposure to hexachloroethane has any effect on other hematological parameters.

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Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after inhalation exposure to hexachloroethane.

There were no treatment-related gross or histopathological lesions of the skeletal muscle or bone in rats, guinea pigs, dogs, or quail exposed to 0, 15, 48, or 260 ppm hexachloroethane 6 hours/day, 5 days/week for 6 weeks (Weeks et al. 1979).

Hepatic Effects. Liver function tests (serum bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase) completed in 11 hexachloroethane workers were within the normal range (Selden et al. 1994). Plasma hexachloroethane levels in these workers, who wore protective equipment, were 7.3 ± 6.04 $\mu\text{g/L}$ at the time of the tests (Selden et al. 1993). Mild skin and mucous membrane irritation were reported in the exposed group, suggesting that exposure may have been through either the inhalation or dermal routes of exposure.

A single 8-hour exposure of rats to 260 ppm hexachloroethane had no effect on relative liver weight or tissue histopathology (Weeks et al. 1979). A single 8-hour exposure to 5,900 ppm did not cause histopathological changes. Organ weights were not determined for the higher exposure concentration.

The relative liver weight was significantly increased (p value not given) in guinea pigs and rats, but not dogs or quail, that were exposed to 260 ppm 6 hours/day, 5 days/week for 6 weeks (Weeks et al. 1979). Since the increase in liver weight was not accompanied by any histological abnormalities, it is classified as a NOAEL rather than a LOAEL in Table 2-1 and Figure 2-1. There were no changes in liver weights or histopathology in any species exposed to concentrations of 15 or 48 ppm for 6 weeks.

Renal Effects. Renal function tests (serum creatinine and urate, urinary hemoglobin, protein and glucose) completed in 11 hexachloroethane workers were within the normal range (Selden et al. 1994). Plasma hexachloroethane levels in these workers, who wore protective equipment, were 7.3 ± 6.04 $\mu\text{g/L}$ at the time of the tests (Selden et al. 1993). Mild skin and mucous membrane irritation were reported in the exposed group, suggesting that exposure may have been through either the inhalation or dermal routes of exposure.

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A single exposure of rats to 260 ppm hexachloroethane had no effect on relative kidney weight or tissue histopathology (Weeks et al. 1979). A single exposure to 5,900 ppm did not cause histopathological changes. Organ weights were not determined for the higher exposure concentration.

The relative kidney weight was significantly increased (p value not stated) in male rats, but not female rats, guinea pigs, dogs, or quail, that were exposed to 260 ppm 6 hours/day, 5 days/week for 6 weeks (Weeks et al. 1979). Since the increase in kidney weight was not accompanied by any histological abnormalities, it is classified as a NOAEL rather than a LOAEL in Table 2-1 and Figure 2-1. There were no changes in kidney weights or histopathology in any species exposed to 15 or 48 ppm hexachloroethane for 6 weeks.

Endocrine Effects. No studies were located regarding endocrine effects in humans after inhalation exposure to hexachloroethane.

There were no histopathological changes in the pancreas or adrenal glands of rats exposed to concentrations of 0, 260, or 5,900 ppm hexachloroethane for 8 hours, or in the pancreas or adrenal glands of rats, guinea pigs, dogs, or quail exposed to 0, 15, 48, or 260 ppm hexachloroethane 6 hours/day, 5 days/week for 6 weeks (Weeks et al. 1979).

Dermal Effects. Hexachloroethane exposed workers reported a slightly higher prevalence of dry skin and dry mucous membranes as well as itching and other skin problems than the unexposed controls (Selden et al. 1994). Clinical examinations of the 11 exposed workers did not reveal signs of abnormal dermatological or mucous membrane status. Plasma hexachloroethane levels in these workers, who wore protective equipment, were 7.3 ± 6.04 $\mu\text{g/L}$ at the time of the examinations (Selden et al. 1993). The investigators indicate that the dermal effects may also have been a result of a local trauma effect of the protective equipment.

Ocular Effects; No studies were located regarding ocular effects in humans after inhalation exposure to hexachloroethane.

Dogs that were exposed to 260 ppm hexachloroethane for 6 hours per day, 5 days per week, kept their eyes closed during each exposure (Weeks et al. 1979). Since this effect occurred throughout the 6-week study, it can be regarded as an acute effect that was most likely the result of vapor contact with the eye. In

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rats, a red exudate appeared about the eyes starting at week 4. This may have been a systemic effect. There were no reported effects on the eyes of guinea pigs or quail during 6 weeks of exposure to hexachloroethane.

Body Weight Effects. Rats exposed to a concentration of 5,900 ppm hexachloroethane for 8 hours had a decreased weight gain over the 14-day, postexposure observation period when compared to controls (Weeks et al. 1979). There were no differences in the weight gain for animals exposed to 260 ppm under the same conditions.

Guinea pigs and male rats had decreased weight gains starting at week 2 or 3 of an intermittent 6-week exposure to 260 ppm hexachloroethane, but there were no effects on dogs or quail (Weeks et al. 1979). Intermittent exposure to 15 or 48 ppm hexachloroethane for 6 weeks had no effect on weight gain in rats, dogs, guinea pigs, or quail.

2.2.1.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans after inhalation exposure to hexachloroethane.

There were no treatment-related gross or histopathological lesions of the thymus and spleen and no changes in spleen weight in rats that were exposed to 260 or 5,900 ppm hexachloroethane for 8 hours, nor were there any effects on thymus and spleen histopathology in rats, guinea pigs, dogs, and quail that were exposed to 260 ppm 6 hours/day, 5 days/week for 6 weeks (Weeks et al. 1979). The relative spleen weight was significantly higher (p value not stated) than that for the controls in young male rats but was not affected in older male rats or any of the other species evaluated. No effects were seen in the 15 and 48 ppm dose groups.

There was an increased incidence of a mycoplasma respiratory tract infection in rats exposed to 260 ppm hexachloroethane for 6 weeks but not in rats exposed to lower doses or in other species. This could indicate compromised immune function or a weakened mucosal barrier along the respiratory epithelium. There were no studies identified that evaluated a wide range of immunological parameters. Therefore, there are no reliable LOAELs or NOAELs for this end point. Increases in spleen weights are not classified as LOAELs since they were not accompanied by histopathological changes.

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2.2.1.4 Neurological Effects

No studies were located regarding neurological effects in humans after inhalation exposure to hexachloroethane.

Acute 8-hour exposures to 5,900 ppm, but not 260 ppm, resulted in a staggering gait in one of six rats (Weeks et al. 1979). Tremors were also noted in pregnant rats exposed 6 hours per day to 260 ppm starting on the 6th day of an 1 l-day exposure period but not in animals exposed to 15 or 48 ppm (Weeks et al. 1979). Based on the NOAEL for neurological effects, an acute inhalation MRL of 6 ppm was calculated, as described in the footnote in Table 2-1.

When male rats were exposed to concentrations of 0, 15,48, or 260 ppm hexachloroethane 6 hours/day, 5 days/week for 6 weeks, foot shock avoidance behavior and spontaneous motor activity were not different from controls when measured at 1 day, 3 weeks, or 6 weeks (Weeks et al. 1979). However, a group of male and female rats exposed to 260 ppm experienced tremors beginning at 4 weeks and persisting for the remainder of the 6-week exposure period. Recovery was evident during the 12 week post-exposure period (Weeks et al. 1978). Tremors were not observed in rats exposed at 48 ppm. Based on the intermediate-duration NOAEL of 48 ppm for neurological effects, an intermediate inhalation MRL of 6 ppm was calculated, as described in the footnote in Table 2-1.

Dogs were apparently quite sensitive to neurological effects during hexachloroethane exposure (Weeks et al. 1979). The animals displayed tremors, ataxia, head bobbing, and fasciculation of the facial muscles with a 260 ppm exposure. Symptoms disappeared in the interval between exposures but returned intermittently over the 6-week exposure period. There were no differences in serum cholinesterase activity between control and exposed animals. Apparently, the levels of the neurotransmitter acetylcholine were not affected by hexachloroethane. There were no neurological responses in guinea pigs or quail with a 260-ppm exposure; and none of the species evaluated showed any overt neurological responses with an intermittent 6-week exposure at 15 or 48 ppm.

The highest NOAEL values and all LOAEL values from each reliable study for neurological effects in each species and duration category are recorded in Table 2- 1 and plotted in Figure 2-1. Acute exposures

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to hexachloroethane appear to cause neurological impairment during exposure of rats and dogs, but symptoms do not persist during the intervals between exposures or after exposure ceases.

2.2.1.5 Reproductive Effects

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

Hexachloroethane was maternally toxic at concentrations of 48 and 260 ppm in rats exposed 6 hours/day on gestation days 6-16, based on significantly decreased (p value not stated) maternal body weight gain, but it was not embryotoxic or fetotoxic (Weeks et al. 1979). There were no treatment-related gross or histopathological lesions of the testes in rats, dogs, guinea pigs, and quail that were exposed to concentrations of hexachloroethane up to 260 ppm 6 hours/day, 5 days/week for 6 weeks. However, relative testes weights were increased in rats when compared to the controls. No other reproductive organs were evaluated (Weeks et al. 1979). The highest NOAEL from each reliable study for reproductive effects in each species and duration category is recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.6 Developmental Effects

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

In animals, hexachloroethane did not cause skeletal or soft tissue abnormalities in offspring of rats that were exposed to vapors of hexachloroethane (1.5-260 ppm) 6 hours/day during gestation days 6-16 (Weeks et al. 1979). The highest NOAEL value from this study for developmental effects in rats is recorded in Table 2-1 and plotted in Figure 2-1.

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after inhalation exposure to hexachloroethane.

Genotoxicity studies are discussed in Section 2.5.

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2.2.1.8 Cancer

One case study was identified where a man who had been occupationally exposed to hexachloroethane was treated for a liver tumor (Selden et al. 1989). Exposure had occurred over a period of 6 years as a result of the presence of hexachloroethane in a degassing agent used during aluminum smelting. However, the hexachloroethane reacted at the 700°C use-temperature, releasing a gas that was 96% hexachlorobenzene with small amounts of other chlorinated compounds. Because there was occupational exposure to a mixture of chlorinated compounds rather than just hexachloroethane, it is highly unlikely that the tumor was the result of hexachloroethane exposure alone. Occupational exposure to mineral oil mists for 20 years was also part of the subject's employment history.

No studies were located regarding cancer incidence in animals after inhalation exposure to hexachloroethane. EPA has derived an inhalation unit risk (cancer slope factor) of 1.4×10^{-7} (mg/kg/day)⁻¹ for hexachloroethane (IRIS 1995). This inhalation unit risk was calculated using data from oral studies (see Section 2.2.2.8) and Figure 2-2.

2.2.2 Oral Exposure

2.2.2.1 Death

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

When hexachloroethane was administered to rats by gavage with corn oil as the solvent, the LD₅₀ value was 5,160 mg/kg for males and 4,460 mg/kg for females (Weeks et al. 1979). Kinkead and Wolfe (1992) reported an LD₅₀ value of 4,489 mg/kg for both male and female rats treated with hexachloroethane in corn oil. When hexachloroethane was dissolved in aqueous methyl cellulose solution, the LD₅₀ was 7,690 mg/kg for male and 7,080 mg/kg for female rats (Weeks et al. 1979). The lower LD₅₀ for the corn oil solvent indicates that the absorption from this hydrophobic medium is greater than that from a hydrophilic medium such as methyl cellulose solution. The LD₅₀ of 4,970 mg/kg for male guinea pigs given hexachloroethane in corn oil is similar to that for rats (Weeks et al. 1979). According to the classification system of Hodge and Sterner (1949), these LD₅₀ values indicate that hexachloroethane is slightly toxic by acute oral exposure.

2. HEALTH EFFECTS

With repeated administration of hexachloroethane, a dose of 750 mg/kg/day in corn oil was lethal to 1 of 5 male rats and 2 of 5 females within 15 days (NTP 1989). The earliest death occurred in a male rat on day 5. All animals died between day 2 and day 8 with doses of 1,500 and 3,000 mg/kg/day.

With 6-week hexachloroethane exposures, there were some deaths among rats given a dose of 1,000 mg/kg/day in corn oil, and all animals died with a dose of 1,780 mg/kg/day (NTP 1977). The number of animals that died at the 1,000 mg/kg/day dose level was not specified and the time of death was not given for any of the doses. There were no deaths in animals given doses of 562 mg/kg/day or lower. Mice were more resistant to hexachloroethane exposure than rats because all of the mice survived doses of 1,000 mg/kg/day in corn oil for 6 weeks (NTP 1977). Some male mice died with a 1,780 mg/kg/day dose, but the exact number was not specified.

With a 13-week exposure duration, doses of 750 mg/kg/day in corn oil were lethal to some male and female rats (NTP 1989). The earliest death occurred among the males at 7 weeks. Between 7 weeks and 13 weeks (the end of the exposure period), 5 of 10 males died; during week 13, 2 of 10 females died.

Chronic (2-year) exposure of male rats to 20 mg/kg/day hexachloroethane and female rats to 160 mg/kg/day had no effect on survival (NTP 1989), but the longevity of rats exposed to doses of 212 and 423 mg/kg/day for 66 weeks was decreased as compared to controls (NTP 1977; Weisburger 1977). The hexachloroethane was given by gavage in corn oil for both studies.

Doses of 750 mg/kg/day and greater can be lethal with both acute- and intermediate-duration exposures, and a chronic intake of 212 mg/kg/day or greater can shorten the lifespan of rats. There were no apparent effects on lifespan with chronic administration of a 160 mg/kg/day dose in female rats or a dose of 20 mg/kg/day in male rats.

All identified LOAEL values for lethality in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic 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.

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

Key ^a to figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect)		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE							
Death							
1	Rat (Sprague-Dawley)	Once (GO)				4489 (LD50)	Kinkead and Wolfe 1992
2	Rat (Sprague-Dawley)	Once ^b (G)				7080 F (LD50)	Weeks et al. 1979
3	Rat (Sprague-Dawley)	Once ^b (G)				7690 M (LD50)	Weeks et al. 1979
4	Rat (Sprague-Dawley)	Once (GO)				5160 M (LD50)	Weeks et al. 1979
5	Rat (Sprague-Dawley)	Once (GO)				4460 F (LD50)	Weeks et al. 1979
6	Gn pig (Hartley)	Once (GO)				4970 M (LD50)	Weeks et al. 1979

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

Key ^a to figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect)		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
Systemic							
7	Rat (Sprague- Dawley)	11d Gd 6-16 (GO)	Resp	100 F	500 F (increased mucus in nasal turbinates; subclinical pneumonitis)		Weeks et al. 1979
			Cardio	500 F			
			Gastro	500 F			
			Musc/skel	500 F			
			Hepatic	500 F			
			Derm	500 F			
			Ocular	500 F			
			Bd Wt	100 F			
8	Rabbit (New Zealand)	12 d (GW)	Resp	1000 M	320M (hepatocellular degeneration) 320M (tubular nephrosis; tubular nephrocalcinosis) 320M (reduced body weight, quantitative data not provided)	1000 M (coagulation; necrosis; hemorrhage)	Weeks et al. 1979
			Cardio	1000 M			
			Gastro	1000 M			
			Musc/skel	1000 M			
			Hepatic	100 ^c M			
			Renal	100 M			
			Endocr	1000 M			
			Ocular	1000 M			
Bd Wt	100 M						

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

Key * to figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect)		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
9	Sheep	Once (GO)	Hepatic		500M (elevated sorbital and glutamate dehydrogenases; ornithine carbamoyl transferase; decreased bromosulphthalein excretion)		Fowler 1969b
Neurological							
10	Rat (Wistar)	Gd 7-17 (GO)		56 F		167 F (decreased motor activity)	Shimizu et al. 1992
11	Rat (Sprague- Dawley)	11d Gd 6-16 (GO)		100 F		500 F (tremors)	Weeks et al. 1979
Reproductive							
12	Rat (Wistar)	Gd 7-17 (GO)		167		500 (increased late-stage fetal resorptions)	Shimizu et al. 1992
13	Rat (Sprague- Dawley)	11d Gd 6-16 (GO)		100 F		500 F (increased fetal resorptions; reduced gestation indices, quantitative data not provided)	Weeks et al. 1979
Developmental							
14	Rat (Wistar)	Gd 7-17 (GO)		167		500 (20-25% decrease in fetal body weights; increase in skeletal anomalies)	Shimizu et al. 1992
15	Rat (Sprague- Dawley)	11d Gd 6-16 (GO)		100	500 (delayed development, details not provided)		Weeks et al. 1979

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

Key ^a to figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect)		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
INTERMEDIATE EXPOSURE							
Death							
16	Rat (Fischer-344)	13 wk 5d/wk (GO)				750 B (death of 5/10 males and 2/10 females)	NTP 1989
17	Rat (Fischer-344)	2-16 d 5d/wk (GO)				750 B (death of 1/5 males and 2/5 females) 1500 B (100% mortality)	NTP 1989
Systemic							
18	Rat (Fischer 344)	16 wk (F)	Resp	62 B			Gorzinski et al. 1985
			Cardio	62 B			
			Gastro	62 B			
			Hemato	62 B			
			Musc/skel	62 B			
			Hepatic	1 ^d B	15M (swelling of hepatocytes in 6/10 males)		
			Renal	1 B		15 M (increased kidney weight 10%; increased relative kidney weight 5.5%; tubular atrophy and hypertrophy)	
			Endocr	62 B			
			Derm	62 B			
			Ocular	62 B			
			Bd Wt	62 B			
19	Rat (Osborne-Mendel)	7 wk 5d/wk (GO)	Hepatic		497M (significantly increased mean liver weight)		Milman et al. 1988; Story et al. 1986

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

Key ^a to figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect)		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
20	Rat (Osborne-Mendel)	6 wk 5d/wk (GO)	Bd Wt	316	562M (decreased body weight gain, amount not specified)	1000 M (38% decreased body weight gain)	NTP 1977
21	Rat (Fischer-344)	2-16 d 5d/wk (GO)	Resp	375 B		750 B (shortness of breath)	NTP 1989
			Renal		187M (hyaline droplet formation; granular cast)		
			Ocular	375 B	750 B (lacrimation)		
			Bd Wt	375 B		750 B (mean body weight decreased 24% in females; males gained 24% of the amount gained by controls)	
22	Rat (Fischer-344)	13 wk 5d/wk (GO)	Resp	750 B			NTP 1989
			Cardio	750 B			
			Gastro	750 B			
			Hepatic	94 B		188 F (centrilobular necrosis and increased liver weight)	
			Renal		47M (hyaline droplets; tubular degeneration)		
			Derm	750 B			
			Ocular	750 B			
			Bd Wt	375 B	750M (19% reduction in mean body weight)		
23	Mouse (B6C3F1)	6 wk 5d/wk (GO)	Bd Wt	1000 B	1780 B (reduced body weight gain, quantitative data not provided)		NTP 1977

