3. HEALTH EFFECTS

3.1   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 hexachloro-
benzene. 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.

3.2   DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near
hazardous waste sites, the information in this section is organized first by route of exposure (inhalation,
oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive,
developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure
periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in
figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or
lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the
studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those
that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory
distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction
or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a
considerable amount of judgment may be required in establishing whether an end point should be
classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be
insufficient data to decide whether the effect is indicative of significant dysfunction. However, the
Agency has established guidelines and policies that are used to classify these end points. ATSDR
believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between
"less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is
considered to be important because it helps the users of the profiles to identify levels of exposure at which

major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

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

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of hexachloro-benzene are indicated in Table 3-2 and Figure 3-2. Because cancer effects could occur at lower exposure levels, Figure 3-2 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 hexachlorobenzene. 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 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

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

3.2.1 Inhalation Exposure

3.2.1.1 Death

No studies were located regarding death in humans or animals following inhalation exposure to hexachlorobenzene.

3.2.1.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, dermal, or ocular effects in humans or animals following inhalation exposure to hexachlorobenzene.

Hepatic Effects.   It is well known that ingestion of hexachlorobenzene can produce porphyria (see Section 3.2.2.2), but there are few data to suggest a similar effect from inhalation exposure. There was a small, but statistically significant, increase (in comparison to matched controls) in total urinary porphyrins in a group of nine workers exposed for 1–19 years to hexachlorobenzene and other organochlorine compounds (including octachlorostyrene, another porphyrinogenic substance [Smith et al. 1986d]) while using hexachloroethane as an aluminum degassing agent at aluminum smelters (Selden et al. 1999). The prevalence of porphyria-related symptoms did not differ between groups. Serum levels of hexachlorobenzene were positively correlated to urine porphyrin levels, but urine porphyrin levels also correlated, to a similar degree, with serum octachlorostyrene levels. Therefore, this study cannot ascertain whether the preclinical porphyria observed in aluminum smelter workers is due to hexachlorobenzene, octachlorostyrene, or both chemicals. End points of hepatic toxicity have been investigated in residents of Flix, Spain, a small village in Catalonia; a nearby electrochemical factory that manufactures organochlorines has been implicated in the village's high environmental levels of hexachlorobenzene ($35 \ ng/m^3$ as a 24-hour average in air). Analysis of blood hexachlorobenzene and urinary porphyrins in 604 residents of Flix, including 185 factory workers, showed that blood hexachlorobenzene levels were roughly 5-fold higher in factory workers than in other residents. However, there were no cases of clinical porphyria cutanea tarda in either group and no evidence that preclinical porphyria was more prevalent in the factory workers than in other residents or that there was any association between urinary porphyrin

levels and blood hexachlorobenzene levels (Herrero et al. 1999; Sala et al. 1999b). A subsequent study verified the 5-fold increase in 75 factory workers, and detected a positive, statistically significant correlation between blood hexachlorobenzene levels and serum γ-glutamyltransferase (Sala et al. 2001a). However, no correlation was seen for serum levels of either aspartate or alanine aminotransferase. The NOAEL for liver effects in the village residents are shown in Table 3-1 and Figure 3-1. In 52 workers employed for 1–25 years at a chemical plant where hexachlorobenzene was the primary byproduct, compared with controls drawn from a local blood bank (Queiroz et al. 1998a), serum aspartate and alanine transaminase levels were increased but no correlation was detected between serum levels of transaminase and blood hexachlorobenzene. The available human data, while suggestive, have not conclusively shown hepatic effects due to inhaled hexachlorobenzene. No studies were located regarding the hepatic effects of airborne hexachlorobenzene in animals.

Renal Effects. Based on analysis of blood samples from 608 individuals, workers (n=189) at an electrochemical factory near Flix, Spain did not have a significantly increased risk of high serum creatinine, a marker for glomerular disease, in comparison to other Flix residents (n=419) despite hexachlorobenzene blood levels that were approximately 5-fold higher (Sala et al. 1999b). However, the power of this study to find an effect was limited by the small sample size (only nine individuals with high serum creatinine were found in the study population). "Marked changes in kidney functions" including microproteinuria were observed in Czechoslovakian workers with high blood hexachlorobenzene levels following occupational inhalation exposure to hexachlorobenzene, originally at 2.1–10.8 mg/m^3 and then at 0.012–0.022 mg/m^3, from 1983 to 1990 (Richter et al. 1994). No studies were located regarding renal effects of inhaled hexachlorobenzene in animals.

Endocrine Effects. Although oral exposure to hexachlorobenzene has been clearly associated with thyroid effects, only limited information is available regarding the endocrine effects of hexachlorobenzene following inhalation exposure. A study of residents (192–558, depending on end point) of Flix, Spain, where a nearby electrochemical factory had resulted in high air and blood levels of hexachlorobenzene, detected statistically significant correlations between increased blood hexachlorobenzene levels and decreased levels of total thyroxine (T4); serum levels of thyroid stimulating hormone (TSH) were not affected (Sala et al. 2001a). Similarly, an earlier study did not detect changes in serum levels of TSH in 189 workers at the factory in comparison to 419 other Flix residents despite 5-fold higher blood hexachlorobenzene levels in the workers (Sala et al. 1999b). Both studies were limited by small sample size,

Table 3-1. Levels of Significant Exposure to Hexachlorobenzene - Inhalation

Key to[a] figure	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL		Reference Chemical Form
					Less serious (mg/m3)	Serious (mg/m3)	
ACUTE EXPOSURE							
Immunological/Lymphoreticular							
1	Rat (Sprague-Dawley)	1-4 d 4 hr/d		4.4 M	33 M (slight impairment of pulmonary immune defenses)		Sherwood et al. 1989

Table 3-1. Levels of Significant Exposure to Hexachlorobenzene - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL		Reference Chemical Form
					Less serious (mg/m3)	Serious (mg/m3)	

INTERMEDIATE EXPOSURE

Immunological/Lymphoreticular

| 2 | Rat (Sprague-Dawley) | 4 wk 4 d/wk 4 hr/d | | | 35 M (slight impairment of pulmonary immune defense) | | Sherwood et al. 1989 |

Table 3-1. Levels of Significant Exposure to Hexachlorobenzene - Inhalation (continued)

Key to[a] figure	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL		Reference Chemical Form
					Less serious (mg/m3)	Serious (mg/m3)	
CHRONIC EXPOSURE							
Systemic							
3	Human	40 yr	Hepatic	0.000035			Herrero et al. 1999

[a]The number corresponds to entries in Figure 3-1.

d = day(s); hr = hours(s); LOAEL = lowest-observable-adverse-effect level; M = male; NOAEL = no-observable-adverse-effect level; wk = week(s); yr = year(s)

Figure 3-1. Levels of Significant Exposure to Hexachlorobenzene - Inhalation
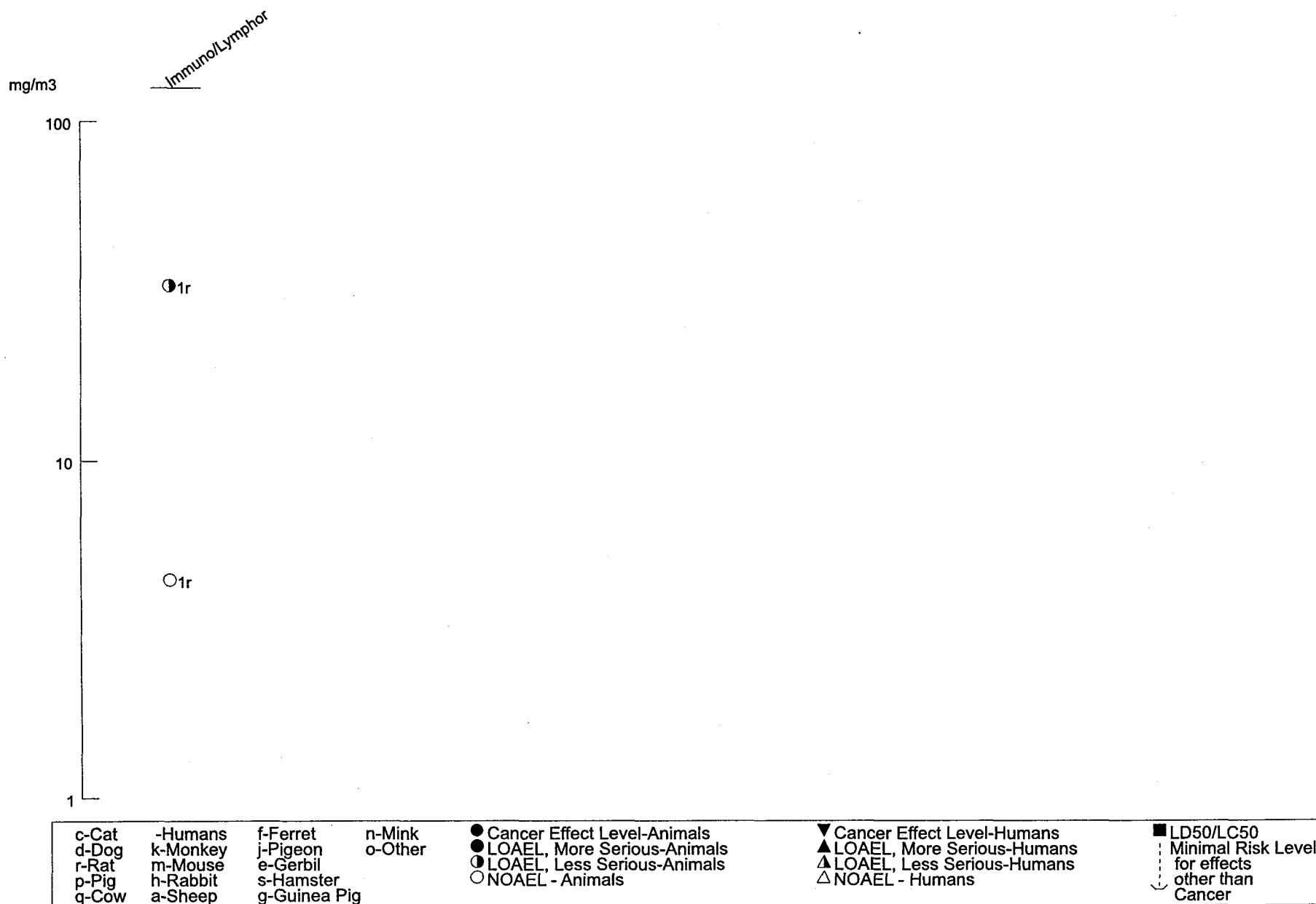
Acute (≤14 days)

Figure 3-1. Levels of Significant Exposure to Hexachlorobenzene - Inhalation (*Continued*)
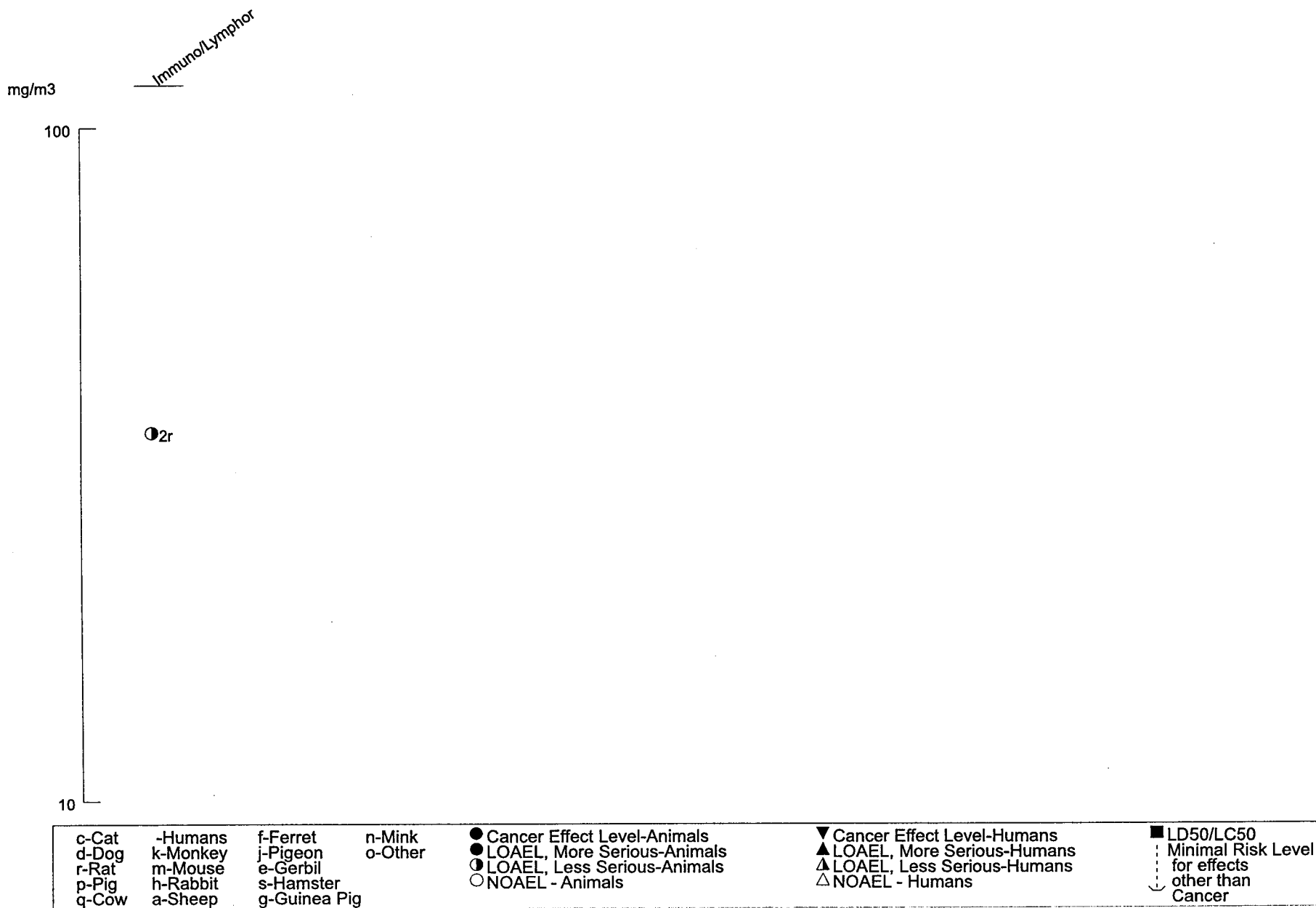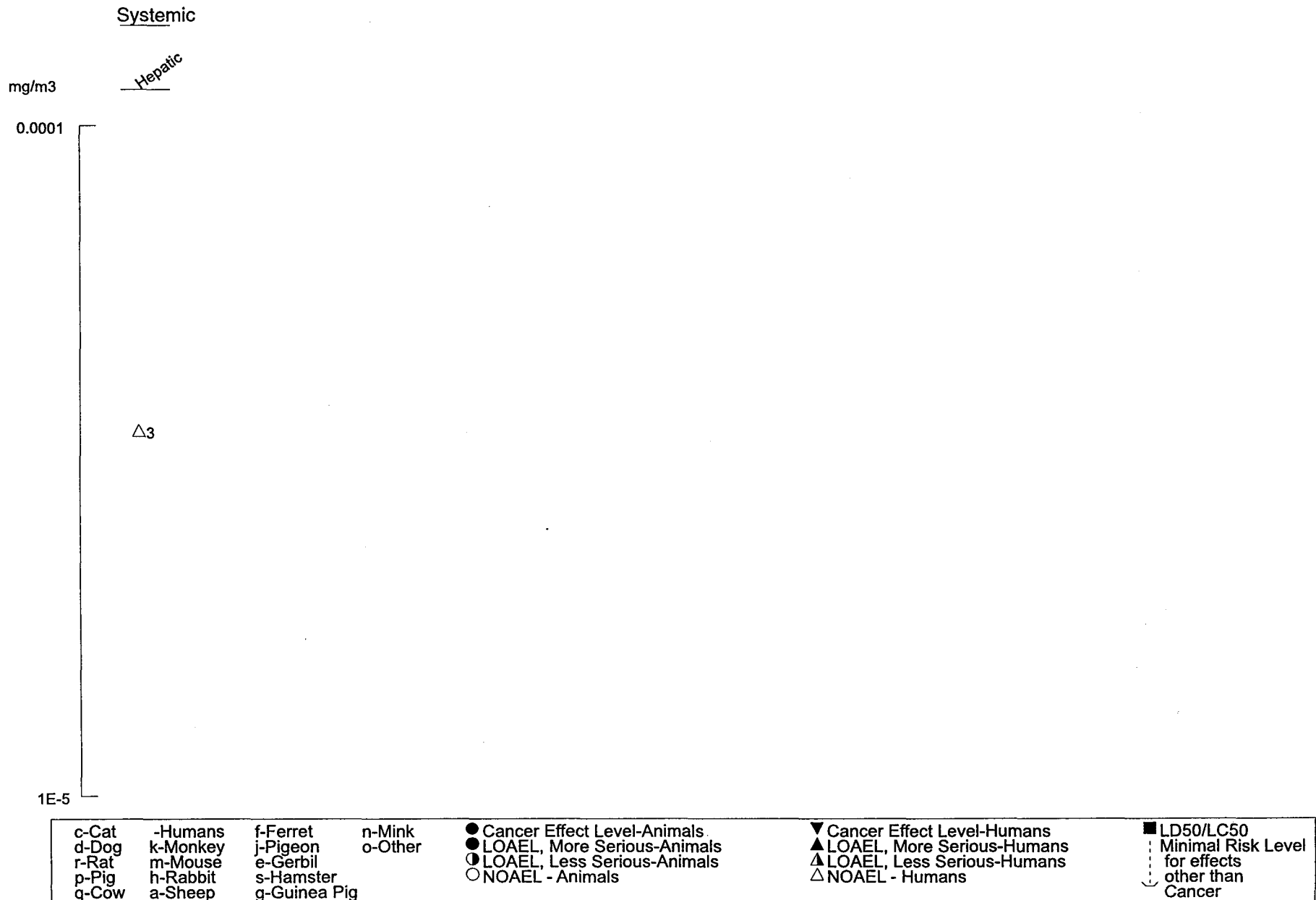
Intermediate (15-364 days)

Immuno/Lymphor

mg/m3

100

O2r

10

c-Cat	-Humans	f-Ferret	n-Mink	● Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	△ LOAEL, Less Serious-Humans	for effects
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer

Figure 3-1. Levels of Significant Exposure to Hexachlorobenzene - Inhalation (*Continued*)

Chronic (≥365 days)

Systemic

Hepatic

mg/m3

0.0001

△3

1E-5

c-Cat	-Humans	f-Ferret	n-Mink	● Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	▲ LOAEL, Less Serious-Humans	for effects
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer

as only 10 cases of elevated TSH were observed in each of the study populations. A larger survey found that the prevalences of goiter and hypothyroidism were similar in ever exposed workers (n=507) and nonexposed residents (n=1,293) (Sala et al. 1999b). No studies were located regarding endocrine effects in animals following inhalation exposure to hexachlorobenzene.

3.2.1.3 Immunological and Lymphoreticular Effects

Occupational studies indicate that inhaled hexachlorobenzene may cause physiological changes in immune parameters, but these effects are not clearly toxic. Queiroz et al. (1997, 1998a, 1998b) studied a group of Brazilian workers (n=51–66) employed for up to 25 years at a chemical plant whose primary waste product was hexachlorobenzene. Although it is not clear from the report, workers were presumably exposed to hexachlorobenzene by inhalation. Findings in blood samples of exposed workers compared to controls selected from a local blood bank were significant decreases in neutrophil chemotaxis, impaired neutrophil cytolytic activity (but not phagocytic activity) and significant increases in serum immunoglobulins (IgG and IgM, but not IgA). Blood hexachlorobenzene levels were elevated in exposed workers, but were not correlated with changes in immunological parameters. Serum immunoglobulins (IgG, IgA, IgM, and IgE, but not IgD) were also increased in Czechoslovakian workers with high blood hexachlorobenzene levels who had been exposed to hexachlorobenzene in the workplace air from 1983 to 1990, originally at 2.1–10.8 mg/m^3 and then at 0.012–0.022 mg/m^3 (Richter et al. 1994). Immunological parameters and serum organochlorine levels were measured in a group of 141 German medical patients presenting with variety of acute symptoms (mainly lack of concentration, exhaustion, and common cold) who had been occupationally exposed (as teachers, construction workers, and telecommunication technicians) for at least 6 months to multiple organochlorines (Daniel et al. 2001). A strong, statistically significant, association was detected between high blood levels of hexachlorobenzene and decreased levels of interferon-γ blood (IFN-γ) levels. Moreover, patients with low overall organochlorine levels had elevated IFN-γ levels. The authors note that IFN-γ is important for increasing the secretion of immunoglobulins by plasma cells, and speculate that decreased IFN-γ might increase susceptibility to infection. It is not clear whether hexachlorobenzene exposure is responsible for the observed effects, because significant cross-correlations were detected between hexachlorobenzene levels and several polycyclic biphenyl compounds.

The animal data provide weak support for an immunological effect of inhaled hexachlorobenzene. Observations in male rats exposed to 33–35 mg/m^3 of hexachlorobenzene aerosol for durations ranging from 4 hours to 4 weeks (4 days/week, 4 hours/day) included a slight decrease in pulmonary macrophage

bactericidal activity to inhaled *Klebesiae pneumoniae*, a slight increase in phagocytic activity of alveolar (but not peritoneal) macrophages *in vitro*, and altered lymphocyte mitogenesis induced by T-cell (phytohemagglutinin [PHA]) and B-cell (*Salmonella typhimurium* lipopolysaccharide) mitogens in lung-associated and mesenteric lymph nodes *in vitro* (Sherwood et al. 1989). No effects were found at 4.4 mg/m^3 (1-day exposure only). The authors concluded that exposure to hexachlorobenzene at about 35 mg/m^3 resulted in slight changes to humoral and pulmonary cellular defenses. However, the reported changes were only marginally different from controls, the magnitude of the reported effects did not generally increase with exposure duration, and some of the results were contradictory (e.g., there was a significant increase in PHA-induced mitogenesis in lung-associated lymph nodes, but a significant decrease in PHA-induced mitogenesis in mesenteric lymph nodes, in rats exposed to hexachlorobenzene for 4 weeks). The NOAEL and LOAEL values from this experiment are recorded in Table 3-1 and plotted in Figure 3-1.

3.2.1.4 Neurological Effects

No clear evidence of neurological effects following inhalation exposure to hexachlorobenzene is available. The prevalence of Parkinson's disease was not significantly increased in workers at an electrochemical factory near Flix, Spain (4/507) compared with other Flix residents (4/1,293) despite 5-fold higher blood hexachlorobenzene levels in the workers (Sala et al. 1999b). The small number of cases is a limitation of this study. A preliminary report (Sala et al. 1999a), based on 63 cases, detected a statistically significant association between prenatal hexachlorobenzene exposure and impaired development of locomotor skills in newborn babies in Flix, compared with those of nearby villages. A different study conducted in Oswego, New York was unable to correlate hexachlorobenzene levels in umbilical blood or breast milk with infant intelligence test results (Darvill et al. 2000). The apparent discrepancy between these studies may be explained by differences in exposure. No studies were located regarding neurological effects in animals following inhalation exposure to hexachlorobenzene.

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans or animals following inhalation exposure to hexachlorobenzene.

3.2.1.6 Developmental Effects

Developmental effects (spontaneous abortions, low birth weight, and congenital malformations) occurred with a similar prevalence among females who ever worked at an electrochemical factory near Flix, Spain (n=46–60 for the different end points) and never exposed female residents (n=719–936), despite 5-fold higher blood hexachlorobenzene levels in the workers (Sala et al. 1999b). The small number of women factory workers is a limitation of this study. No studies were located regarding developmental effects in animals by inhalation exposure to hexachlorobenzene.