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

Key ^a to figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect)		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
Neurological							
24	Rat (Fischer-344)	13 wk 5d/wk (GO)		47 B	94 B (post-gavage hyperactivity)	375 B (convulsions)	NTP 1989
CHRONIC EXPOSURE							
Death							
25	Rat (Osborne- Mendel)	66 wk 5d/wk (GO)				212 B (26/50 males and 23/50 females died before the end of the study)	NTP 1977; Weisburger 1977
Systemic							
26	Rat (Osborne- Mendel)	66 wk 5d/wk (GO)	Renal			212 B (tubular necrosis; interstitial nephritis; regenerative epithelium; fibrosis and casts)	NTP 1977; Weisburger 1977
			Bd Wt			212 M (30% reduction of weight gain)	
27	Rat (Fischer-344)	2 yr 5d/wk (GO)	Resp	160 F			NTP 1989
			Cardio	160 F			
			Gastro	160 F			
			Hepatic	160 F			
			Renal		10M (mineralization of renal papillae; renal tubule hyperplasia)	20 M (necrosis; regenerative epithelium; interstitial fibrosis)	
Derm	160 F						

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

Key ^a to figure	Species (strain)	Exposure duration/ frequency (specific route)	System	NOAEL (mg/kg/day)	LOAEL (effect)		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
28	Mouse (B6C3F1)	78 wk 5d/wk (GO)	Renal			590 B (tubular nephropathy; degeneration of the tubular epithelium; inflammation, fibrosis; calcium deposits)	NTP 1977; Weisburger 1977
			Bd Wt	590 B			
			Cancer				
29	Rat (Fischer-344)	2 yr 5d/wk (GO)				20 M (CEL: renal tubular adenoma or adenocarcinoma in 7/50) 10 M (CEL: pheochromocytomas in adrenal gland 28/45)	NTP 1989
30	Mouse (B6C3F1)	78 wk 5d/wk (GO)				590 B (CEL: hepatocellular carcinomas 35/50)	NTP 1977; Weisburger 1977

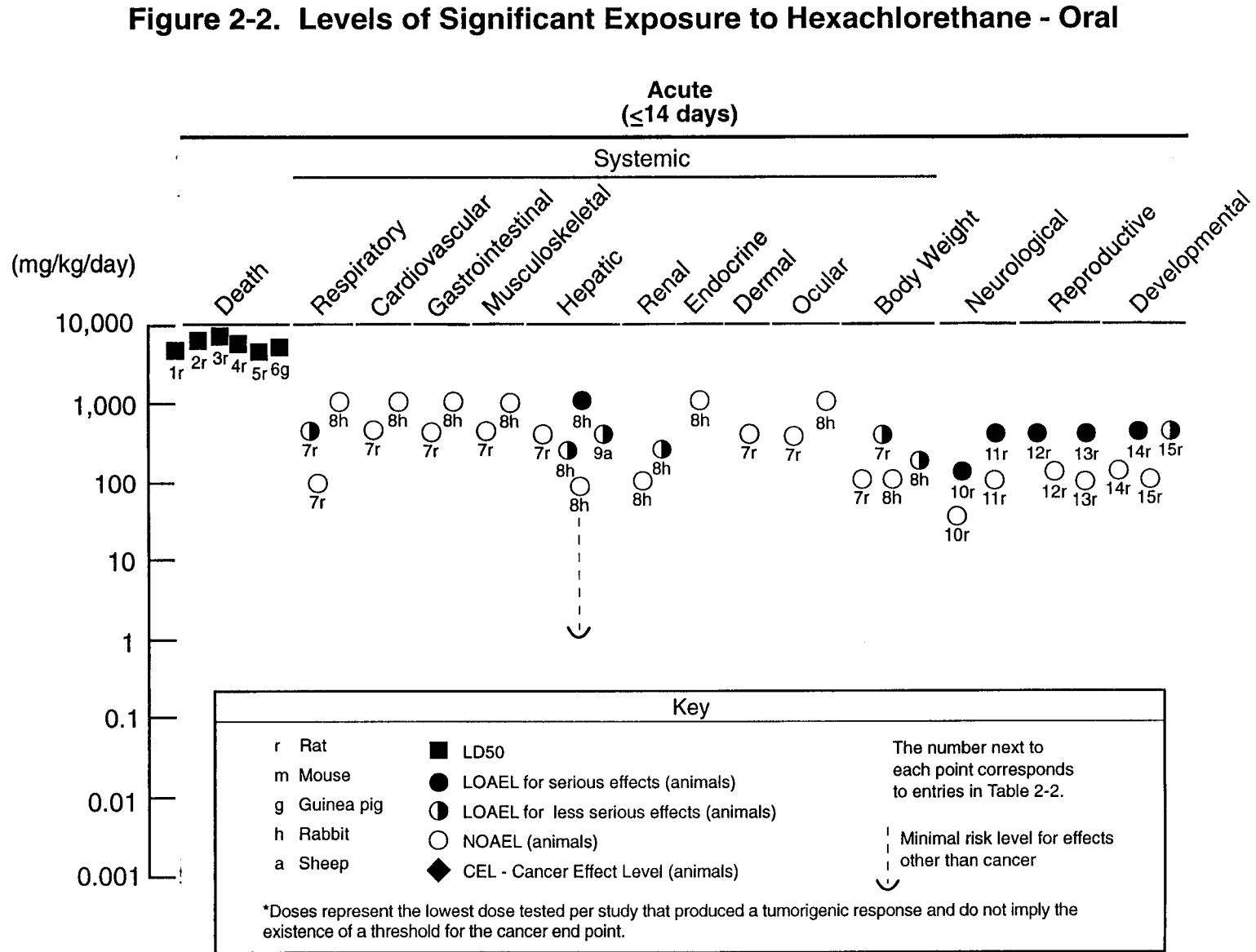
^aThe numbers correspond to entries in Figure 2-2.

^bAdministered in methylcellulose solution.

^cUsed to derive an acute-duration oral minimal risk level (MRL) of 1 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

^dUsed to derive an intermediate-duration oral MRL of 0.01 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

B = both; Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Derm = dermal; Endocr = endocrine; F = female; (F) = food; (G) = gavage; Gastro = gastrointestinal; Gd = gestation day(s); Gn pig = guinea pig; (GO) = gavage oil; (GW) = gavage water; Hemato = hematological; LD50 = lethal dose (50% kill); LOAEL = lowest-observed-adverse-effect level; M = male;; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); yr = year(s)



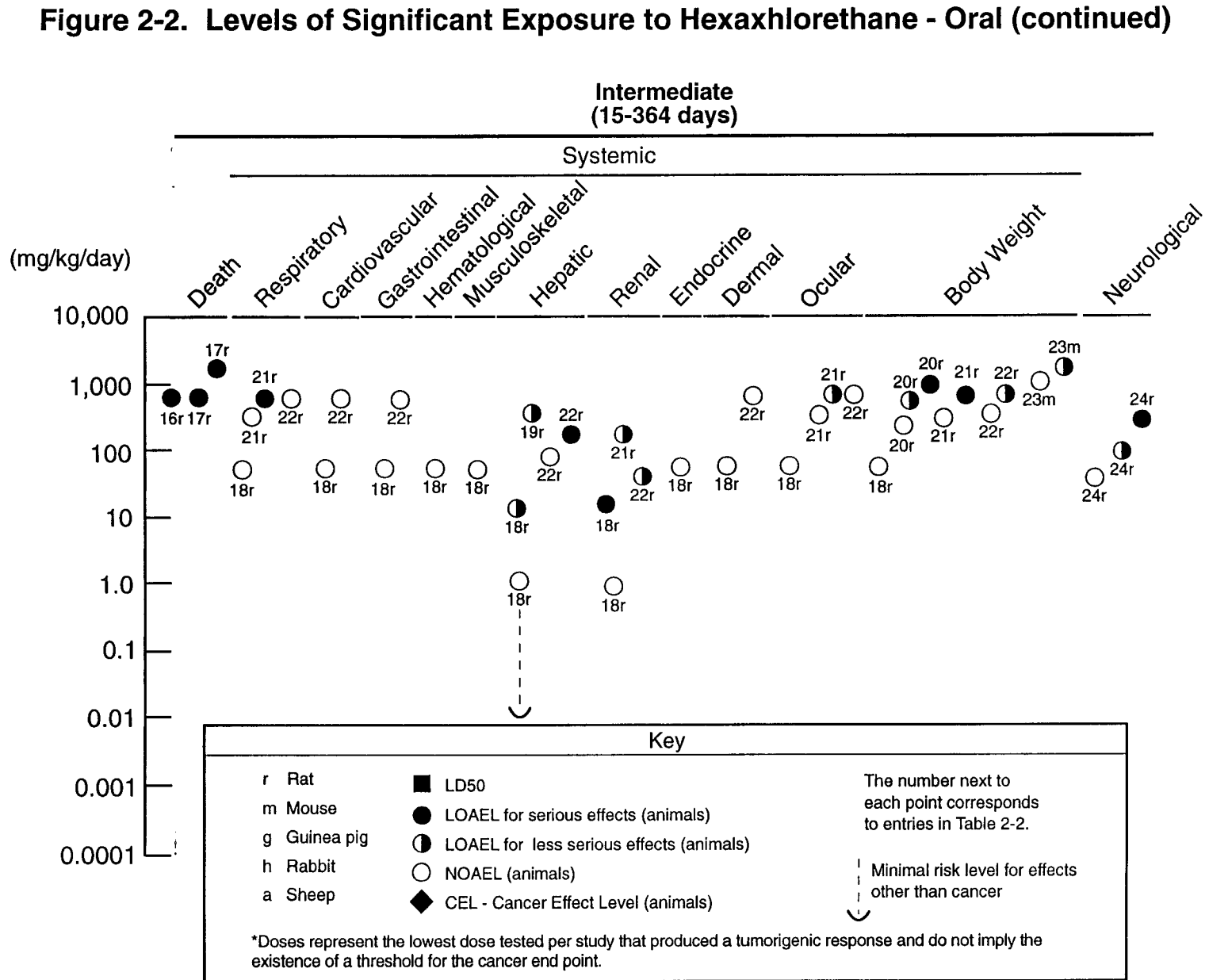
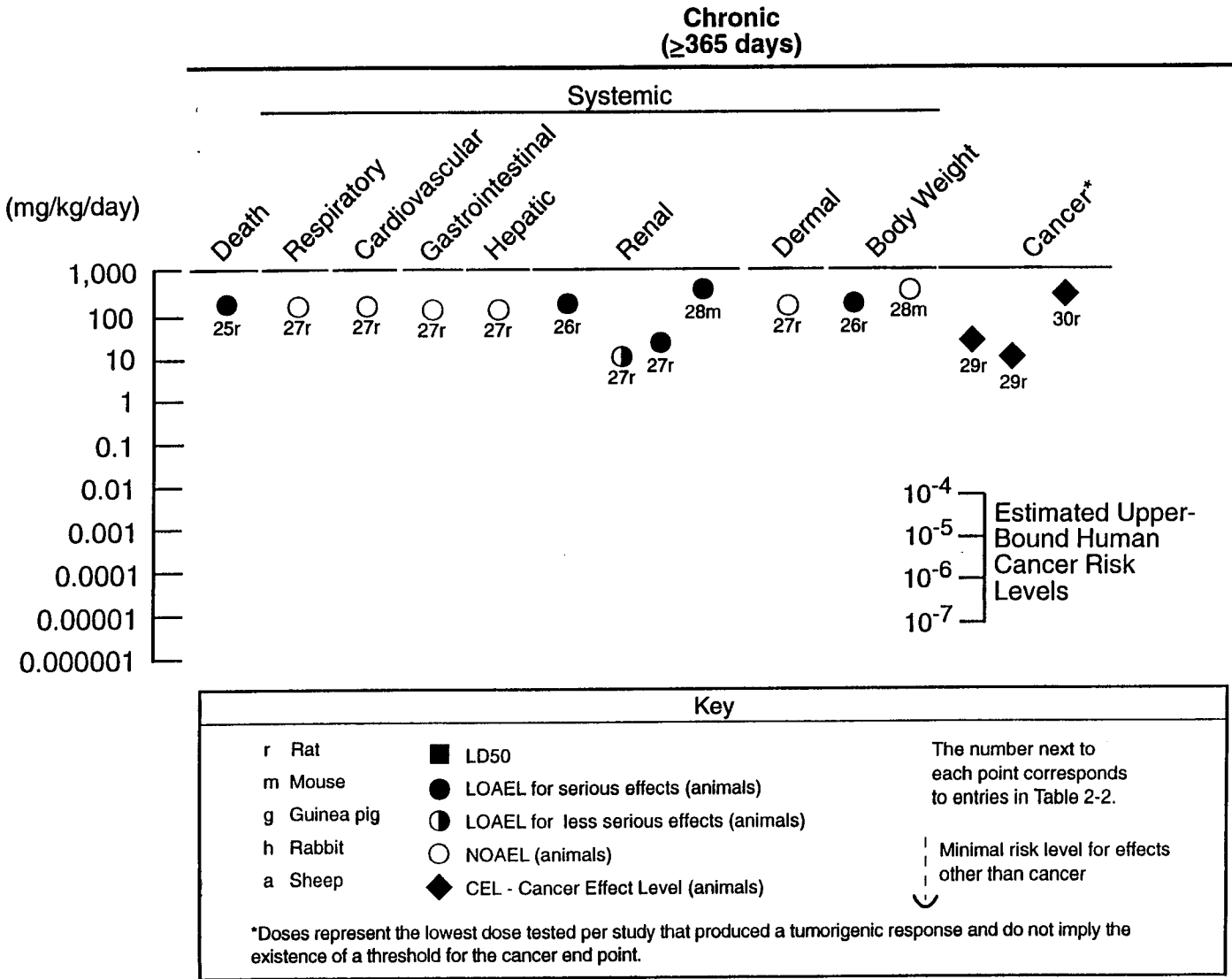


Figure 2-2. Levels of Significant Exposure to Hexachlorethane - Oral (continued)



2. HEALTH EFFECTS

Respiratory Effects. No studies were located regarding respiratory effects in humans after oral exposure to hexachloroethane.

Pregnant female rats were given 100 or 500 mg/kg/day hexachloroethane in corn oil by gavage for 11 days (gestation days 6-16) (Weeks et al. 1979). In the high dose group, 75% of the animals showed an increased incidence of upper respiratory tract irritation compared to only 10% of the controls. Subclinical pneumonitis was evident in 20% of the animals in the high dose group as was increased mucous in the nasal turbinates. There were no effects on the respiratory tract for the animals exposed to 100 mg/kg/day when compared to the controls.

No changes in lung histopathology or in lung weights were observed in rabbits exposed by gavage to 0, 100,320, or 1,000 mg/kg/day hexachloroethane in methyl cellulose solution for 12 days (Weeks et al. 1979).

When rats were exposed to 750 mg/kg/day or less hexachloroethane in corn oil by gavage for 13 weeks, there were no effects on the histopathology of the nasal cavity, nasal turbinates, larynx, trachea, bronchi, or lungs (NTP 1989). There were also no changes in the trachea or lungs of rats when hexachloroethane was fed in the diet at a dose of 62 mg/kg/day for 16 weeks (Gorzinski et al. 1985). Doses of 20 mg/kg/day for males and 160 mg/kg/day for females had no effects on the nasal cavity, nasal turbinates, larynx, trachea, bronchi, or lungs when given to rats by gavage in corn oil over their lifetimes (NTP 1989). It appears that hexachloroethane can cause irritation of the respiratory passages in rats even when given orally. The presence of mycoplasma in the animal colony may have contributed to the appearance of lesions and, thus, the lesions may be the result of a synergistic interaction between the microorganism and the hexachloroethane. Based on the data from inhalation exposures as well as the findings of upper respiratory tract infections in pregnant rats that were orally exposed to hexachloroethane (Weeks et al. 1979), it appears that hexachloroethane may weaken resistance to bacterial infections.

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans after oral exposure to hexachloroethane.

There were no histopathological changes in the hearts of rabbits that were exposed by gavage to doses of 0, 100,320, or 1,000 mg/kg/day hexachloroethane in methyl cellulose solution for 12 days (Weeks et al.

2. HEALTH EFFECTS

1979). This was also true for rats that were exposed to doses of up to 62 mg/kg/day in the diet for 16 weeks (Gorzinski et al. 1985) or rats exposed to doses of up to 750 mg/kg/day by gavage in corn oil for 13 weeks (NTP 1989). There were significant increases in heart weight for male rats receiving doses of 188-750 mg/kg/day and female rats receiving 750 mg/kg/day for 13 weeks (NTP 1989). Since these changes were not accompanied by any histopathological lesions, they are regarded in Table 2-2 and Figure 2-2 as NOAEL values rather than LOAELs. The heart does not appear to be a target organ when hexachloroethane is administered orally.

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects in humans after oral exposure to hexachloroethane.

There were no histopathological changes in the stomach, small intestines, or large intestines of rabbits exposed by gavage to 1,000 mg/kg/day hexachloroethane in methyl cellulose solution for 12 days (Weeks et al. 1979). No effects were noted in rats that were exposed by gavage to concentrations of up to 750 mg/kg/day hexachloroethane in corn oil or 62 mg/kg/day in feed for 13 or 16 weeks (Gorzinski et al. 1985; NTP 1989). Lifetime doses of up to 20 mg/kg/day for male and 160 mg/kg/day for female rats were without effect on the histopathology of the stomach or intestines (NTP 1989). Hexachloroethane appears to have no effects on the gastrointestinal system when administered orally.

Hematological Effects. No studies were located regarding hematological effects in humans after oral exposure to hexachloroethane.

There were no significant changes in red cell counts, hemoglobin concentration, or white cell counts in rats fed doses of 0, 1, 15, or 62 mg/kg/day in the diet for 16 weeks (Gorzinski et al. 1985).

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after oral exposure to hexachloroethane.

There were no treatment-related gross or histopathological lesions of the skeletal muscle or bone in rabbits exposed by gavage to 100-1,000 mg/kg/day hexachloroethane in water for 12 days (Weeks et al. 1979). Histopathological changes were not observed in the skeletal muscle of rats exposed to hexachloroethane in drinking water at 62 mg/kg/day for 16 weeks (Gorzinski et al. 1985).

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Hepatic Effects. No studies were located regarding hepatic effects in humans after oral exposure to hexachloroethane.

The liver appeared to be a target organ for hexachloroethane following oral administration. When one dose of 500 mg/kg was administered in an olive oil aqueous emulsion to male sheep, the levels of glutamate dehydrogenase, sorbitol dehydrogenase, ornithine carbamyl transferase, and aspartate aminotransferase in serum increased in the 2-day period after compound administration and then normalized (Fowler 1969b). Hexachloroethane had no effect on bromsulphthalein uptake from the blood by liver cells, but the transfer of this dye to bile was reduced in sheep exposed to doses of 500-1,000 mg/kg/day.

On the other hand, in rats, a single dose of 6,156 mg/kg hexachloroethane in mineral oil had no effects on a different set of biochemical indicators of liver function (microsomal protein, oxidative demethylase, NADP-NT reductase, glucose-6-phosphatase, or lipid conjugated diene concentration) when measured 2 hours after compound administration (Reynolds 1972). Each of these parameters is an indicator of microsomal function. The authors postulated that the observed lack of effects could have been the result of slow uptake of hexachloroethane by the liver in a 2-hour period. Gastrointestinal absorption of hexachloroethane in mineral oil is probably minimal because, unlike olive oil, mineral oil cannot be digested. Dissolved lipophilic materials could be excreted in the feces soon after administration because mineral oil can act as a laxative. Thus, the author's hypothesis that minimal hexachloroethane would reach the liver in 2 hours is reasonable.

In rabbits, relative liver weights were increased by a dose of 1,000 mg/kg/day hexachloroethane in methyl cellulose solution when given by gavage for 12 days (Weeks et al. 1979). Doses of 320 and 1,000 mg/kg/day were associated with hepatic necrosis, fatty degeneration, hemosiderin-laden macrophages, eosinophilic change, hemorrhage, and coagulation necrosis. The occurrence and severity of each effect at each dose was not presented in the published report of this study. There were also nonsignificant increases in the serum levels of alkaline phosphatase, aspartate aminotransferase, and bilirubin with the 1,000 mg/kg/day dose. No effects were seen with a dose of 100 mg/kg/day. As described in the footnote of Table 2-2, this NOAEL was used to calculate an MRL of 1 mg/kg/day for acute oral exposures.

Liver weights were increased with doses of 15-497 mg/kg/day in rats and exposure durations of 7-16 weeks (Gorzinski et al. 1985; Milman et al. 1988; NTP 1989; Story et al. 1986). The lowest LOAEL

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was 15 mg/kg/day from a 16-week study where the hexachloroethane was fed in the diet (Gorzinski et al. 1985). At this LOAEL, hepatocytes were visibly enlarged in 6 of 10 males. At a 62 mg/kg/day dose, 8 males had enlarged hepatocytes and liver weights were increased 10%. No hepatic effects were noted in rats fed 1 mg/kg/day in this study. Based on this value, an intermediate oral MRL of 0.01 mg/kg/day was calculated as described in the footnote in Table 2-2. In a different study, hepatic necrosis in the centrilobular area was seen in 40% of the female rats with a dose of 188 mg/kg/day and in both sexes at doses of 375 mg/kg/day and greater when hexachloroethane was given by gavage in corn oil for 13 weeks (NTP 1989). With the 750 mg/kg/day dose, 40% of the males and 80% of the females were affected.

No effects were seen on liver histopathology in male rats given up to 20 mg/kg/day hexachloroethane for their 2-year lifetime or females given up to 160 mg/kg/day (NTP 1989).

The liver is sensitive to hexachloroethane following both acute and longer term exposure scenarios. Evidence of effects on the liver include increased weight and centrilobular necrosis in rats and rabbits and increased serum levels of liver enzymes in sheep. There can also be fatty degeneration of the tissues and hemorrhage when damage is severe.

Renal Effects. No studies were located regarding renal effects in humans after oral exposure to hexachloroethane.

Male New Zealand rabbits displayed nephrosis of the convoluted tubules and nephrocalcinosis when given doses of 320 and 1,000 mg/kg/day hexachloroethane in methyl cellulose solution for 12 days (Weeks et al. 1979). Kidney weights were increased significantly for the 1,000 mg/kg/day dose. There were no observed effects on the kidney with a dose of 100 mg/kg/day.

In male rats, hyaline droplets could be seen in tubular epithelial cells after 12 gavage doses of 187-750 mg/kg/day in corn oil over a 16-day period (NTP 1989). No adverse histopathologic effects were seen in the kidneys of females. Hyaline droplet formation, tubular regeneration, and tubular casts were present with doses of 47-750 mg/kg/day when the hexachloroethane was administered in corn oil by gavage for 13 weeks (NTP 1989). Renal tubular necrosis and papillary necrosis were present in five males that died during weeks 7-12 when given 750 mg/kg/day hexachloroethane. The kidneys of the five survivors were not examined. Kidney weights were increased significantly in males at doses of

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94 mg/kg/day or greater and in females at 375 and 750 mg/kg/day. Hemorrhagic necrosis of the urinary bladder was present in the males from the highest dose group.

When hexachloroethane was given in the diet for 16 weeks, male rats showed a dose-related increase in tubular hypertrophy, dilation, atrophy, peritubular fibrosis, and tubular degeneration (Gorzinski et al. 1985). These signs of nephropathy were present in all of the males at the 62 mg/kg/day dose and 70% of the males at the 15 mg/kg/day dose. Kidney weights were significantly increased for the 62 mg/kg/day dose group. Renal effects were also present in female rats, but they were less severe than the effects seen in males and occurred at higher doses. A dose of 62 mg/kg/day in the diet for 16 weeks was associated with atrophy and degeneration of the tubules in 60% of the females (Gorzinski et al. 1985).

Chronic exposure of both rats and mice resulted in tubular nephropathy in both males and females. In rats, lesions were present in 45-66% of the males when they were sacrificed at 110 weeks after receiving 212 and 423 mg/kg/day hexachloroethane for 66 weeks of a 78-week exposure period (NTP 1977; Weisburger 1977). The renal lesions were characterized by hyperchromic regenerative epithelium, necrosis, interstitial nephritis, fibrosis, focal pyelonephritis, tubular ectasis, and hyaline casts. Lesions were also present in females but had a lower incidence (18% and 59%) for the two dose groups. Two-year exposures of male rats to much lower doses (10 and 20 mg/kg/day) resulted in similar effects on the kidneys (NTP 1989). Minimal to mild nephropathy was present in females for doses of 80 and 160 mg/kg/day. Over 90% of the male and female mice exposed to 590 and 1,179 mg/kg/day hexachloroethane for 78 weeks displayed tubular nephropathy when sacrificed at 90 weeks (NTP 1977; Weisburger 1977). Regenerative tubular epithelium was visible and degeneration of the tubular epithelium occurred at the junction of the cortex and the medulla. Hyaline casts were present in the tubules, and fibrosis, calcium deposition, and inflammatory cells were noted in the kidney tissues.

Male rats are sensitive to renal tubular nephropathy after exposure to hexachloroethane. The lesions observed are characteristic of hyaline droplet nephropathy. They are most likely the result of hexachloroethane or one of its metabolites binding to the excretory protein $\alpha_2\mu$ -globulin, altering its kidney transport, and leading to the formation of hyaline droplets. This protein is synthesized by male rats and accounts for 26% of their urinary protein excretion (Olson et al. 1990). It is not excreted in female rats except in minimal quantities. Since some effects are also seen in kidneys of female rats and in male and female mice that do not synthesize $\alpha_2\mu$ -globulin hexachloroethane must also have milder adverse effects on the kidney through a different mechanism.

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Endocrine Effects. No studies were located regarding endocrine effects in humans after oral exposure to hexachloroethane.

Histopathological changes were not observed in the pancreas or adrenal glands of pregnant rats treated by gavage with hexachloroethane in corn oil for 11 days at doses up to 500 mg/kg/day, or in the pancreas of male rabbits treated for 12 days at doses up to 1,000 mg/kg/day (Weeks et al. 1979). Following 16 weeks of dietary treatment, hexachloroethane did not result in histopathologic changes in the pancreas, adrenal glands, thyroid, or parathyroid glands of rats treated at doses up to 62 mg/kg/day (Gorzinski et al. 1985).

An increased incidence of pheochromocytomas in the adrenal gland was observed in male rats treated by gavage with hexachloroethane in corn oil at 10 and 20 mg/kg/day 5 days/week for 2 years (NTP 1989). This effect is discussed further in Section 2.2.2.8, Cancer.

Dermal Effects. No studies were located regarding dermal effects in humans after oral exposure to hexachloroethane.

Histopathological changes in the skin were not observed in pregnant rats given hexachloroethane in corn oil by gavage for 11 days at doses up to 500 mg/kg/day (Weeks et al. 1979). Treatment of rats with doses of hexachloroethane in the diet for 13 weeks at 62 mg/kg/day (Gorzinski et al. 1985), by gavage for 13 weeks at 750 mg/kg/day, and by gavage for 2 years at 20 mg/kg/day for males and 160 mg/kg/day for females (NTP 1989) did not result in histopathologic changes in the skin.

Ocular Effects. No studies were located regarding ocular effects in humans after oral exposure to hexachloroethane.

An oral dose of 750 mg/kg/day of hexachloroethane for 12 of 16 days resulted in lacrimation in rats during exposure, -but this effect was not mentioned for this dose in the discussion of the results following 13-weeks of treatment (NTP 1989). There were no histopathological effects of hexachloroethane on the eyes of rats at doses up to 750 mg/kg/day for 13 weeks or for lifetime administration of doses of 10 or 20 mg/kg/day to male rats and 80 or 160 mg/kg/day to females rats (NTP 1989).

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Body Weight Effects. Rabbit weight gain was reduced by doses of 320 and 1,000 mg/kg/day hexachloroethane in methyl cellulose solution given by gavage for 12 days (Weeks et al. 1979). Body weight gain was also reduced in rats exposed by gavage to 750 mg/kg/day hexachloroethane in corn oil for 12 of 16 days, but not in males receiving doses of 375 mg/kg/day and lower (NTP 1989). Females in the 375 mg/kg/day dose groups gained only $67 \pm 7\%$ of the weight gained by the controls, and the females in the 750 mg/kg/day dose group lost $25 \pm 2\%$ of their initial body weight.

With exposures of 6 to 16 weeks, doses of 562 mg/kg/day and greater were associated with decreased weight gain in rats (NTP 1977, 1989). No effects on weight were seen with doses of 375 mg/kg/day and lower (Gorzinski et al. 1985; NTP 1977, 1989). Mice were more resistant to effects on weight gain with a NOAEL of 1,000 mg/kg/day and an LOAEL of 1,760 mg/kg/day for a 6-week exposure (NTP 1977).

With chronic exposures, the doses that had no effect on weight gain in rats were 160 mg/kg/day or lower (NTP 1989). A dose of 212 mg/kg/day was associated with a 30% reduction of weight gain in males (NTP 1977). Chronic exposure of mice to doses as high as 1,179 mg/kg/day had no apparent effect on weight gain (NTP 1977).

2.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans after oral exposure to hexachloroethane.

There were no treatment-related gross or histopathological lesions of the thymus, spleen, or lymph nodes in animals that were exposed to hexachloroethane over any duration (Gorzinski et al. 1985; NTP 1977, 1989; Weeks et al. 1979). For acute exposures, doses of 1,000 mg/kg/day or less were given to rabbits for 12 days (Weeks et al. 1979). In the intermediate-duration exposure category, doses of 750 mg/kg/day or less were tested in rats (Gorzinski et al. 1985; NTP 1989) while for chronic exposures, doses of 20 mg/kg/day or less were given to male rats and 160 mg/kg/day or less were given to female rats (NTP 1989). No studies were identified that evaluated a wide range of immunological parameters; therefore, there is no reliable LOAEL or NOAEL for this end point.

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2.2.2.4 Neurological Effects

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

Acute exposure of sheep to 500 mg/kg hexachloroethane resulted in tremors of the facial muscles immediately after the exposure (Fowler 1969b). In sheep that were suffering from liver fluke infections, the neurotoxicity of hexachloroethane was even more pronounced. A dose of 170 mg/kg given for treatment of the fluke infection rendered 2 of 15 sheep immobile and unable to stand on the day after treatment, and a dose of 338 mg/kg affected 6 of 15 animals. Tremors of the facial muscles, neck, and forelimbs were apparent. The animals that were able to stand had a staggering gait, and when they fell, they were unable to return to their feet (Southcott 1951). Treatment with calcium borogluconate relieved most of the neuromuscular symptoms although the twitches of the facial muscles persisted.

Tremors were also noted in pregnant rats exposed to 500 mg/kg/day for 11 days during gestation (Weeks et al. 1979). Decreased motor activity was observed in pregnant rats treated with oral doses of 167 mg/kg/day hexachloroethane on gestation days 7-17 (Shimizu et al. 1992). No effects were observed at 56 mg/kg/day. Male and nonpregnant female rats exposed to 750 mg/kg/day for 12 of 16 days suffered from ataxia and prostration (NTP 1989). When exposures were carried out for 13 weeks, a dose of 94 mg/kg/day was associated with postgavage hyperactivity and doses of 375 and 750 mg/kg/day were associated with convulsions (NTP 1989). There were no effects noted on brain histopathology for doses of 750 mg/kg/day or less in rats given hexachloroethane by gavage in corn oil for 13 weeks, but brain weights were increased significantly in both sexes at this dose (NTP 1989).

Chronic exposure of male rats to doses of 20 mg/kg/day or less and of female rats to doses of 160 mg/kg/day or less had no effect on the histopathology of the brain or spinal cord (NTP 1989). Hyperactivity was reported in females, but it was not clear if one or both dose groups were affected.

There has been no comprehensive evaluation of neurological function in animals after oral exposure to hexachloroethane. The data are limited primarily to clinical signs immediately after exposure and to histopathological evaluations of the brain tissues, which showed no effects. The highest NOAEL values and all LOAEL values from each reliable study for neurological effects in each species and duration category are recorded in Table 2-2 and Figure 2-2.

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2.2.2.5 Reproductive Effects

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

In rats, fertility was adversely affected in dams that were administered 500 mg/kg/day (highest dose tested) as evidenced by a reduction in gestation indices and the number of live fetuses per dam. Similar effects were not seen in concurrent vehicle controls. Also, fetal resorption rates were higher at this dose than in the control group (Weeks et al. 1979). Maternal body weight gain was also suppressed. It should be noted that quantitative data were not provided for evaluation. No effects on the number of corpora lutea, the number of implants, or the number of live fetuses were observed in rats treated with hexachloroethane by gavage at 500 mg/kg/day on gestation days 7-17 (Shimizu et al. 1992). The number of late gestation resorptions was increased at 500 but not 167 mg/kg/day. The 500-mg/kg/day dose also resulted in maternal body weight gains that were about 35% less than those of the controls. 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 hexachloroethane.

A slowing of fetal development was observed in offspring of rats that were exposed to a dose of 500 mg/kg/day hexachloroethane during gestation days 6-16; however, no effects were seen at doses of 100 mg/kg/day or less (Weeks et al. 1979). Hexachloroethane was not teratogenic under the conditions of this study. It should be noted that the authors did not provide quantitative data for evaluation. These results have been confirmed in a study by Shimizu et al. (1992) in which decreased fetal body weights and delayed ossification were observed in the offspring of rats treated with hexachloroethane at 500 mg/kg/day on gestation days 7-17. This dose also resulted in maternal body weight gains that were about 35% less than those of the controls. An increase in fetal anomalies was not observed, with no significant fetal effects at 167 mg/kg/day. The highest NOAEL values and all LOAEL values from each reliable study in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

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2.2.2.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after oral exposure to hexachloroethane.

Genotoxicity studies are discussed in Section 2.5

2.2.2.8 Cancer

No studies were located regarding cancer in humans after oral exposure to hexachloroethane.

There have been three bioassays of hexachloroethane; two were conducted using rats as the test species and one used mice. In the first rat bioassay, there were no statistically significant increases in tumors that could be attributed to compound administration (NTP 1977; Weisburger 1977). Time weighted average doses of 212 or 423 mg/kg/day were given in corn oil by gavage 5 days per week for 66 of 78 weeks. The animals were not exposed to hexachloroethane for 32 weeks before they were sacrificed at 110 weeks.

The total number of tumors for the exposed animals was 22/49 for the males in the low-dose group and 12/50 for the high-dose group, compared to a value of 13/120 for the controls (Weisburger 1977). In the females, the number of tumors for the controls was 22/20; for the low-dose group, 50/50; and for the high-dose group, 27/49. When evaluated by tumor type there were no statistically significant patterns apparent. Tumors seen in control and treated animals were thyroid adenomas and carcinomas; pituitary adenomas; adrenal tumors; mammary fibromas, fibroadenomas, and carcinomas; and kidney tumors.

A significant and dose-related increase in adenomas and adenocarcinomas of the kidney was observed in male rats given doses of 0, 10, or 20 mg/kg/day for 2 years (NTP 1989). The combined incidence of adenomas and adenocarcinomas was 1/50 for the vehicle controls, 2/50 for the low-dose group, and 7/50 for the high-dose group. These tumors are considered to be unique to male rats and are not indicative of tumorigenic potential in other species because they were associated with hyaline droplet nephropathy. There was no increase in renal adenomas and carcinomas for the female rats, even though they were given doses of 80 and 160 mg/kg/day. In this study, there was also an increased incidence of pheochromatomas in the adrenal glands of male rats when compared to the controls. In the vehicle controls, tumor incidence

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was 30% (15/50), in the low-dose group it was 62% (28/45), and in the high-dose group it was 43% (21/49).

In mice, there was an increase in hepatocellular carcinomas when the animals were sacrificed at 90 weeks after being exposed to doses of 590 and 1,179 mg/kg/day for 78 weeks (NTP 1977; Weisburger 1977). The total number of tumors for the exposed animals was 4/20 for the male controls, 17/50 for the males in the low-dose group, and 37/49 for the males in the high-dose group. In females, the number of tumors was 9/20 for the controls, 40/50 for the low-dose group, and 30/49 for the high-dose group. When evaluated by tumor type, there was a dose-related trend of 3/20, 15/50, and 29/49 for liver hepatocellular carcinomas in males but not in females, where the corresponding values were 2/20, 20/50, and 15/49. Other tumors seen in control and experimental animals included lung adenomas and carcinomas and histolytic lymphomas.

An investigation of the incidence of gamma glutamyl transpeptidase (GGT+) lesions in the liver indicates that hexachloroethane is a promoter of carcinogenicity rather than an initiator. These lesions are markers for preneoplastic cellular changes. When male rats were given a single dose of 497 mg/kg hexachloroethane followed by treatment with a known promoter (phenobarbital) for 7 weeks, there was no increase in number of liver GGT+ foci (Milman et al. 1988). However, when a single dose of a known initiator (dimethylnitrosamine) was followed by 7 weeks of dosing with 497 mg/kg/day hexachloroethane, the number of GGT+ foci was four times the number seen with a single dose of dimethylnitrosamine in the absence of hexachloroethane treatment.