3.2.1.7 Cancer

In comparison to the surrounding Province of Tarragona, the incidences of thyroid cancer and soft-tissue sarcoma were significantly increased, and brain tumors marginally increased, for the years 1980–1989 in male residents of Flix, Spain, where a nearby organochlorine factory had produced high levels of hexa-chlorobenzene in the ambient air for decades (40 measurements in 1989–1992 averaged 35 ng/m^3, and the researchers suspected concentrations had been higher in years past) (Grimalt et al. 1994). Tumor incidences were not elevated in female Flix residents, but exposures of the females (few of whom worked at the factory) may have been considerably lower than those of the males (many of whom, including all those with tumors, worked at the factory). The findings in males were based on very small numbers of observed cases (2–4 for the various tumor types), and were not duplicated in a companion analysis of cancer mortality reported in the same paper. Therefore, this study was not conclusive. Hepatocellular carcinoma was diagnosed in 1985 in a 65-year-old male who had been exposed to airborne hexachloro-benzene and, to a lesser extent, other organochlorine compounds (e.g., chlorinated benzenes, chlorophenols, dioxins, and dibenzofurans) at an aluminum smelter from 1967 to 1973 while using hexachloroethane as an aluminum degassing agent (Selden et al. 1989). This finding is suggestive, but does not provide rigorous evidence for an association between tumor development and inhalation exposure to hexachlorobenzene. No other data were located specifically associating cancer with exposure to hexachlorobenzene, but several studies have investigated an apparent association between porphyria and subsequent development of liver cancer in humans. These data are relevant because hexachloro-benzene is porphyrogenic in humans. However, factors such as cancer evolution times, liver pathology (hepatitis viral infection, fibrosis, or cirrhosis), and age were found to be better predictors of subsequent tumor development than porphyrin status in these studies (Axelson 1986; Keczkes and Barker 1976; Salata et al. 1985; Siersema et al. 1992; Topi et al. 1980; Waddington 1972).

Animal data regarding the carcinogenicity of hexachlorobenzene via the inhalation route were not
located, although there is evidence that hexachlorobenzene produces liver tumors in animals by the oral
route (see Section 3.2.2.8).

3.2.2 Oral Exposure

3.2.2.1 Death

Evidence of human lethality following oral exposure to hexachlorobenzene is derived mainly from
epidemiologic data from from 1955 to 1959. An estimated 3,000–4,000 people ingested bread prepared
from grain treated with fungicides composed of 10% hexachlorobenzene, at an estimated 2 kg/1,000 kg
wheat. There was an extremely high rate of mortality in breast fed children (under 2 years of age) of
mothers known to have ingested this bread. All children born to porphyric mothers during that epidemic
died (Gocmen et al. 1989; Peters et al. 1982) and an estimated 1,000–2,000 infants died due to a condition
known as *pembe yara* or "pink sore" because of the associated skin lesions (blistering and epidermolysis
and annular erythema) (Cripps et al. 1984; Peters et al. 1982, 1987). Although a 10% rate of mortality in
exposed adults has been reported, it is not clear how that figure relates to the expected mortality rates for
comparable cohorts (Peters et al. 1982, 1987). No quantitation of exposure (dose and duration) was
presented in any of these clinical reports. However, an estimated dose of 0.05–0.2 g/day
(0.7–2.9 mg/kg/day for a 70-kg person) is considered to be reliable by the original investigators of the
Turkey epidemic (Cam and Nigogosyan 1963). Analysis of human milk from exposed women and
unexposed controls in this epidemic showed hexachlorobenzene concentrations of 0.51 and 0.07 ppm,
respectively (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987). A report on an
epidemiological study in New South Wales, Australia, which gave hexachlorobenzene concentrations
ranging from trace amounts to 8.2 ppm in human body fat and #0.41 ppb in whole blood, found no
adverse health effects or mortality associated with these levels of body burden (Brady and Siyali 1972;
Siyali 1972).

The acute lethality of ingested hexachlorobenzene in animal studies is relatively low. LD_{50} data are
limited to a report from the Russian literature of single-dose oral LD_{50} values of 3,500 mg/kg for rats,
4,000 mg/kg for mice, 2,600 mg/kg for rabbits, and 1,700 mg/kg for cats (Savitskii 1964, 1965).
Although these LD_{50} values were determined under unknown experimental conditions, they are consistent
with the remainder of the database showing low acute lethality of hexachlorobenzene. One death was
observed among 10 rats given a single dose of 600 mg/kg by gavage in corn oil (Lecavalier et al. 1994),

but it is not clear from the report that the death was due to hexachlorobenzene. Lethal levels in animal studies are progressively lower as exposure duration is increased. Lethal levels ranged from 19 to 245 mg/kg/day in most (the exception is discussed below) intermediate-duration feeding studies (Cuomo et al. 1991; De Matteis et al. 1961; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Kennedy and Wigfield 1990; Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977; Ockner and Schmid 1961) and from 6 to 16 mg/kg/day in chronic oral studies (Cabral et al. 1977, 1979; Gralla et al. 1977a). Death in these studies was closely associated with occurrence of neurological symptoms (e.g., tremors, paresis, weakness, convulsions) and, in some cases, weight loss (Cabral et al. 1977; Cuomo et al. 1991; De Matteis et al. 1961; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Gralla et al. 1977a; Ockner and Schmid 1961).

Aside from duration of exposure, other factors that appear to influence susceptibility to hexachloro-benzene-induced mortality include species, strain, sex, age, pregnancy status, diet, nutritional status (fasting verus normal diet), and dosing protocol (including the vehicle used and the method of exposure). A direct comparison of multiple species was performed by De Matteis et al. (1961), who treated rats, mice, guinea pigs, and rabbits with 5,000 ppm of hexachlorobenzene in the diet, providing estimated doses of 250, 650, 200, and 245 mg/kg/day, respectively. Guinea pigs, with the lowest estimated daily dose, and mice, with the highest dose, were the most severely affected of the species tested, with severe neurological effects and death occurring as soon as 8–10 days after the start of exposure. Rats and rabbits also developed neurological symptoms and died, but only after $8 weeks of exposure. The severe effects in guinea pigs despite the low dose suggest that this species may be especially sensitive to hexachloro-benzene; however, additional supporting data are lacking. Although death was reported in both male and female animals in various studies, studies that included both sexes generally reported a higher incidence of mortality in females than in males treated with the same doses (Gralla et al. 1977a; Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977). Pregnant females in particular seem to be especially susceptible to hexachlorobenzene-induced mortality. Two pregnant rats fed hexachlorobenzene during gestation were much more severely affected than the nonpregnant rats in the De Matteis et al. (1961) study, with one dying before giving birth and the other dying 4 weeks after giving birth. Grant et al. (1977) observed death of pregnant dams at doses as low as 8 mg/kg/day in an intermediate-duration reproduction study, which is considerably lower than the lethal dose range to nonpregnant animals in other intermediate-duration studies (19–245 mg/kg/day).

Toxicity of hexachlorobenzene is enhanced by use of an oil vehicle in animal studies. This was demonstrated by Kennedy and Wigfield (1990), who fed female Wistar rats a diet to which hexachloro-

benzene was added either in corn oil or as crystalline chemical. At 1,000 ppm (50 mg/kg/day), 2 deaths occurred within 23 days of the start of exposure in the corn oil group and the remaining 27 animals were removed from the study 2 days later due to obvious ill health. No deaths occurred in the crystalline chemical group (n=27) fed the same dose for 56 days. The increased toxicity of hexachlorobenzene fed with corn oil appeared to be related to increased accumulation of the chemical in the body (measured in liver, kidney, and spleen) under these conditions.

Reliable LOAEL values for mortality in each species and duration category are presented in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

The available data in humans and laboratory animals indicate that the liver, and specifically, the heme biosynthesis pathway, is the major systemic target of hexachlorobenzene toxicity. Human data have also shown effects on other systemic targets, including the skin, bone, and thyroid. These effects were less common than inhibition of heme biosynthesis in exposed people. No information was located regarding respiratory, cardiovascular, gastrointestinal, hematological, renal, ocular, or body weight effects in humans following oral exposure to hexachlorobenzene. However, animal data are available for these systemic effects and suggest that the blood, lungs, and kidneys may be additional systemic targets of hexachlorobenzene.

The highest NOAEL values and all reliable LOAEL values for systemic effects in each species and duration category are presented in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects.   No studies were located regarding respiratory effects of oral hexachloro-benzene exposure in humans.

Animal studies have shown that ingested hexachlorobenzene can produce pathological effects in the lungs. The most widely reported lesions were hypertrophy and proliferation of the lining endothelial cells of the pulmonary venules and intra-alveolar accumulation of foamy-looking macrophages. The foamy appearance of macrophages is a result of increased lipid content (Goldstein et al. 1978). These

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
ACUTE EXPOSURE							
Death							
1	Mouse (NS)	10 d (F)				650 F (death)	De Matteis et al. 1961
2	Gn Pig (NS)	10 d (F)				200 F (death)	De Matteis et al. 1961
Systemic							
3	Rat (Wistar)	1, 2, 3, or 4 wks 5 d/wk (GW)	Hepatic		1000 F (increased porphyria, altered hepatic enzyme levels)		Billi de Catabbi et al. 2000a
4	Rat (Wistar)	1 or 7 wks, # of doses per wk unknown (GW)	Hepatic		1000 F (increased urinary porphyrin excretion)		Billi de Catabbi et al. 2000a
5	Rat (Sprague-Dawley)	2 wk 5 d/wk 1 x/d (GO)	Renal		100 M (incr kidney wt, degenerative and regenerative foci in tubules, accumulation of protein droplets in tubular cells)		Bouthillier et al. 1991
6	Rat (Sprague-Dawley)	5 d 1 x/d (GO)	Endocr		50 F (decr serum thyroxine)		Foster et al. 1993
7	Rat (CD)	1 wk (F)	Hepatic	5 F	16 F (incr hepatic ALA-S activity)		Goldstein et al. 1978
			Bd Wt	159 F			

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene ‑ Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
8	Rat (Wistar)	1 d (F)	Hepatic		50 F (incr highly carboxylated porphyrins in liver)		Kennedy and Wigfield 1990
9	Rat (Wistar)	Gd 6-21, 6-16, 6-9 or 10-13 1x/d (GO,GW)	Bd Wt	40 F		80 F (loss of body weight by pregnant rats)	Khera 1974a
10	Rat (Sprague-Dawley)	2x (GW)	Hepatic	700 F	1400 F (incr ornithine decarboxylase in the liver)		Kitchin and Brown 1989
11	Rat (Wistar)	7 d 1 x/d (GW)	Hepatic	250 F	500 F (decr hepatic URO-D activity)		Kleiman de Pisarev et al. 1990
			Endocr		250 F (decr serum T4 levels)		
12	Rat (Sprague-Dawley)	2-16 d 1 x/d (GO)	Hepatic		25 F (incr urinary and hepatic porphyrins)		Krishnan et al. 1991
13	Rat (Sprague-Dawley)	6 d 1 x/d (GO)	Hepatic		10 M (incr liver wt)		Mehendale et al. 1975
			Bd Wt	25 M			
14	Rat (Brown-Norway)	7 or 21 d daily (GW)	Hepatic		45 F (increased liver weight)		Michielsen et al. 2001
15	Rat (NS)	5 d 1 x/d (GO)	Bd Wt	221 M			Simon et al. 1979

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
16	Rat (Wistar)	2 wk 3 d/wk 1 x/d (GW)	Endocr Bd Wt	484 M 997 M	740 M (decr serum total and free T4)		van Raaij et al. 1993a
17	Mouse (CD-1)	Gd 7-16 (GO)	Hepatic Bd Wt	100 F	100 F (incr liver wt in pregnant dams)		Courtney et al. 1976
18	Gn Pig (NS)	10 d (F)	Hepatic Renal		200 F (fatty change) 200 F (incr ammonia in blood)		De Matteis et al. 1961
Neurological							
19	Rat (Wistar)	Gd 6-21, 6-16, 6-9 or 10-13 1x/d (GO,GW)		40 F		80 F (hyperesthesia, tremors, and convulsions in pregnant rats)	Khera 1974a
20	Gn Pig (NS)	10 d (F)				200 F (marked weakness, hyperexcitability, tremors, convulsions)	De Matteis et al. 1961
Reproductive							
21	Rat (Sprague-Dawley)	5 d 1 x/d (GO)			50 F (incr serum progesterone)		Foster et al. 1993
22	Rat (NS)	5 d 1 x/d (GO)		70 M	221 M (decr male impregnation of females)		Simon et al. 1979

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Developmental							
23	Rat (Sprague-Dawley)	4 d 1x/d (GO)			2.5[b] (hyperactivity in young pups)		Goldey and Taylor 1992
24	Rat (Wistar)	Gd 6-21, 6-16, 6-9 or 10-13 1x/d (GO,GW)			40 (incr skeletal variations)		Khera 1974a
25	Mouse (CD-1)	Gd 7-16 (GO)				100 (incr abnormal fetuses per litter)	Courtney et al. 1976

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
INTERMEDIATE EXPOSURE							
Death							
26	Rat (Wistar)	80 d (F)				100 F (60/90 died)	Cuomo et al. 1991
27	Rat (Wistar)	13 wk (F)				19 F (4/9 died)	Den Besten et al. 1993
28	Rat (Sprague-Dawley)	4 gen (F)				8 F (1/20 dams died)	Grant et al. 1977
29	Rat (Wistar)	56 d (F)				50 F (2/29 died)	Kennedy and Wigfield 1990
30	Rat (Sherman)	4 mo (F)				25 (2/10 males and 14/20 females died)	Kimbrough and Linder 1974
31	Rat (Wistar)	15 wk 3-4 d/wk 1 x/d (GO)				50 F (5/19 died)	Koss et al. 1978
32	Rat (COBS)	15 wk (F)				32 F (incr mortality in females)	Kuiper-Goodman et al. 1977
33	Rat (Sprague-Dawley)	56 d (F)				100 M (13/33 died within 1 month)	Ockner and Schmid 1961
34	Rabbit (NS)	12 wk (F)				245 F (4/4 died)	De Matteis et al. 1961

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene ‑ Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
35	Pig (SPF)	90 d (F)				50 M (5/5 died)	Den Tonkelaar et al. 1978
Systemic							
36	Monkey (Cynomolgus)	90 d 1 x/d (C)	Hemato	10 F			Foster et al. 1995a
			Bd Wt	10 F			
37	Monkey (Rhesus)	60 d 1x/d (GW)	Resp	128 F			Iatropoulos et al. 1976
			Cardio	128 F			
			Gastro	128 F			
			Musc/skel	128 F			
			Hepatic		8 F (hepatocellular hypertrophy, cloudy swelling)		
			Renal		8 F (vacuolization of proximal renal tubules)		
			Endocr	128 F			
			Ocular	128 F			
38	Monkey (Cynomolgus)	90 d 1x/d (C)	Hepatic	0.1 F	1 F (hepatocellular vacuolation, intrahepatic cholestasis)		Jarrell et al. 1993
			Endocr	1 F	10 F (incr adrenal weight)		
			Bd Wt	10 F			
39	Monkey (Rhesus)	60 d 1 x/d (GW)	Hemato	128 F			Knauf and Hobson 1979
			Hepatic	64 F	128 F (incr serum AST)		
			Renal	64 F	128 F (incr blood urea nitrogen)		
			Bd Wt		8 F (unspecified weight loss)		

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
40	Rat (Fischer 344)	5 wk 5 d/wk 1 x/day (GO)	Hepatic	0.1	1 M (incr liver wt)		Andrews et al. 1988
			Renal	25 M			
			Endocr		10 M (incr serum 1,25-dihydroxy- vitamin D3 and parathyroid hormone)		
41	Rat (Fischer 344)	15 wk 5 d/wk 1 x/d (GO)	Musc/skel	0.1 M	1 M (incr femur density)		Andrews et al. 1989
			Hepatic	1 M	10 M (incr liver wt)		
			Renal	1 M	10 M (incr kidney wt, incr urinary LDH and alkaline phosphatase)		
			Endocr	0.1 M	1 M (incr serum 1,25-dihydroxy- vitamin D3)		
			Bd Wt	25 M			
42	Rat (Fischer 344)	15 wk 5 d/wk 1 x/d (GO)	Musc/skel	0.1 M	1 M (incr femur density and cortical area)		Andrews et al. 1990
			Renal	0.1 M	1 M (incr urinary LDH)		
			Endocr	1 M	10 M (incr serum parathyroid hormone)		
43	Rat (Wistar)	1, 2, 3, or 4 wks 5 d/wk (GW)	Hepatic		1000 F (increased porphyria, altered hepatic enzyme levels)		Billie de Catabbi et al. 2000a
44	Rat (Wistar)	1 or 7 wks # of doses per wk unknown (GW)	Hepatic		1000 F (increased urinary porphyrin excretion)		Billie de Catabbi et al. 2000a

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg)	LOAEL		Reference Chemical Form
					Less serious (mg/kg)	Serious (mg/kg)	
45	Rat (Wistar)	1x/d 5 d/wk 7, 14, 21, or 28 d (GW)	Hepatic		50 F (hepatic porphyria changes in hepatic sphingolipid levels)		Billie de Catabbi et al. 2000b
46	Rat (Chbb THOM)	1x/d 5 d/wk 7, 14, 21, or 28 d (GW)	Hepatic		50 F (hepatic porphyria changes in hepatic sphingolipid levels)		Billie de Catabbi et al. 2000b
47	Rat (Sprague-Dawley)	7 wk 5 d/wk 1 x/d (GO)	Renal		50 M (incr kidney wt, glucosuria, proteinuria, degenerative and regenerative foci in tubules, accumulation of protein droplets in tubular cells)		Bouthillier et al. 1991
48	Rat (Wistar)	80 d (F)	Hepatic		100 F (incr liver wt, decr hepatic URO-D, incr hepatic porphyrins, incr lipid peroxidation)		Cantoni et al. 1990
49	Rat Chbb THOM)	1x/d 5 d/wk 1, 2, 3, 4, or 8 wks (GW)	Hepatic		1000 F (Hepatic porphyria)		Cochon et al. 2001

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg)	LOAEL Less serious (mg/kg)	LOAEL Serious (mg/kg)	Reference Chemical Form
50	Rat (Wistar)	1x/d 5 d/wk 1, 2, 3, 4, or 8 wks (GW)	Hepatic		1000 F (Hepatic porphyria)		Cochon et al. 2001
51	Rat (Wistar)	80 d (F)	Hepatic		100 F (porphyria, incr liver wt, fatty degeneration)		Cuomo et al. 1991
			Dermal		100 F (photosensitive skin lesions)		
			Bd Wt	100 F			
52	Rat (Wistar)	13 wk (F)	Hepatic		9.5 F (incr liver wt, incr urinary and liver porphyrins)		Den Besten et al. 1993
			Renal	9.5 F	19 F (incr kidney wt, basophilic renal tubules, protein casts)		
			Endocr	9.5 F	19 F (adrenal: incr wt, cortical hypertrophy and hyperplasia thyroid: decr serum T4 and T3)		
			Dermal		9.5 F (skin lesions)		
			Bd Wt	9.5 F		19 F (severe wt loss in 4/9)	
53	Rat (Wistar)	107 d (F)	Hepatic		150 F (marked decr in hepatic URO-D activity, massive incr in hepatic porphyrin content)		Elder and Urquhart 1986
54	Rat (Wistar)	4 wk 5 d/wk 1 x/d (GW)	Renal		1000 F (porphyria and lipid peroxidation in cortex)		Fernandez-Tome et al. 2000

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to figure [a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
55	Rat (Sprague-Dawley)	30 d 1 x/d (GO)	Endocr		1 F (decr serum corticosterone)		Foster et al. 1995b
			Bd Wt	100 F			
56	Rat (CD)	4 mo (F)	Resp	4 F	12 F (hypertrophy and proliferation of endothelial cells, incr macrophages)		Goldstein et al. 1978
			Cardio	36 F			
			Hepatic	4 F	12 F (incr urinary and hepatic porphyrins, enlarged hepatocytes)		
			Endocr	36 F			
			Dermal	12 F	36 F (small sores)		
			Bd Wt	36 F			
57	Rat (Fischer- 344)	daily 5 wks (GO)	Hepatic		28 M (liver enlargement, porphyria, increased enzyme expression, increased protooncogene expression)		Gustafson et al. 2000
58	Rat (Wistar)	56 d (F)	Hepatic		5 F (incr highly carboxylated porphyrins in liver)		Kennedy and Wigfield 1990
			Renal		5 F (incr highly carboxylated porphyrins in kidney)		
			Bd Wt	5 F		50 F (large decr body wt gain)	

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
59	Rat (Sherman)	4 mo (F)	Resp		5 (incr macrophages, focal areas of fibrosis)	25 F (extensive intra-alveolar hemorrhage, edema in females)	Kimbrough and Linder 1974
			Cardio	5		25 (fibrosis, degeneration)	
			Hemato		5 (decr hemoglobin and hematocrit)		
			Hepatic		5 (incr liver wt, enlarged hepatocytes)	25 (necrosis, fibrosis)	
			Renal	5	25 (incr kidney wt)		
			Endocr		5 (hyperplasia of the adrenal cortex)		
			Dermal	5	25 (skin eruptions)		
			Bd Wt	5	25 (decr body wt gain in males)		
60	Rat (Sprague-Dawley)	1 gen (F)	Resp		3 F (intraalveolar foamy histiocytes, hypertrophy and proliferation of endothelial cells of pulmonary venules in dams)		Kitchin et al. 1982
			Bd Wt	7 F			
61	Rat (Wistar)	4 wk 1 x/d (GW)	Endocr		1000 F (decr serum T4, incr T4 metabolism, incr serum TSH, incr thyroid iodine uptake)		Kleiman de Pisarev et al. 1989
			Bd Wt	1000 F			
62	Rat (Wistar)	30 d 1 x/d (GW)	Endocr	125 F	500 F (decr serum T4 levels)		Kleiman de Pisarev et al. 1989

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
63	Rat (Wistar)	8 wk 7 d/wk 1 x/d (GW)	Hepatic		1000 F (incr liver wt, decr URO-D activity, incr hepatic and urinary porphyrins, incr dehalogenation of T4)		Kleiman de Pisarev et al. 1990
			Endocr		1000 F (decr serum T4, incr serum TSH)		
64	Rat (Wistar)	4 wk 7 d/wk 1 x/d (GW)	Hepatic		1000 F (incr liver wt)		Kleiman de Pisarev et al. 1995
			Endocr		1000 F (decr serum T4, incr serum TSH)		
			Bd Wt	1000 F			
65	Rat (Wistar)	15 wk 3-4 d/wk 1 x/d (GO)	Hepatic		50 F (incr liver wt, massive incr liver and urine porphyrins)		Koss et al. 1978
			Renal		50 F (incr kidney wt)		
			Endocr		50 F (incr adrenal wt)		
			Dermal		50 F (rough appearance of fur, hair loss, skin lesions)		
			Bd Wt	50 F			
66	Rat (Sprague-Dawley)	3-6 wk 5 d/wk 1x/d (GO)	Hepatic		50 (incr urinary and hepatic porphyrins)		Krishnan et al. 1991
67	Rat (Sprague-Dawley)	6 wk 5 d/wk 1 x/d (GO)	Hepatic		50 F (incr hepatic and urinary porphyrins)		Krishnan et al. 1992

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
68	Rat (COBS)	15 wk (F)	Hemato	8	32 F (decr red blood cell count, hematocrit, and hemoglobin; leukocytosis in females)		Kuiper-Goodman et al. 1977
			Musc/skel	32			
			Hepatic	0.5	2 (basophilic clumping in hepatocytes)		
			Renal	8	32 (incr kidney wt)		
			Dermal	8	32 (alopecia)		
			Bd Wt	8	32 M (13% decr in terminal body wt in males)		
69	Rat (Wistar)	30 d (F)	Resp		22 F (cuboidal venule endothelial cells, perivascular infiltrate, accumulation of alveolar macrophages)		Michielsen et al. 1997
			Hepatic		22 F (incr liver wt, hepatocyte hypertrophy with cytoplasmic inclusions)		
			Renal	45 F			
			Dermal		22 F (gross skin lesions, epidermal hyperplasia, inflammatory infiltrate in the dermis)		
			Bd Wt	45 F			

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to figure [a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
70	Rat (Lewis)	29 d (F)	Resp		8 F (cuboidal venule endothelial cells, perivascular infiltrate, accumulation of alveolar macrophages)		Michielsen et al. 1997
			Hepatic		8 F (incr liver wt, hepatocyte hypertrophy with cytoplasmic inclusions)		
			Renal	22 F			
			Dermal		8 F (gross skin lesions, epidermal hyperplasia, inflammatory infiltrate in the dermis)		
			Bd Wt	22 F			
71	Rat (Brown Norway)	28 d (F)	Resp		8 F (cuboidal venule endothelial cells, perivascular infiltrate, accumulation of alveolar macrophages)		Michielsen et al. 1997
			Hepatic		8 F (incr liver wt, hepatocyte hypertrophy with cytoplasmic inclusions)		
			Renal	22 F			
			Dermal		8 F (gross skin lesions, epidermal hyperplasia, inflammatory infiltrate in the dermis)		
			Bd Wt	22 F		45 F (sudden weight loss)	