All CELs from each reliable study are included in Table 2-2 and plotted in Figure 2-2. EPA has derived an oral slope factor of $1.4 \times 10^{-2} \text{ (mg/kg/day)}^{-1}$ for hexachloroethane based on hepatocellular carcinomas in male mice (IRIS 1995). Doses that correspond to excess cancer risks of 10^{-4} to 10^{-7} are shown in Figure 2-2.

2.2.3 Dermal Exposure

2.2.3.1 Death

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

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In rabbits, a dermal LD₅₀ of greater than 32,000 mg/kg for a 24-hour exposure to a water paste of hexachloroethane was reported. Ataxia, tremors, and convulsions were noted in those animals that died from exposure (Weeks et al. 1979). The LD₅₀ value is reported in Table 2-3.

2.2.3.2 Systemic Effects

No studies were located regarding cardiovascular, gastrointestinal, or musculoskeletal effects after dermal exposure to hexachloroethane. The highest NOAEL values and all reliable LOAEL values in each species and duration category are recorded in Table 2-3.

Respiratory Effects. Pulmonary function tests (vital capacity, forced expiratory volume at 1 second) were in the normal range in 11 workers occupationally exposed to hexachloroethane at 0.5-2.1 ppm for 5 weeks while wearing protective equipment including respiratory protection (Selden et al. 1994). Plasma hexachloroethane levels were 7.3 ± 6.04 yg/L at the time of testing indicating that despite protective equipment, low-level exposure occurred (Selden et al. 1993). Mild skin and mucous membrane irritation were reported in the exposed group, suggesting that exposure may have been through either the inhalation or dermal routes of exposure.

No studies were located regarding respiratory effects in animals after dermal exposure to hexachloroethane.

Hematological Effects. Routine blood parameters (hemoglobin, erythrocyte, leukocyte and thrombocyte levels) measured in 11 hexachloroethane workers that wore protective clothing did not differ from those of the controls (Selden et al. 1994). Mild skin and mucous membrane irritation were reported in the exposed group, suggesting that exposure may have been through either the inhalation or dermal routes of exposure.

No studies were located regarding hematological effects in animals after dermal exposure to hexachloroethane.

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

Species/ (strain)	Exposure duration/ frequency (Specific route)	System	NOAEL	LOAEL (effect)		Reference
				Less Serious	Serious	
ACUTE EXPOSURE						
Death						
Rabbit (New Zealand)	24 hr				>32 M (LD50) g/kg	Weeks et al. 1979
Systemic						
Rabbit (New Zealand)	24 hr	Derm	132 M mg/kg			Weeks et al. 1979
Rabbit (New Zealand)	Once	Ocular		100 mg M (corneal opacity; iritis)		Weeks et al. 1979
INTERMEDIATE EXPOSURE						
Immunological/Lymphoreticular						
Gn pig (Hartley)	3 wk		1000 M ppm	(no skin sensitization)		Weeks et al. 1979

Derm = dermal; Gn pig = guinea pig;; hr = hour(s); LD50 = lethal dose (50% kill); LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; wk = week(s)

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Hepatic Effects. Liver function tests (serum bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase) completed in 11 hexachloroethane workers who wore protective clothing were within the normal range (Selden et al. 1994). Mild skin and mucous membrane irritation were reported in the exposed group, suggesting that exposure may have been through either the inhalation or dermal routes of exposure.

No studies were located regarding hepatic effects in animals after dermal exposure to hexachloroethane.

Renal Effects. Renal function tests (serum creatinine and urate, urinary hemoglobin, protein and glucose) completed in 11 hexachloroethane workers who wore protective clothing were within the normal range (Selden et al. 1994). Mild skin and mucous membrane irritation were reported in the exposed group, suggesting that exposure may have been through either the inhalation or dermal routes of exposure. No studies were located regarding renal effects in animals after dermal exposure to hexachloroethane.

Dermal Effects. Hexachloroethane-exposed workers reported a slightly higher prevalence of dry skin and dry mucous membranes as well as itching and other skin problems than the unexposed controls (Selden et al. 1994). Clinical examinations of the 11 exposed workers did not reveal signs of abnormal dermatological status. Plasma hexachloroethane levels in these workers, who wore protective equipment, were 7.3 ± 6.04 $\mu\text{g/L}$ at the time of the examinations (Selden et al. 1993). The investigators indicate that the dermal effects may also have been a result of a local trauma effect of the protective equipment.

Hexachloroethane had no effects on intact or abraded skin of rabbits when 500 mg was applied to shaved skin as the pure solid (Weeks et al. 1979). There was only a slight redness at the application site when it was applied as a water paste. All redness disappeared after 72 hours. The NOAEL for dermal effects in rabbits (132 mg/kg) is reported in Table 2-3.

Ocular Effects. No studies were located regarding ocular effects in humans after dermal exposure to hexachloroethane.

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Contact with crystalline hexachloroethane (100 mg) caused swelling, iritis, corneal opacity, and discharge when placed in rabbit eyes overnight. All signs of ocular irritation were reversed 72 hours later (Weeks et al. 1979). This LOAEL for ocular effects in rabbits is reported in Table 2-3.

Contact with hexachloroethane vapors at a concentration of 260 ppm was apparently irritating to the eyes of dogs because the animals kept their eyes closed during all exposure periods (Weeks et al. 1979). In rats, a red exudate was observed about the eyes after 4 weeks of exposure to hexachloroethane vapors, and in rabbits, after a single dermal exposure to a water paste of hexachloroethane (Weeks et al. 1979). The red exudate did not appear until after 4 weeks of exposure to hexachloroethane vapor and, thus, may be a systemic effect rather than the effect of direct contact of the eye with hexachloroethane.

2.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans following dermal exposure to hexachloroethane.

Hexachloroethane did not act as a sensitizer in guinea pigs when a challenge dose was given 2 weeks after the end of a 3-week sensitization period (Weeks et al. 1979). Accordingly, it did not stimulate antibody formation during sensitization. The NOAEL for dermal sensitization is reported in Table 2-3.

2.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans following dermal exposure to hexachloroethane.

Rats that died after dermal exposure to unspecified doses of hexachloroethane during an LD₅₀ test protocol displayed ataxia, tremors, and convulsions before death (Weeks et al. 1979).

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

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

Genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

No studies were located regarding cancer in humans or animals after dermal exposure to hexachloroethane.

2.3 TOXICOKINETICS

Hexachloroethane has been found in the plasma of workers wearing protective clothing and respiratory protection suggesting that hexachloroethane can be absorbed following inhalation and/or dermal exposure. Based on the minimal effects seen on target tissues (liver and kidney) in animal studies, absorption from the lungs seems to be limited. Dermal absorption was also estimated to be low based on calculated dermal penetration rates.

Data on absorption across the gastrointestinal tract indicate that hexachloroethane is absorbed, but the percentage of a dose that is absorbed varies. Absorption estimates based on excretory products in rabbits suggest that a moderate portion of a 500 mg/kg dose (perhaps 40-50%) is absorbed. Data on excretory products from rats and mice indicate that a much larger portion (62-88%) of this same dose is absorbed.

Hexachloroethane distributes preferentially to the adipose tissue. Relatively high concentrations are also found in male rat kidneys. Moderate concentrations of hexachloroethane are found in the liver, female kidney, and blood and small amounts in muscle, lungs, and brain. If the hexachloroethane is generated endogenously from carbon tetrachloride, the concentration in the rat liver exceeds that in the kidneys.

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Hexachloroethane is metabolized by the mixed function oxidase system by way of a two-step reduction reaction involving cytochrome P-450 and either reduced nicotinamide adenine dinucleotide phosphate (NADPH) or cytochrome b, as an electron donor. The first step of the reduction reaction results in the formation of the pentachloroethyl free radical. In the second step, tetrachloroethene is formed as the primary metabolite. Two chloride ions are released. Pentachloroethane is a minor metabolic product that is generated from the pentachloroethyl free radical.

The primary metabolites of hexachloroethane are eventually oxidized to form trichloroethanol and trichloroacetic acid. These ultimate metabolites are excreted along with unchanged hexachloroethane, tetrachloroethene, and pentachloroethane. A small amount of the absorbed hexachloroethane is oxidized completely to carbon dioxide. Hexachloroethane and its metabolites are removed from the body in exhaled air, urine, and bile. In rats and mice, 60-70% of the radiolabeled hexachloroethane was in exhaled air and was present as volatiles other than carbon dioxide.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

No information was located regarding absorption in either humans or animals after inhalation exposure to hexachloroethane.

The minor effects of hexachloroethane on organs other than the lungs in animal studies indicates that absorption does occur, but is probably minimal. Given the lipophilic nature of hexachloroethane, absorption across the lung epithelium is possible.

2.3.1.2 Oral Exposure

No studies were located regarding absorption in humans following oral exposure to hexachloroethane. When sheep were administered a dose of 500 mg/kg hexachloroethane dissolved in olive oil and emulsified in water, absorption was slow based on the appearance of hexachloroethane in the venous blood (Fowler 1969b). The maximum concentration in blood was observed 24 hours after compound administration in one sheep.

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Based on the amount of label found in rabbit urine and exhaled air, 19-29% of a 500 mg/kg dose was absorbed (Jondorf et al. 1957). Since some hexachloroethane would be excreted in bile and found in fecal matter, the actual amount absorbed was larger than 30%, perhaps 40-50%.

Data from studies in rats and mice using ¹⁴C-radiolabeled hexachloroethane suggest that much higher proportions of a 500 mg/kg/day dose of hexachloroethane were absorbed (Mitoma et al. 1985). Rats exhaled 65% of the radiolabel in expired air and 6% in the excreta. This indicates that more than 65-70% of the hexachloroethane was absorbed. Comparable data from mice given 999 mg/kg/day indicate that more than 72-88% of the dose was absorbed. The radiolabel in expired air was 72% of the dose in mice and there was 16% of the label in the excreta (Mitoma et al. 1985).

Hexachloroethane is apparently absorbed to a greater extent when administered in corn oil than when administered in an aqueous medium, based on the fact that the LD₅₀ values for hexachloroethane dissolved in methyl cellulose solution are higher than those for a corn oil solvent in both male and female rats (Weeks et al. 1979). The ratio of LD₅₀ values suggests that about one-third less material is absorbed from an aqueous medium.

2.3.1.3 Dermal Exposure

Despite wearing protective equipment that included disposable overalls and compressed-air-fed visors or full-facepiece masks with filters for dusts and vapors, hexachloroethane was detected in the plasma of exposed workers (Selden et al. 1993). After 5 weeks of exposure, plasma levels of hexachloroethane in 12 workers were $7.3 \pm 6 \mu\text{g/L}$. Mild dermal irritation was also noted. If the skin irritation was a response to hexachloroethane rather than trauma from the protective clothing, the irritation suggests that the principal exposure route may have been dermal. Absorption of a saturated hexachloroethane solution across human skin was estimated to be $0.0230 \text{ mg/cm}^2/\text{hour}$ based on the physical properties of hexachloroethane (Fiserova-Bergerova et al. 1990).

No information was located regarding absorption of hexachloroethane in animals after dermal exposure.

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2.3.2 Distribution

2.3.2.1 Inhalation Exposure

No information was located regarding distribution in humans or animals following inhalation exposure to hexachloroethane.

2.3.2.2 Oral Exposure

No information was located regarding distribution in humans following oral exposure to hexachloroethane.

After oral exposure of rats to hexachloroethane for 8-16 weeks, the largest concentration of hexachloroethane was found in the adipose tissues (Gorzinski et al. 1985; Nolan and Karbowski 1978). The kidneys of male rats, but not females, also contained high concentrations of hexachloroethane. When rats were given doses of 3,30, or 100 mg/kg/day for 110-111 days, the concentration in the male kidney was four times larger than that in the female kidney at the lowest dose and 48 times larger at the highest dose (Nolan and Karbowski 1978). These proportions are very much like the relationship found with doses of 1, 15, or 62 mg/kg/day given to rats where the male kidney contained four times as much label as the female kidney at the low dose and 45 times as much as the female kidney at the high dose (Gorzinski et al. 1985).

Hexachloroethane is also found in the liver and blood after oral exposure to hexachloroethane, although the levels found in these tissues are much lower than those found in adipose tissue and male rat kidney (Gorzinski et al. 1985; Nolan and Karbowski 1978). With a dose of 1 mg/kg/day, adipose tissue samples from male rats contained 3.15 µg/g; the kidneys contained 1.36 µg/g; the liver, 0.29 µg/g; and the blood, 0.08 µg/g after 16 weeks of exposure (Gorzinski et al. 1985). In the female rat, the adipose tissue contained 2.59 µg/g; the kidneys, 0.39 µg/g; the liver, 0.26 µg/g; and the blood, 0.07 µg/g. As the doses were increased, the concentrations in the tissues also increased.

There is a relatively rapid turnover of hexachloroethane in the tissues. In studies where doses of 62 or 100 mg/kg/day hexachloroethane were fed in the diet for about 8 weeks, the level in the tissue decayed

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with a half-life of 2.3-2.7 days following first order kinetics (Gorzinski et al. 1985; Nolan and Karbowski 1978).

In sheep fed hexachloroethane in olive oil emulsified in water, the hexachloroethane was found primarily in the liver, kidneys, and adipose tissue 8 hours after exposure; much smaller amounts were found in brain and muscle 8 hours after exposure. The maximum concentration of hexachloroethane in blood occurred 24 hours after dosing (Fowler 1969b).

2.3.2.3 Dermal Exposure

No information was located regarding distribution in humans or animals after dermal exposure to hexachloroethane.

2.3.2.4 Other Routes of Exposure

The tissue distribution of intraperitoneal ¹⁴C hexachloroethane in male rats differed from that in male mice based on the concentrations that were bound to DNA, RNA, and protein (Lattanzi et al. 1988). In both species the highest concentrations of label were found in the kidney, followed by the liver, lungs, and stomach in descending order. The amount of bound label in the mice, however, was about twice that in the rat for both kidney and liver. The higher concentration of label in mouse liver may help to explain why hepatocellular cancer has been seen in mice but not in rats.

Hexachloroethane can be generated endogenously from exposure to carbon tetrachloride. Hexachloroethane is formed in the liver through the union of two trichloromethyl free radicals. The tissue distribution of endogenously generated hexachloroethane differed from that of exogenous hexachloroethane. After oral administration of 1 mL/kg carbon tetrachloride to rabbits, adipose tissue contained the highest concentration of hexachloroethane (4.1 ± 1.2 , 16.5 ± 1.6 , and 6.8 ± 2.4 ng/g) at 6, 24, and 48 hours (Fowler 1969a). This was similar to the distribution found after oral and intraperitoneal exposure to hexachloroethane (Gorzinski et al. 1985; Lattanzi et al. 1988; Nolan and Karbowski 1978). However, the amount in the liver was about twice that in the kidney at both 6 and 24 hours. At 6 hours, the liver contained 1.6 ± 0.5 ng/g and the kidney 0.7 ± 0.2 ng/g, while at 24 hours the liver contained 4.2 ± 1.8 ng/g and the kidney contained 2.2 ± 1.1 ng/g (Fowler 1969a). Only small amounts were found in the muscle.

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2.3.3 Metabolism

Most of the information on the metabolism of hexachloroethane has been collected by *in vitro* techniques using rat liver slices or rat liver microsomes. Figure 2-3 summarizes the results of these studies. The identification of tetrachloroethene and pentachloroethane as the initial metabolites of hexachloroethane metabolism *in vitro* agrees with *in vivo* data from sheep that were orally exposed to doses of 500-1,000 mg/kg hexachloroethane (Fowler 1969b).

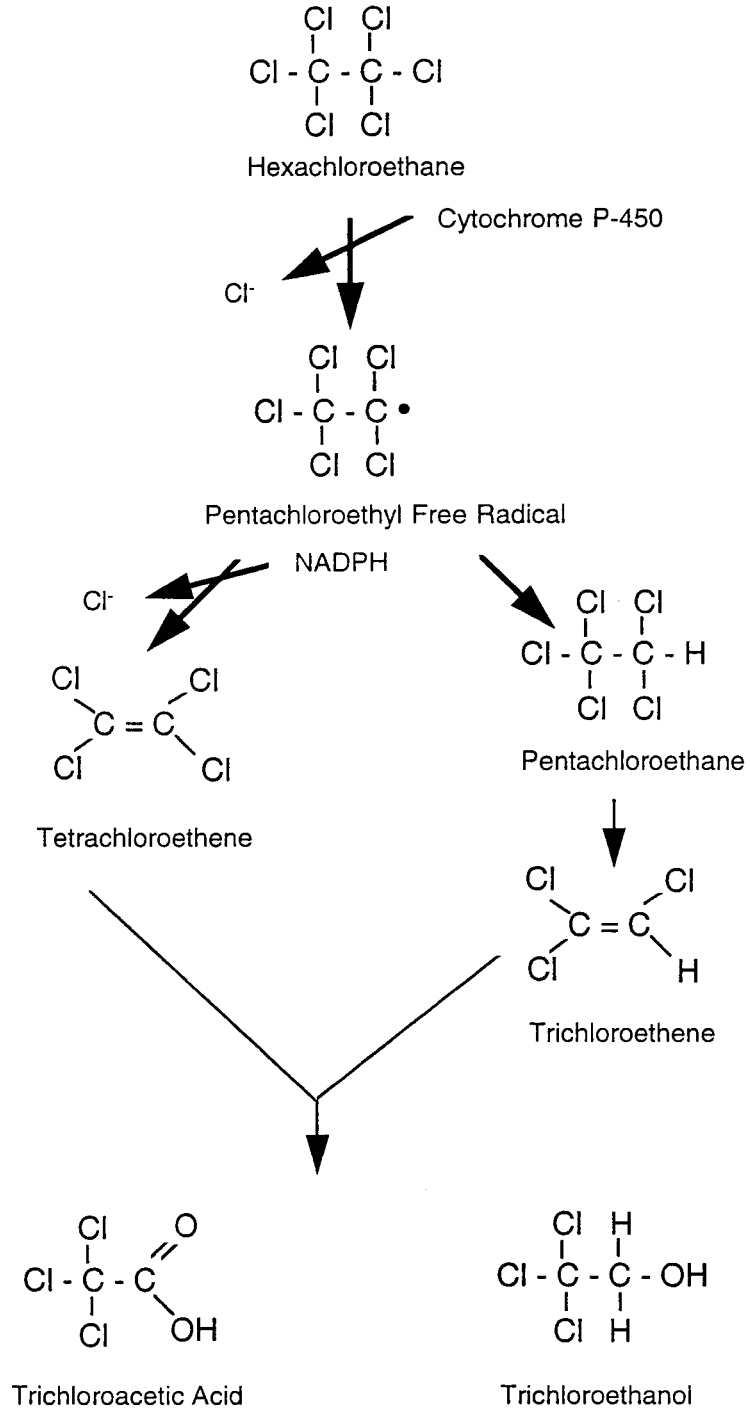
The initial steps of hexachloroethane metabolism take place in liver microsomes under anaerobic conditions (Nastainczyk et al. 1982a, 1982b; Salmon et al. 1981, 1985; Town and Leibman 1984). Cytosolic enzymes are minimally involved with hexachloroethane metabolism (Town and Leibman 1984). Hexachloroethane is dechlorinated in a two-step reduction reaction. In the first step, cytochrome P-450 contributes one electron to hexachloroethane, leading to the loss of a chloride ion and the formation of a pentachloroethyl free radical. In the second step, a second electron is contributed by either NADPH or cytochrome b₅ and a second chloride is lost, producing tetrachloroethene (Nastainczyk et al. 1982a). A smaller amount of the pentachloroethyl free radical becomes pentachloroethane by abstraction of a hydrogen atom from a hydrogen donor.

In studies using liver microsomes, approximately 99.5% of the hexachloroethane was converted to tetrachloroethene at physiological pHs (Nastainczyk et al. 1982b). When the reaction occurred at higher pHs (8.4-8.8), the ratio of pentachloroethane to tetrachloroethene was increased. The specific cytochrome P-450 involved in this series of reactions was stimulated by phenobarbital and not by 3-methylchloanthrene (Nastainczyk et al. 1982a; Salmon et al. 1985; Thompson et al. 1984; Town and Leibman 1984).

Both tetrachloroethene and pentachloroethane undergo subsequent hepatic metabolism. Pentachloroethane is reductively dechlorinated by microsomes to yield trichloroethene. (Reductive dechlorination was favored when there were three chlorines on one carbon and at least one chlorine on the vicinal carbon [Thompson et al. 1984], a characteristic shared by hexachloroethane and pentachloroethane). Trichloroethene and tetrachloroethene were then oxidized by hepatic enzymes to form trichloroethanol and trichloroacetic acid as terminal reaction products. Apparently additional dechlorination reactions can occur since labeled dichloroethanol, dichloroacetic acid, monochloroacetic acid, and oxalic acid have been

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FIGURE 2-3. Metabolism of Hexachloroethane



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found in the urine of animals given an oral dose of labeled hexachloroethane (Jondorf et al. 1957; Mitoma et al. 1985). Some hexachloroethane (about 2%) was completely dechlorinated and metabolized to carbon dioxide in rats and mice (Mitoma et al. 1985).

2.3.4 Excretion

2.3.4.1 I Inhalation Exposure

No data were located regarding excretion in humans or animals after inhalation exposure to hexachloroethane.

2.3.4.2 Oral Exposure

Orally ingested hexachloroethane is exhaled and excreted in urine and fecal matter. The portion of the hexachloroethane found in fecal matter is the result of excretion in bile. The results of studies that measured the amount of residual hexachloroethane in excreta can be misleading, since much of the absorbed hexachloroethane is metabolized to other compounds. Measurement of ¹⁴C label after exposure to labeled compound presents a more complete picture of ultimate hexachloroethane fate and excretion than measurement of hexachloroethane.

In rats and mice, 65-70% of an oral dose of radiolabeled hexachloroethane (500 mg/kg/day for rats and 999 mg/kg/day for mice) was present in exhaled air (Mitoma et al. 1985). Only about 2% of this amount was exhaled as carbon dioxide. The remainder was present as other volatile compounds. In rabbits, a much smaller portion of the label was found in exhaled air (14-24%) after oral administration of 500 mg/kg hexachloroethane. The amount of labeled carbon dioxide was not determined (Jondorf et al. 1957).

Relatively little hexachloroethane, pentachloroethane, and tetrachloroethene was found in the urine of sheep after oral administration of 500 mg/kg hexachloroethane (Fowler 1969b), and relatively little label (5%) was found in the urine of rabbits given 500 mg/kg (Jondorf et al. 1957). The major urinary metabolites were trichloroethanol and trichloroacetic acid in rats, rabbits, and mice (Jondorf et al. 1957; Mitoma et al. 1985). In rabbits, smaller amounts of dichloroethanol, dichloroacetic acid, monochloroacetic acid, and oxalic acid were also present (Jondorf et al. 1957).

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In sheep, 80% of the hexachloroethane, tetrachloroethene, and pentachloroethane fecal excretions were excreted within 24 hours (Fowler 1969b). Some of this was unabsorbed hexachloroethane and the remainder was material that had been absorbed and was excreted with the bile. Hexachloroethane was present in bile within 15 minutes of dosing and the concentration in bile was 8-10 times greater than that in blood at that time. Traces of hexachloroethane, tetrachloroethene, and pentachloroethane were present in the 48-72 hour fecal collections.

2.3.4.3 Dermal Exposure

No studies were located regarding excretion by humans or animals after dermal exposure to hexachloroethane.

2.4 Mechanisms of Action

The kidney and liver are the primary target organs for hexachloroethane based on the results of toxicity testing and supported by toxicokinetic information from tissue distribution and binding studies (Lattanzi et al. 1988). Male rats were more susceptible to kidney damage than female rats (NTP 1989), and the kidneys of male rats contained 4-45% more hexachloroethane radiolabel than the kidneys of female rats (Gorzinski et al. 1985). However, there were some effects on kidneys of both sexes.

The mechanism of toxicity leading to tubular nephropathy and renal tumorigenesis in male rats is related to the synthesis and excretion of the protein $\alpha_2\mu$ -globulin. This protein is synthesized in the liver and secreted into the blood. It is filtered through the glomeruli of the kidneys and partially reabsorbed through the proximal tubules where it is partially hydrolyzed (Swenberg 1993). The remainder is excreted, comprising 26% of the urinary protein (NTP 1989; Olson et al. 1990). Other species of laboratory animals, female rats, and humans produce minimal amounts of an $\alpha_2\mu$ -globulin-type protein. In the presence of hexachloroethane and other nonpolar hydrocarbons or their metabolites, $\alpha_2\mu$ -globulin accumulates in hyaline droplets in the tubular epithelium. The accumulation of hyaline droplets damages the epithelial cells, leading to exfoliation and the appearance of hyaline casts in the urine. Regenerative repair of the epithelium leads to hyperplasia and increases the risk for tumors when mutated cells divide before DNA repair can occur.

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Although binding of hexachloroethane to $\alpha_2\mu$ -globulin can explain kidney damage in male rats, it does not explain the less severe kidney changes in female rats (NTP 1989). Thus, other mechanisms must be involved in the nephrotoxicity of hexachloroethane. When DNA, RNA, and protein were isolated from kidney cells of male rats, it was found that hexachloroethane was bound more strongly to RNA and protein than to DNA (Lattanzi et al. 1988). The highest concentrations were found bound to RNA. Epigenetic interference with protein synthesis and cell function could lead to the kidney nephropathy seen in female rats and contribute to the damage in male rats. However, no studies were identified that would support this hypothetical mechanism.

Liver necrosis is another concern following hexachloroethane exposure. Hexachloroethane is metabolized in the centrilobular area of the liver by way of the microsomal mixed function oxidase system. The relatively nonpolar pentachloroethyl free radical is an intermediate in this pathway. The reaction of the free radical with unsaturated lipids in the cellular or organelle membranes could contribute to hepatocyte damage and necrosis.

Conjugated dienes and malondialdehyde serve as markers for free radical-induced lipid peroxidation. There was a uniform increase in malondialdehyde in eight assays of rat liver microsomes that were incubated with hexachloroethane (Town and Leibman 1984). Conjugated dienes were increased in some, but not all, of the samples. No changes were seen in the concentration of conjugated dienes in the hepatic endoplasmic reticulum of male rats, 2 hours after hexachloroethane exposure (Reynolds 1972). The authors hypothesized that the poor solubility of hexachloroethane in body fluid and the use of a mineral oil solvent limited the concentration of hexachloroethane in the liver at 2 hours and, thus, the lack of its effects on conjugated dienes could not be used to eliminate the possibility of free radical cellular damage at a later point in time. Although limited, the data provide some support for a free radical mechanism for the hepatic toxicity of hexachloroethane.

Clinical signs of neurotoxicity (tremors and ataxia) have been observed in sheep, dogs, and rats during or immediately after both oral and inhalation exposure. Sometimes tremors developed early in the treatment regime and other times the tremors became apparent only after repeated exposures. Fluke-infected sheep experienced tremors of the facial muscles, neck, and forelimbs and were unable to stand after treatment with hexachloroethane. They were successfully treated with calcium borogluconate. This suggests that the neurological action of hexachloroethane may be the result of interference with the availability of calcium within excitable cells.

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2.5 RELEVANCE TO PUBLIC HEALTH

Hexachloroethane is a solid crystalline material that has entered the environment as a result of its use in military pyrotechnics and as a component of smoke-producing devices used for screening or signaling purposes. It is an intermediate in the production of fluorocarbons, cleaning agents, and refrigerants and was once used in veterinary medicine to control liver flukes in sheep. It can be found at military disposal sites and at hazardous waste sites. In addition, hexachloroethane can be formed during incineration of chlorinated organic compounds and during chlorination of drinking water. Accordingly, there is some risk that humans can be exposed to this material.

Respiratory, hematological, liver, and renal effects were not observed in 11 hexachloroethane-exposed workers. The identification of hexachloroethane in the plasma of these workers confirmed exposure, although the workers were wearing protective equipment. Mild dermal irritation was noted that may have been from exposure or a result of a local trauma effect of the protective equipment.

Animal studies identify the kidney and liver as the primary target organs for hexachloroethane. Renal problems were most severe in male rats and were associated with $\alpha_2\mu$ -globulin/hyaline droplet nephropathy. Minimal to mild lesions were also seen in female rat kidneys and in male and female mice, indicating that some mechanism, in addition to hyaline droplet formation, is involved in renal toxicity. The liver responds to hexachloroethane exposure with increases in liver weight, increases in serum levels of liver enzymes, centrilobular necrosis, fatty degeneration, hemosiderin-laden macrophages, and hemorrhage. Effects on the liver and kidneys were mild with inhalation exposure and more pronounced with oral exposure. No data were available for effects on the liver and kidneys by the dermal exposure route.

Hexachloroethane vapors and ingested hexachloroethane act as irritants on the lining of the lung, nasal cavity, trachea, and other tissues of the respiratory tract. Pulmonary irritation was associated with an increased incidence of mycoplasma infection in rats. Hexachloroethane exposure can also irritate the eyes. The irritation of the eye and respiratory tract are reversible once exposure has ceased.

Both oral and inhalation exposures to high concentrations of hexachloroethane were associated with hyperactivity, ataxia, convulsions, and/or prostration in rats, sheep, and dogs. The mechanism for these

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neurological effects is not clear since there were no apparent histopathological lesions in the brains of the affected animals. Neurological effects were only noted with the high-dose exposures.

There has been no comprehensive evaluation of the reproductive and developmental effects of hexachloroethane. Limited data indicate that it is maternally toxic and retards fetal development. It does not appear to be a teratogen.

Minimal Risk Levels for Hexachloroethane

Inhalation MRLs

- An MRL of 6 ppm has been derived for acute inhalation exposure to hexachloroethane. This MRL is based on a study in pregnant female rats exposed to concentrations of 0, 15, 48, or 260 ppm hexachloroethane for 6 hours/day on gestation days 6-16 (Weeks et al. 1979). Tremors were observed in the 260-ppm dose group during exposure starting on day 12 and persisting through day 16. Excess mucus was present in the nasal turbinates of all of the dams in the 260-ppm dose group and 85% of the dams in the 48-ppm dose group. This effect was not observed at 48 ppm in the 6-week study, and the endemic mycoplasma infection that was present in the colony of rats used in the Weeks et al. (1979) study may have contributed to this effect. Based on the NOAEL of 48 ppm for neurological effects observed in the teratology study by Weeks et al. (1979), an acute inhalation MRL was calculated by adjusting to a Human Equivalent Concentration (HEC) of 181 ppm using reference ventilation rates (rat, 0.22 m³/day; human, 20 m³/day) and body weights (rat, 0.204 kg; human, 70 kg) from EPA (1988a) and by dividing by an uncertainty factor of 30. A factor of 3 was used to extrapolate from animals to humans, and a factor of 10 was used to account for human variability.
- An MRL of 6 ppm has been derived for intermediate-duration inhalation exposure to hexachloroethane. This MRL is based on the 6-week study in rats by Weeks et al. (1979) in which tremors were observed at 260 ppm but not at 48 ppm. Based on the NOAEL of 48 ppm for neurological effects observed in the 6-week study (Weeks et al. 1979), an intermediate inhalation MRL was calculated by adjusting the NOAEL to an HEC of 174 ppm using reference ventilation rates (rat, 0.245 m³/day; human, 20 m³/day) and body weights (rat, 0.236 kg; human, 70 kg) from EPA (1988a) and by dividing by an uncertainty factor of 30. A factor of 3 was used

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to extrapolate from animals to humans, and a factor of 10 was used to account for human variability.

A chronic MRL for inhalation exposure has not been derived because no data were located on the effects of long-term exposures in humans or animals.

Oral MRLs

- An MRL of 1 mg/kg/day has been derived for acute oral exposure to hexachloroethane. This MRL was derived from a NOAEL of 100 mg/kg/day in a study where groups of five male New Zealand rabbits were given doses of 0, 100, 320, and 1,000 mg/kg/day hexachloroethane dissolved in methyl cellulose solution by gavage for 12 days (Weeks et al. 1979). Dose-dependant liver degeneration and necrosis were noted at dose levels of 320 and 1,000 mg/kg/day. Effects were characterized as fatty degeneration, coagulation necrosis, hemorrhage, ballooning degeneration, eosinophilic change, hemosiderin-laden macrophages and giant cells. Comparable effects were not seen in the 100-mg/kg/day dose group. Liver weights increased at the highest dose tested; however, quantitative data were not provided. The NOAEL of 100 mg/kg/day was divided by an uncertainty factor of 100, 10 each for interspecies and intraspecies variability.
- Toxic tubular nephrosis and minimal nephrocalcinosis of the convoluted tubules were seen at dose levels of 320 and 1,000 mg/kg/day, however comparable effects were not seen at the lowest dose tested. Kidney weights increased significantly ($p < 0.05$) at the highest dose tested. For the most part, serum clinical parameters (blood urea nitrogen, protein bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and sodium) were not affected significantly. Levels of potassium and glucose were decreased significantly at dose levels of 320 mg/kg/day or greater. Body weights were reduced significantly ($p < 0.05$) at exposure levels of 320 and 1,000 mg/kg/day. Quantitative data were not provided for any of the effects noted in this study, although the degree of significance and the dose-related nature of the effects were included in the discussion of the results.
- An MRL of 0.01 mg/kg/day has been derived for intermediate oral exposure to hexachloroethane. The MRL was derived from a NOAEL of 1 mg/kg/day in a study where

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groups of 10 male and 10 female rats were given hexachloroethane at 1, 15, or 65 mg/kg/day in the diet for 16 weeks (Gorzinski et al. 1985). All rats survived the 16-week exposure period and there were no clinical signs of compound toxicity. Organ weights were not significantly different than those of the controls except for absolute and relative liver weights and kidney weights of males treated at the highest dose. Swelling of the hepatocytes was present in males at the two highest doses. At the 15-mg/kg dose, swollen hepatocytes were noted in 6 of 10 males, and 8 of 10 males were affected at the 62-mg/kg/day dose. Swollen hepatocytes were seen in four control males and in three males from the lowest dose group. Hepatocyte size was not affected in females, but absolute and relative liver weights were increased in the highest dose group. The male rats exhibited renal tubular atrophy, hypertrophy, dilation, and degeneration for both the 15- and 62-mg/kg/day doses. Atrophy and tubular degeneration was also present in 6 of 10 females at the 62-mg/kg/day dose and 2 of 10 females at the 15 mg/kg/day dose. The 15 mg/kg/day dose was identified as the LOAEL in this study with the 1 -mg/kg/day dose as the NOAEL. This NOAEL was used with an uncertainty factor of 100 (10 each for interspecies and intraspecies variability) to derive the MRL.

Insufficient information was available to derive a chronic-duration oral exposure MRL for hexachloroethane.

Death. No studies were located regarding lethality in humans after exposure to hexachloroethane. LD₅₀ values for animals range from 4,460 to 5,160 mg/kg when hexachloroethane is administered by gavage in corn oil and from 7,080 to 7,690 mg/kg when administered in an aqueous methyl cellulose solution (Weeks et al. 1979). The higher LD₅₀ value for the aqueous solution indicates that absorption from this medium is lower than from a digestible food oil. When exposures occurred by the inhalation route, 1 of 6 rats died during an 8-hour exposure to 5,900 ppm (Weeks et al. 1979). At this concentration, the inhalation chamber contained crystalline hexachloroethane as well as hexachloroethane vapors. The dermal LD₅₀ was greater than 32,000 mg/kg when hexachloroethane was applied to shaved rabbit skin for 24 hours (Weeks et al. 1979). This suggests poor dermal absorption of hexachloroethane and agrees with a calculated low dermal absorption rate of 0.023 mg/cm²/hr based on physical properties (Fiserova-Bergerova et al. 1990).

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There were some deaths among dogs, rats, and guinea pigs with exposure to 260 ppm in air for 6 weeks (Weeks et al. 1979). Following oral exposure, death occurred in rats at doses of 750 mg/kg/day (NTP 1989) and in mice at doses of 1,780 mg/kg/day (NTP 1977). Lower doses were nonlethal.

Chronic oral exposure to 212 mg/kg/day shortened the life expectancy of male and female rats (NTP 1977), but doses of 20 mg/kg/day in males and 160 mg/kg/day in females did not (NTP 1989). Although mice were exposed to oral doses of 590 and 1,170 mg/kg/day hexachloroethane for 78 weeks, poor survival among male controls made it difficult to evaluate the effects of hexachloroethane. Survival for the high-dose females was slightly less than that for vehicle controls, but the differences were not significant (NTP 1977).

LD₅₀ values and the lowest lethal doses for acute- and intermediate-duration exposures classify hexachloroethane as slightly toxic (Hodge and Sterner 1949). It is unlikely that exposures to hexachloroethane at levels found at hazardous waste sites would cause death in humans.

Systemic Effects

There are no data for cardiovascular, gastrointestinal, musculoskeletal, endocrine, or ocular effects in humans following exposure to hexachloroethane by any route. Data are available for inhalation and oral exposures in several animal species. The only available dermal exposure data apply to dermal and ocular effects.