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
72	Rat (Brown-Norway)	daily 4 wks (F)	Hepatic		67 F (increased liver weight)		Michielsen et al. 2000
			Resp		67 F		
			Dermal		67 F (dermal lesions-hyperplasia, deep venules with activated endothelium, inflammation)		
73	Rat (Brown-Norway)	7 or 21 d daily (GW)	Hepatic		45 F (increased liver weight)		Michielsen et al. 2001
			Dermal			45 F mg/kg (lesions with inflammatory response)	
			Resp			45 F mg/kg (airway hyperresponsiveness, granuloma (non-neoplastic lesions) formation)	
74	Rat (Brown-Norway)	7 or 21 d daily (GW)	Hemato		45 F (increased total IgE)		Michielsen et al. 2001
			Dermal			45 F mg/kg (lesions, inflammatory cellular imune response)	
			Resp			45 F mg/kg (airway hyperresponsiveness, granuloma (non-neoplastic lesions) formation)	
75	Rat (Wistar)	60 d (F)	Hepatic		430 M (incr serum ALT, bilirubin, and glutamate dehydrogenase)		Nikolaev et al. 1986

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
76	Rat (Sprague-Dawley)	56 d (F)	Hemato	100 M			Ockner and Schmid 1961
			Musc/skel		100 M (porphyrin accumulation in cortex of long bones)		
			Hepatic		100 M (porphyria, hepatomegaly, hepatocellular degeneration)		
77	Rat (Wistar)	3 wk (F)	Hepatic		25 M (incr liver wt)		Schielen et al. 1993
			Dermal		25 M (transient lesions on face and ears)		
			Bd Wt	50 M			
78	Rat (Wistar)	3 wk (F)	Dermal		50 (skin lesions)		Schielen et al. 1995b
			Bd Wt	50			
79	Rat (Agus)	112 d (F)	Hepatic		5 F (incr hepatic porphyrin content, decr hepatic URO-D activity, incr hepatic ALA-S activity)		Smith et al. 1979
80	Rat (Porton-Wistar)	112 d (F)	Hepatic	5 F			Smith et al. 1979

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
81	Rat (Fischer- 344)	15 wk (F)	Hepatic		10 (incr liver wt, hepatocellular hypertrophy, incr hepatic porphyrin levels in females)		Smith et al. 1985
			Renal		10 F (incr renal porphyrin levels in females)		
82	Rat (Wistar)	60 d 1 x/d (GW)	Hepatic		1000 F (decr URO-D activity in liver)		Sopena de Kracoff et al. 1994
			Endocr		1000 F (decr serum T4, incr serum TSH)		
83	Rat (Wistar)	6 wk (F)	Hepatic	8 M	22 M (incr liver wt)		Van Loveren et al. 1990
84	Rat (Wistar)	4 wk 3 d/wk 1 x/d (GW)	Hepatic		1000 M (incr liver wt)		van Raaij et al. 1993b
			Endocr		1000 M (decr serum T4 levels)		
85	Rat (Wistar)	3 wk (F)	Hemato		25 M (incr extramedullary hematopoiesis in spleen; neutrophilia)		Vos et al. 1979b
			Hepatic		25 M (incr liver wt, liver cell hypertrophy and cytoplasmic hyalinization)		
			Renal	100 M			
			Endocr	100 M			
			Bd Wt	100 M			

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
86	Rat (BD VI)	5 wk 7 d/wk 1 x/d (GW)	Hepatic		1000 (decr URO-D activity in males and females; incr hepatic and urinary porphyrins in females)		Wainstok de Calmanovici et al. 1991
87	Mouse (Balb/c)	6 wk (F)	Resp	22 M			Loose et al. 1977
			Hepatic		22 M (incr liver wt, hepatocellular hypertrophy)		
88	Mouse (C57BL/ 10ScSn)	7 wk (F)	Hepatic		26 M (incr hepatic porphyrin levels)		Vincent et al. 1989
89	Dog (Beagle)	21 d 1or 2 x/d (C)	Resp	100 F			Sundlof et al. 1981
			Cardio	100 F			
			Hemato	100 F			
			Hepatic		50 F (fatty changes in liver, swollen hepatocytes and hepatomegaly)		
			Renal	100 F			
			Endocr	100 F			

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
90	Rabbit (NS)	12 wk (F)	Hemato	245 F			De Matteis et al. 1961
			Musc/skel			245 F (necrosis, degeneration, and focal calcification in muscle, incr porphyrins in bone)	
			Hepatic			245 F (incr urinary and hepatic porphyrins, fatty change, necrosis)	
			Bd Wt			245 F (weight loss)	
91	Pig (SPF)	90 d (F)	Hemato	50 M			Den Tonkelaar et al. 1978
			Hepatic	0.05 M	0.5 M (hepatocellular hypertrophy)		
			Renal	0.5 M	5 M (incr kidney wt)	50 M (degeneration of proximal tubules and loop of Henle)	
			Endocr	0.5 M	5 M (incr thyroid wt)		
			Bd Wt	5 M		50 M (depressed growth)	

Immunological/Lymphoreticular

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
92	Monkey (Rhesus)	60 d (GW)			8 F (thymic cortical atrophy)		Iatropoulos et al. 1976
93	Rat (Wistar)	56 d (F)		5 F	50 F (incr spleen weight, incr highly carboxylated porphyrins in spleen)		Kennedy and Wigfield 1990
94	Rat (Wistar)	15 wk 3-4 d/wk 1 x/d (GO)			50 F (incr spleen wt)		Koss et al. 1978

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
95	Rat (COBS)	15 wk (F)		8	32 (incr lymphocyte count, incr spleen wt, congestive splenomegaly in females)		Kuiper-Goodman et al. 1977
96	Rat (Wistar)	30 d (F)			22 F (incr spleen wt, "high endothelial" venules in popliteal lymph node, incr serum IgM)		Michielsen et al. 1997
97	Rat (Lewis)	29 d (F)			8 F ("high endothelial" venules in popliteal lymph node)		Michielsen et al. 1997
98	Rat (Brown Norway)	28 d (F)			8 F (incr spleen wt, "high endothelial" venules in popliteal lymph node; incr serum IgM autoantibodies)		Michielsen et al. 1997
99	Rat (Brown-Norway)	daily 4 wks (F)			67 F (increased spleen weight)		Michielsen et al. 2000
100	Rat (Brown-Norway)	7 or 21 d daily (GW)			45 F (Increased spleen weight)		Michielsen et al. 2001
101	Rat (Wistar)	3 wk (F)			25 M (incr IgM autoantibodies)		Schielen et al. 1993
102	Rat (Wistar)	13 wk (F)			8 F (incr spleen wt, incr total serum IgM, incr serum IgM autoantibodies)		Schielen et al. 1995a

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
103 Rat	(Wistar)	3 wk (F)			50 (incr spleen and lymph node wt, altered size distribution of splenocytes, selective activation of splenic B-1 cells)		Schielen et al. 1995b
104 Rat	(Wistar)	6 wk (F)			8 M (decr NK cell activity in lung)		Van Loveren et al. 1990
105 Rat	(Wistar)	3 wk (F)			25 M (incr wt and proliferation of high-endothelial venules in lymph nodes, enlarged white pulp [marginal zones and follicles] in spleen, incr neutrophil count)		Vos et al. 1979b
106 Mouse		6 wk (F)			22 (incr susceptibility to hepatitis infection)		Carthew et al. 1990
107 Mouse	(Balb/c)	6 wk (F)			22 M (immunosuppression, decr antibody production)		Loose et al. 1977
108 Mouse	(Balb/c)	6 wk (F)			22 M (incr susceptibility to bacterial endotoxin and protozoan infection)		Loose et al. 1978
109 Mouse	(Balb/c)	18 wk (F)		22 M			Loose et al. 1981

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL Less serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
110	Mouse	18 wk (F)			0.6 M (incr susceptibility to tumor challenge in vivo, decr killing of tumor cells in vitro)		Loose et al. 1981
111	Mouse (C57BL/6)	40 wk (F)			22 M (decr graft-host activity)		Silkworth and Loose 1981
112	Pig (SPF)	90 d (F)		5 M	50 M (atrophy of lymph nodes)		Den Tonkelaar et al. 1978

Neurological

113	Monkey (Rhesus)	60 d 1 x/d (GW)		32 F		64 F (severe tremors, muscular weakness)	Knauf and Hobson 1979
114	Rat (Wistar)	1x/d 5 d/wk 1, 2, 3, 4, or 8 wks (GW)			1000 F (altered phospholipid levels in brain)		Cochon et al. 2001
115	Rat (Chbb THOM)	1x/d 5 d/wk 1, 2, 3, 4, or 8 wks (GW)			1000 F (altered phospholipid levels in brain)		Cochon et al. 2001
116	Rat (CD)	4 mo (F)		12 F		36 F (excessive irritability)	Goldstein et al. 1978
117	Rat (Sprague-Dawley)	4 gen (F)		4		8 F (convulsions in dams)	Grant et al. 1977

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
118	Rat (Wistar)	56 d (F)		5 F		50 F (lethargy, tremor, convulsions)	Kennedy and Wigfield 1990
119	Rat (Sherman)	4 mo (F)		5		25 (tremor, hyperexcitability)	Kimbrough and Linder 1974
120	Rat (Wistar)	15 wk 3-4 d/wk 1 x/d (GO)				50 F (muscle fasciculations, tremors)	Koss et al. 1978
121	Rat (COBS)	15 wk (F)		8		32 (tremors, ataxia, hind limb paralysis)	Kuiper-Goodman et al. 1977
122	Rat (Wistar)	60 d (F)				430 M (clonic convulsions, tremors, hyperexcitability)	Nikolaev et al. 1986
123	Rat (Sprague-Dawley)	56 d (F)				100 M (ataxia, tremor, paralysis)	Ockner and Schmid 1961
124	Rat (Sprague-Dawley)	20 wk (F)			40 M (decr nerve conduction velocity)		Sufit et al. 1986
125	Dog (Beagle)	21 d 1or 2 x/d (C)				50 F (dysrhythmic electro-encephalogram)	Sundlof et al. 1981
126	Rabbit (NS)	12 wk (F)				245 F (tremor, paresis)	De Matteis et al. 1961
127	Pig (SPF)	90 d (F)		5 M		50 M (tremors, unsteady gait)	Den Tonkelaar et al. 1978

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

| Key to[a] figure | Species (Strain) | Exposure/ duration/ frequency (Specific route) | System | NOAEL (mg/kg/day) | LOAEL | | Reference Chemical Form |
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
Reproductive							
128	Monkey (Cynomolgus)	90 d 1 x/d (C)			0.1 F (cellular degeneration of ovarian surface epithelium)		Babineau et al. 1991
129	Monkey (Cynomolgus)	90 d 1 x/d (C)			0.01[c] F (mitochondrial degeneration in developing ovarian follicles)		Bourque et al. 1995
130	Monkey (Cynomolgus)	13 wk 7 d/wk 1 x/d (C)		0.1 F	1 F (decr serum progesterone levels during the luteal phase of menstruation)		Foster et al. 1992a
131	Monkey (Cynomolgus)	90 d 1 x/d (C)		1 F	10 F (incr length of menstrual cycle, decr ovulatory levels of estradiol)		Foster et al. 1995a
132	Monkey (Rhesus)	60 d (GW)			8 F (degeneration of the germinal epithelium, reduced number of primary follicles, and multiple follicular cysts in the ovaries)		Iatropoulos et al. 1976
133	Monkey (Cynomolgus)	90 d 1x/d (C)			0.1 F (degenerative lesions in oocytes)		Jarrell et al. 1993
134	Monkey (Rhesus)	24 d 1 x/d (GW)				4 F (blocked ovulation)	Muller et al. 1978

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
135	Monkey (Cyno-molgus)	12 wk 7 d/wk 1 x/d (C)			0.1 F (altered morphology of ovary surface epithelium cells)	1 F (necrosis of ovary surface epithelium cells, denuding of ovary)	Sims et al. 1991
136	Rat (Wistar)	30 d 1 x/d (GW)			1000 F (incr estrus duration, altered hormone levels, reduced ovulation, degenerative ovarian lesions)		Alvarez et al. 2000
137	Rat (Sprague-Dawley)	21 d 1 x/d (GO)			1 F (incr serum progesterone levels)		Foster et al. 1992b
138	Rat (Sprague-Dawley)	4 gen (F)		8		16 (decr fertility and litter size)	Grant et al. 1977
139	Rat (Sprague-Dawley)	daily 28 d pre-mating - weaning (GO)	Bd Wt	0.008 F			Nakashima et al. 1997
140	Pig (SPF)	90 d (F)		5 M	50 M (retarded development of the testes)		Den Tonkelaar et al. 1978

Developmental

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	Less serious (mg/kg/day)	Serious (mg/kg/day)	Reference Chemical Form
141	Monkey (Rhesus)	22-60 d 1 x/d (GW)				64 (2/3 infants died with lethargy, ataxia, and listlessness)	Iatropoulos et al. 1978

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene ‑ Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL				Reference Chemical Form
					Less serious (mg/kg/day)		Serious (mg/kg/day)		
142 Rat	(Sprague-Dawley)	4 gen (F)		2	4	(decr pup weight gain)	8	(decr pup viability)	Grant et al. 1977
143 Rat	(Sprague-Dawley)	1 gen (F)			3	(decr pup weight gain)	4	(decreased survival)	Kitchin et al. 1982
144 Rat	(Wistar)	2 gen (F)		0.6 M	1.3 M	(reduced efficiency of pups in operant behavior task)			Lilienthal et al. 1996
145 Rat	(Wistar)	gestation + lactation + 2 wks post-weaning (F)			2.5	(decr resistance to infection, incr IgG response to tetanus toxoid, and proliferation of high endothelial venules in lymph nodes in pups)			Vos et al. 1979a
146 Rat	(Wistar)	gestation + lactation + 2 wks or 7 mo post-weaning (F)			0.2	(incr IgG and IgM response to tetanus toxoid, incr delayed-type hypersensitivity reaction to ovalbumin, and accumulation of foamy macrophages in the lung in offspring)	5	(high pup mortality)	Vos et al. 1983

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
CHRONIC EXPOSURE							
Death							
147	Mouse (Swiss)	120 wk (F)				24 (decr survival)	Cabral et al. 1979
148	Hamster (Syrian)	lifespan (F)				16 (decr lifespan)	Cabral et al. 1977
149	Dog (Beagle)	1 yr 1x/d (C)				11 F (2/6 females died)	Gralla et al. 1977
Systemic							
150	Rat (Sprague- Dawley)	2 gen (F)	Hemato	2			Arnold et al. 1985
			Hepatic	0.08	0.4 M (incr liver wt)		
			Renal	2			
			Bd Wt	2			
151	Rat (Fischer- 344)	daily 5 wks (F)	Hepatic	6.25	6.25 (increased liver weight, centrilobular hypertrophy, foci of GST-P)		Kishima et al. 2000
152	Rat (Sprague- Dawley)	1 yr (F)	Hepatic	0.05	0.25 (mitochondrial swelling and elongation)		Mollenhauer et al. 1975
153	Rat (Agus)	90 wk (F)	Hepatic		7 F (porphyria)		Smith and Cabral 1980
			Dermal		7 F (alopecia)		
			Bd Wt		7 F (decr body wt gain)		

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene ⁻ Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
154	Rat (Fischer- 344)	90 wk (F)	Hepatic		10 (decr URO-D activity, incr hepatic porphyrin levels, hepatocyte hypertrophy, fatty degeneration, bile duct hyperplasia)		Smith et al. 1985
			Renal		10 (incr kidney wt, incr renal porphyrin levels, nephrosis in males)		
			Endocr	10			
			Bd Wt		10 (decr body wt)		
155	Rat (Fischer- 344)	65 wk (F)	Hepatic		10 F (incr liver wt; biliary hyperplasia; incr liver porphyrins; induction of microsomal enzymes and glutathione S-transferase)		Smith et al. 1993
			Bd Wt	10 F			
156	Mouse (C57BL/10Sc Sn)	18 mo (F)	Hepatic		13 M (hepatocyte hypertrophy)		Smith et al. 1989
			Bd Wt		13 M (decr body wt)		
157	Hamster (Syrian golden)	lifespan (F)	Bd Wt			16 M (marked decr in wt gain)	Cabral et al. 1977

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

| Key to[a] figure | Species (Strain) | Exposure/ duration/ frequency (Specific route) | System | NOAEL (mg/kg/day) | LOAEL | | Reference Chemical Form |
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
158	Dog (Beagle)	1 yr (C)	Cardio	11		110 (arteriopathy)	Gralla et al. 1977a
			Gastro	1		11 (diarrhea, necrotic and inflammatory lesions of the omentum and abdominal serosa)	
			Hemato	1	11 (neutrophilia)	110 (anemia)	
			Hepatic	1		11 (hepatomegaly, bile duct hyperplasia, pericholangitis, periportal fibrosis, incr serum alkaline phosphatase and AST)	
			Bd Wt	1		11 (loss of body weight)	

Immunological/Lymphoreticular

| 159 | Dog (Beagle) | 1 yr (C) | | | 0.1 (incr severity of nodular hyperplasia of the gastric lymphoid tissue) | | Gralla et al. 1977a |

Neurological

| 160 | Mouse (Swiss) | 120 wk (F) | | | | 24 (tremors, convulsions) | Cabral et al. 1979 |

Reproductive

| 161 | Rat (Sprague-Dawley) | 2 gen (F) | | 2 | | | Arnold et al. 1985 |

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
Developmental							
162 Rat	(Sprague-Dawley)	2 gen (F)			0.016[d] M (peribiliary lymphocytosis and fibrosis of the liver in F1 adult males)	2 (decr neonatal viability of F1 pups; incr neoplastic liver nodules, parathyroid adenoma, and adrenal pheochromocytoma in F1 adults)	Arnold et al. 1985
Cancer							
163 Rat	(Sprague-Dawley)	104 wk (F)				4 (CEL: hepatoma, bile-duct adenoma, hepatocarcinoma, renal adenoma)	Ertürk et al. 1986
164 Rat	(Agus)	90 wk (F)				7 F (CEL: liver-cell tumors)	Smith and Cabral 1980
165 Rat	(Wistar)	75 wk (F)				5 F (CEL: liver-cell tumors)	Smith and Cabral 1980
166 Rat	(Fischer- 344)	90 wk (F)				10 (CEL: hepatocarcinoma)	Smith et al. 1985
167 Rat	(Fischer- 344)	65 wk (F)				10 F (CEL: liver tumors)	Smith et al. 1993
168 Mouse	(Swiss)	120 wk (F)				12 (CEL: liver tumors)	Cabral et al. 1979

Table 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (continued)

Key to[a] figure	Species (Strain)	Exposure/ duration/ frequency (Specific route)	System	NOAEL (mg/kg/day)	LOAEL		Reference Chemical Form
					Less serious (mg/kg/day)	Serious (mg/kg/day)	
169 Hamster (Syrian)		lifespan (F)				4 (CEL: hepatoma, liver hemangioendothelioma, thyroid alveolar adenoma)	Cabral et al. 1977

[a]The number corresponds to entries in Figure 3-2.

[b]Used to derive an acute oral minimal risk level (MRL) of 0.008 mg/kg/day; dose divided by uncertainty factor of 300 (10 for extrapolation from rats to humans, 10 for human variability, and 3 for use of a minimal LOAEL).

[c]Used to derive an intermediate oral MRL of 0.0001 mg/kg/day; dose divided by an uncertainty factor of 100 (3 for extrapolation from monkeys to humans, 10 for human variability, and 3 for use of a minimal LOAEL).

[d]Used to derive a chronic oral MRL of 0.00005 mg/kg/day; dose divided by an uncertainty factor of 300 (10 for extrapolation from rats to humans, 10 for human variability, and 3 for use of a minimal LOAEL).

ALA-S = delta-aminolevulinic acid synthetase; AST = aspartate aminotransferase; Bd wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); CNA = central nervous system; Decr = decreased; Endocr = endocrine; F = female; (F) = feed; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; gen = generation; (GO) = gavage in oil; (GW) = gavage in water; Hemato = hematological; Incr = increased; LD_{50} = lethal dose, 50% kill; LDH = lactose dehydrogenase; LOAEL = lowest-observable-adverse effect level; M = male; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observable-adverse-effect level; Resp = respiratory; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone; URO-D = uroporphyrinogen decarboxylase; wk = week(s); wt = weight; x = times; yr = year(s)

Figure 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral

Acute (≤14 days)

Figure 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (*Continued*)

Intermediate (15-364 days)

Figure 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (*Continued*)

Intermediate (15-364 days)

Systemic

mg/kg/day

Musculoskeletal

Hepatic

10000

1000 — ①43r ①44r ①49r ①50r ①63r ①64r ①82r ①84r ①86r
①75r

●90h ●90h
100 — ○37k ①76r ①39k ①48r ①53r ①76r
○39k ①51r ①72r
○68r ①89d ①45r ①46r ①65r ①66r ①67r ①73r
①87m ①88m ①57r ●59r ①69r ①77r ①83r ①85r
10 — ①37k ①41r ①52r ①56r ①70r ①71r ①81r ○83r
○56r ①58r ①59r ①79r ○80r
①68r

1 — ①41r ①42r ①38k ①40r ○41r ○68r ①91p

0.1 — ○41r ○42r ○38k ○40r ○91p

0.01

0.001

0.0001

---

c-Cat	-Humans	f-Ferret	n-Mink	● Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		① LOAEL, Less Serious-Animals	▲ LOAEL, Less Serious-Humans	for effects
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer

Figure 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (*Continued*)

Intermediate (15-364 days)

Systemic

Renal   Endocrine   Dermal

mg/kg/day

10000 —

1000 —
Renal: ◐54r
Endocrine: ◐61r ◐63r ◐64r ◐82r ◐84r
◐62r

100 —
Renal: ○89d ◐39k ○39k ◐47r ◐65r ○69r ○85r ◐91p ○40r ◐52r ◐59r ◐68r ○70r ○71r ◑37k ◐41r ○52r ○68r ◑81r ◐58r ○59r
Endocrine: ○89d ○37k ○62r ○85r ◐65r ○56r ◐52r ◐38k ◐40r ◐42r ○52r ○59r ◐91p
Dermal: ◐51r ◐65r ◐72r ◐56r ◐73r ●74r ◐78r ○59r ◐68r ◐77r ◐69r ◐52r ○56r ○68r ◐70r ◐71r ○59r

10 —

1 —
Renal: ○41r ◐42r ○91p
Endocrine: ○38k ◐41r ○42r ◐55r ○91p

0.1 —
Renal: ○42r
Endocrine: ○41r

0.01 —

0.001 —

0.0001 —

c-Cat	-Humans	f-Ferret	n-Mink	●Cancer Effect Level-Animals	▼Cancer Effect Level-Humans	■LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	●LOAEL, More Serious-Animals	▲LOAEL, More Serious-Humans	┊ Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		◐LOAEL, Less Serious-Animals	▲LOAEL, Less Serious-Humans	┊ for effects
p-Pig	h-Rabbit	s-Hamster		○NOAEL - Animals	△NOAEL - Humans	└ other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer

Figure 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (*Continued*)
Intermediate (15-364 days)

c-Cat	-Humans	f-Ferret	n-Mink	● Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		◐ LOAEL, Less Serious-Animals	△ LOAEL, Less Serious-Humans	for effects
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer

Figure 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (*Continued*)

Intermediate (15-364 days)

mg/kg/day

c-Cat	-Humans	f-Ferret	n-Mink
d-Dog	k-Monkey	j-Pigeon	o-Other
r-Rat	m-Mouse	e-Gerbil	
p-Pig	h-Rabbit	s-Hamster	
q-Cow	a-Sheep	g-Guinea Pig	

● Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
△ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
┆ Minimal Risk Level
┆ for effects
┘ other than
   Cancer

Figure 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (*Continued*)

Chronic (≥365 days)

Systemic

mg/kg/day

Death Cardiovascular Gastrointestinal Hematological Hepatic Renal Endocrine Dermal Body Weight