Respiratory Effects. Pulmonary function tests were not affected in workers exposed to hexachloroethane for 5 weeks while wearing protective equipment (Selden et al. 1994). Acute exposure of rats to 5,900 ppm hexachloroethane (a combination of gaseous and microcrystalline material) resulted in interstitial pulmonary pneumonitis (Weeks et al. 1979). These pulmonary lesions were seen after a 14-day recovery period. The entrapment of solid hexachloroethane particles in the lungs could have contributed to the symptoms observed.

Excess mucus in the nasal turbinates, irritation of the epithelium, and increased incidence of a mycoplasma respiratory infection were seen in rats with inhalation exposure to 260 ppm for 6 weeks and in pregnant rats with inhalation exposure to 48 ppm for 11 days. Pulmonary irritation was also present in pregnant rats treated with an oral dose of 500 mg/kg/day for 11 days (Weeks et al. 1979). Effects on the respiratory

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epithelium were not apparent in the tissue of the lungs, nasal cavity, nasal turbinates, larynx, trachea, or bronchi based on histopathological examination (NTP 1977, 1989; Weeks et al. 1979). Exposure to hexachloroethane, especially in its vaporous state, may weaken the effectiveness of respiratory tract mucus as an antimicrobial barrier and, thus, increase the incidence of pulmonary infections in exposed animals. Alternatively, it may weaken disease resistance by some other mechanism. Humans exposed to hexachloroethane vapors in the environment could experience an increased risk of respiratory tract infections.

Cardiovascular Effects. There were no histopathological effects on the heart after inhalation or oral exposure at any concentration tested (15-5,900 ppm for the inhalation route and 1-750 mg/kg/day for the oral route) and with acute, intermediate, or chronic exposure durations (Gorzinski et al. 1985; NTP 1977, 1989; Weeks et al. 1979). The risk that humans will experience adverse effects on the cardiovascular system as the result of exposure to hexachloroethane through the environment seems to be relatively low.

Gastrointestinal Effects. There were no histopathological effects on the stomach, small intestines, or large intestines with inhalation or oral exposure to hexachloroethane at any concentration tested (15-5,900 ppm for the inhalation route and 1-750 mg/kg/day for the oral route) and with acute, intermediate, or chronic exposure durations (Gorzinski et al. 1985; NTP 1977, 1989; Weeks et al. 1979). The risk that humans will experience adverse effects on the gastrointestinal system as the result of exposure to hexachloroethane in the environment seems to be relatively low.

Hematological Effects. Routine blood parameters (hemoglobin, erythrocyte, leukocyte and thrombocyte levels) measured in 11 hexachloroethane-exposed workers who wore protective clothing did not differ from those of the controls (Selden et al. 1994). Hexachloroethane plasma levels in these workers were $7.3 \pm 6 \mu\text{g/L}$ (Selden et al. 1993).

The effects of acute exposures to hexachloroethane on hematological parameters were not evaluated in animals. Inhalation doses of 260 ppm for 6 weeks had no effect on erythrocyte counts in dogs (Weeks et al. 1979) and oral exposures of up to 62 mg/kg/day for 16 weeks had no effect on red cell counts, hemoglobin concentrations, or white cell counts in rats (Gorzinski et al. 1985). These results suggest that hexachloroethane does not affect hematological parameters, but there are relatively few data upon which to base this conclusion. On the basis of the existing data, the occurrence of hexachloroethane at hazardous waste sites should not pose a significant hematological risk for humans.

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Musculoskeletal Effects. Neither inhalation nor oral exposures to hexachloroethane were associated with histopathological changes in skeletal muscle or bone in rats following acute-, intermediate-, or chronic duration exposures with inhalation exposure concentrations of 15-5,900 ppm or oral exposure doses of 1-750 mg/kg/day (Gorzinski et al. 1985; NTP 1977, 1989; Weeks et al. 1979). More comprehensive data pertaining to the musculoskeletal system were not identified. Based on the data available, there appears to be no risk for musculoskeletal effects for those who live or work near a hazardous waste site.

Hepatic Effects. Liver function tests (serum bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase) were not affected in 11 hexachloroethane-exposed workers who wore protective clothing (Selden et al. 1993).

Animal studies indicate that hepatic tissues are moderately vulnerable to exposure to hexachloroethane especially when exposure occurs by the oral route. With acute- and intermediate-duration inhalation exposures, the only effects noted were an increase in liver weight in rats and guinea pigs, but not dogs or quail, after 6 weeks of exposure to 260 ppm (Weeks et al. 1979). There were no observable histopathological changes in the tissues that accompanied the organ weight change and no histopathological changes with acute exposure to an even higher hexachloroethane concentration (5,900 ppm).

When exposures occurred by the oral route, increased liver weights, increases in serum liver enzyme levels, centrilobular necrosis, fatty degeneration, hemosiderin-laden macrophages, and hemorrhage were noted in animals following acute- and intermediate-duration exposures (Fowler 1969b; Gorzinski et al. 1985; NTP 1989; Weeks et al. 1979). The lowest LOAEL for these effects was a dietary dose of 15 mg/kg/day for 16 weeks which was associated with enlargement of the hepatocytes in males (Gorzinski et al. 1985). However, there were no observable adverse effects on tissue histopathology in male rats given 20 mg/kg/day for 2 years or in females given 160 mg/kg/day for the same period of time (NTP 1989). Organ weights were not determined for the chronic exposures. These data suggest that there is a potential for individuals who might be exposed to hexachloroethane from a contaminated drinking water supply to experience hepatic effects. The risk from other exposure routes (inhalation or dermal) due to contaminated hazardous waste sites is probably minimal.

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Renal Effects. No effects on renal function tests (serum creatinine and urate, urinary hemoglobin, protein and glucose) were noted in 11 hexachloroethane-exposed workers who wore protective clothing (Selden et al. 1994).

Acute exposure to concentrations of 260-5900 ppm hexachloroethane had minimal effects on the kidney. There was an increase in kidney weights in male rats exposed to 260 ppm hexachloroethane for 6 weeks but no discernable effects on tissue histopathology (Weeks et al. 1979). This same exposure concentration had no effect on female rats or on male or female dogs, guinea pigs, or quail under parallel exposure conditions.

Acute-, intermediate-, and chronic-duration oral exposures of male rats to doses of 10 mg/kg/day or greater were associated with renal tubular nephropathy (Gorzinski et al. 1985; NTP 1977, 1989; Weeks et al. 1979). Affected animals displayed tubular necrosis, hyaline droplets in tubular epithelial cells, regenerative tubular epithelium, interstitial nephritis, and fibrosis. The severity of the renal lesions varied with the dose and the duration of exposure.

Hexachloroethane is a member of a family of compounds that bind to the male rat excretory protein $\alpha_2\mu$ -globulin and form hyaline droplets in the tubular epithelium leading to necrosis and repair hyperplasia (Borghoff 1993; Olson et al. 1990). The hexachloroethane metabolites tetrachloroethene and pentachloroethane are also members of this family of compounds (Borghoff 1993; Swenberg 1993). Female rats and laboratory animals from other species synthesize only minimal quantities of this protein and, thus, have a lower risk for renal effects. In male rats, $\alpha_2\mu$ -globulin accounts for 26% of the urinary protein, and chemicals that bind with it have a strong tendency to accumulate in the kidney causing cellular damage.

Mild to moderate nephropathy in female rats exposed to 80 or 160 mg/kg/day for 2 years, a high incidence of nephropathy in mice exposed to 590 or 1,179 mg/kg/day for 78 weeks, and nephrosis in rabbits exposed to 320 or 1,000 mg/kg/day for 12 days, indicate that hexachloroethane has an effect on the kidney that is independent of $\alpha_2\mu$ -globulin (NTP 1977, 1989; Weeks et al. 1979). Thus, the public health risk for renal effects should be considered when evaluating the possible effects of human exposure to hexachloroethane at hazardous waste sites.

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Endocrine Effects. Histological changes have not been observed in the pancreas or adrenal glands of rats, guinea pigs, dogs, or quail following inhalation exposure to hexachloroethane at concentrations up to 260 ppm for 6 weeks (Weeks et al. 1979). Intermediate-duration oral treatment of rats with hexachloroethane at doses up to 62 mg/kg/day also did not result in histological changes in the pancreas, adrenal glands, thyroid, or parathyroid glands (Gorzinski et al. 1985). Chronic gavage treatment of male rats with hexachloroethane at 10 or 20 mg/kg/day did result in an increased incidence of pheochromocytomas in the adrenal gland (NTP 1989). The relevance of this effect to humans is not clear.

Dermal Effects. Hexachloroethane-exposed workers reported a slightly higher prevalence of dry skin and dry mucous membranes as well as itching and other skin problems than the unexposed controls (Selden et al. 1994). Clinical examinations of the 11 exposed workers did not reveal signs of abnormal dermatological status. The investigators indicate that the dermal effects may also have been a result of a local trauma effect of the protective equipment.

There was no evidence that crystalline hexachloroethane affected the skin of animals with either inhalation or oral exposures of acute, intermediate, or chronic durations (Fowler 1969b; Gorzinski et al. 1985; NTP 1977, 1989; Weeks et al. 1979). When a water paste was placed on the shaved skin of rabbits for 24 hours, there was only a slight redness as the result of contact (Weeks et al. 1979).

The concentrations of hexachloroethane that might be found at hazardous waste sites are unlikely to act as skin irritants in humans.

Ocular Effects. Inhalation and oral exposure of animals to hexachloroethane caused lacrimation and reddening of the eyes after oral exposure (NTP 1977, 1989), or closing of the eyes as an avoidance mechanism during inhalation exposure (Weeks et al. 1979). Overnight, direct contact of the eyes with crystalline hexachloroethane resulted in corneal opacity and iritis in rabbits, but recovery was complete 3 days later (Weeks et al. 1979). Direct eye contact with hexachloroethane at hazardous waste sites may result in an eye irritation.

Body Weight Effects. Decreased weight gains occurred in rats in response to both acute inhalation exposure to a high concentration of hexachloroethane (5,900 ppm) and intermediate-duration exposures to a lower concentration (260 ppm) (Weeks et al. 1979). Oral exposures were also associated with decreased weight gains with doses of 320 mg/kg/day or greater for 12 days in rabbits (Weeks et al. 1979) and with

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562 mg/kg/day or greater for 6-16 weeks in rats (NTP 1977, 1989). Female rats exposed to 750 mg/kg/day for 16 days actually lost 25% of their initial body weight (NTP 1989). Decreased weight gain occurred in mice at doses of 1,760 mg/kg/day (NTP 1977). In light of these findings, the concentrations of hexachloroethane found at hazardous waste sites are unlikely to be of great enough magnitude to have an effect on body weight in humans.

Immunological and Lymphoreticular Effects. No studies were located regarding immunological effects in humans after exposure to hexachloroethane. In addition, there were no data from comprehensive studies of immune response in animals for exposure by any route and for any duration. When the tissue histopathology of the spleen, thymus, and, in one case, lymph nodes were evaluated, no abnormalities were noted (Gorzinski et al. 1985; NTP 1989; Weeks et al. 1979). After 6-week inhalation exposures to 260 ppm hexachloroethane, the relative spleen weight was increased in young, but not in older, male rats. Data on dermal sensitization in guinea pigs indicate that exposure to low levels of hexachloroethane does not elicit antibody formation leading to an allergic dermatological response (Weeks et al. 1979).

An increased incidence in mycoplasma infections in rats exposed to 260 ppm hexachloroethane for 6 weeks suggests that hexachloroethane might weaken resistance to infection (Weeks et al. 1979). This could be the result of either a change in the quantity or consistency of the respiratory tract mucus or a systemic weakening of the immune system. The data are inadequate to formulate any hypothesis regarding the mechanism for diminished host resistance or to postulate whether hexachloroethane in the environment might lower the resistance of humans to respiratory infections.

Neurological Effects. No studies were located regarding neurological effects in humans after exposure to hexachloroethane. Inhalation, oral, and dermal exposure of animals to moderate or high doses (260 ppm, 5,900 ppm, 375 mg/kg/day, 750 mg/kg/day) resulted in hyperactivity, tremors, fasciculation of the facial muscles, ataxia, convulsions, and/or prostration (Fowler 1969b; NTP 1977, 1989; Southcott 1951; Weeks et al. 1979). Reduced motor activity has also been observed following oral exposure of pregnant rats (167 mg/kg/day) (Shimizu et al. 1992). Inhalation exposure of rats to 260 ppm for 6 weeks did not have any effect on spontaneous motor activity or shock avoidance behavior (Weeks et al. 1979).

Ataxia, tremors, and prostration in sheep given hexachloroethane (170 or 338 mg/kg) for a liver fluke infection were successfully ameliorated with calcium as calcium borogluconate. This suggests that the neurological action of hexachloroethane may be the result of interference with the availability of calcium

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within excitable cells. This mechanism would explain the transient nature of the hexachloroethane neurotoxicity and is compatible with the low affinity that hexachloroethane shows for brain tissue (Fowler 1969b).

There were no effects at any of the doses tested on the histopathology of the brain for any duration or route of exposure (Gorzinski et al. 1985; NTP 1977, 1989; Weeks et al. 1979). This observation is consistent with tissue distribution studies which indicate that hexachloroethane has no particular affinity for the brain tissues (Fowler 1969b).

Based on the available data, the concentrations of hexachloroethane at hazardous waste sites are unlikely to reach levels that would elicit a neurological response in humans. However, there have not been any comprehensive studies of brain or nerve function after exposure to hexachloroethane.

Reproductive Effects. No studies of reproductive effects in humans were located. In animals, hexachloroethane adversely affected fertility following oral exposure, but no effects were reported following inhalation exposure (Weeks et al. 1979). The absence of quantitative data on reproductive parameters, as well as evaluation of parameters that are pertinent to the assessment of reproductive risk, precludes any meaningful determination of the potential for hexachloroethane to cause adverse effects on human reproduction.

Developmental Effects. No studies were located regarding the developmental effects of hexachloroethane in humans. Fetal body weights were reduced, late-gestation resorptions were increased, and the degree of ossification was reduced in offspring from pregnant rats treated orally with hexachloroethane at 500 mg/kg/day on gestation days 7-17 (Shimizu et al. 1992) or gestation days 6-16 (Weeks et al. 1979). The 500-mg/kg/day dose also caused a significant decrease in maternal body weights. No effect on the number of fetuses with anomalies was observed, and no fetal or maternal effects were observed at lower doses. These studies suggest that in the absence of maternal effects, developmental effects in humans are unlikely.

Genotoxic Effects. No studies were located regarding the genotoxic effects of hexachloroethane in humans after inhalation, oral, or dermal exposure. *In vitro* studies of hexachloroethane using microbial, fungal, and rodent cell assays are summarized in Table 2-4. Tests of prokaryotic cell systems failed to detect gene mutation (Haworth et al. 1983; Roldan-Arjona et al. 1991; Simmon and Kauhanen 1978;

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

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
<i>Salmonella typhimurium</i> (TA98, TA100, TA1535, TA1537)	Gene mutation	-	-	Haworth et al. 1983
<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Gene mutation	-	-	Weeks et al. 1979
<i>S. typhimurium</i> (BA13)	Gene mutation	-	-	Roldan-Arijona et al. 1991
<i>S. typhimurium</i> (TA98, TA100, TA1535, TA1537, TA1538)	Gene mutation	-	-	Simmon and Kauhanen 1978
<i>S. typhimurium</i> (TA1535/psk1002)	DNA damage	-	-	Nakamura et al. 1987
Eukaryotic organisms:				
Fungi:				
<i>Saccharomyces cerevisiae</i> D ₄	Gene mutation	-	-	Weeks et al. 1979
<i>S. cerevisiae</i> D ₃	DNA damage/repair	-	-	Simmon and Kauhanen 1978
<i>S. cerevisiae</i>	Gene mutation	-	-	Bronzetti et al. 1989
<i>Aspergillus nidulans</i> diploid strains P1	Chromosomal abberation	-	Not tested	Crebelli et al. 1988
Mammalian cells:				
Chinese hamster ovary	Chromosomal aberration	-	-	Galloway et al. 1987
Chinese hamster ovary	Sister chromatid exchange	+	-	Galloway et al. 1987
Mouse (Balb/C-3T3)	Cell transformation	Not tested	-	Tu et al. 1985

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

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Weeks et al. 1979) or DNA damage (Nakamura et al. 1987) following hexachloroethane treatment. Similar results were reported for eukaryotic cells. Hexachloroethane did not cause gene mutation in cells harvested from the stationary growth phase (Bronzetti et al. 1989) or DNA damage in yeast (*Saccharomyces cerevisiae*) (Simmon and Kauhanen 1978), chromosomal aberrations in fungi (*Aspergillus nidulans*) (Crebelli et al. 1988), chromosomal aberrations in Chinese hamster ovary cells (Galloway et al. 1987), or cell transformations in mouse cells (Tu et al. 1985). Hexachloroethane did cause sister chromatid exchanges in Chinese hamster ovary cells in the presence of activation; however, the overall importance of this response is reduced since these effects occurred at doses that were cytotoxic (e.g., induced cell cycle delay) (Galloway et al. 1987). Similarly, hexachloroethane induced a significant ($p < 0.01$) increase of gene conversion in *S. cerevisiae* cells harvested from the logarithmic growth phase. Similar effects were not seen in stationary growth phase cells, both with and without metabolic activation (Bronzetti et al. 1989). Because cells of this sort contain a high level of cytochrome P-450, it is plausible that the positive responses were due to metabolites rather than the parent compound.

Cancer. Only one report was located regarding an association between hexachloroethane and cancer in humans (Selden et al. 1989). In this study a liver tumor was found in an adult male who had used a product containing hexachloroethane at work for 6 years. However, under the conditions of use, the hexachloroethane reacted to form hexachlorobenzene and other chlorinated compounds which were as likely, or more likely, to have contributed to the tumorigenesis as the hexachloroethane.

Lifetime exposure of rats to hexachloroethane resulted in renal carcinomas and adenomas in Fischer-344 male rats (NTP 1989). The incidence of adenomas was 1/50 for the controls, 2/50 for animals at a dose of 10 mg/kg/day, and 4/50 for animals at a dose of 20 mg/kg/day. In the animals from the high-dose group, there were also 3/50 renal carcinomas. The number of tumors (carcinomas and adenomas) was significantly greater in exposed rats than in both controls and historical controls using the Fisher Exact Test (NTP 1989). No tumors were seen in the female rats.

In an earlier study, there were renal tubular cell adenomas in 5/50 Osborne-Mendel rats receiving doses of 212 mg/kg/day but no tumors in 49 animals receiving 423 mg/kg/day or in 20 vehicle control rats (Weisburger 1977). Despite the lack of tumors, there was a high incidence of nephropathy (18-66%) in exposed male and female rats.