1000

100   ●158d (Cardiovascular)   ●158d (Hematological)

10   ●147m ●148s ●149d   ○158d   ●158d   ◑158d   ●158d ◐156m ○151r ◑151r ◑154r ◑153r ●155r   ◑154r   ○154r   ◑153r   ●157s ◑158d ◐156m ◑153r ◑154r ○155r

1   ○150r ○158d   ○158d   ○158d   ○150r   ○150r ○158d

0.1   ◑150r ◑152r

0.01   ○150r ○152r

0.001

0.0001

1E-5

1E-6

1E-7

1E-8

Figure 3-2. Levels of Significant Exposure to Hexachlorobenzene - Oral (*Continued*)

Chronic (≥365 days)

mg/kg/day

Immuno/Lymphor   Neurological   Reproductive   Developmental   Cancer*

```
1000

 100

  10            ●160m                              ◆168m        ◆166r  ◆167r
                                                        ◆164r
                                                    ◆169s ◆163r  ◆165r
   1                     ○161r          ●162r

 0.1    ◑159d

0.01                                    ◑162r

0.001

0.0001

1E-5                                                                    10⁻⁴
                                                                             Estimated
                                                                        10⁻⁵ Upper-Bound
1E-6                                                                         Human Cancer
                                                                             Risk Levels
                                                                        10⁻⁶
1E-7    *Doses represent the lowest dose tested per study that produced a tumorigenic
        response and do not imply the existence of a threshold for the cancer end point.  10⁻⁷

1E-8
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c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	⁞ Minimal Risk Level
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	△ LOAEL, Less Serious-Humans	for effects
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	other than
q-Cow	a-Sheep	g-Guinea Pig				Cancer

lesions, typically occurring together, were found in six different strains of rats and both sexes, at doses as low as 3 mg/kg/day in intermediate-duration feeding studies (Goldstein et al. 1978; Kimbrough and Linder 1974; Kitchin et al. 1982; Michielsen et al. 1997, 1999, 2001). They were seen at doses as low as 1 mg/kg/day, with a NOAEL of 0.2 mg/kg/day, in rats that received both pre- and postnatal exposure (Vos et al. 1979a, 1983). (The Vos studies are included as developmental toxicity studies in Table 3-2 and Figure 3-2.) Michielsen et al. (1997) hypothesized an immunomodulated etiology for these lesions, but the lesions occurred to a similar extent in five rat strains (Wistar, Lewis, Brown Norway; athymic and euthymic WAG/Rij) with very different responses to immunomodulating agents and did not correlate with observed immune changes, providing no support for this hypothesis (Michielsen et al. 1997, 1999, 2001). More severe pulmonary effects have also been observed in rats at higher doses. In addition to macrophage accumulation and focal areas of interstitial fibrosis, which they observed in male and female rats exposed to $5 mg/kg/day in the diet for 4 months, Kimbrough and Linder (1974) also observed extensive intra-alveolar hemorrhage, inflammation, and edema, accompanied by an increase in lung weight, in females exposed to 25 or 50 mg/kg/day. Michielsen et al. (2001) observed an adverse effect on respiratory function, and airway hyperreponsiveness, as well as granulomatous lung inflammation, in rats exposed to 45 mg/kg/day. Only limited pulmonary histopathology data are available for other species, and pulmonary lesions were not seen in available studies on monkeys, dogs, or mice (Iatropoulos et al. 1976; Loose et al. 1977; Sundlof et al. 1981).

Cardiovascular Effects.   No studies were located regarding cardiovascular effects of oral hexa-chlorobenzene exposure in humans.

There have been a few reports of cardiovascular lesions in animals exposed to hexachlorobenzene. Gralla et al. (1977a) described an arteriopathy affecting multiple organs in dogs treated with 110 mg/kg/day of hexachlorobenzene for 1 year. The lesion was characterized by inflammation of small arteries and arterioles, with focal proliferative endartitis, fibrinoid necrosis, and thrombosis, and occasionally involved fibrosis and inflammation adjacent to the arterioles in the heart and the liver. Although features of the lesion suggested a hypersensitivity reaction, an immune etiology was not supported by serum electrophoretic data. The arteritis was seen in 4 of 12 beagle dogs treated with 110 mg/kg/day, a dose that produced weight loss, mortality, and other frank toxic effects, but was not seen in dogs treated with lower doses. It is not known if these effects were produced by a direct effect of hexachlorobenzene or were secondary to general poor health of the dogs in this study. However, similar observations in the heart were made by Kimbrough and Linder (1974) in rats. These researchers found fibrosis and degeneration of muscle fibers in the heart of rats exposed to 25 mg/kg/day or more in the diet for 4 months.

Degenerated tissue was infiltrated by inflammatory cells. Other studies that included pathological examination of cardiovascular tissues in dogs, rats, and monkeys did not find treatment-related lesions (Goldstein et al. 1978; Iatropoulos et al. 1976; Sundlof et al. 1981).

Gastrointestinal Effects. No studies were located regarding gastrointestinal effects of oral hexachlorobenzene exposure in humans.

Gastrointestinal effects have not been commonly reported in animal studies of hexachlorobenzene. Dogs given $11 mg/kg/day by capsule for 1 year experienced intermittent episodes of diarrhea (Gralla et al. 1977a). Necropsy revealed necrotic and inflammatory lesions of the omentum and abdominal serosa, but there were apparently no findings in the stomach, small intestines, or large intestines. Pathological examination of female pigs exposed to 0.025 or 0.5 mg/kg/day for 212 days throughout mating, gestation, and lactation showed gastrointestinal lesions ranging from catarrhal exudation to mild ulceration, but microscopic signs of gastritis were also found in some control group animals, suggesting that the observed lesions were not an effect of hexachlorobenzene (Hansen et al. 1979). Gastrointestinal lesions were not observed in female Rhesus monkeys given oral hexachlorobenzene at doses of up to 128 mg/kg/day for 60 days (Iatropoulos et al. 1976).

Hematological Effects. No data were located on the hematological effects of hexachlorobenzene in humans.

Animal data suggest that hexachlorobenzene can produce anemia and leukocytosis. Several studies reported decreases in hemoglobin, hematocrit, and/or red blood cell count in rats at doses ranging from 5 to 32 mg/kg/day in 1–4-month studies (Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977; Richter et al. 1981). Female rats were much more sensitive than male rats in these studies (Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977). Other studies in rats did not find changes in these parameters, but used lower doses (Arnold et al. 1985), a much shorter exposure period (Lecavalier et al. 1994), or only the less sensitive male rats (Ockner and Schmid 1961). Other reported findings in rats consistent with the hypothesis that hexachlorobenzene can produce anemia were increased extramedullary hematopoiesis in the spleen, which was seen at doses as low as 5 mg/kg/day with combined pre- and postnatal exposure (Vos et al. 1979a, 1979b, 1983), and reduced medullary area in the femur, which was found at 10 mg/kg/day and above in a 15-week study (Andrews et al. 1990). Limited data are available regarding hematological effects in other species. Anemia was observed in dogs exposed to 110 mg/kg/day for 1 year (Gralla et al. 1977a), but not in dogs exposed to 100 mg/kg/day for only

3 weeks (Sundlof et al. 1981), in rabbits exposed to 245 mg/kg/day for 12 weeks (De Matteis et al. 1961), in pigs exposed to 50 mg/kg/day for 13 weeks (Den Tonkelaar et al. 1978), or in monkeys exposed to 128 mg/kg/day for 60 days (Knauf and Hobson 1979) or 10 mg/kg/day for 90 days (Foster et al. 1995a).

Many of the same studies that reported anemia and related findings also reported neutrophilia and/or leukocytosis. In rats, the white blood cell increases were found at the same or higher doses than the red cell changes ($25 mg/kg/day in 3–16 week feeding studies), and there appeared to be less of a disparity in response between males and females (Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977; Vos et al. 1979b). In dogs, afebrile neutrophilia was observed at 11 mg/kg/day, while anemia was found only at the high dose of 110 mg/kg/day (Gralla et al. 1977a). Negative studies in these species used lower doses (Arnold et al. 1985) or a much shorter exposure period (Lecavalier et al. 1994; Sundlof et al. 1981). Although values remained within normal ranges, Den Tonkelaar et al. (1978) identified a tendency towards leukocytosis and relative neutrophilia in male pigs treated with dietary doses as low as 0.05 mg/kg/day for 90 days. However, studies in monkeys given doses up to 10 mg/kg/day for 90 days (Foster et al. 1995a) or doses up to 128 mg/kg/day for 60 days (Knauf and Hobson 1979) were negative.

Musculoskeletal Effects.   Hexachlorobenzene has been associated with painless arthritis (swelling of the joints distinct from rheumatoid arthritis), osteoporosis, and small distinctive hands in patients exposed to the chemical from consumption of bread prepared from contaminated grain. Although there was severe shortening of digits due to osteoporosis in the bones of the hands (phalangeal, carpal, and metacarpal), particularly at the ends, no limitation of movement was reported. Painless arthritic changes were also reported in the patients (Cripps et al. 1984; Peters et al. 1982, 1987).

Effects on both bone and muscle have been reported in animal studies of hexachlorobenzene. Detailed studies of bone effects were conducted by Andrews et al. (1989, 1990) in male rats treated by gavage in corn oil for up to 15 weeks. These researchers found significant, dose-related increases in femur density (osteosclerosis) at doses of 1 mg/kg/day and above, and identified a NOAEL of 0.1 mg/kg/day for this effect. Femur length, weight, volume, and cross-sectional area were not consistently altered, indicating no effect on the rate of bone growth. The other dose-related changes in bone were an increase in cortical area at 1 mg/kg/day and above and a corresponding decrease in medullary area at 10 mg/kg/day and above. Other pertinent findings were decreases in serum alkaline phosphatase and increases in serum 1,25-dihydroxy-vitamin D3 and parathyroid hormone (hyperparathyroidism). The joint findings of osteosclerosis, increased cortical and reduced medullary area without a change in the rate of bone growth, and decreased serum alkaline phosphatase are consistent with a mechanism involving reduced resorption

of bone. The increases in serum 1,25-dihydroxy-vitamin D3 and parathyroid hormone, both of which are involved in calcium regulation and bone resorption, suggest that the hypothesized effect on bone resorption is probably secondary to hyperparathyroidism. The decrease in medullary area (bone marrow cavity) that ultimately results from these changes may, in turn, contribute to hematological and immune effects associated with hexachlorobenzene.

Studies conducted at higher doses that produced marked interference with heme metabolism found substantial accumulation of porphyrins in bone cortex, but not marrow. This was observed in rats treated with 100 mg/kg/day in the diet for 56 days (Ockner and Schmid 1961) and rabbits treated with 245 mg/kg/day in the diet for 84 days (De Matteis et al. 1961). No bone (or liver or kidney) accumulation of porphyrins was observed by Andrews et al. (1989), but the highest dose in this study was only 25 mg/kg/day.

Skeletal muscle lesions have been reported in animals exposed to hexachlorobenzene, but only with repeated exposure to high doses. Rabbits treated with 245 mg/kg/day of hexachlorobenzene for 12 weeks were observed to have necrosis, degeneration, and focal calcification in skeletal muscle (De Matteis et al. 1961). Skeletal muscle lesions were not found in rats fed up to 32 mg/kg/day for 15 weeks (Kuiper-Goodman et al. 1977).

Hepatic Effects.   The major evidence that oral exposure to hexachlorobenzene by humans can result in hepatopathology is derived from studies of an outbreak of porphyria in Turkey attributed to the consumption of bread prepared from hexachlorobenzene-contaminated grain from 1955 to 1959 (Cam and Nigogosyan 1963; Cripps et al. 1984; Peters et al. 1982, 1987). These adverse hepatic effects were mainly characterized by porphyria. The appearance of abnormal levels of porphyrin precursors in the urine suggests that hexachlorobenzene disturbed the body's porphyrin metabolism in the liver, which caused histopathologic changes in the liver. Uroporphyrin and δ-aminolevulinic acid (d-ALA) synthase increased in the urine, and uroporphyrin and coproporphyrin increased in the stool of patients who had ingested hexachlorobenzene-contaminated bread (Cripps et al. 1984; Peters et al. 1982, 1987). No quantitation of exposure (dose and duration) was presented in any of these clinical reports. However, an estimated dose of 0.05–0.2 g/day (0.7–2.9 mg/kg/day for a 70-kg person) is considered to be reliable by the original investigators of the Turkey epidemic (Cam and Nigogosyan 1963).

Studies in animals have confirmed that the liver is an important target organ for hexachlorobenzene following ingestion. Hepatic effects associated with oral exposure to hexachlorobenzene in animal

studies include disruption of heme synthesis (culminating in porphyria), induction of microsomal enzymes, hepatomegaly, and cellular damage.

Disruption of hepatic heme synthesis by hexachlorobenzene has been well studied in rats (see below). Hexachlorobenzene inhibits the activity of hepatic uroporphyrinogen decarboxylase, an enzyme in the heme biosynthesis pathway, leading to build up of heme precursors (porphyrins) in the liver and other tissues and their excessive excretion in the urine (porphyria). The activity of other enzymes in the heme biosynthesis pathway may also be altered; in particular, an increase in the activity of δ-aminolevulinic acid synthestase has been reported in some studies (see Section 3.5, Mechanisms of Action). This pattern of effects is very similar to what is seen in human porphyria cutanea tarda.

Following acute exposure, the lowest dose reported to induce outright porphyria in an animal study was 25 mg/kg/day in an 8-day study in female rats in which hexachlorobenzene was administered by gavage in corn oil; the rats were monitored for urinary and hepatic porphyrins after 35–50 days (Krishnan et al. 1991). Goldstein et al. (1978) observed a statistically significant increase in hepatic δ-aminolevulinic acid synthestase activity in female rats fed $16 mg/kg/day of hexachlorobenzene in the diet for 1 week, but there was little or no effect on hepatic porphyrin levels at that time, possibly because of insufficient latency time for the effect to develop. (Time course studies have shown that there may be a delay of approximately 4 weeks between treatment with hexachlorobenzene and development of porphyria [Krishnan et al. 1991; Mylchreest and Charbonneau 1997; Billi de Catabbi et al. 2000a], although there may be little or no delay if the hexachlorobenzene is administered in a form that is readily absorbed [e.g., predissolved in corn oil] at high doses [Kennedy and Wigfield 1990].) There was no increase in hepatic δ-aminolevulinic acid synthestase activity at 5 mg/kg/day in the Goldstein et al. (1978) study. The dose level at which hexachlorobenzene will produce porphyria depends on the exposure protocol. When hexa-chlorobenzene was administered to female rats by gavage for 7 days in an aqueous suspension rather than oil, no effect on hepatic uroporphyrinogen decarboxylase (monitored at 7 days) was observed even at 250 mg/kg/day, and only doses of 500 mg/kg/day or higher were effective (Kleiman de Pisarev et al. 1990), reflecting the fact that hexachlorobenzene administered in water is only minimally absorbed from the gastrointestinal tract, and also perhaps, the short latency time. Even at 1,000 mg/kg/day in this study, uroporphyrinogen decarboxylase activity was decreased only 25%, while δ-aminolevulinic acid synthestase activity and liver porphyrin levels were unchanged from controls. Billi de Catabbi et al. (2000a) observed that acute (5-day) exposure above a threshold (1 g/kg) caused persistent porphyria (lasting at least as long as the 20-week observation period).

Hexachlorobenzene doses as low as 5–12 mg/kg/day have been reported to produce porphyrinogenic effects, such as increased liver weight, inhibition of hepatic uroporphyrinogen decarboxylase, accumulation of porphyrins in liver, excretion of porphyrins in urine, and increased hepatic δ-aminolevulinic acid synthestase activity, in female rats exposed for intermediate durations (Alvarez et al. 2000; Den Besten et al. 1993; Goldstein et al. 1978; Kennedy and Wigfield 1990; Kuiper-Goodman et al. 1977; Smith et al. 1979, 1985; Sweeney et al. 1986). One of these studies, Goldstein et al. (1978), identified a NOAEL of 4 mg/kg/day for increases in liver porphyrins and δ-aminolevulinic acid synthestase activity after a 4-month dietary exposure. In male rats, there was little or no evidence of porphyria at doses up to 25 mg/kg/day (Andrews et al. 1989; Kuiper-Goodman et al. 1977; Smith et al. 1985), but mild changes were noted at 32–50 mg/kg/day (Krishnan et al. 1991; Kuiper-Goodman et al. 1977) and severe porphyria was observed at 100 mg/kg/day (Ockner and Schmid 1961). The reduced sensitivity of male rats in comparison to females may be related to differences in metabolism of hexa-chlorobenzene between the sexes in this species, particularly with regard to glutathione conjugation (D'Amour and Charbonneau 1992; Richter et al. 1981; Rizzardini and Smith 1982), which have been linked to the presence of estradiol in the females (Legault et al. 1997). Among female rats, strain-related differences in sensitivity have also been reported (Billi de Catabbi et al. 2000a; Michielsen et al. 1997), possibly related, at least in one case, to differences in nonheme iron content of the liver between strains (Smith et al. 1979). In other species, there was no evidence of porphyria in female monkeys treated with up to 10 mg/kg/day by capsule for 3 months (Jarrell et al. 1993) or in male pigs exposed to up to 50 mg/kg/day in the diet for 3 months (Den Tonkelaar et al. 1978); these results appear to reflect species differences, but may be influenced by the lack of oil vehicle to enhance absorption.

In studies of chronic exposure duration, hexachlorobenzene doses of 7–10 mg/kg/day in the feed produced complete inhibition of uroporphyrinogen decarboxylase and high levels of porphyrins in the liver and urine in both male and female rats (Smith and Cabral 1980; Smith et al. 1985, 1993). Although uroporphyrinogen decarboxylase activity was completely inhibited in both sexes, liver accumulation of porphyrins was 5-fold higher in females than in males (Smith et al. 1985). Chronic rat studies that employed lower dose levels did not monitor porphyrin levels (Arnold et al. 1985; Mollenhauser et al. 1975). Male mice exposed to slightly higher dietary doses of hexachlorobenzene (13 mg/kg/day) showed only a modest transitory increase in hepatic porphyrin levels after 6 months of treatment, which was not found at subsequent sacrifices at 12 and 18 months (Smith et al. 1989). There was no evidence of porphyrin accumulation in the liver or other tissues of dogs treated with hexachlorobenzene doses as high as 110 mg/kg/day for 1 year (Gralla et al. 1977a, 1977b).

As in human porphyria cutanea tarda, porphyria in rats produced by hexachlorobenzene typically occurs along with other effects on the liver, such as induction of microsomal enzymes, increased liver weight, hepatocellular hypertrophy, cytoplasmic vacuolation, fatty degeneration, and biliary hyperplasia (Cuomo et al. 1991; Den Besten et al. 1993; Kimbrough and Linder 1974; Koss et al. 1978; Kuiper-Goodman et al. 1977; Michielsen et al. 1997; Smith et al. 1985, 1993; Sweeney et al. 1986; Vos et al. 1979b). The relationship between porphyria and these other hepatic effects is uncertain. In some instances, effects on liver weight, enzymes, and/or histopathology occurred in rats at lower doses than porphyria or in the absence of porphyria (e.g., Arnold et al. 1985; Gustafson et al. 2000; Kishima et al. 2000; Mehendale et al. 1975; Mollenhauer et al. 1975; Michielsen et al. 2000). Liver lesions have also been observed in species, such as monkeys, dogs, and pigs, where there is no evidence of a porphyrinogenic effect (Den Tonkelaar et al. 1978; Gralla et al. 1977a; Iatropoulos et al. 1976; Jarrell et al. 1993). Kishima et al. (2000) reported that the hepatotoxicity of hexachlorobenzene was increased by an energy-restricted diet. The most sensitive hepatic end points following acute, intermediate, and chronic exposure, respectively, were increased liver weight and induction of microsomal enzymes in male rats exposed to 10 mg/kg/day for 6 days (Mehendale et al. 1975), hepatocellular hypertrophy in male pigs exposed to 0.5 mg/kg/day for 90 days (Den Tonkelaar et al. 1978), and peribiliary lymphocytosis and fibrosis in F_1 adult male rats exposed to 0.016 mg/kg/day *in utero* and throughout their lifetime in a 2-generation study (Arnold et al. 1985; listed as a developmental effect in Table 3-2 and the basis for the chronic oral MRL of 0.00005 mg/kg/day, as described in the footnote to Table 3-2 and in Appendix A).

Renal Effects.   No studies were found regarding renal effects in humans following oral exposure to hexachlorobenzene.

Animal studies have demonstrated that the kidney is a target for hexachlorobenzene. Renal effects that have been widely reported in animal studies are increased kidney weight, accumulation of porphyrins in association with disruption of heme metabolism (as in the liver), and direct and indirect evidence of renal tissue damage. Increases in kidney weight have been observed in many studies, primarily those involving $7 weeks of exposure (Andrews et al. 1989; Bouthillier et al. 1991; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Kimbrough and Linder 1974; Koss et al. 1978; Kuiper-Goodman et al. 1977; Smith et al. 1985). Most studies shorter than 7 weeks in duration did not find increases in kidney weight, even using doses as high as 100 mg/kg/day (Andrews et al. 1988; Richter et al. 1981; Sundlof et al. 1981; Vos et al. 1979b). This includes interim sacrifices in longer duration studies that did eventually show increases in kidney weight (Andrews et al. 1989). Multiple-dose feeding studies of 12–16 weeks identified LOAEL values of 19–32 mg/kg/day and NOAEL values of 5–9.5 mg/kg/day for increased

kidney weight in male and female rats (Den Besten et al. 1993; Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977). Lower critical values were obtained when exposure was by gavage in oil rather than in the diet (Andrews et al. 1989, 1990), when pigs were tested rather than rats (Den Tonkelaar et al. 1978), and when exposure duration was extended over 1 year (Smith et al. 1985). The lowest LOAEL and NOAEL for increased kidney weight in any study were 1 and 0.1 mg/kg/day, respectively (Andrews et al. 1989). It has been proposed that increased kidney weight in animals exposed to hexachlorobenzene may result from induction of renal microsomal enzymes (Bouthillier et al. 1991). Renal pathology (described below) may have been a contributing factor in some studies.

Among the available studies, the most sensitive indication of renal pathology due to hexachlorobenzene was increased urine enzyme levels. Male rats treated with $1 mg/kg/day of hexachlorobenzene by gavage in oil for 15 weeks had increased concentrations of alkaline phosphatase and/or lactate dehydrogenase in the urine, an effect that was not found at 0.1 mg/kg/day (Andrews et al. 1989, 1990). These effect levels correspond with those for changes in kidney weight in the same studies. Increased urinary levels of these enzymes suggest the occurrence of either glomerular damage allowing the enzymes to leak into the urine from the serum or tubular cell damage where the enzymes are released directly from the damaged cells into the urine. There was an apparent increase in calcium excretion in a higher dose group that could be interpreted to indicate impaired reabsorption of calcium by the distal tubules, which would support the hypothesis that damaged tubular cells were responsible for the observed enzymuria.

Studies that included histopathological examination of the kidneys provided more direct evidence of damage to renal tubule cells. Bouthillier et al. (1991) observed degenerative and regenerative foci of epithelial cells in the proximal tubules and accumulation of protein droplets in proximal tubular cells in male rats treated with 50 mg/kg/day for 7 weeks or 100 mg/kg/day for 2 weeks. These lesions were accompanied by glucosuria, proteinuria, and an 11-fold increase in $\alpha_{2\mu}$-globulin levels. The nature of the effects in males and near-absence of effects in females (just glucosuria and an increase in urinary γ-glutamyl transpeptidase were found) led the researchers to suggest that hexachlorobenzene produces a male rat specific protein droplet nephropathy, as is seen with some other chlorinated benzenes. Support for a nephrotoxic effect in rats is provided by Smith et al. (1985), who found mild to severe nephrosis in 12/12 surviving male rats treated with 10 mg/kg/day for 90 weeks, but 0/10 surviving male controls and only 1/10 surviving treated females. Similarly, Arnold et al. (1985) observed chronic nephrosis in male, but not female, rats with combined pre- and postnatal lifetime exposure to 2 mg/kg/day of hexachloro-benzene.

3. HEALTH EFFECTS

Renal lesions, however, have been reported in female rats treated with hexachlorobenzene. Basophilic renal tubules and protein casts were seen in female rats exposed to 19 mg/kg/day for 13 weeks (Den Besten et al. 1993). Renal accumulation of porphyrins has been reported in female rats exposed to doses as low as 5 mg/kg/day for 56 days (see below). Renal effects have also been noted in other animal species. Female monkeys treated with $8 mg/kg/day for 60 days developed vacuolization of proximal tubules, and at 128 mg/kg/day, thickening of the basement membranes, glomerular hyperemia, and increased BUN were also found (Iatropoulos et al. 1976; Knauf and Hobson 1979). Male pigs exposed to 50 mg/kg/day in the diet all died prior to scheduled sacrifice and were found upon necropsy to have degeneration of the proximal tubules and loop of Henle (Den Tonkelaar et al. 1978). Blood ammonia levels were increased 3-fold in female guinea pigs treated with 200 mg/kg/day (De Matteis et al. 1961). There is also a brief paper by Ertürk et al. (1986), lacking detail regarding experimental methods or results, that reports marked renal lesions, including severe hyperemia, necrotic tubular degeneration, hemorrhage, and nephritis in male and female rats, mice, and hamsters given 90-day dietary exposure to 10–33 mg/kg/day (although the effects were most severe in male rats, they were seen in all groups). The occurrence of renal effects in female rats and in other species of laboratory animal shows that the nephrotoxic effects of hexachlorobenzene are not limited to the $\alpha_{2\mu}$-globulin nephropathy demonstrated by Bouthillier et al. (1991), and suggests that this chemical may produce renal effects by multiple mechanisms.

Renal accumulation of porphyrins has been related to disruption of heme metabolism and lipid peroxidation. Female rats treated with 1,000 mg/kg/day of hexachlorobenzene by gavage in aqueous Tween 20 had a significant decrease in uroporphyrinogen decarboxylase (URO-D) activity in the renal cortex after 3 weeks of exposure and a subsequent increase in porphyrin levels in the renal cortex, but not the renal medulla or papilla, after 4 weeks of exposure (Fernandez-Tome et al. 2000). Lipid peroxidation, indicated by measurement of conjugated dienes and malondialdehyde (MDA), was significantly increased throughout most of the exposure period in the renal cortex, but was not increased at all in the renal medulla or papilla. Based on these findings, the researchers suggested that disruption of heme metabolism and accumulation of porphyrins in the renal cortex are secondary to lipid peroxidation produced by hexachlorobenzene in this tissue. Distribution of porphyria in kidney is consistent with fact that enzymes of heme metabolism are localized in the renal cortex; the occurrence of lipid peroxidation in the renal cortex is consistent with its relative susceptibility to oxidative stress, compared to the papilla or medulla. The use of such a high dose in this study reflects the fact that hexachlorobenzene is not well absorbed from water. Renal accumulation of porphyrins was observed at much lower doses (as low as 5 mg/kg/day) in feeding studies in female rats (Kennedy and Wigfield 1990; Smith et al. 1985),

particularly when hexachlorobenzene was added to the diet in an oil vehicle (Kennedy and Wigfield 1990). No experimental evidence was found for renal accumulation of porphyrins in male rats treated with up to 25 mg/kg/day for 15 weeks in the diet or by gavage in oil (Andrews et al. 1989; Smith et al. 1985).

Despite the numerous data supporting an effect of hexachlorobenzene on the kidney, it should be noted that several well-conducted investigations of kidney histopathology failed to find any treatment-related lesions in either male or female rats, even with high exposures (up to 50 mg/kg/day for 4 months) that, based on the database as a whole, would have been expected to produce tissue damage (Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977; Michielsen et al. 1997; Vos et al. 1979b).

Endocrine Effects.   Human data suggest that hexachlorobenzene adversely affects the endocrine system; specifically, the thyroid is a target organ. Two follow-up studies conducted 25 (Peters et al. 1982) and 20–30 years (Cripps et al. 1984) after patients (n=161–225) were exposed as children to bread contaminated with hexachlorobenzene in Southeast Turkey detected thyromegaly in 60% of women and 25% of men (Cripps et al. 1984; Peters et al. 1982). The background incidence for this area was 5%. Additionally, hirsutism and small stature were observed in 47 and 44%, respectively, of the study population. However, a study of serum hormone and organochlorine levels in a cohort of 110 Swedish or Latvian men who consumed Baltic sea fish (Hagmar et al. 2001) found no age-adjusted correlation between hexachlorobenzene levels and any of the measured serum hormones (follicle-stimulating hormone, luteinizing hormone, prolactin, plasma thyrotropin, free and total T3, free and total T4, and free testosterone). The authors considered the fish to be a diet high in persistent organohalogens, but did not quantitate intake.

Animal studies have demonstrated that hexachlorobenzene has multiple endocrine effects; the most striking are the induction of hypothyroidism and hyperparathyroidism in rats. Limited evidence suggests that hexachlorobenzene also affects serum retinoid levels, the adrenal gland and serum levels of corticosterone and cortisol (see below). Moreover, studies have shown that hexachlorobenzene affects serum levels of estrogen and progesterone (discussed in Section 3.2.2.5, Reproductive Effects).

Multiple studies have demonstrated that serum T4 levels decrease rapidly in rats following gavage treatment with hexachlorobenzene (Den Besten et al. 1993; Den Tonkelaar et al. 1978; Foster et al. 1993; Kleiman de Pisarev et al. 1989, 1990, 1995; Smith et al. 1986b; Sopena de Kracoff et al. 1994; van Raaij et al. 1993a, 1993b). Effects on serum TSH levels (both increases and decreases) are delayed and appear

secondary to decreases in T4. The most sensitive acute study observed statistically significant decreases in T4 levels in female rats at doses as low as 50 mg/kg/day for 5 days (Foster et al. 1993). A time-course in female Wistar rats gavaged with 1,000 mg/kg/day of hexachlorobenzene in corn oil found that serum T4 levels rapidly decreased, reaching a steady-state after 8 days, approximately 75% below controls. In contrast, serum TSH levels reached a steady state after 30 days, at 80% below controls (Sopena de Kracoff et al. 1994). Similarly, experiments in female Wistar rats gavaged with 1,000 mg/kg/day for at least 8 weeks observed significantly decreased serum T4 and protein-bound iodine and elevated TSH levels and thyroid weight (Kleiman de Pisarev et al. 1989, 1990, 1995).

The most sensitive intermediate-duration studies were conducted in male Syrian hamsters (Smith et al. 1986b, 1987), which responded differently than rats to hexachlorobenzene. In hamsters exposed to 10 mg/kg/day in feed for 28 weeks (the lowest effective intermediate-duration dose identified) or to a higher dose for a shorter time (50 mg/kg/day or more for 6 weeks), thyroid gland weights were significantly increased ($2.5-fold), serum T3 levels were decreased, and sodium iodide uptake was increased (~3-fold), while serum T4 levels were unchanged. Thyroid weight increased significantly, and correlated with histopathological observations of large and irregularly shaped follicles in the thyroid. Notably, two other studies did not observe treatment-related thyroid histopathology at effective doses; Den Besten et al. (1993) observed decreased T3 and T4 levels in female Wistar rats exposed to hexa-chlorobenzene in the feed at doses up to 19.0 mg/kg/day for 13 weeks, and Den Tonkelaar et al. (1978) detected significantly increased thyroid weight in male Landvarken pigs fed 5 mg/kg/day of hexachloro-benzene for 12 weeks.

Increased hepatic metabolism and hepatic excretion (into bile) appear to be important to the mode-of-action for the thyroid effects of hexachlorobenzene. Kleiman de Pisarev et al. (1989, 1990, 1995) observed that gavage administration of 1,000 mg/kg/day of hexachlorobenzene in corn oil to female Wistar rats for 28–30 days not only significantly decreased serum T4 levels, but also increased both the metabolism (deiodination) and fecal excretion of T4. In male Wistar (WAG/MBL) rats orally dosed with 120 mg/kg/day of hexachlorobenzene 3 times/week for 4 weeks, levels of T4 glucuronide increased but levels of serum T4 and nonconjugated T4 decreased (van Raaij et al. 1993b). Hepatic T4 UDP-glucuro-nyltransferase (UDPGT) activity was increased while T4 iodothyronine deiodinase activity was decreased. Bile flow and T4 excretion were increased. However, serum T3 levels were unaffected. Taken together, these data indicate that hexachlorobenzene induces liver activity that results in decreased serum levels of T4. Treatment of female Sprague-Dawley rats with 50 mg/kg/day of hexachlorobenzene by gavage for 5 days significantly decreased serum T4 levels and free thyroxine index without affecting

T3 uptake (Foster et al. 1993). However, following gonadotropin pretreatment to induce superovulation, 3-day treatment with 50 mg/kg/day of hexachlorobenzene significantly decreased both serum T4 levels and serum T3 uptake. The authors concluded that the acute induction of hypothyroidism was augmented by ovulation (Foster et al. 1993). Gavage treatment of inbred male Wistar (WAG/RIJ) rats with 27 mg/kg/day pentachlorophenol was more effective than 780 mg/kg/day of hexachlorobenzene at reducing free and total T4 levels (van Raaij et al. 1993a). The authors concluded that metabolites of hexachlorobenzene, rather than hexachlorobenzene itself, may be involved in the reduction of serum thyroid hormones.

Andrews et al. (1988, 1989, 1990) have conducted detailed studies into hexachlorobenzene-induced hyperparathyroidism and osteoporosis in male Fischer 344 rats. Gavage treatment of rats with doses as low as 1.0 mg/kg for 5 weeks, 5 days/week, significantly increased serum levels of vitamin D_3 and urinary levels of phosphorous. Treatment with at least 10 mg/kg significantly increased serum levels of PTH, increased urinary levels of calcium, and decreased serum levels of alkaline phosphatase (an enzyme important in bone mineralization). The combination of high PTH and vitamin D_3 is expected to cause calcium resorption from bone and calcium conservation by the kidneys, and this is consistent with the adverse skeletal effects seen by authors at 1.0 mg/kg (see Section 3.2.2.2, Musculoskeletal Effects).

Animal studies have shown that hexachlorobenzene affects the adrenal gland (weight, histopathology, and hormone levels). Adrenal gland weight was significantly increased in male and female Sherman rats fed at least 25 mg/kg/day of hexachlorobenzene for 4 months (Kimbrough and Linder 1974). Adrenal gland weight was also significantly increased in Wistar rat pups exposed to at least 2.5 mg/kg/day of hexa-chlorobenzene (starting early in pregnancy and continuing through gestation and lactation, and an additional 2 weeks after weaning) (Vos et al. 1979a). In female Wistar rats that were fed 9.5 or 19.0 mg/kg/day of hexachlorobenzene for 13 weeks (Den Besten et al. 1993), adrenal gland weight (69%) was significantly increased in the high-dose group. Histopathology (reported only for the high-dose group) observed adrenal cortex hypertrophy, hyperplasia, occasional hemorrhaging, cortical cell vacuolation, and inflammatory cell infiltrates. In female ovariectomized Sprague-Dawley rats gavaged for 30 days with 1, 10, or 100 mg/kg/day of hexachlorobenzene, corticosterone was decreased at all doses, but serum cortisol levels were only decreased by 100 mg/kg/day (Foster et al. 1995a). Moreover, no effect was seen on serum progesterone and aldosterone levels or adrenal weight. Koss et al. (1978) detected statistically significant increases in relative adrenal weight (up to 43%) in female Wistar rats that were treated with 50 mg/kg hexachlorobenzene every other day by gavage for 9–15 weeks; this effect had reversed after a 38-week posttreatment period. In another experiment, single adult female monkeys

received 0, 8, 32, or 64 mg/kg and two monkeys received 128 mg/kg of hexachlorobenzene by gavage in methylcellulose daily for 60 days (Iatropoulos et al. 1976). One of the monkeys given 128 mg/kg exhibited moderate adrenal medullary hyperplasia and the monkey given 64 mg/kg/ exhibited slight hyperplasia of the adrenal zona fasciculata; however, these findings are not conclusive due to the small numbers of animals used.

Limited animal evidence suggests that hexachlorobenzene affects retinoid levels. In blood samples taken from 101 polar bears from Svalbard, Norway, statistically significant correlations were observed between higher blood levels of hexachlorobenzene and both lower levels of retinol and a lower ratio of total thyroxine (T4) to free triiodothyronine (T3) (Skaare et al. 2001). In female Wistar rats fed 19 (but not 9.5) mg/kg/day of hexachlorobenzene for 13 weeks (Den Besten et al. 1993), significant increases were seen in adrenal gland weight (69%) and both liver retinol and retinyl palmitate levels. Plasma retinol levels were not affected at 1 week, but were significantly increased at 13 weeks by the high dose.

Dermal Effects.   Studies of humans exposed to hexachlorobenzene in bread prepared from contaminated grain in Turkey demonstrated that hexachlorobenzene can produce skin lesions following oral exposure. It is well known that ingestion of hexachlorobenzene can produce porphyria (see Section 3.2.2.2, Hepatic Effects). The porphyrias are a class of inherited and acquired diseases caused by enzymatic defects in heme biosynthesis, leading to the generation of porphyrins. One of the most serious symptoms of porphyria is photosensitivity; porphyrins accumulated in the skin absorb radiation (maximally at 400–410 nm) and then generate reactive oxygen species, causing tissue damage (Lim and Cohen 1999; Meola and Lim 1993; Sandberg et al. 1982). Skin lesions occur most commonly on areas exposed to sunlight, such as the hands and face. Porphyria cutanea tarda, a specific type of vesiculobullous porphyria, was widespread in southeast Anatolia, Turkey in the late 1950s (approximately 1955–1959). The disease was traced to ingestion of bread made from seed grain that had been treated with hexachlorobenzene as a fungicide (Cam and Nigogosyan 1963). The ingested dose of hexachlorobenzene by exposed persons was estimated to be in the range of 0.05–0.2 g/day (0.7–2.9 mg/kg/day for a 70-kg person) during the episode (Cam and Nigogosyan 1963). Symptoms of what was called *kara yara* or "black sore" appeared after approximately 6 months of exposure (Gocmen et al. 1989). The disease was observed most frequently in children between the ages of 6 and 15 years, although some younger children and adults were also affected (Cam and Nigogosyan 1963; Dogramaci 1964). The initial lesions resembled comedones (blackhead acne) and milia (small whitish epidermal cysts caused by hair follicle and sweat gland obstruction) with photosensitivity and the development of erythema on sun-exposed areas; moreover, the skin was sensitive to minor trauma. These lesions

progressed to include large bullous lesions that ulcerated and healed leaving severe mutilating scars, hyperpigmentation that was most prominent on exposed areas but usually affected the entire skin, and hypertrichosis (hirsutism) that occurred principally on the forehead, cheeks, arms, and legs but occasionally involved the whole body (Gocmen et al. 1989). Infants who had been breast fed by mothers who had ingested the contaminated bread displayed a condition known as *pembe yara* or "pink sore" because of the associated skin lesions (annular erythema) (Peters et al. 1966, 1982). The medical history of people who were exposed to hexachlorobenzene in the Turkish poisoning episode was followed for up to 30 years (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987). A total of 252 patients who had become porphyric during the Turkish epidemic were studied over a 10-year period from 1977 to 1987 (Gocmen et al. 1989). Subjects had an average age of 7.6 years at onset of symptoms and an average age at follow-up of 35.7 years. Thirty years after onset, clinical findings persisted in most subjects, including severe scarring (83.7%), hyperpigmentation (65%), hypertrichosis (44.8%), pinched face appearance with perioral scarring (40.1%), and fragile skin (33.7%). Dermal lesions were more prominent in sun-exposed areas of the skin.

Studies into the mode of action of porphyria (unrelated to hexachlorobenzene-exposure) have suggested that the dermal toxicity of porphyrins is exacerbated by involvement of the immune system; following irradiation, porphyrins may activate the complement system and stimulate mast cells and neutrophils to damage nearby tissues (Lim and Cohen 1999; Meola and Lim 1993). Additionally, an increased risk of porphyria cutanea tarda has been associated with human immunodeficiency virus (HIV) infection (Drobacheff et al. 1998; Egger et al. 2002).

Although several animal studies have demonstrated that oral exposure to hexachlorobenzene (for at least 4 weeks) results in dermal lesions and causes immunological effects with dermal lesions, none established a casual relationship (Koss et al. 1978; Michielsen et al. 1997, 2000; Schielen et al. 1995a; Torinuki et al. 1981). Additionally, it is unclear whether porphyrin-induced phototoxicity occurs in rats; the combination of hexachlorobenzene and sunlight exposure induced dermal lesions in rats similar to those reported in people (Torinuki et al. 1981). However, hexachlorobenzene-treated rats have exhibited skin lesions without detectable dermal porphyrin accumulation (assayed with fluorescence microscopy) (Michielesen et al. 1997). Dermal lesions in rats following hexachlorobenzene exposure have been seen most frequently on the head, neck, and shoulders (similar to humans) although the rats' exposure to sunlight was presumably limited by experimental design and body fur (Koss et al. 1978; Michielsen et al. 1997, 2000). These data seem to suggest that additional modes-of-action are important.

Dermal effects were noted in a study of female Wistar rats that were gavaged with 50 mg/kg hexachloro-benzene in olive oil every other day for 9–15 weeks, followed by a 38-week observation period (Koss et al. 1978). The fur had a roughened appearance during treatment, and round, ulcerous lesions on the head, ears, throat, and shoulders, with diameters of 2–20 mm, were observed in 10% of treated animals after 4 weeks and 50% of animals after 9 weeks. These lesions resolved 12–16 weeks after discontinuation of treatments. At 15 weeks only, spleen weight was significantly increased. A subsequent study, using lower doses and more sensitive end points, observed strain specificity in rats fed hexachlorobenzene for 4 weeks (Michielsen et al. 1997). Brown Norway and Lewis rats received 18 or 53 mg/kg/day, while Wistar rats were fed 50 or 97 mg/kg/day. Increased incidence of skin lesions were statistically significant for all treatment groups compared to controls. In Brown Norway rats, skin lesions were very severe, and their incidence was correlated with signs of immunomodulation (increased IgG, IgE, and IgM levels, spleen weight, and lung inflammation). In Lewis rats, skin lesions were moderate, and correlated less strongly with evidence of immune effects. In Wistar rats, lesions were observed only at the high dose, and were considered negligible; immune effects were also minor. Grossly, the lesions were found in the head and neck region and ranged from redness only to large exudating sores with crusts. Histopathology of both lesional and non-lesional skin observed epidermal hyperplasia, deep dermal venules with activated endothelial cells, and deep inflammatory cell infiltrates. A subsequent experiment (Michielson et al. 2000) verified that doses as low as 58 mg/kg/day in the feed of female Brown Norway rats for 4 weeks induced skin lesions in the head and neck areas, the severity of which increased with time. Likewise, a study in female Wistar rats fed 15 or 30 mg/kg/day of hexachlorobenzene for 13 weeks observed statistically significant increases in the incidence of wounds appearing by weeks 5–7 on the face, neck, shoulders, and behind the ears of treated animals (Schielen et al. 1995a). The incidence (but not severity) increased with increasing dose. Treatment also induced porphyria and increased serum levels of IgM, IgA, and autoantigen-specific IgM. Torinuki et al. (1981) treated rats for 2 months with hexachlorobenzene with repeated exposure to sunlight. In addition to porphyria, gross pathology of the skin observed erythema, erosion, crusting, skin thickening, and scarring. Histopathology found acanthosis (abnormal dermal thickening), vacuolization of malpighian cells, subepidermal vesicles, blood vessel dilation, and perivascular cell infiltration of lymphocytes, histiocytes, and mast cells. In addition to lesions, hexachlorobenzene has induced dermal effects that are not clearly toxic. In female Wistar rats, skin cytochrome P450-dependent 7-ethoxyresorufin-O-deethylase was induced after 60 or 70 days (but not 10 days) of feeding with 50 mg/kg/day of hexachlorobenzene (Goerz et al. 1978, 1994). No dermal lesions were observed in female Agus Wistar rats fed diets containing 5 mg/kg/day of hexachlorobenzene for 90 weeks, although treated animals "possessed less hair than controls" (Smith and Cabral 1980).

Ocular Effects.   No studies were found regarding adverse ocular effects in humans following oral exposure to hexachlorobenzene.

Only one relevant animal study was identified. In adult female Rhesus monkeys treated by gavage with hexachlorobenzene in methylcellulose at doses up to 32 mg/kg/day for 60 days, gross pathology and histopathology of the eyes did not detect any adverse effects (Iatropoulos et al. 1976).

Body Weight Effects.   No studies were located regarding body weight effects in humans following oral exposure to hexachlorobenzene.

Decreases in body weight following oral exposure of animals to hexachlorobenzene were not observed following acute exposure and were observed in intermediate- and chronic-duration experiments only in the presence of other adverse effects such as mortality, clinical evidence of weakness or lethargy, increased incidences of tumors, or liver toxicity (see below and Section 3.3.2). The available data suggest that body weight loss may be secondary to organ-specific toxicity.

No acute studies have reported changes in body weight. Body weight was not affected in rats dosed once by gavage with 400 or 600 mg/kg of hexachlorobenzene in corn oil (Lecavalier et al. 1994) or in male rats gavaged with doses up to 221 mg/kg/day for 5 consecutive days (Simon et al. 1979).

Similarly, many intermediate-duration experiments did not detect significant changes in body weight in Wistar rats fed up to 1,000 mg/kg/day of hexachlorobenzene for 4 weeks (Alvarez et al. 2000; Kleiman de Pisarev et al. 1995; Michielsen et al. 1997; Schielen et al. 1993, 1995b; Vos et al. 1979b), in Brown Norway rats and Lewis rats fed up to 45 mg/kg/day for 4 weeks (Michielsen et al. 1997, 2000), in ovariectomized adult female Sprague-Dawley rats gavaged with doses up to 100 mg/kg/day of hexa-chlorobenzene in corn oil for 30 days (Foster et al. 1995b), in female CD rats fed up to 36 mg/kg/day for 4 months (Goldstein et al. 1978), in female Wistar rats fed 5 mg/kg/day of hexachlorobenzene in arachis oil for 75 weeks (Smith and Cabral 1980), or in male BALB/c mice fed 22 mg/kg/day for 6 weeks (Loose et al. 1977). Intermediate-duration experiments that did report statistically significant weight loss in rats (Kuiper-Goodman et al. 1977; Smith and Cabral 1980; Smith et al. 1985) or mice (Cabral et al. 1979; Shirai et al. 1978) usually considered it to be slight-to-moderate. The most rapid onset of weight loss was a slight decrease at doses as low as 22 mg/kg/day in male Wistar rats for 6 weeks, in the presence of increased liver, spleen, and lymph node weight and decreased natural killer cell activity (Van Loveren et al. 1990). Although one study reported statistically significant decreases in body weight in male

Fisher 344 rats at doses as low as 0.1 mg/kg of hexachlorobenzene by gavage in corn oil 5 days/week for 5 weeks (Andrews et al. 1988), subsequent studies using the same protocols in 15-week experiments saw no effects on body weight (Andrews et al. 1989, 1990). Oral intermediate-dosing studies that observed hexachlorobenzene-induced mortality also found significant weight loss. In a 13-week feeding study, four of nine female Wistar rats receiving the high dose of 19 mg/kg/day of hexachlorobenzene in corn oil for 13 weeks were euthanized during the study due to severe weight loss and distress, while no body weight effects were seen in surviving or lower dose animals (Den Besten et al. 1993). Male Syrian golden hamsters fed 16 (but not lower doses up to 8) mg/kg/day of hexachlorobenzene in the diet for life, males exhibited "marked weight reduction" and significantly increased mortality (Cabral et al. 1977). However, similar mortality was observed in treated female hamsters without corresponding weight loss.