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The male rat kidney is susceptible to the induction of tumors because of $\alpha_2\mu$ -globulin excretion (Borghoff 1993; Olson et al. 1990). This protein is not made by female rats, other laboratory species, or humans in significant quantities, but large amounts are synthesized and excreted by male rats. EPA (1991a) has concluded that renal tumors in male rats that are associated with $\alpha_2\mu$ -globulin should not be used in assessing the potential for any chemical to cause renal tumors in humans. Compounds that bind to $\alpha_2\mu$ -globulin lead to the formation of hyaline droplets in the kidney causing cell damage and regenerative hyperplasia (Borghoff 1993; Olson et al. 1990).

A statistically significant increase in hepatocellular carcinomas was seen in male and female mice that were dosed with 590 and 1,179 mg/kg/day hexachloroethane in corn oil by gavage for 78 continuous weeks (Weisburger 1977). The incidence of tumors in the exposed mice was greater than that in controls on the basis of both the Fisher Exact test and the Cochran-Armitage test. There were no hepatic tumors in male or female rats with chronic exposure to doses of 10423 mg/kg/day (NTP 1977, 1989; Weisburger 1977).

Hexachloroethane may function as a promoter rather than an inducer of hepatic tumors. When male rats were given a single dose of 497 mg/kg hexachloroethane followed by daily treatment with a known promoter (phenobarbital) for 7 weeks, there was no increase in the number of GGT+ foci in the liver (Milman et al. 1988). GGT+ foci are markers for precarcinogenic cell changes. When a single dose of a known initiator dimethylnitrosamine was followed by 7 weeks of dosing with 497 mg/kg/day hexachloroethane, the number of GGT+ foci was four times the number seen with a single dose of dimethylnitrosamine in the absence of hexachloroethane treatment. The fact that hexachloroethane does not appear to be mutagenic in short-term tests of genetic toxicity and that it has a low tendency to bind to DNA (Lattanzi et al. 1988) is consistent with classifying it as a promoter rather than a direct acting carcinogen.

NTP determined that there was clear evidence of carcinogenicity in male rats based on the increased incidence of renal neoplasms and no evidence of carcinogenic activity in female rats (NTP 1989). The EPA classified hexachloroethane as a possible human carcinogen (Group C). The slope factor calculated by EPA is $1.4 \times 10^{-2} \text{ (mg/kg/day)}^{-1}$ for both the oral and inhalation routes of exposure (IRIS 1995). IARC has determined that hexachloroethane is not classifiable as to human carcinogenicity (Group 3).

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2.6 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).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. 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/NCR 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 hexachloroethane are discussed in Section 2.6.1.

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 hexachloroethane are discussed in Section 2.6.2.

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

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biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.8, "Populations That Are Unusually Susceptible."

2.6.1 Biomarkers Used to Identify or Quantify Exposure to Hexachloroethane

Based on results from animal studies, urinary and fecal excretion of hexachloroethane can be used to identify recent exposures (Fowler 1969b; Jondorf et al. 1957). Recent exposure to hexachloroethane can also be determined by measuring the amount of hexachloroethane in the blood, but this would be a more invasive procedure than analyzing urine or fecal matter (Fowler 1969b). The concentrations of hexachloroethane in fecal matter were higher than those in urine in sheep for the 24-hour period following exposure (Fowler 1969b). Thus, fecal matter might be better for analysis than urine.

Both hexachloroethane and its lipophilic metabolites can distribute to body fat. Only hexachloroethane can be used to confirm compound exposure by way of a fat biopsy, since some of its metabolites are also produced from other chlorinated hydrocarbons or are present as contaminants in the environment. Based on one worker occupationally exposed to hexachloroethane, Selden et al. (1993) estimated that the plasma half-life in humans was several days, but less than one week. A clearance half-life in rats of 2.5 days was reported for hexachloroethane absorbed from the diet (Gorzinski et al. 1985). Therefore, similar to measurement of hexachloroethane in blood, urine, and feces, hexachloroethane in body fat is representative of current exposures rather than exposures that occurred weeks or months before testing.

2.6.2 Biomarkers Used to Characterize Effects Caused by Hexachloroethane

No information was located regarding adverse health effects of hexachloroethane in humans; therefore, no judgment can be made concerning possible biomarkers of exposure in humans.

Animal data suggest that renal and liver effects may occur in humans exposed to high doses of hexachloroethane. Kidney and liver effects are not specific to hexachloroethane. Lesions of the kidney (nephropathy, linear mineralization, and hyperplasia) were reported at 10 mg/kg/day or greater in male rats (NTP 1989). Urinalysis also revealed granular and cellular casts in rats exposed to hexachloroethane (47 mg/kg/day or greater) for 13 weeks (NTP 1989). Because other compounds cause similar effects and because some of these effects are unique to male rats, they are not valuable as biomarkers for human hexachloroethane exposure.

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The liver is also a target of hexachloroethane toxicity, but the effects are not as severe as for the kidneys. For the most part, effects in rats were confined to swelling of hepatocytes which occurred at dose levels of 15 mg/kg/day or greater following oral exposure (Gorzinski et al. 1985). Certain biochemical parameters that are commonly associated with chemically-induced liver damage were assessed in rabbits exposed to hexachloroethane by gavage for 12 days (Weeks et al. 1979) and in sheep given a single dose of 500 mg/kg (Fowler 1969b). There were no statistically significant alterations in serum enzymes (alanine amino transferase, aspartate aminotransferase, and alkaline phosphatase) or bilirubin in rabbits, but serum values were increased as compared to controls (Weeks et al. 1979). Plasma sorbitol dehydrogenase, glutamate dehydrogenase, and ornithine carbamoyl transferase concentrations increased in sheep (Fowler 1969b). Because these effects can also be caused by other chemicals, they cannot be considered specific biomarkers for hexachloroethane.

2.7 INTERACTIONS WITH OTHER SUBSTANCES

Hexachloroethane is commonly used by the military for pyrotechnics and smoke screens. Hexachloroethane-containing, smoke-producing devices combine hexachloroethane with zinc oxide (Gordon et al. 1991). Small quantities of other materials such as calcium silicide can also be present. Hexachloroethane is generally about 44-47% of the reaction mixture. When a smoke pot or grenade is ignited, hexachloroethane reacts with zinc oxide to produce zinc chloride. Only small amounts (0.3-5%) of hexachloroethane remain. Other products of the reaction are tetrachloroethylene, carbon tetrachloride, phosgene, and hexachlorobenzene (Gordon et al. 1991). The environmental residues from smoke generation vary with the configuration of the device and its position when it ignites (upright or prone) (Schaeffer et al. 1988).

A number of studies of the toxicity of zinc oxide/hexachloroethane smoke have been conducted (Brown et al. 1990; Karlsson et al. 1986; Mans et al. 1983). These studies demonstrate that smoke exposure results in pulmonary inflammation and irritation. When male Porton Wistar rats were exposed to hexachloroethane/zinc oxide smoke for 60 minutes, the lungs showed pulmonary edema, alveolitis, and areas of macrophage infiltration 3 days later. At 14 days, there was interstitial fibrosis and macrophage infiltration. At 28 days, increased fibrosis and macrophage infiltration were noted. However, these same symptoms occurred when the animals inhaled zinc chloride; there was no apparent synergism between the zinc chloride and residual hexachloroethane (Brown et al. 1990; Richard et al. 1989). This is consistent

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with the fact that smoke contains little hexachloroethane and the observation that acute exposure to 260 ppm hexachloroethane had no effects on the lungs of rats (Weeks et al. 1979).

Environmental agents that influence microsomal reactions will influence hexachloroethane toxicity. The production of tetrachloroethene as a metabolite is increased by agents like phenobarbital that induce certain cytochrome P-450 isozymes (Nastainczyk et al. 1982a; Thompson et al. 1984). Exposure to food material or other xenobiotics that influence the availability of mixed function oxidase enzymes and/or cofactors will change the reaction rate and end products of hexachloroethane metabolism and thus influence its toxicity.

No other studies of interactions of hexachloroethane with other chemicals were identified in the published literature. However, the primary metabolites of hexachloroethane (tetrachloroethene and pentachloroethane) are themselves toxic and would be expected to exacerbate hexachloroethane toxicity if they were present in a mixture with hexachloroethane. Concurrent carbon tetrachloride exposure would also be expected to exacerbate hexachloroethane toxicity. Both hexachloroethane and carbon tetrachloride are processed by microsomes to generate free radicals, and carbon tetrachloride also forms endogenous hexachloroethane in the liver (Fowler 1969a).

2.8 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to hexachloroethane than will most persons exposed to the same level of hexachloroethane 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 may 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 on clearance rates and any resulting end-product 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."

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No studies were located regarding populations that are unusually susceptible to hexachloroethane toxicity. Because the kidney and liver are the primary target tissues, individuals with compromised liver or kidney function would have an increased risk from exposure. Susceptibility to pulmonary infections could be increased by exposure to hexachloroethane vapors and, thus, individuals that suffer from chronic respiratory problems could also have an increased risk from hexachloroethane exposure.

The risk to overweight individuals consuming a high fat diet is likely to be greater than that for lean individuals. Excess deposits of body fat increase physiological exposure durations due to the affinity of the adipose tissue for hexachloroethane. Hexachloroethane collects in the adipose deposits during exposure and is released slowly to circulatory fluids after the exposure has ceased. Individuals consuming a high fat diet are likely to absorb increased quantities of hexachloroethane when exposure occurs through the oral route; absorption from a lipid matrix is favored over absorption from an aqueous medium.

2.9 METHODS FOR REDUCING TOXIC EFFECTS

This section describes clinical practice and research concerning methods for reducing toxic effects of exposure to hexachloroethane. 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 hexachloroethane. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.9.1 Reducing Peak Absorption Following Exposure

Humans can be exposed to hexachloroethane by inhalation, ingestion, or skin contact. There are no specific treatments for hexachloroethane overexposure. However, treatments available for halogenated hydrocarbons may be useful.

When individuals have been exposed to vapors of hexachloroethane, they should be moved to fresh air. Additional treatment with oxygen may be beneficial.

If hexachloroethane has been ingested, treatments designed to minimize absorption of halogenated hydrocarbons are appropriate. If the victim is alert, can swallow, and appears to have a good gag reflex, water (1-2 glasses) may be administered after ingestion of small amounts of hexachloroethane (Bronstein

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and Currance 1988; Stutz and Ulin 1992). Because many hydrocarbons may cause spontaneous vomiting, induced emesis is not recommended since it may result in aspiration of gastric contents. If large amounts of hexachloroethane have been ingested, gastric lavage may be useful if performed soon after exposure. Activated charcoal can be administered to bind hexachloroethane in the gastrointestinal tract and minimize absorption. Activated charcoal can be combined with cathartics to speed fecal excretion. Because hexachloroethane is lipid soluble, the administration of a fat-based substance or whole milk are not recommended as they may cause increased absorption.

In order to minimize absorption through the skin, all contaminated clothing should be removed and the skin should be washed with mild soap and water (Bronstein and Currance 1988; Stutz and Ulin 1992). In cases where the compound has been splashed into the eyes, irrigation with large amounts of water for 15-30 minutes has been recommended.

2.9.2 Reducing Body Burden

Hexachloroethane that is absorbed appears rapidly in the systemic circulation. It is distributed widely throughout the body, with the highest concentration in fat and kidney and the lowest in the muscle (Fowler 1969b; Gorzinski et al. 1985). There are no specific treatments available for reducing the body burden if hexachloroethane is absorbed. Because hexachloroethane causes renal injury, hemodialysis may be useful to reduce the plasma levels of hexachloroethane should renal failure occur in exposed persons.

2.9.3 Interfering with the Mechanism of Action for Toxic Effects

No information is available on the adverse health effects of hexachloroethane in humans. Animal studies revealed that hexachloroethane primarily causes liver and kidney toxicity. Effects on the nervous system and lungs have also been reported. The mechanism by which these effects are mediated is not well characterized. Reductive metabolism by cytochrome P-450 and production of a free radical intermediate have been suggested as factors in hexachloroethane-induced hepatotoxicity (Nastainczyk et al. 1982a; Thompson et al. 1984; Town and Leibman 1984). Accordingly, one possible approach may be to reduce free radical injury. To that end, oral administration of N-acetylcysteine can be used as a means of reducing free radical injury. Also, oral administration of vitamin E and vitamin C may be of value since they are free radical scavengers.

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The mechanism of renal toxicity is not clear. Because the spectrum of kidney lesions observed in male rats (Gorzinski et al. 1985; NTP 1989) resembled those for $\alpha_2\mu$ -globulin nephropathy, hexachloroethane-induced kidney lesions may, in part, be due to hexachloroethane binding to this protein. On the other hand, renal toxicity was observed in female rats and did not present the same sequence of lesions. This suggests the effects in males may not be totally due to $\alpha_2\mu$ -globulin. Specific methods to minimize renal toxicity, based on mechanism of action, cannot be proposed at this time.

2.10 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 hexachloroethane 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 hexachloroethane.

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.

2.10.1 Existing Information on Health Effects of Hexachloroethane

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to hexachloroethane are summarized in Figure 2-4. The purpose of this figure is to illustrate the existing information concerning the health effects of hexachloroethane. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need." A data need, as defined in ATSDR's Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

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As indicated by Figure 2-4, data available on the health effects of hexachloroethane in humans is extremely sparse. A study (Selden et al. 1994) of 11 workers who wore protective equipment while being exposed to hexachloroethane for 5 weeks showed no respiratory, hematological, liver or kidney effects at plasma levels of $7.3 \pm 6 \mu\text{g/L}$ (Selden et al. 1993). Because of skin irritation, the investigators suggest that the principal exposure route may have been dermal, although the dermal effects may also have been a result of trauma from the protective equipment. Because of the protective equipment it is not possible to determine exposure levels. There is one case study of a liver tumor in an individual who had used a hexachloroethane-containing degassing agent in his work for a period of 6 years (Selden et al 1989). However, during use the hexachloroethane reacted to form hexachlorobenzene and small amounts of other chlorinated compounds. Exposure to hexachloroethane was minimal compared to exposure to the reaction products.

There are more data available concerning the effects of hexachloroethane in animals, particularly for exposure by the inhalation and oral routes. These studies identify the liver and kidney as target organs for hexachloroethane. There have been no studies of chronic exposure by the inhalation route. Although there are some data on neurological and immunological effects, there have been no well-designed, comprehensive studies of these systems. This is also true for reproductive and developmental effects. The data are limited and there has been no comprehensive multigeneration study of reproductive processes and only two studies of developmental effects.

In vivo testing for mutagenic potential has also not been conducted. The carcinogenic potential for hexachloroethane has only been evaluated for the oral route.

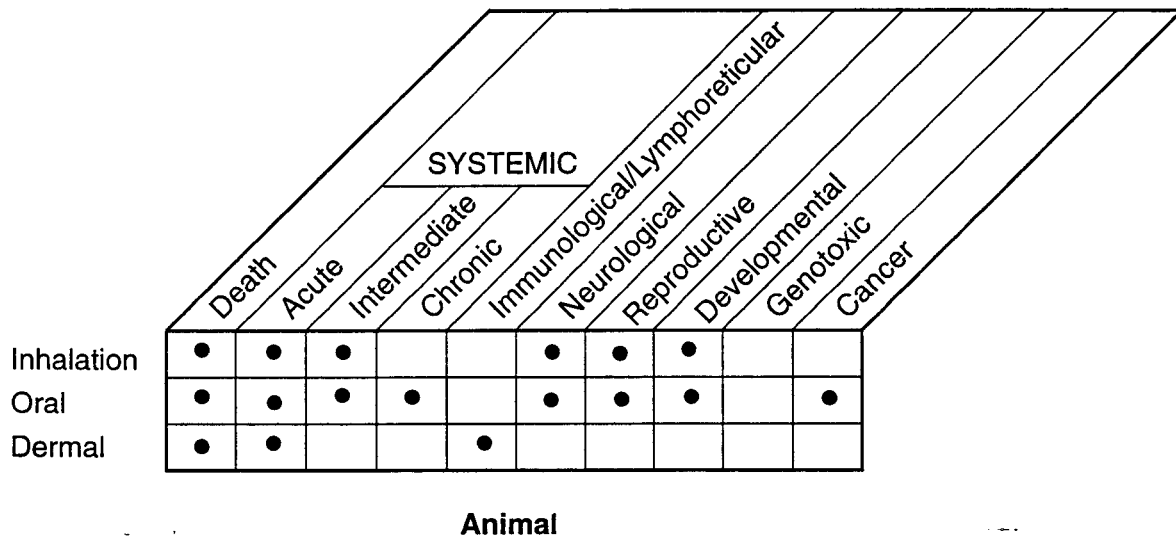
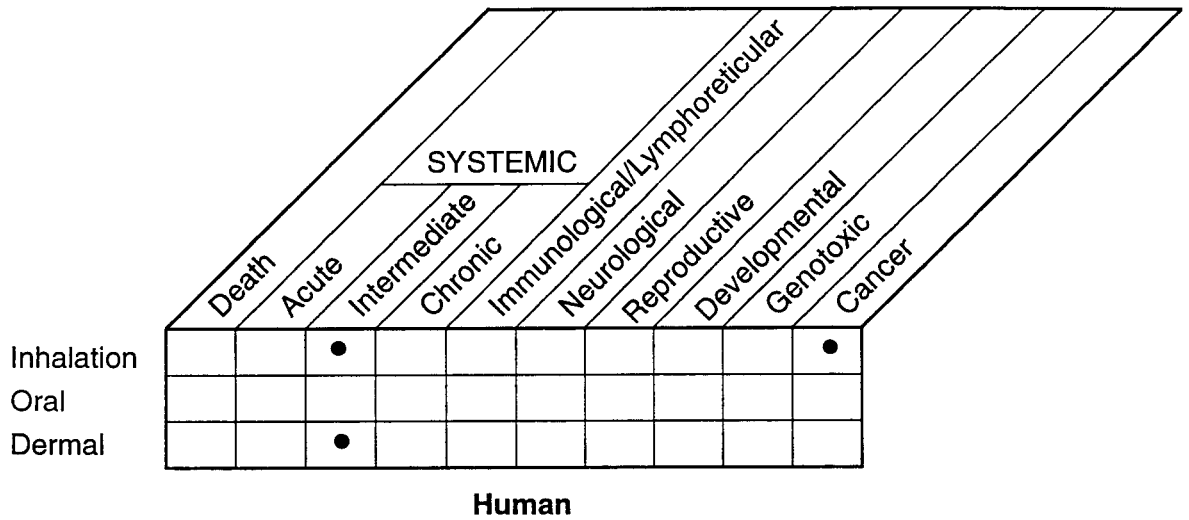
Data for the dermal route are limited to an LD_{50} study and data on dermal/ocular effects. A theoretical estimation of dermal transport of hexachloroethane indicated that absorption is low.