Similar findings have also been demonstrated in dogs, monkeys, and pigs. No treatment-related effects on body weight were observed in adult female beagle dogs that received hexachlorobenzene in corn oil orally in capsules for 3 weeks at doses up to 100 mg/kg/day (Sundlof et al. 1981) or in male and female beagle dogs fed capsules of hexachlorobenzene at doses up to 110 mg/kg/day for up to 12 months (Gralla et al. 1977a). Similarly, experiments in which female Cynomolgus monkeys were dosed with hexa-chlorobenzene in gelatin capsules at doses up to 10 mg/kg/day for 90 days, did not detect changes in body weight (Foster et al. 1992a, 1995a; Jarrell et al. 1993). In contrast, unspecified weight loss was reported beginning at 4 weeks in adult female Rhesus monkeys given hexachlorobenzene by daily gavage (in aqueous methylcellulose) for 60 days at doses as low as 8 mg/kg/day; renal and neurological effects were also reported (Knauf and Hobson 1979). All male SPF pigs fed 50 mg/kg/day (but not lower doses up to 5 mg/kg/day) for 90 days exhibited depressed growth (beginning at week 4) prior to death between weeks 8 and 12 (Den Tonkelaar et al. 1978).

In fetal and immature pups, hexachlorobenzene-induced changes body weight were observed only in the presence of maternal toxicity. No decreases in maternal or pup body weight were observed at doses as high as 100 mg/kg/day on gestation days 7–16 in CD-1 mice or as long as *in utero* and lifetime exposure of Sprague-Dawley rats to 2 mg/kg/day (Arnold et al. 1985; Courtney et al. 1976; Goldey and Taylor 1992; Lilienthal et al. 1996; Taylor and Goldey 1990; Vos et al. 1979a, 1983). In Wistar rats, maternal and fetal body weight were decreased by gavage doses of at least 80 mg/kg/day of hexachlorobenzene in corn oil on gestation days 6–21 (Khera et al. 1974a); however, slight changes in the dosing protocols (fewer treatment days, use of aqueous gum tragacanth instead of corn oil) prevented weight loss. Likewise, Sprague-Dawley rat pup weight and viability were significantly reduced at birth, 5 days, and 24 days, following lifetime dietary exposure of both male and female parent rats to at least 4 mg/kg/day

(Grant et al. 1977). Maternal signs reported by these two studies (Grant et al. 1977; Khera et al. 1974a) included mortality, convulsions, and reduced fertility.

3.2.2.3 Immunological and Lymphoreticular Effects

Although epidemiological studies have suggested that consumption by mothers of fish high in organochlorines may affect the immune system of their infants, oral exposure of people to hexachloro-benzene has not been clearly associated with immunological effects. The levels of hexachlorobenzene, dieldrin, and *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) in milk samples collected shortly after the birth of Canadian Inuit infants correlated with increased risk of otitis media in their first year of life, but not with other serum immune parameters (immunoglobulins, cytokines, lymphocyte activation markers) (Dewailly et al. 2000). A similar study of immune parameters in umbilical blood of Canadian mothers with high or low consumption of fish from the Lower-North-Shore of the St. Lawrence River detected a statistically significant association only between decreased lymphocyte secretion of cytokine IL-10 and increased levels of hexachlorobenzene, *p,p'*-DDE, and mercury (Belles-Isles et al. 2000). However, evidence suggests that hexachlorobenzene may indirectly affect the immune system by inducing porphyria (see Section 3.2.2.2 Hepatic Effects). Mode-of-action studies of individuals with inherited and acquired porphyria (unrelated to hexachlorobenzene) have found that irradiated porphyrins may activate the complement system and stimulate mast cells and neutrophils to damage nearby tissues (Lim and Cohen 1999; Meola and Lim 1993). Additionally, an increased risk of porhyria cutanea tarda has been associated with HIV infection (reviewed Drobacheff et al. 1998; Egger et al. 2002).

The effects of oral exposure to hexachlorobenzene to the immune system of animals appear to be species- and strain- (Michielsen et al. 1997) dependent, with immunosuppression observed in mice (see below), monkeys (Iatropoulos et al. 1976) and bears (Bernhoft et al. 2000), and at least a partial stimulation of the immune system in rats (see below) and dogs (Gralla et al. 1977a). Additionally, a number of animal studies have observed inflammation and immune cell infiltration in tissues such as the liver (Arnold et al. 1985; Ertürk et al. 1986), respiratory tract (Goldstein et al. 1978; Kimbrough and Linder 1974; Kitchin et al. 1982; Michielsen et al. 1997, 1999, 2001; Vos et al. 1979a, 1983), and skin (Koss et al. 1978; Michielsen et al. 1997, 2000; Schielen et al. 1993, 1995b; Torinkui et al. 1981) following oral exposure to hexachlorobenzene. The lowest dose to cause an immune response was 0.016 mg/kg/day, which induced peribiliary lymphocytosis in F_1 Sprague-Dawley rats exposed for life to dietary doses (Arnold et al. 1985). Because the mode-of-action is unclear, it is not known if these immune effects are secondary following toxicity to target organs or if they are involved in the etiology of disease in these organs.

Mode-of-action studies in rats have suggested that the immune effects of hexachlorobenzene may be secondary to the accumulation of porphyrins produced by the liver in the spleen or other organs of immunological importance (Kennedy and Wigfield 1990; Kuiper-Goodman et al. 1977) or by the metabolic products of hexachlorobenzene (Schielen et al. 1995a).

Immunosuppression has been observed in male mice following oral exposure to hexachlorobenzene. The most sensitive effects were seen at dietary doses as low as 0.6 mg/kg/day; 6 weeks of exposure significantly reduced spleen cell cytocidal activity (Loose et al. 1981) and 18 weeks of exposure decreased the mean survival time of mice following challenge with injected ascites tumor cells. Immune effects were seen as early as 3 weeks; feeding of 13 mg/kg/day decreased survival time following ascites tumor cell challenge (Loose et al. 1981), and feeding with 22 mg/kg/day decreased resistance to bacterial endotoxin (*Salmonella typhosa* lipopolysaccharide [LPS]) and to malarial infection (*Plasmodium berghei*) (Loose et al. 1978). Feeding at the same dose (22 mg/kg/day) for 6 weeks reduced primary and secondary plaque-forming cell responses to sheep red blood cells, reduced serum IgG, IgM, and IgA levels, increased spleen weight (Loose et al. 1977), increased susceptibility to infection by hepatis (but not cytomegalovirus or pneumonia) virus (Carthew et al. 1990), and reduced ability of spleen cells to lyse P388 tumor cells (Silkworth and Loose 1981). Feeding of 22 mg/kg/day for 37 (but not 13 or fewer) weeks of treatment decreased graft-versus-host response (measured by injecting harvested spleen cells into neonatal BDF1 mice) (Silkworth and Loose 1981). In addition to the effects seen in adult male mice, similar effects were observed in a developmental study. Pups of pregnant BALB/c mice fed doses as low as 0.5 mg/kg/day on gestation days 1–18 exhibited a marked, significant decrease in delayed type hypersensitivity (DTH) response when tested on postnatal day 45 (Barnett et al. 1987). Analyses of collected spleen cells found that 5 (but not 0.5) mg/kg/day decreased mixed lymphocyte response and decreased B cell numbers; neither dose affected spleen blastogenesis induced by T- or B-cell mitogens.

In contrast to the immunosuppression observed in mice exposed to hexachlorobenzene, studies in rats have generally found stimulation of the immune system, as indicated by such effects as increased spleen and lymph node weights, increased neutrophil counts, and increased serum immunoglobulins. In feeding studies with male rats, exposure to at least 25 mg/kg/day of hexachlorobenzene for 3 weeks caused increased neutrophil counts, elevated popliteal lymph node weight with a corresponding proliferation of popliteal lymph node high-endothelial venules (Vos et al. 1979b), and spleen histopathology consisting of extramedullary hematopoiesis, enlarged marginal zones and follicles, and increased macrophage density in the marginal zones (Schielen et al. 1993; Vos et al. 1979b). In Wistar rats fed doses as low as 50 mg/kg/day for 3 weeks, immunological effects observed included increased spleen and lymph node

(popliteal, inguinal, and mediastinal, but not parathymic) weight; increased basophil, monocyte, and total leukocyte counts (Kennedy and Wigfield 1990; Koss et al. 1978; Schielen et al. 1995b; Vos et al. 1979a, 1979b); increased splenic (but not lymph nodal) cell size; selectively activated B cell (but not T cell) subpopulations (Schielen et al. 1995b); increased splenic and serum levels of total IgM; and increased serum levels of specific IgM (anti-phosphatidylcholine IgM, and anti-single stranded DNA IgM) without increases in IgM against foreign antigens (tetanus toxoid, sheep erythrocytes, and bovine serum albumin) (Schielen et al. 1993, 1995b). These reported changes in B cell populations and IgM levels strongly suggest an autoimmune response. Remarkably, no changes were seen immune function tests for thymus-independent (*Escherichia coli* lipopolysaccharides) and thymus-dependent (tetanus toxoid) antibody responses, cell-mediated immunity (rejection of skin transplants, resistance to *Listeria monocytogenes* infection, or delayed-type hypersensitivity to *Mycobacterium tuberculin*), phagocytic competence (clearance of carbon particles), the mitogenic response of peripheral blood lymphocytes (to pokeweed mitogen, concanavalin A, and phytohemagglutinin), or susceptibility to *E. coli* endotoxin in Wistar rats at doses up to 100 mg/kg/day for 3 weeks (Vos et al. 1979b). Feeding of male Wistar rats with doses as low as 8 mg/kg/day for 6 weeks reduced natural killer cell activity (Van Loveren et al. 1990). Atrophy of the thymus was observed in male rats fed 100 mg/kg/day for 3 weeks (Vos et al. 1979b) and in female Wistar rats fed 15 mg/kg/day for 13 weeks (Schielen et al. 1995a).

Two developmental studies in rats by the same laboratory reported hexachlorobenzene effects on humoral and cellular immunity (Vos et al. 1979a, 1983). The first study reported that hexachlorobenzene exposure strongly enhanced humoral immunity (antibody response to tetanus toxoid), but slightly depressed cellular immunity (as evaluated by susceptibility to infection, skin graft rejection time, and response to mitogens) in pups whose mothers were fed 2.5 or 7.5 mg/kg/day of hexachlorobenzene from early pregnancy and continuing through gestation and lactation; after weaning pups were fed the same doses for an additional 2 weeks prior to testing (Vos et al. 1979a). In both treatment groups, resistance to infection (*L. monocytogenes* and *Trichinella spiralis*) was reduced, the IgG response to tetanus toxoid was significantly increased, and histopathology found proliferation of high-endothelial venules in the paracortex of the lymph nodes. At 7.5 mg/kg/day, the IgG response to *Trichinella* was increased, increases were seen in blood levels of eosinophils, basophils, IgM and IgG, and histopathology found accumulation of foamy macrophages in the lung and increased extramedullary hematopoiesis in the spleen. Neither dose affected rejection of skin transplant, passive cutaneous anaphylaxis, IgM response to LPS, mitogenic responsiveness of lymphocytes, clearance of carbon particles, or clearance of *Listeria monocytogenes*. However, the follow-up study found that hexachlorobenzene stimulated both humoral and cell-mediated immunity in the Wistar rat pups whose mothers were fed 0.2, 1, or 5 mg/kg/day of

hexachlorobenzene from early pregnancy through lactation; weaning pups were fed the same doses up to 7 months (Vos et al. 1983). Cytotoxicity of spleen cells (to injected lymphoma cells) was not affected by treatment. Treatment with at least 0.2 mg/kg/day significantly increased the IgG and IgM response to tetanus toxoid and delayed-type hypersensitivity reaction to ovalbumin, and induced accumulation of foamy macrophages in the lungs. At 1 mg/kg/day and above, increased popliteal lymph node weight with a corresponding increased in lymph node cellular proliferation were observed, and cell proliferation was also detected in the endothelial cells lining pulmonary capillaries and venules. At 5 mg/kg/day, increases were observed in spleen, lung, and mesenteric lymph node weight; serum IgM (but not IgG) levels; the relative number of basophils in the blood; and extramedullary hematopoiesis of the spleen. These effects demonstrated stimulation of both humoral and cell-mediated immunity.

A strain-dependent correlation between immunological and dermal effects was observed in female rats (Michielsen et al. 1997). For 28 days, Wistar rats were fed 22 or 45 mg/kg/day, while Lewis rats were fed 8 or 22 mg/kg/day, and Brown Norway rats were fed 8, 22, or 45 mg/kg/day of hexachlorobenzene. Brown Norway rats were the most sensitive to hexachlorobenzene exposure, and correlations were observed between the incidence and severity of immune responses and dermal lesions. In contrast, Wistar rats were the most resistant, and correlations were not apparent, while some correlations were seen in Lewis rats. Hexachlorobenzene induced skin lesions in all treatment groups, most severe in Brown Norway rats and least severe in Wistar rats, characterized by epidermal hyperplasia, inflammatory infiltrate in the dermis, and activation (due to hypertrophy and proliferation) of endothelial cells in dermal vessels. Relative spleen weights were significantly increased in a dose-related fashion in all three strains while popliteal lymph node weight was increased in the high-dose Lewis and Brown Norway rats, but not in Wistar rats. All strains showed increases in IgM, but only Brown Norway rats exhibited increases in serum IgE and IgG. However, lung pathology was not strain dependent; histopathology observed lung lesions consisting of venules lined with unusually plump endothelial cells and surrounded by large perivascular infiltrate and accumulation of alveolar macrophages. The authors concluded that the inflammatory responses in the skin and lungs were of different etiologies, and speculated an involvement of the immune system in the observed dermal lesions.

Studies of the immunological effects of hexachlorobenzene in nonrodents observed evidence of immunosuppression in monkeys and bears, and stimulation of the immune system in dogs. In female Rhesus monkeys, gavage with hexachlorobenzene in methylcellulose at doses as low as 8 mg/kg for 60 days caused thymic cortical atrophy, consisting of a reduction or absence of individual lobules, increased numbers of thymic corpuscles, and medullar hyperplasia or reticular cells, macrophages, plasma

cells, and lymphocytes. However, the use of only one or two monkeys in this study diminishes the reliability of these data (Iatropoulos et al. 1976). A significant correlation between hexachlorobenzene levels and decreased IgG was observed in an analysis of sera from 56 polar bears in Svalbard, Norway, observed (Bernhoft et al. 2000). Because similar correlations were also observed for three polychlorinated biphenyl congeners (99, 194, and 206), this effect cannot be clearly attributed to hexa-chlorobenzene. Although no immunologically-related gross pathology or histopathology were observed in a 21-day oral study in female dogs at doses as high as 150 mg/kg/day (Sundlof et al. 1981), nodular hyperplasia of gastric lymphoid tissue was found in all beagle dogs given hexachlorobenzene in gelatin capsules daily for 12 months at doses as low as 10 mg/kg/day and an increased incidence of infebrile neutrophilia (increased numbers of blood neutrophils without fever) was observed at 10 and 110 mg/kg/day (Gralla et al. 1977a).

The highest NOAEL values and all reliable LOAEL values for immunologic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

The evidence of neurotoxicity in humans following oral exposure to hexachlorobenzene was provided by studies of people in southeast Turkey who consumed contaminated bread in the late 1950s. The ingested dose of hexachlorobenzene by exposed persons was estimated to be in the range of 0.05–0.2 g/day (0.7–2.9 mg/kg/day for a 70-kg person) during the episode (Cam and Nigogosyan 1963).

Neurological symptoms included loss of appetite, tremors, convulsions, and "weakness that often made it impossible to eat with a knife and fork, rise from a squat, or climb stairs" (Gocmen et al. 1989; Peters et al. 1982). Follow-up studies of 25 (Peters et al. 1982) and 30 years (Cripps et al. 1984) included 161 and 204 patients, respectively. They found that neurological symptoms persisted in adults who had been exposed as children, and included weakness (62–66%), paresthesia (spontaneous tingling or burning sensations, 55%), sensory shading (graded sensory loss that diminishes upon testing more proximally and is indicative of polyneuropathy, 61–63%), myotonia (delayed muscle relaxation after an initial contraction, 38–50%), and cogwheeling (irregular jerkiness of movement due to increased muscle tone as seen in Parkinson's disease, 29–41%). During the grain poisoning epidemic, there was an extremely high (95%) rate of mortality in infants under 2 years of age, who had been breast fed by mothers who had ingested the contaminated bread; these children exhibited convulsions, tremors, and progressive weakness prior to death (Cripps 1990; Peters et al. 1966). Analysis of human milk from exposed women and

unexposed controls in this epidemic showed hexachlorobenzene concentrations of 0.51 and 0.07 ppm, respectively (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987). A study investigating the potential effects of consuming fish from the Great Lakes was unable to correlate hexachlorobenzene levels in umbilical blood or breast milk with infant intelligence test results (Darvill et al. 2000).

Multiple studies have shown that hexachlorobenzene induces serious neurological effects such as convulsions, tremors (intermittent and constant), lethargy, and progressive weakness in rats, mice, rabbits, pigs, monkeys, and quail (Cabral et al. 1979; Cripps 1990; De Matteis et al. 1961; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Grant et al. 1977; Hahn et al. 1988; Kennedy and Wigfield 1990; Kimbrough and Linder 1974; Khera 1974a; Knauf and Hobson 1979; Ockner and Schmid 1961; others). In several studies, these effects were only seen prior to death or in treatment groups with significant mortality. The lowest dose to cause such serious effects was 8 mg/kg/day in Sprague-Dawley rats as part of a multigeneration study, with convulsions preceding death (Grant et al. 1977). In comparison, no tremors were seen in female Agus or Wistar rats fed diets containing 5 mg/kg/day of hexachlorobenzene for 75–90 weeks (Smith and Cabral 1980). Similar effects have been seen in other species. Mice orally exposed to 26 mg/kg/day of hexachlorobenzene for up to 17 weeks exhibited severe tremors prior to death (Hahn et al. 1988). Male SPF pigs fed 50 mg/kg/day of hexachlorobenzene for 90 days exhibited tremors, panting, and unsteady gait without histopathology (Den Tonkelaar et al. 1978). Adult female Rhesus monkeys given oral doses of hexachlorobenzene for 60 days suffered severe tremors and muscular weakness at doses as low as 64 mg/kg/day and marked lethargy and weakness were observed at 128 mg/kg/day (Knauf and Hobson 1979). Two of three infant Rhesus monkeys whose mothers were treated with 64 mg/kg/day for up to 60 days during lactation displayed hypoactivity, lethargy, and ataxia, and subsequently died (Iatropoulos et al. 1978). The offspring of pregnant rats feed 200 (but not 5) mg/kg/day for an unspecified period during gestation exhibited convulsions, tremors, and progressive weakness (Cripps 1990).

Other studies have investigated more subtle neurological effects resulting from oral exposure to hexa-chlorobenzene. Electrophysiological changes (dysrhythmic electroencephalogram) in the central nervous system were demonstrated in dogs receiving doses of $50 mg/kg/day for 21 days (Sundlof et al. 1981). Another functional experiment observed axonal effects in the sciatic nerve (fibrillations, repetitive or pseudomyotonic discharges, and mild slowing of conduction velocities) in rats fed 40 mg/kg/day of hexa-chlorobenzene for 20 weeks or at least 3.75 mg/kg/day for 2 years (Sufit et al. 1986). In male Wistar rats gavaged with doses as low as 317 mg/kg/day for 4 weeks, significant decreases were observed in the rate of T4 uptake into cerebrospinal fluid and brain tissue (van Raaij et al. 1994). Additionally, gavage

treatment of Chbb THOM and Wistar rats with 1,000 mg/kg/day of hexachlorobenzene in a water-Tween suspension for up to 28 days induced changes in brain phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositiol, phosphatidylserine, and sphingomyelin) without affecting brain porphyrin levels (Billi de Catabbi et al. 2000b; Cochon et al. 2001). Because these effects were seen prior to the onset of porphyrin accumulation in the liver, the authors concluded that the effects of hexachlorobenzene on phospholipids in the brain were different from its effects on phospholipids of the liver or Harderian gland, which reportedly occur following porphyrin accumulation.

One of two developmental studies in rats that investigated the neurobehavioral effects of hexachloro-benzene observed hyperactivity. Two weeks prior to mating, female Sprague-Dawley rats were gavaged for 4 days with 2.5 or 25 mg/kg/day (Goldey and Taylor 1992; Taylor and Goldey 1990). In the first 3 weeks postnatally, both treatment groups of pups exhibited a significantly increased level of hyper-activity compared to controls. Specifically, treated pups reoriented themselves significantly more quickly in a negative geotaxis test, required less time in an olfactory discrimination test, and demonstrated increased exploratory activity in a motor activity test (postnatal days 15–20). No significant effects on learning (swim T-maze) or motor activity (measured in older offspring on postnatal days 40 and 50, respectively) were detected. Pups exposed to 25 mg/kg/day exhibited decreased acoustic startle response (ASR) on postnatal day 23 and increased ASR on postnatal day 90. Another study fed female Wistar rats hexachlorobenzene for 90 days prior to mating through lactation and offspring were maintained on the same diet until postnatal day 150 (Lilienthal et al. 1996). In pups exposed to 1.3 mg/kg/day (but not lower doses up to 0.64 mg/kg/day), significant decreases were seen in operant learning ("post-reinforcement pause" and "index of curvature") on postnatal day 150. However, because the rats were exposed both developmentally and as adults, the development significance of changes in operant learning is unclear. No changes were seen in an open field activity test (a measure of early locomotor skills) on day 21 or an active avoidance learning test on day 90.

The highest NOAEL values and all reliable LOAEL values for neurological effects in each species for intermediate duration category are shown in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

Epidemiological studies suggest that hexachlorobenzene may cause spontaneous abortion in women. In Southeastern Turkey, consumption of bread made from grain treated with hexachlorobenzene resulted in widespread poisoning between 1955 and 1959. Although no quantitation of exposure was presented in any of these clinical reports, an estimated dose of 0.05–0.2 g/day (0.7–2.9 mg/kg/day for a 70-kg person) is considered to be reliable by the original investigators of the Turkey epidemic (Cam and Nigogosyan 1963). A follow-up study conducted between 1977 and 1981 identified 42 porphyric mothers who had been exposed as children, with 188 pregnancies (Peters et al. 1982, 1987). Of these, 15 were fetal deaths (13 miscarriages and 2 stillbirths), and 31 produced children who died in the first several years of life. Similarly, another follow-up study conducted 20–30 years after initial exposure identified 57 porphyric mothers, who had a total of 276 pregnancies (Gocmen et al. 1989). Of these, 23 were fetal deaths, and 54 produced children who died in the first several years of life. Porphyric mothers had an average of 0.51 ppm hexachlorobenzene in their breast milk, compared to 0.07 ppm in unexposed controls (Gocmen et al. 1989). The degree to which these two exposed populations overlap and the expected frequencies of adverse pregnancy outcomes for the unexposed cohorts were unclear. Surviving offspring of porphyric mothers were clinically normal, and had urine and stool porphyrin levels similar to control children.

A subsequent retrospective study, conducted 40 years after initial exposure, compared three groups of 42 women (controls from outside the exposed area and women from the hexachlorobenzene-exposed region either with or without a diagnosis of porphyria cutanea tarda) (Jarrell et al. 1998). The incidence of women with blood levels of hexachlorobenzene exceeding 1 ng/mL was greater in women with porphyria cutanea tarda or women living in the contaminated region than country-wide controls and correlated (across exposure-groups) with an increased risk of spontaneous abortion. Notably, blood levels did not correlate with the number of pregnancies, sex ratio of born children, or onset of menopause. Statistically significant increases in the levels of inhibin (a hormone secreted by ovarian granulosa cells to decrease the release of follicle-stimulating hormone [FSH] from the pituitary) were observed in women diagnosed with porphyria cutanea tarda. Because no exposure-related differences were seen for FSH or estradiol, the biological significance is unclear, but ovarian effects would be consistent with animal studies (see below).

Studies of other populations with exposure to multiple organochlorines did not find significant differences in blood hexachlorobenzene levels between controls and cases of spontaneous abortion in Italy (Leoni et al. 1986, 1989) or Germany (Gerhard et al. 1998). Average maternal blood levels of

hexachlorobenzene were 1.6 and 0.679 ng/mL, respectively. Similarly, no changes in reproductive outcomes in Xixin, China, were detected following the cessation of agricultural uses of hexachlorobenzene (Huang et al. 1989).

For hexachlorobenzene, animal studies have identified adverse effects in the ovaries at doses as low as 0.01 mg/kg/day (Alvarez et al. 2000; Babineau et al. 1991; Bourque et al. 1995; Foster et al. 1992a, 1992b, 1995a, 1995b; Jarrell et al. 1993; Lecavalier et al. 1994; Iatropoulos et al. 1976; Knauf and Hobson 1979; MacPhee et al. 1993; Muller et al. 1978; Sims et al. 1991), in the testes at doses as low as 10 mg/kg/day (Gralla et al. 1977a; Smith et al. 1985), and on reproductive function with doses of at least 16 mg/kg/day (Grant et al. 1977; Simon et al. 1979).

The ovaries are a sensitive target organ for hexachlorobenzene. Distribution studies have identified the ovaries as a site of hexachlorobenzene accumulation (Foster et al. 1993; Sitarska et al. 1995; others). Studies have reported changes in organ weight; histological (light microscopy) and ultrastructural (electron microscopy) degenerative changes; and altered serum levels of gonadal hormones (estrogen and progesterone). Investigations into the mode-of-action generally found disruptions in steroidogenesis.

The acute data for ovarian effects are limited (Foster et al. 1993; Lecavalier et al. 1994). Mild morphological changes in the ovary were detected in female Sprague-Dawley rats given a single gavage dose of at least 400 mg/kg in corn oil (Lecavalier et al. 1994). Serum progesterone levels were increased in female Sprague-Dawley rats that were superovulated prior to gavage treatment with 50 mg/kg/day of hexachlorobenzene in corn oil for 5 days but not in normally-cycling rats similarly treated (Foster et al. 1993).

A 90-day assay in adult female Cynomolgus monkeys observed ovarian toxicity at the lowest dose tested, 0.001 mg/kg/day of hexachlorobenzene (Bourque et al. 1995). Ultrastructural analyses of developing ova detected mitochondrial changes, which increased in frequency and severity with dose. Swelling of the cristae resulting in abnormal intracristae spaces was seen at 0.01 mg/kg/day; mitochondrial matrices became more coarsely granular and exhibited occasional irregular morphology at 0.1 mg/kg/day; and mitochondria had "electron-lucent" matrices and reduced membrane integrity at 10 mg/kg/day. A similar increase in frequency and severity was observed for lesions in follicular cells: abnormal nuclei were seen in "a few cells" at 0.01 mg/kg/day, while nuclear membrane infolding was clearly apparent at 0.1 mg/kg/day. Abnormal spaces between follicular cells were observed at 1 mg/kg/day, and follicular cells exhibited abnormal lipid accumulation and deeply folded and indented nuclear membranes at

10 mg/kg/day. Thecal cells exhibited deformed nuclei only at 10 mg/kg/day. Since mitochondrial changes may represent nonspecific cell injury, the specific mode of action of degenerative ovarian changes remains unclear. The LOAEL of 0.01 mg/kg/day from this study was used to calculate an intermediate oral MRL of 0.0001 mg/kg/day as described in the footnote to Table 3-2 and in Appendix A.

Supporting data are provided by previous 90-day studies in female Cynomolgus monkeys that had dose-related degenerative changes in oocytes and ovaries at all doses tested, 0.1–10 mg/kg/day (Babineau et al. 1991; Foster et al. 1992a, 1995a; Jarrell et al. 1993; Sims et al. 1991). Increasing in frequency and severity, oocyte effects progressed from increased numbers of lysosomal elements and irregularly arranged thecal layer cells to altered oocyte morphology (shape irregularities, increased granularity and density, less distinct membrane), cytoplasmic vacuolation, lysomal aggregation, and pyknosis of follicular granulosa cells. Similarly, ovarian changes progressed from cellular hypertrophy, increasing columnarization of normally cuboidal cells, and small lipid inclusions, to cellular necrosis and separation of epithelium from connective tissue, stratification and elongation of epithelial cells, and increased numbers of lysosomes, vesicles, and lipid inclusions. Additionally, luteal phase progesterone levels were reduced at 1 mg/kg/day, ovulatory surge estrogen levels were decreased at 10 mg/kg/day, and menstrual cycle length variability was increased at 10 mg/kg/day (Foster et al. 1992a, 1995a). Remarkably, doses up to 10 mg/kg/day did not affect fertility (measured in oocytes by *in vitro* tests), serum inhibin levels, or numbers or size of oocytes, follicles, or corpora lutea. Notably, liver lesions (cholestasis, accentuated zonation, increased portal density, and mid-zone vacuolation) were only seen at the higher doses of 1 and 10 mg/kg/day.

Earlier studies in female Rhesus monkeys observed similar lesions. In female Rhesus monkeys given hexachlorobenzene for 60 days, ovarian effects seen at 8 mg/kg/day included cortical degeneration, reduced numbers of primary follicles with a concurrent increase in relative corpora lutea volume, multiple follicular cysts, and a thickening of the ovarian germinal epithelium with cells exhibiting a columnar appearance progressing to pseudostratification (Iatropoulos et al. 1976). These effects increased in incidence and severity with dose. At 32 mg/kg/day and above, epithelial nuclei were pyknotic (condensed) and karyorrhectic (fragmented) and at 128 mg/kg/day, ovarian cortices were predominated by dense stroma. These ovarian changes were similar to those normally seen in menopause, and indicate that the corpora lutea were not producing steroids. A subsequent study in female Rhesus monkeys found that serum cholesterol was increased by gavage doses of 8 mg/kg/day of hexachlorobenzene in methyl cellulose for 60 days (Knauf and Hobson 1979); this effect may be secondary to changes in ovarian steroidogenic activity. These findings are supported by a study in which hexachlorobenzene blocked

ovulation (estrogen and progesterone remained low, LH and FSH continued to climb, and menstruation was delayed) in one of four female Rhesus monkeys gavaged with 4 mg/kg/day of hexachlorobenzene in aqueous methyl cellulose for up to 78 days (Muller et al. 1978). A lower-dose study found no changes in serum levels of estrogen, progesterone, FSH, or LH in female Rhesus monkeys fed 0.03 mg/kg/day of hexachlorobenzene in monkey chow for 11 months (Rozman et al. 1978). The difference between this study and the one in Cynomolgus monkeys that observed changes in estrogen and progesterone levels (Foster et al. 1992a, 1995a) may reflect strain specificity or differences in absorption following disparate methods of oral exposure.

No evidence of histopathology was found in the ovaries of female beagle dogs given hexachlorobenzene in gelatin capsules with corn oil at doses up to 100 mg/kg/day for 21 days; data for other reproductive organs were not reported (Sundlof et al. 1981).

Although rat studies have not investigated doses as low as those used in monkey studies, they have identified histological evidence of degeneration and ultrastructural changes in the ovaries of animals treated with hexachlorobenzene. Gavage treatment of female Wistar rats with 1,000 mg/kg/day of hexachlorobenzene for 30 days caused degenerative lesions (increased numbers of atresic follicles, inflammatory infiltration of primary follicles, stratification and proliferation of ovarian surface epithelial cells, and irregular nuclei in epithelial cells) and changes in hormone and hormone receptor levels (decreased serum estradiol and prolactin, increased FSH, decreased estrogen receptor levels) (Alvarez et al. 2000). Similarly, a 21-day study detected increased serum progesterone levels in female Sprague-Dawley rats gavaged with at least 1 mg/kg/day of hexachlorobenzene (Foster et al. 1992b). In treated animals, the number of ova produced per rat decreased significantly and the length of estrus increased significantly. Another study in female Sprague-Dawley rats observed suggestive evidence of ovarian lesions (increased prominence of Golgi complexes, smooth endoplasmic reticulum, and free polysomes) at 10 (but not 1 or 100) mg/kg/day (MacPhee et al. 1993). The differences observed between rats and monkeys for changes in progesterone levels may be related to differences in their cycle lengths.

To investigate the contributions of adrenal steroidogenesis, adult female Sprague-Dawley rats were ovariectomized prior to treatment (Foster et al. 1995a). Gavage treatment for 30 days at doses as low as 1 mg/kg/day decreased serum corticosterone, while serum cortisol was decreased only at 100 mg/kg/day; no effects were seen on aldosterone or progesterone levels. The authors concluded that hexachloro-benzene induced alterations in steroidogenesis of cells of the inner zone of the adrenal cortex.

Some intermediate-duration experiments have demonstrated that hexachlorobenzene adversely affects reproductive performance. In a multigenerational study in which male and female Sprague-Dawley rats were fed hexachlorobenzene at doses of 0.5–32 mg/kg/day through premating and two series of mating, gestation, and lactation for up to four generations, statistically significant decreases in fertility and increases in the number of stillborns were observed at doses as low as 16 mg/kg/day and average litter size was decreased at 8 mg/kg/day and above (Grant et al. 1977). A study which dosed only male rats were dosed with 70 mg/kg/day of hexachlorobenzene for 5 consecutive days prior to 14 weeks of consecutive mating found decreased male reproductive performance (mating index), but no changes in fertility (numbers of inseminated females made pregnant) (Simon et al. 1979). In contrast, in two studies in which only females were dosed, no reproductive toxicity was observed, either in female Sprague-Dawley rats fed 7 mg/kg/day continuously from 96 days prior to first mating through gestation of two successive litters (Kitchin et al. 1982) or in female Cynomolgus monkeys given 10 mg/kg/day orally for 90 days (Jarrell et al. 1993).

Similarly, no reproductive toxicity was observed in a chronic assay in which both male and female Sprague-Dawley rats were fed doses of hexachlorobenzene up to 2 mg/kg/day from 3 months prior to mating through weaning (Arnold et al. 1985).

The other chronic data available are pertinent to testicular damage (Gralla et al. 1977a; Smith et al. 1985). In male Fischer 344/N rats fed 10 mg/kg/day of hexachlorobenzene in arachis oil for 90 weeks, testicular weight was significantly increased and testicular interstitial cell tumors were more severe (although incidence was not affected) compared to controls (Smith et al. 1985). Slight testicular degeneration, with numerous spermatogonic giant cells and incomplete complement of spermatogonia in the seminiferous tubules, was observed in two of six male beagle dogs given 110 mg/kg/day of hexachlorobenzene in gelatin capsules with corn oil for 12 months (Gralla et al. 1977a). Additionally, retarded sexual maturation of the testes was observed in male SPF pigs fed 50 (but not lower doses up to 5 mg/kg/day of hexachlorobenzene for 90 days (Den Tonkelaar et al. 1978).

The highest NOAEL values and all reliable LOAEL values for reproductive effects in each species and duration category are shown in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

Human and animal studies have demonstrated that hexachlorobenzene crosses the placenta to accumulate in fetal tissues; additionally, hexachlorobenzene is concentrated in milk and can be transferred to the suckling neonate (for more information, see Sections 3.4.1 and 3.4.2).

Oral exposure to hexachlorobenzene has been associated with serious developmental toxicity in a study of a poisoning epidemic (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1966, 1982, 1987). In southeast Anatolia, Turkey, ingestion of an estimated 0.7–2.9 mg/kg/day of hexachlorobenzene between 1955 and 1959 (in bread made from grain treated with hexachlorobenzene as a fungicide) resulted in dramatic developmental effects, including a 95% mortality rate in infants under 2 years of age who had been breast fed by exposed mothers (Peters et al. 1966). These infants were diagnosed with a condition known as *pembe yara* or "pink sore" because of associated skin lesions consisting of blistering, epidermolysis, and annular erythema. The cause of death in these infants was cardiorespiratory failure; weakness and convulsions were also seen frequently. Older children, between the ages of 6 and 15 years, exhibited a condition known as *kara yara* or "black sore" more frequently than younger children or adults. The symptoms of this disease began with photosensitivity and progressed within 6 months to include hyperpigmentation, dermal fragility (resulting in ulcerating lesions and severe mutilating scars), and hirsutism (Cam and Nigogosyan 1963; Dogramaci 1964; Gocmen et al. 1989). Mortality was 10% among *kara yara* patients. These skin lesions (pink sore and black sore) have been diagnosed as porphyria cutanea tarda, a specific type of vesiculobullous porphyria. The porphyrias are a class of inherited and acquired diseases caused by enzymatic defects in heme biosynthesis, leading to the generation of porphyrins, which may cause tissue damage, especially in the skin (for more information, see Sections 3.2.2.2, Hepatic Effects and Dermal Effects, and 3.5.2, Mechanisms of Toxicity). Similar dermal lesions, but no increase in mortality incidence, were reported for exposed adults, who also exhibited neurological disorders (weakness and diminished muscle control).

Follow-up studies have found persistent symptoms of developmental toxicity in a cohort of 252 adults (162 men and 90 women) who had been exposed as children in the poisoning epidemic (average age of the cohort at the time of exposure was 7.6 years) (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982). Short stature was seen in 42.1% of the patients, considered striking in comparison to their unexposed siblings. Additionally, 66.6% of the exposed patients exhibited osteoporosis of bones in the hands, associated with distinctive small hands, painless swelling, and spindling of fingers. Osteoporosis and osteosclerosis have been observed in adult hexachlorobenzene-exposed rats (see Section 3.2.2.2,

Musculoskeletal Effects). In addition to the profound weakness and decreased muscle control observed in exposed adults (see Section 3.2.2.4, Neurological Effects), this cohort also presented parestheisa (spontaneous tingling or burning sensations, in 53.6% of patients) and graded sensory loss indicative of polyneuropathy (in 60.6% of patients).

Two follow-up investigations have found potential reproductive effects in women exposed as children to hexachlorobenzene in the Turkish epidemic (Gocmen et al. 1989; Jarrell et al. 1998). A study conducted 20–30 years after initial exposure identified 57 porphyric mothers with 23 fetal deaths and 54 children who died in the first several years of life from a total of 276 pregnancies (Gocmen et al. 1989). Porphyric mothers had an average of 0.51 ppm hexachlorobenzene in their breast milk, compared to 0.07 ppm in unexposed controls. The results of this study were inconclusive because no information was provided regarding the expected incidence of fetal deaths and newborn-death in this population. Another study found a statistically significant increased risk of abortion among a subset of exposed women who exhibited porphyria cutanea tarda and had blood levels of hexachlorobenzene above 1.0 ng/mL (Jarrell et al. 1998).

A German case-control study found that adipose hexachlorobenzene levels in 18 male patients who underwent orchidopexy to correct unilateral or bilateral undescended testis (mean age 4.2 years) were 3-fold higher compared to a group of 30 male control patients (mean age 3.5 years); this difference was highly significant statistically (Hosie et al. 2000). These results were inconclusive because a similar correlation was also observed for heptachloroepoxide (but not for other organochlorines measured). This study is also limited by its small study size and lack of age-adjustment.

Although epidemiological studies have suggested that consumption by mothers of fish high in organochlorines may affect the immune system of their infants, perinatal exposure to hexachlorobenzene has not been clearly associated with immunological effects. The levels of hexachlorobenzene, dieldrin, and *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE) in milk samples collected shortly after the birth of Canadian Inuit infants correlated with increased risk of otitis media in their first year of life, but not with other serum immune parameters (immunoglobulins, cytokines, lymphocyte activation markers) (Dewailly et al. 2000). A similar study of immune parameters in umbilical blood of Canadian mothers with high or low consumption of fish from the Lower-North-Shore of the St. Lawrence River detected a statistically significant association only between decreased lymphocyte secretion of cytokine IL-10 and increased levels of hexachlorobenzene, *p,p'*-DDE, and mercury (Belles-Isles et al. 2000).

Acute-duration developmental studies have verified that hexachlorobenzene impaired neurological development at doses as low as 2.5 mg/kg/day in rats (Goldey and Taylor 1992) and produced teratogenic abnormalities at doses as low as 40 mg/kg/day (Courtney et al. 1976; Khera 1974a). Intermediate-duration developmental studies in rats have seen immunodevelopmental effects at 0.5 mg/kg/day (Barnett et al. 1987); neurodevelopmental effects at 1.3 mg/kg/day (Lilienthal et al. 1996); and reduced neonatal viability and growth, and organ weight changes at 4–5 mg/kg/day (Grant et al. 1977; Kitchin et al. 1982; Vos et al. 1979a, 1983). In Rhesus monkey pups, death (accompanied by neurological effects, lung edema, and liver damage) resulted from nursing for 15–38 days from female monkeys exposed to 64 mg/kg/day of hexachlorobenzene (Bailey et al. 1980; Iatropoulos et al. 1978). One chronic study was found (Arnold et al. 1985). In a multigenerational reproductive study in Sprague-Dawley rats, hexachlorobenzene induced minor liver effects (peribiliary lymphocytosis and fibrosis) at doses as low as 0.016 mg/kg/day in F_1 adults and induced more serious effects (decreased F_1 neonatal viability; tumor formation in F_1 adults) at doses as low as 2 mg/kg/day of hexachlorobenzene.

The most sensitive acute study evaluated neurodevelopmental end points and detected evidence of hyperactivity in Sprague-Dawley rat pups (Goldey and Taylor 1992; Taylor and Goldey 1990). This study was considered acute because virgin female Sprague-Dawley rats were gavaged for 4 days with 2.5 or 25 mg/kg/day of hexachlorobenzene 2 weeks prior to mating. Compared to controls, pups from both treatment groups reoriented themselves significantly more quickly in a negative geotaxis test, required less time in an olfactory discrimination test (postnatal days 6, 8, and 10), and demonstrated increased exploratory activity in a motor activity test (postnatal days 15–20). Pups exposed to 25 mg/kg/day exhibited decreased acoustic startle response (ASR) on postnatal day 23 and increased ASR on postnatal day 90. The LOAEL of 2.5 mg/kg/day from this study has been used to calculate an acute oral MRL of 0.008 mg/kg/day as described in the footnote to Table 3-2 and in Appendix A.

Other acute studies have used higher doses to investigate traditional end points of developmental toxicity. An acute single-dose study found an increase in the overall incidence of fetal abnormalities (but not any specific abnormality) in the fetuses of pregnant female CD-1 mice gavaged with 100 mg/kg/day of hexachlorobenzene on gestation days 7–16; cleft palate and renal agenesis were the most common anomalies noted (Courtney et al. 1976). Three acute (3–10 days) and one intermediate (15 days) developmental toxicity experiments in pregnant Wistar rats observed increases in the incidences of sternal variations and the 14[th] rib formation at $40 mg/kg/day (Khera 1974a). At $80 mg/kg/day, decreased fetal and maternal body weights were seen with other maternal effects.

The most sensitive intermediate-duration study evaluated immunodevelopmental toxicity. Pups of pregnant BALB/c mice fed doses as low as 0.5 mg/kg/day on gestation days 1–18 exhibited a marked, significant decrease in delayed type hypersensitivity (DTH) response when tested on postnatal day 45 (Barnett et al. 1987). Splenic effects (decreased B cell numbers and decreased mixed lymphocyte responses) were seen at 5 mg/kg/day (but not 0.5 mg/kg/day). Studies in Wistar rats have also demonstrated immunodevelopmental toxicity. In pups born to dams exposed through gestation and lactation to hexachlorobenzene and continued on the same diets, 0.2 mg/kg/day, increased immune responses (IgM and IgM responses to tetanus toxin, delayed-type reaction to ovalbumin, and pulmonary accumulation of foamy macrophages) were noted (Vos et al. 1983); exposure to 2.5 mg/kg/day decreased resistance to infection and caused lymph node endothelial proliferation. Additional signs of developmental toxicity were seen in these pups at higher doses: pup mortality, liver weight, and popliteal lymph node weights were increased at 5 mg/kg/day; and adrenal weight and serum immunoglobulin concentrations (IgM and IgG) were increased at 7.5 mg/kg/day (Vos et al. 1979a, 1983). Similar developmental effects were observed in an intermediate-duration reproductive study which fed female Sprague-Dawley rats were fed hexachlorobenzene for 96 days prior to mating through 2 rounds of breeding (Kitchin et al. 1982). In both F_{1a} and F_{1b} pups, doses as low as 3 mg/kg/day decreased body weight and doses as low as 4 mg/kg/day decreased survival (Kitchin et al. 1982).

The only animal developmental study in non-rodents used Rhesus monkey mothers (Bailey et al. 1980; Iatropoulos et al. 1978). Only one of three infant monkeys (between 21 and 118 days of age) nursing from mothers fed 64 mg/kg/day of hexachlorobenzene by daily gavage survived; the durations of dosing were 15 and 38 days (for the mortalities) and 60 days (for the survivor). Although the mothers were asymptomatic, both infant mortalities exhibited neurological effects (listlessness, lethargy, depression, and ataxia) and lung edema prior to death. Microscopic findings included mild hepatocellular hypertrophy in the infant that survived, and hepatic fatty changes, slight renal proximal tubule vacuolation, and mild cerebral gliosis in one or both infants that died.

Two chronic-duration reproductive studies in Sprague-Dawley rats observed signs of developmental toxicity (Arnold et al. 1985; Grant et al. 1977). In one study male and female rats were fed hexachloro-benzene from 3 months prior to mating through weaning, and pups were continued on the same diet for their entire lifetime. Only the highest dose tested, 2 mg/kg/day, decreased pup survival. When examined as adults (week 130), peribiliary lymphocytosis and fibrosis were observed in F_1 males at the lowest dose tested, 0.016 mg/kg/day, and hepatic basophilic chromogenesis was seen at $0.4 mg/kg/day (Arnold et al. 1985). A four-generation assay found increased liver weight and hepatic aniline hydroxylase activity at

2 mg/kg/day (F_{1a} and F_{3a} animals), consistently decreased pup weight at $4 mg/kg/day (all pup generations), and decreased pup viability at 8 mg/kg/day (F_{1a} and F_{1b} animals) (Grant et al. 1977). The LOAEL of 0.016 mg/kg/day from the lower-dose study (Arnold et al. 1985) has been used to calculate a chronic oral MRL of 0.00005 mg/kg/day as described in the footnote to Table 3-2 and in Appendix A.

The highest NOAEL values and all reliable LOAEL values for developmental effects in each species and duration category are presented in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

The Department of Human Health Services (DHHS) considers the evidence for the carcinogenicity of hexachlorobenzene in experimental animals sufficient, and this chemical is reasonably anticipated to be a carcinogen in humans (NTP 2001). A cancer assessment for hexachlorobenzene is available on Integrated Risk Information System (IRIS 2001) in which the chemical is assigned to U.S. EPA cancer weight-of-evidence Group B2, probable human carcinogen, on the basis that oral administration of hexa-chlorobenzene has been shown to induce tumors in the liver, thyroid, and kidney in three rodent species. IRIS (2001) presents an oral slope factor of 1.6 per (mg/kg)/day and inhalation unit risk of 0.00046 per ($\mu g/m^3$) for hexachlorobenzene based on hepatocellular carcinoma in orally exposed female rats (Ertürk et al. 1986). This EPA assessment is currently undergoing re-evaluation.

The available epidemiology reports taken together do not support an association between hexachloro-benzene exposure and increased cancer incidence, but their limitations (including small study sizes, similar tissue hexachlorobenzene levels between cancer and control groups, and potentially confounding effects of other organochlorines) preclude considering them evidence of noncarcinogenicity. Case-control studies (ranging in size from 20 cancer cases and 20 controls to 304 cases and 186 controls) investigating organochlorine levels in serum or breast tissue samples surgically removed from groups of patients either with breast cancer or with benign breast tumors were unable to detect statistically significant differences in hexachlorobenzene levels (Dorgan et al. 1999; Falck et al. 1992; Guttes et al. 1998; Liljegren et al. 1998; Mendonca et al. 1999; Moysich et al. 1998; Mussalo-Rauhammaa et al. 1990; Zheng et al. 1999). The exception was a prospective study by Dewailly et al. (1994) in which serum and adipose organochlorine levels were measured in 41 Canadian women (ages 40-69) undergoing breast biopsy. Mammary adenocarcinoma was diagnosed in 20 women and benign breast tumors in 17 others; the former group had statistically significantly higher serum (but not adipose) levels of hexachloro-benzene compared to the latter group. No association of blood hexachlorobenzene levels with cancer