2.10.2 Identification of Data Needs

Acute-Duration Exposure. No studies were located on the effects of hexachloroethane in humans after acute exposure by any route. Acute inhalation exposure in animals caused respiratory effects, staggered gait, and reduced body weight gain, but these effects occurred at a concentration that was lethal (Weeks et al. 1979). There were no histological changes or changes in organ weights in the liver and kidneys after inhalation exposure to hexachloroethane (Weeks et al. 1979). Reproductive and

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



● Existing Studies

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developmental effects were not observed in rats exposed to hexachloroethane on gestation days 6-16 (Weeks et al. 1979). Tremors were observed at the high concentration in pregnant rats, and an acute inhalation MRL of 6 ppm was calculated based on the NOAEL for neurological effects observed in the developmental study (Weeks et al. 1979).

Acute oral exposure of animals was associated with tubular nephrosis and nephrocalcinosis in the kidneys (Weeks et al. 1979), hepatocellular degeneration, coagulation necrosis, hemorrhage in the liver (Weeks et al. 1979), and elevated liver enzymes in the serum (Fowler 1969b). An MRL of 1 mg/kg was derived for acute oral exposures based on a NOAEL for the absence of liver damage in rabbits (Weeks et al. 1979). Reduced body weight gain and neurological effects (tremor, decreased motor activity) have also been observed following acute oral exposure to hexachloroethane (Shimizu et al. 1992; Weeks et al. 1979). Increased fetal resorptions, decreases in fetal body weight, and an increase in skeletal anomalies have also been observed in rats treated orally with hexachloroethane during organogenesis (Shimizu et al. 1992; Weeks et al. 1979). As the dose following inhalation exposure is limited by the amount of hexachloroethane vapor that can be formed at standard temperatures (Weeks et al. 1979), oral studies may be more useful. Additional oral exposure studies to delineate the threshold for acute liver effects and to help clarify the indices that are predictive of liver damage would be especially useful. Studies of kidney effects in female rats and other laboratory animals using the oral route would also be helpful to differentiate between lesions associated with α_2 μglobulin and those produced by other mechanisms. Hexachloroethane caused reversible corneal injury in rabbits following ocular contact, but contact with the skin for 24 hours resulted in no dermal effects (Weeks et al. 1979). The physical properties of hexachloroethane suggest that absorption across human skin would be limited (Fiserova-Bergerova et al. 1990). Therefore, unless dermal absorption studies indicate that this prediction is incorrect, there is no need for additional studies of acute dermal toxicity.

Intermediate-Duration Exposure. A study (Selden et al. 1994) of 11 workers who wore protective equipment while-being exposed to hexachloroethane for 5 weeks showed no respiratory, hematological, liver, or kidney effects at plasma levels of 7.3 f 6 ug/L (Selden et al. 1993). Because mild dermal effects were noted, the principal exposure route may have been dermal. The dermal effects may also have been a result of trauma from the protective equipment. Because of the protective equipment, it is not possible to determine exposure levels.

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Inhalation of 260 ppm hexachloroethane, but not 48 ppm, was associated with an increased incidence of a respiratory tract mycoplasma infection that was endemic in the rat colony (Weeks et al. 1979). Ocular irritation, reduced body weight gain, and tremors were also noted in rats at 260 ppm. Reduced body weight gain in guinea pigs and ocular irritation and tremors in dogs were noted at 260 ppm, but not 48 ppm (Weeks et al. 1979). Based on the 48 ppm concentration for the lack of neurological effects in rats (Weeks et al. 1979), an intermediate inhalation MRL of 6 ppm was calculated.

Following intermediate oral exposure to hexachloroethane, decreased body weight gain has been observed in rats and mice (NTP 1977) and kidney effects, including increased kidney weight, tubular atrophy and hypertrophy, and hyaline droplet formation (Gorzinski et al. 1985; NTP 1989), and post-treatment hyperactivity and convulsions have been observed in rats (NTP 1989). Following intermediate exposure, liver enlargement has also been observed in rats treated with hexachloroethane (Gorzinski et al. 1985; Milman et al. 1988; Story et al. 1986). Enlargement of hepatocytes in male rats following oral exposures to 15 mg/kg/day hexachloroethane, but not 1 mg/kg/day (Gorzinski et al. 1985), was used as the basis of an MRL of 0.01 mg/kg/day for intermediate-duration oral exposures. Because there is potential for persons living near hazardous waste sites to be exposed to hexachloroethane through contaminated drinking water, additional studies of liver damage in male and female rats and kidney damage in female rats by the oral route would be useful. Hexachloroethane appears to be less bioavailable in an aqueous medium than in a lipid medium (Weeks et al. 1979).

In an intermediate-duration study, dermal treatment of rats did not result in skin sensitization (Weeks et al. 1979). Additional intermediate-duration dermal studies were not available. The physical properties of hexachloroethane suggest that absorption across human skin would be limited (Fiserova-Bergerova et al. 1990). Therefore, unless dermal absorption studies indicate that this prediction is incorrect, there is no need for additional studies of intermediate dermal toxicity.

Chronic-Duration Exposure and Cancer. No studies were located in humans following chronic duration exposure to hexachloroethane for any exposure route. No chronic animal studies were conducted using the inhalation route of exposure. In oral studies with rats, the kidney was identified as a primary target organ in males and females (NTP 1989). The kidney damage in male rats was the result of hyaline droplet nephropathy and, accordingly, was not suitable as the basis for an oral MRL. In contrast to acute and intermediate-duration oral exposure, liver toxicity was not evident in rats following chronic oral exposure. There were no studies of chronic dermal exposure to hexachloroethane.

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Studies using the inhalation route might be useful to determine the potential human health risk in populations that may be occupationally exposed to hexachloroethane vapors for long periods. Additional chronic oral studies may be useful to help further clarify the dose-response relationships and better characterize thresholds. Studies by the dermal route would not be useful until the rate and extent of absorption have been better characterized.

The carcinogenic potential of hexachloroethane has not been evaluated following chronic inhalation or dermal exposure. Hexachloroethane increased the incidence of renal tumors in male rats (NTP 1989) following chronic oral exposure. However, these tumors were associated with renal hyaline droplets and, thus, are unique to male rats. Although kidney damage was present in female rats after lifetime exposures to 80 and 160 ppm hexachloroethane, there was no increase in renal tumors. Liver lesions and liver tumors were found in mice following long-term oral exposure (NTP 1977).

A bioassay in mice (oral route) using current good laboratory practices may be useful in order to clarify whether or not hexachloroethane is a hepatic carcinogen. The suspected hepatic carcinogenicity of the hexachloroethane metabolites pentachloroethane and tetrachloroethene strengthens the justification for additional investigations of the carcinogenic potential of hexachloroethane. Studies of the mechanism of hexachloroethane carcinogenicity would also be useful to determine if effects observed in mice are applicable to humans. To further characterize risk to persons occupationally exposed to hexachloroethane, inhalation studies of the carcinogenic potential of hexachloroethane may be useful. The concentrations used in the inhalation studies would be limited to about 670-700 ppm, the saturated vapor concentration at 20°C (Weeks et al. 1979). The physical properties of hexachloroethane suggest that absorption across human skin would be limited (Fiserova-Bergerova et al. 1990). Therefore, unless dermal absorption studies indicate that this prediction is incorrect, there is no need for carcinogenic studies following dermal exposure.

Genotoxicity. Hexachloroethane did not exhibit mutagenic activity *in vitro* in prokaryotic cells (Haworth et al. 1983; Nakamura et al. 1987; Roldan-Arjona et al. 1991; Simmon and Kauhanen 1978; Weeks et al. 1979) or in eukaryotic cells (Galloway et al. 1987; Tu et al. 1985). *In vivo* data from animals, such as the results of a micronucleus assay, would be of value.

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The mutagenic potential of hexachloroethane has not been evaluated in humans. Because hexachloroethane has been detected at hazardous waste sites, it would be useful to evaluate the potential for hexachloroethane to induce mutagenic effects in human cells (e.g., peripheral lymphocytes).

Reproductive Toxicity. The effects of hexachloroethane on human reproduction have not been evaluated for any route of exposure. Data were available from animal studies using the inhalation and oral routes of exposure, but there were no data from studies using the dermal route. Following inhalation exposure to 260 ppm hexachloroethane, signs of maternal toxicity (decreased weight gain and clinical signs of neurotoxicity) were noted, but there was no evidence of embryotoxicity or fetotoxicity (Weeks et al. 1979). Reduced fertility, as characterized by reduced gestation indices and the number of live fetuses, occurred in pregnant rats following oral exposure during gestation (Weeks et al. 1979). Increased late-stage fetal resorptions were observed in rats treated orally with hexachloroethane at doses that resulted in maternal body weight gain 35% less than the controls (Shimizu et al. 1992). A comprehensive one-generation study focusing on a broad range of predictive parameters regarding reproductive success would be useful. Because inhalation exposure is limited by the properties of hexachloroethane, higher doses could be achieved using oral exposure. If an oral study is negative for reproductive effects at high doses, an inhalation study may not be necessary.

Developmental Toxicity. No studies on developmental effects in humans using any route of exposure were located. Rats were exposed to hexachloroethane during gestation using both the oral and inhalation routes and there were no soft tissue or skeletal effects in the pups (Weeks et al. 1979). Decreased fetal body weights and delayed ossification were observed in offspring of rats treated orally with hexachloroethane at doses that resulted in maternal body weight gain 35% less than the controls (Shimizu et al. 1992). Malformations were not observed in either control or hexachloroethane-treated fetuses. Confirmation of a lack of developmental effects in a second species following oral exposure would be useful. The inclusion of a developmental component to the suggested one-generation study would also provide useful data. As inhalation and dermal exposure are limited because of the physical properties of hexachloroethane that prevent exposure to high concentrations of vapor and that limit dermal absorption, studies by these routes of exposure may not be necessary.

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Immunotoxicity. No studies are available for any exposure route on the potential for hexachloroethane to cause immunotoxic effects in humans. Data in animals are limited to studies that evaluated lymphoid organs (e.g., spleen and thymus) as part of a comprehensive histopathological examination following oral and inhalation exposure to hexachloroethane (Gorzinski et al. 1985; Weeks et al. 1979). Adverse effects were not reported for these organs.

Effects on immune function have not been evaluated in animals following any route of exposure. There was an increased frequency of pulmonary tract infections in animals following inhalation and oral exposures (Weeks et al. 1979). Responses of this sort may be due, in part, to compromised immune functions. Studies in animals, using a battery of *in vitro* and short-term *in vivo* studies of immunotoxicity following inhalation and oral exposure, may enhance our overall understanding of the effects of hexachloroethane on disease resistance.

Neurotoxicity. No information is available on neurotoxic effects of hexachloroethane in humans following any route of exposure. Acute inhalation exposure in rats caused staggering gait after exposure to high concentrations (5,900 ppm) (Weeks et al. 1979). The usefulness of this data is limited since this concentration was lethal. Tremors have been reported at 260 ppm but not 48 ppm following inhalation exposure of rats in a developmental study and in a study of 6-weeks duration (Weeks et al. 1979). The lack of tremors at 48 ppm in the developmental study serves as the basis for the acute inhalation MRL, and the lack of tremors at 48 ppm in the 6-week study serves as the basis for the intermediate inhalation MRL. One study that evaluated spontaneous motor activity and avoidance behavior in rats during 6 weeks of exposure to 260 ppm hexachloroethane vapors did not reveal adverse effects of hexachloroethane on these neurobehavioral functions (Weeks et al. 1979).

Acute oral doses (500 mg/kg) given to healthy sheep caused tremors of the facial muscles (Fowler 1969b); several liver-fluke-infected sheep experienced prostration with even lower doses (170 or 338 mg/kg) (Southcott 1951). Treatment of sheep with calcium relieved the clinical signs of neurotoxicity, suggesting that cellular availability of calcium ion may be related to the neuromuscular symptoms noted (Southcott 1951). Therefore, mechanistic studies of neuromuscular impulse transmission and cognitive function in animals would be useful. These neurological studies should examine the effects of different concentrations of hexachloroethane in several species.

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Postgavage hyperactivity was noted in rats with an oral dose of 375 mg/kg/day (NTP 1989) and tremors occurred following a dose of 500 mg/kg/day (Weeks et al. 1979). Other available animal data are limited to the findings of histological examination of the brain and other nervous tissue following inhalation, oral, and dermal exposures (Gorzinski et al. 1985; NTP 1977, 1989; Weeks et al. 1979). No lesions were reported.

Epidemiological and Human Dosimetry Studies. No epidemiological studies of hexachloroethane exposure were identified. Epidemiological studies including endpoints such as immunotoxicity, neurotoxicity, liver enzymes, and kidney function in individuals who handle hexachloroethane in the production of military pyrotechnics and smoke-producing devices would be useful. Epidemiological studies of exposure to the hexachloroethane-generated smoke might be of little value because most of the hexachloroethane is consumed in the smoke-generating reaction. Epidemiological studies will be of greatest value when mixtures of other chlorinated hydrocarbons are not present with hexachloroethane in the occupational environment because there are similarities in metabolism and possible synergistic demands on microsomal enzymes.

Biomarkers of Exposure and Effect

Exposure. Hexachloroethane has been measured in the plasma of occupationally exposed humans (Selden et al. 1993). Because these workers were wearing protective equipment, it is not possible to relate exposure concentrations to plasma levels of hexachloroethane. Based on animal data, exposure to hexachloroethane can be determined by analyzing blood, urine, and fecal matter for the presence of hexachloroethane within 24 hours of exposure (Fowler 1969b). After 24 hours, most of the hexachloroethane has been metabolized to compounds that are not unique to hexachloroethane metabolism. Additional studies of biomarkers of exposure in animals are not needed at this time.

Effect. No data on biomarkers of effect in humans were identified. In animals, kidney and liver effects have been reported (Fowler 1969b; Gorzinski et al. 1985; NTP 1989; Weeks et al. 1979). Hyaline casts were present in urine but they can be caused by other chemicals as well as hexachloroethane (Borghoff 1993). Biochemical tests (e.g., blood urea nitrogen) to detect renal damage were negative (Weeks et al. 1979). However, the usual battery of urinary tests (i.e., glucose, protein, enzymes, creatinine, electrolytes, and urine output) has not been applied. Additional studies that monitor these indices would be useful and

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could demonstrate whether or not hexachloroethane has an effect on glomerular filtration and/or tubular resorption.

In rabbits, biochemical indices commonly used to assess liver damage (e.g., aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin, and protein) were elevated but within normal ranges (Weeks et al. 1979). On the other hand, plasma enzyme concentrations (sorbitol dehydrogenase, glutamate dehydrogenase, and ornithine carbamoyl transferase) increased in sheep (Fowler 1969b). Because people living near hazardous waste sites are likely to be exposed for long periods, it may be useful to evaluate these parameters in hexachloroethane-exposed animals to determine if there is consistent elevation of these biomarkers of liver damage with chronic exposure.

Absorption, Distribution, Metabolism, and Excretion. There are no mechanistic or quantitative studies of hexachloroethane absorption from the lungs or across the gastrointestinal tract or skin. However, absorption does occur following oral exposure based on the appearances of hexachloroethane and its metabolites in blood, urine, and exhaled air (Fowler 1969b; Gorzinski et al. 1985; Jondorf et al. 1957; Mitoma et al. 1985; Nolan and Karbowski 1978). The observation of toxic effects following inhalation exposure and dermal exposure (Weeks et al. 1979) indicates that hexachloroethane is absorbed through the respiratory tract. Quantitative studies that examine the absorption of hexachloroethane following inhalation and dermal exposure would be useful.

Oral kinetic studies in rats indicate that hexachloroethane distributes preferentially to the adipose tissue (Gorzinski et al. 1985; Nolan and Karbowski 1978). Relatively high concentrations are also found in male rat kidneys (Nolan and Karbowski 1978). Moderate concentrations of hexachloroethane are found in the liver, kidneys of female rats, and blood, and small amounts in muscle, lungs, and brain (Gorzinski et al. 1985).

The metabolism of hexachloroethane is relatively well defined and involves dehalogenation followed by oxidation (Fowler 1969b; Gorzinski et al. 1985; Jondorf et al. 1957; Nastainczyk et al. 1982a, 1982b; Nolan and Karbowski 1978; Salmon et al. 1981; Thompson et al. 1984; Town and Leibman 1984). Urinary by-products such as trichloroacetic acid and trichloroethanol are consistent with metabolism (Jondorf et al. 1975; Mitoma et al. 1985) and do not require additional evaluation at this time. A small portion of the exhaled radioactive label from hexachloroethane is exhaled as carbon dioxide (Mitoma et al. 1985). A more thorough investigation of the other hexachloroethane metabolites removed from the body

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in exhaled air would be useful, along with quantification of biliary excretion and identification of the biliary metabolites.

Comparative Toxicokinetics. There are differences in the effects of hexachloroethane on different species and between sexes. Male rats are particularly susceptible to kidney damage following hexachloroethane exposure because of the binding of hexachloroethane to $\alpha_2\mu$ -globulin (NTP 1989). However, nephrotoxicity is also seen in female rats and in mice and rabbits (Gorzinski et al. 1985; NTP 1977, 1989; Weeks et al. 1979) and should be examined in greater detail to identify the mechanisms for the lesions observed. Additional information on hepatic lesions in species other than the rat and mouse would be useful in evaluating the risk to humans for both noncarcinogenic and carcinogenic effects from hexachloroethane exposure.

An increased risk of respiratory infections was seen in rats exposed to 260 ppm hexachloroethane for 6 weeks (Weeks et al. 1979). A similar occurrence was not noted in dogs or guinea pigs. Additional information on the susceptibility of species other than the rat to similar infections would be useful in determining the significance of this observation to human health. Comparative studies of the infection incidence should await elucidation of the mechanism for this effect.

Dogs were more susceptible to neurotoxicity from hexachloroethane vapors than rats, and both species were more sensitive than guinea pigs and quail (Weeks et al. 1979). Pregnant rats were more sensitive to tremors than nonpregnant rats (Weeks et al. 1979). Once the mechanism of neurotoxicity has been determined, the advisability of examining differences in species response can be evaluated.

Methods for Reducing Toxic Effects. There are no compound-specific methods for reducing the toxic effects of hexachloroethane. The mitigation procedures suggested (Bronstein and Currance 1988; Stutz and Ulin 1992) are applicable to exposure to volatile chlorinated hydrocarbons as a class and are not specific for hexachloroethane.

Neither the mechanism of absorption nor the mechanism of distribution for hexachloroethane has been established. There are indications that free radical reactions may be responsible for some of the toxic effects of hexachloroethane in the liver (Town and Leibman 1984), but the data are not conclusive. When additional data on absorption, distribution and mechanism are available, compound-specific studies on methods for mitigation of toxic effects can be designed.

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2.10.3 On-going Studies

No on-going studies of the toxicity of hexachloroethane were identified.