incidence was observed in a study of 108 pancreatic cancer patients in the San Francisco Bay area
(Hoppin et al. 2000), or in a study of 154 endometrial cancer patients in Sweden (Weiderpass et al. 2000).
Additionally, studies analyzing hexachlorobenzene in the bone marrow of 13 German leukemia patients
(Scheele et al. 1996) and in the adipose of 4 male Swedish patients with Ewing's sarcoma of the bone
(Hardell et al. 1997) did not find any statistically significant increases compared to controls. No evidence
of cancer was reported in a 25-year follow-up study of 161 people (Peters et al. 1982) or a 20–30-year
follow-up study of 204 people (Cripps et al. 1984) who consumed hexachlorobenzene-contaminated grain
in Turkey from 1955 to 1959. However, these two studies did not examine patients for internal cancer
and were not designed to detect increases in cancer incidence.

Those follow-up studies (Cripps et al. 1984; Peters et al. 1982) did detect porphyria in some adults
(17/204 and 33/161, respectively) who had been exposed as children to hexachlorobenzene (see
Section 3.2.2.2 Hepatic Effects). This is relevant to cancer formation in humans because other
epidemiology studies (unrelated to hexachlorobenzene) have found statistically significant associations
between porphyria and increased risk of liver cancer. Fracanzani et al. (2001) reported that the presence
of porphyria conferred a 5-fold increased risk of liver cancer. Linet et al. (1999) reported that porphyria
cutanea tarda and acute intermittent porphyria were associated, respectively, with 20- and 70-fold
increases in liver cancer and 3-fold increases in lung cancer. However, porphyria and liver cancer in the
general population share common etiologies, so the association may not be casual (Axelson 1986; Salata
et al. 1985; Topi et al. 1980; Waddington 1972).

Oral exposure of rats, mice, and hamsters to hexachlorobenzene has induced tumors in the liver ("liver
cell" tumor, hepatocellular carcinoma, hepatoma, hemangiohepatoma, hemangioendothelioma, bile duct
tumor) (see below). Individual studies have also reported statistically significant increases in the
incidences of kidney (renal cell adenoma), thyroid (alveolar adenoma), parathyroid (adenoma), and
adrenal gland (pheochromocytoma) tumors, as well as the induction of lymphosarcoma (non-Hodgkin's
lymphoma) (Arnold et al. 1985; Ertürk et al. 1986). Female rats (Ertürk et al. 1986; Pereira et al. 1982;
Smith and Cabral 1980) and mice (Cabral et al. 1979) appear to be more susceptible than males to the
hepatocarcinogenic effects of hexachlorobenzene and limited evidence suggests that males are more
susceptible to renal cancer (Ertürk et al. 1986). The cause of these gender specificities are unclear.

Chronic oral exposure to hexachlorobenzene induces liver tumors in rats, with female rats appearing more
susceptible than males. Ertürk et al. (1986) fed Sprague-Dawley rats 4 or 8 mg/kg/day of hexachloro-
benzene for up to 2 years, with 9 interim sacrifices. Hexachlorobenzene induced statistically significant

increases in the incidences of hepatoma, hepatocarcinoma, and renal carcinoma in both genders. In the liver, degenerative lesions were seen after 2–3 weeks, preneoplastic changes were detected after 200 days, and hepatocarcinomas were detected beginning at 300 days. Hepatomas, hemangiohepatomas, and hepatocellular carcinomas were significantly more common in females than in males. Bile duct adenomas (statistically significant increased incidence) and bile duct adenocarcinomas (not significant) were seen only in treated females. In contrast, renal adenomas and renal cell carcinomas were more frequent in males. Smith and Cabral (1980) detected liver cell tumors in all (14/14) female Agus rats and in a majority of female Wistar rats (4/6) fed hexachlorobenzene for 90 weeks at doses of 6–8 and 5 mg/kg/day, respectively. Moreover, in those treated Wistar rats without tumors (2/6), evidence of preneoplastic changes (hepatocellular hypertrophy) was observed. Similar results were seen in a subsequent study (Smith et al. 1985). In Fischer 344/N rats fed 10 mg/kg/day of hexachlorobenzene for 90 weeks, all surviving females had multiple liver tumors and at least 50% exhibited hepatocellular carcinomas. In contrast, only 16% of males exhibited liver tumors, which were smaller and limited to one per animal. Liver tumors stained heavily for gamma-glutamyl transpeptidase. In a tumor promotion study, feeding of 10 mg/kg/day of hexachlorobenzene for 45 days induced significantly more preneo-plastic changes (hepatic foci positive for gamma-glutamyl transpeptidase) in the initiated livers of female Sprague-Dawley rats than of males (Pereira et al. 1982). In female (but not male) F_1 Sprague-Dawley rats with lifetime exposure to 2 mg/kg/day of hexachlorobenzene, the incidence of liver neoplastic nodules was significantly increased compared to controls (Arnold et al. 1985).

Hexachlorobenzene also induced liver tumors in mice and hamsters, but gender specificity was apparent only in mice. Syrian golden hamsters were fed 0, 4, 8, and 16 mg/kg/day of hexachlorobenzene in the diet for life (Cabral et al. 1977). Statistically significant increases in incidence were seen for hepatomas and hemangioendotheliomas (liver and spleen) in all treatment groups, with a slightly higher incidence in males than in females. Thyroid tumors were not seen in control groups, but were found in all treatment groups except low dose males; however, the increased incidence was significant only for males at 16 mg/kg/day. Outbred Swiss mice were fed 6, 12, or 25 mg/kg/day of hexachlorobenzene for up to 120 weeks (Cabral et al. 1979). Statistically significant increases in the incidence of liver cell tumors were seen at 12 and 25 (but not 6) mg/kg/day for both genders. Liver tumor incidence was significantly more common in females than in males treated with 25 mg/kg/day. Tumor multiplicity and size increased with increasing dose, while the latency period decreased.

A brief paper by Ertürk et al. (1986) reported the induction of liver and other tumor types in Sprague-Dawley rats, Swiss mice, and Syrian golden hamsters after only 90 days of feeding the animals 20 or

40 mg/kg/day of hexachlorobenzene. However, the report is limited by its lack of methodology and quantitative results. Liver effects included hepatomas, metaplasia, and stromal activation. Some support for the findings of a rapid onset of liver tumor formation is found in intermediate oral dosing (10 days to 24 weeks) experiments with hexachlorobenzene that induced hepatocellular hypertrophy in rats (Den Besten et al. 1993; Smith et al. 1985), mice (Shirai et al. 1978), pigs (Den Tonkelaar et al. 1978), dogs (Sundlof et al. 1981), and Rhesus monkeys (Iatropoulos et al. 1976; Knauf and Hobson 1979). However, other intermediate-duration studies (13 weeks to 1 year) in rats did not detect neoplasia or preneoplastic effects in the liver or other organs (Goldstein et al. 1978; Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977; Mollenhauer et al. 1975; Smith et al. 1979, 1985, 1986a).

The mode-of-action for the hepatocarcinogenicity of hexachlorobenzene has been investigated. Multiple studies have indicated that non-heme iron potentiates the hepatocarcinogenic effects of hexachloro-benzene (Adjarov 1990; Elder and Urquhart 1986; Hahn et al. 1988; Smith and Francis 1983; Smith et al. 1989, 1993; Vincent et al. 1989). Two studies in rats provided limited evidence that hexachlorobenzene is a promoter but not an initiator of liver cancer. As noted above, treatment of intact Sprague-Dawley rats with 10 mg/kg/day of hexachlorobenzene for 45 days did not induce hepatic foci positive for gamma-glutamyl transpeptidase, but foci were induced following liver initiation by partial hepatectomy with and without diethylnitrosomaine (Pereira et al. 1982). This finding suggests that hexachlorobenzene may act as an promoter at doses insufficient to initiate tumors. No evidence of altered foci were detected in male Fisher 344 rats pretreated with partial hepatectomy and treated with a single gavage dose of 5,000 mg/kg hexachlorobenzene followed by liver tumor promotion with carbon tetrachloride and cholic acid for 12 weeks (Tsuda et al. 1993).

Reported incidences of other tumor types have also increased following oral exposure to hexachloro-benzene. A group of five adult female Rhesus monkeys were dosed by gavage with hexachlorobenzene in methylcellulose daily for 60 days (Iatropoulos et al. 1976). Single monkeys received 0, 8, 32, or 64 mg/kg and two monkeys received 128 mg/kg. In one of the high dose monkeys (but not in the others), a benign mammary fibroadenoma was detected. This evidence is inconclusive, because of the low statistical power of the study. The other monkey given 128 mg/kg exhibited moderate adrenal medullary hyperplasia and the monkey given 64 mg/kg exhibited slight hyperplasia of the adrenal zona fasciculata; these findings support observations made in rats. In F_1 Sprague-Dawley rats exposed to 2 mg/kg/day for their entire lives (including *in utero* exposure and approximately 130 weeks *ex utero*, as part of a 2-generation reproductive study), statistically significant increases in adrenal pheochromocytomas were seen in both males and females, while a significantly increased incidence of parathyroid adenomas was

observed only in females (Arnold et al. 1985). The biological significance of the adrenal effect is also supported by observations of adrenal gland cortical hyperplasia in female Wistar rats at doses as low as 9.5 mg/kg/day for 13 weeks (Den Besten et al. 1993) and in male and female Sherman rats exposed to hexachlorobenzene in the diet at doses at low as 5 mg/kg/day for 4 months (Kimbrough and Linder 1974). No other studies have reported any parathyroid histopathology caused by oral exposure to hexachloro-benzene, but parathyroid effects (changes in hormone levels) have been observed in male Fischer 344 rats (Andrews et al. 1988, 1989, 1990). The observations made during the 90-day study in which Sprague-Dawley rats, Swiss mice, and Syrian golden hamsters were fed 20 or 40 mg/kg/day of hexachloro-benzene have not been confirmed (Ertürk et al. 1986). Renal damage with metaplastic regenerative changes were seen in treated animals; renal tumors were "most frequent in rats" and "more frequent in males." Lymphosarcomas (detected in the thymus, spleen, and lymph nodes) were apparently common in all treatment groups, with frequent lymphohematopoietic hyperplasia and lymphocytic infiltrations. These lesions occurred 2–4-fold (depending on organ) more frequently in female mice than in male mice.

The CEL (i.e., lowest dose that produced a tumorigenic response for each species), the duration category of exposure to hexachlorobenzene, and the estimated upper-bound risk levels from 10^{-4} to 10^{-7} are shown in Table 3-2 and plotted in Figure 3-2.

3.2.3 Dermal Exposure

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

3.2.3.1 Death
3.2.3.2 Systemic Effects
3.2.3.3 Immunological and Lymphoreticular Effects
3.2.3.4 Neurological Effects
3.2.3.5 Reproductive Effects
3.2.3.6 Developmental Effects

3.2.3.7 Cancer

3.3   GENOTOXICITY

An increased incidence of micronuclei was observed in the peripheral lymphocytes of 41 chemical workers in San Paulo, Brazil, who had been exposed to a mixture of chlorinated solvents that included hexachlorobenzene, as well as carbon tetrachloride and perchlorethylene (da Silva Augusto et al. 1997). The usefulness of this study is limited by the confounding effect of multiple chemical exposure.

No studies were located regarding genotoxic effects in animals following inhalation exposure to hexachlorobenzene.

No studies were located regarding the genotoxic effects of hexachlorobenzene in humans following oral exposure.

In vivo studies in rats revealed the lack of significant genotoxic activity in mammals following oral exposures to hexachlorobenzene. Negative results were observed in two dominant lethal mutation assays that orally exposed rats at doses ranging from 60 to 221 mg/kg (Khera 1974a; Simon et al. 1979). No evidence of genotoxicity was observed in mouse liver, lung, kidney, spleen, or bone marrow after oral dosing (Sasaki et al. 1997). An oral exposure study to test the DNA induction potential of hexachlorobenzene in Wistar rats provided equivocal evidence that hexachlorobenzene reacts directly with DNA (Gopalaswamy and Nair 1992). Male rats were untreated or pretreated with phenobarbital (0.1% sodium phenobarbital in drinking water for 2 weeks) and then administered 25 mg/kg (specific activity 14.0 mCi/mmole) hexachlorobenzene in 0.1 mL refined peanut oil for 24 hours. The animals were sacrificed and DNA obtained from liver extracts. Upon analysis, the DNA was found to be bound by hexachlorobenzene (2.23 ± 0.27 pmoles/mg DNA for phenobarbital untreated animals and 3.56 ± 0.18 pmoles/mg DNA for phenobarbital pretreated animals). The comparative values for lindane in the same study were 5.82 ± 0.31 and 6.90 ± 0.14 pmoles/mg DNA , respectively. No hexachlorobenzene untreated control values were provided in the study report. It is notable, however, that there is evidence (Jackson et al. 1993) that phenobarbital is mutagenic *in vitro* in several test systems. Other studies have likewise failed to observed gene mutations or unscheduled DNA repair in microbial assays (Gopalaswamy and Nair 1992; Haworth et al. 1983; Siekel et al. 1991).

3. HEALTH EFFECTS

No studies were located regarding genotoxic effects in humans or animals following dermal exposure to hexachlorobenzene.

Hexachlorobenzene did not produce chromosomal aberrations in human peripheral lymphocytes *in vitro* (Siekel et al. 1991). However, hexachlorobenzene produced weak positive results in assays for DNA fragmentation and micronuclei formation in primary cultures of human hepatocytes (Canonero et al. 1997). Treatment with hexachlorobenzene induced only minimal formation of DNA adducts in cultured human Hep G2 hepatoma cells (Dubois et al. 1997).

The micronucleus assay, but not the DNA fragmentation assay, was positive in cultured rat hepatocytes (Canonero et al. 1997). The researchers concluded that hexachlorobenzene is a weak genotoxic carcinogen and that negative responses in standard genotoxicity assays were due to limitations in the ability of exogenous metabolic activation systems to duplicate the complex interactions of the intact liver cell. Hexachlorobenzene was also positive in an assay for replicative DNA synthesis in mouse hepatocytes (Miyagawa et al. 1995). Treatment with hexachlorobenzene induced only minimal formation of DNA adducts in fetal hepatocytes from rats and quail (Dubois et al. 1997).

Hexachlorobenzene tested negative or ambiguous in reverse mutation assays in *S. typhimurium* (Gopalaswamy and Aiyar 1986; Gopalaswamy and Nair 1992; Haworth et al. 1983; Siekel et al. 1991) and *E. coli* (Siekel et al. 1991) with and without metabolic activation, although an assay for reverse mutation in the yeast *Saccharomyces cerevisiae* was positive (Guerzoni et al. 1976). Hexachlorobenzene also tested negative in a DNA repair assay in *E. coli* (Siekel et al. 1991).

Data from a study in male Long Evans rats suggested that the metabolism of hexachlorobenzene to penta-chlorobenzene and other more polar metabolites proceed either through a free-radical mechanism or by initial formation of an arene oxide. These reactive intermediates may form covalent bonds with cellular constituents (such as protein amino acids or DNA nucleic acids) leading to irreversible cell damage (Lui and Sweeney 1975). Several other studies have also found evidence of binding to cellular proteins by reactive electrophilic metabolites of hexachlorobenzene formed by cytochrome P-450 system (Gopalaswamy and Nair 1992; Rajamanickam and Padmanaban 1974; van Ommen et al. 1985).

Key *in vivo* genotoxicity studies are presented in Table 3-3 and *in vitro* genotoxicity studies are presented in Table 3-4.

3.4 TOXICOKINETICS

In humans, inhalation accounts for an unknown, but probably low, amount of exposure due to the low vapor pressure of hexachlorobenzene. Current information indicates that human absorption of inhaled hexachlorobenzene is poor; approximately two orders of magnitude less than the exposure estimate for the oral route (Burns et al. 1974; Burton and Bennett 1987; Currier et al. 1980). Other data of absorption following inhalation exposure come from studies of occupational exposures (Queiroz et al. 1997, 1998a, 1998b; Richter et al. 1994; Selden et al. 1997) and citizens of Flix, Spain, who have been exposed to airborne hexachlorobenzene from an organochlorine factory (Grimalt et al. 1994; Herrero et al. 1999; Sala et al. 1999b; To-Figueras et al. 1997). Based on information from an epidemic resulting from ingestion of hexachlorobenzene-contaminated bread in Turkey, ingested hexachlorobenzene is moderately absorbed from the gastrointestinal tract (Albro and Thomas 1974; Cam and Nigogosyan 1963; Gocmen et al. 1989; Peters et al. 1982). However, most of the hexachlorobenzene body burden in the U.S. population derives from dietary intake of fatty foods (Burton and Bennett 1987). Schlummer et al. (1998) estimated that 85.4% of ingested hexachlorobenzene will be absorbed when the blood contains no hexachlorobenzene, and that this percentage will be reduced by 0.2% for each ng of hexachlorobenzene per g lipid in blood, and hypothesized a "fat-flush" theory of hexachlorobenzene absorption: temporary increases in lipid content in the gut dilute hexachlorobenzene concentrations and increase the diffusion gradient from the gut into the lymph and blood. Data from animal studies indicate that the gastrointestinal absorption of hexachlorobenzene is quite variable, depending upon the solvent vehicle used for administration, ranging from 6% when administered in aqueous solution to 82% when administered with squalene in cottonseed oil (Albro and Thomas 1974), olive oil (Freeman et al. 1989;

Table 3-3. Genotoxicity of Hexachlorobenzene *In Vivo*

End point	Species (test system)	Exposure route	Results	Reference
Mammalian systems: Dominant lethals	Rat	Oral	–	Khera 1974a
	Rat	Oral	–	Simon et al. 1979
DNA binding	Rat (Wistar)	Oral	±	Gopalaswamy and Nair 1992

– = negative result; ± = weakly positive; DNA = deoxyribonucleic acid

Table 3-4. Genotoxicity of Hexachlorobenzene *In Vitro*

Species (test system)	End point	Results With activation	Without activation	Reference
Prokaryotic organisms: Reverse mutation				
Salmonella typhimurium	Gene mutation	–	–	Haworth et al. 1983
S. typhimurium	Gene mutation	±	–	Gopalaswamy and Nair 1992
S. typhimurium	Gene mutation	–	–	Siekel et al. 1991
Escherichia coli	Gene mutation	–	–	Siekel et al. 1991
DNA Repair Assays				
E. coli	DNA repair	–	–	Siekel et al. 1991
Eukaryotic cells:				
Human peripheral blood lymphocytes	Chromosomal aberration	–	–	Siekel et al. 1991
Mammalian system:				
Rat (Wistar)	DNA binding	±	–	Gopalaswamy and Nair 1992

– = negative result; ± = weakly positive; DNA = deoxyribonucleic acid

Goldey et al. 1990; Knauf and Hobson 1979; Koss and Koransky 1975; Mehendale et al. 1975; Sundlof et al. 1982; Villeneuve and Hierlihy 1975), or peanut oil (Ingebrigtsen and Nafstad 1983; Ingebrigtsen et al. 1981). The lymphatic system has been shown to play an important role in the gastrointestinal uptake of hexachlorobenzene in animals (Iatropoulos et al. 1975). Although no empirical data are available on the dermal absorption of hexachlorobenzene in humans, data from a rat study were used to develop a compartment model for application to a 70-kg worker. Using these data, the dermal absorption constant for hexachlorobenzene was calculated as 1.4×10^{-3} per hour (Koizumi 1991).

Information on distribution in people following inhalation exposure to hexachlorobenzene is limited (Ataniyazova et al. 2001; Sala et al. 2001b) and no information is available on the distribution of inhaled hexachlorobenzene in animals. However, orally absorbed hexachlorobenzene distributes widely in mammalian tissue, rapidly partitioning to blood, liver, breast milk, adipose tissue, endocrine organs, bone marrow, and ovarian follicular fluid (Ellenhorn and Barceloux 1988; Foster et al. 1995a; Ingebrigtsen 1986; Ingebrigtsen and Nafstad 1983; Knauf and Hobson 1979; Wickstrom et al. 1983), and preferentially distributing to adipose tissue or organs with high fat content (Burton and Bennett 1987; Cohn et al. 1978; Koss and Koransky 1975; Lecavalier et al. 1994; Mehendale et al. 1975; Robinson et al. 1990; Teufel et al. 1990; van Raaij et al. 1993a; Verschueren 1983). Animal studies of oral dosing have showed that levels of hexachlorobenzene increase in a dose-dependent manner in all tissues up to 100 mg/kg/day (Foster et al. 1995a; Jarrell et al. 1993; Sundlof et al. 1982). Hexachlorobenzene body burden is readily transferred from pregnant mother to the fetus through the placenta in animals (Courtney and Andrews 1985; Courtney et al. 1979; Cripps 1990; Goldey et al. 1990; Nakashima and Ikegari 2000; Nakashima et al. 1997, 1999; Villeneuve and Hierlihy 1975; Villeneuve et al. 1974a). Additionally, hexachlorobenzene is concentrated in the milk and can be transferred to the suckling neonate (Bailey et al. 1980; Cripps 1990; Goldey et al. 1990; Nakashima and Ikegari 2000; Nakashima et al. 1997, 1999). Evidence from animal studies indicates that protein-poor diets may promote the preferential partitioning of ingested hexachlorobenzene to fatty tissue (Rodrigues et al. 1991). In a survey of the U.S. population, it was found that concentration of hexachlorobenzene tended to increase with increasing age, a testimony to the propensity of hexachlorobenzene to bioaccumulate in mammalian tissue (Robinson et al. 1990). In a group of 350 German children, blood hexachlorobenzene levels (and levels of other organochlorines) correlated strongly with the length of breast-feeding (Karmaus et al. 2001). Weak associations were seen between decreased blood hexachlorobenzene levels and increased child body mass index (above 18 kg/m^2), and between increased hexachlorobenzene levels and both maternal age at birth (36–45-year-old group only) and late birth order (3rd or later, with spacing between children of at least 4 years). These data suggest that increased size may dilute hexachlorobenzene in the body, and that levels of hexachloro-

benzene in mothers may increase with age. No correlations were seen for mothers who smoked during pregnancy, or the age and gender of the child.

Hexachlorobenzene is slowly metabolized in mammals, and the majority of hexachlorobenzene is excreted unchanged (in feces). Reductive dechlorination of hexachlorobenzene—catalyzed by enzymes located in the microsomal fraction of liver, lung, kidney, and intestine—appears to be an important pathway for the metabolism of hexachlorobenzene (Ingebrigtsen et al. 1986). It has been suggested that epoxide formation also occurs in this metabolism (Lui et al. 1976). PCP has been identified in human liver preparations incubated with hexachlorobenzene (Koss et al. 1986). In animals, hexachlorobenzene is slowly metabolized to PCP by the hepatic cytochrome P-450 system, conjugated with glutathione to yield pentachlorothiophenol, or reductively dechlorinated to form pentachlorobenzene. Other metabolites include less chlorinated benzenes, chlorophenols, S-conjugated phenols, and benzenes. PCP is subsequently converted to TCHQ (Hahn et al. 1988, 1989; Linko et al. 1986; Mehendale et al. 1975; Rozman et al. 1977a). The feces contain mostly unchanged parent compound, and about 1% pentachloro-benzene and traces of PCP after oral hexachlorobenzene exposure in mammals, while urinary excretion consists of mostly the metabolites, pentachlorobenzene, 2,4,5-trichlorophenol, *N*-acetyl-*S*(pentachloro-phenyl)cysteine, mercaptotetrachlorothioanisole, and tetrachlorobenzene, 2,3,5,6-tetrachlorobenzene-1,4-diol; and unchanged parent compound (Koss et al. 1978; Mehendale et al. 1975; Rizzardini and Smith 1982; Rozman et al. 1978). Pentachlorothiophenol, PCP, methylthiopentachlorobenzene, 1,4-bis-(methylthio)-2,3,5,6-tetrachlorobenzene, chlorophenols, S-conjugated phenols and benzenes, and less chlorinated benzenes have also been identified in the liver following oral exposure in animals (D'Amour and Charbonneau 1992; Engst et al. 1976a; Ingebrigtsen et al. 1981, 1986; Jansson and Bergman 1978; Koss et al. 1976, 1979; Lui and Sweeney 1975; Renner 1988; Richter et al. 1981; Stewart and Smith 1986; To-Figueras et al. 1992; van Ommen et al. 1985, 1989; Yang et al. 1978). Sex differences in the metabolism of hexachlorobenzene in the adult animals have been reported. Urinary excretion of PCP, 2,3,5,6-tetrachlorobenzene-1,4-diol, and pentachlorothiophenol was greater in females than in males in this study (Rizzardini and Smith 1982).

To-Figueras et al. (2000) observed a high correlation between fecal and blood levels of hexachloro-benzene in 53 people highly exposed to airborne hexachlorobenzene. No information is available on the excretion of hexachlorobenzene following inhalation exposure in animals or following dermal exposure in humans or animals. In humans, ingested hexachlorobenzene is excreted in the urine mainly as its metabolites, PCP and pentachlorothiophenol (To-Figueras et al. 1992). In animals, the excretion of hexa-chlorobenzene appears to be quite variable, depending upon the solvent vehicle used (Albro and Thomas

1974; Rozman et al. 1977a). Based on decreasing concentrations in the liver, the biological half-life of hexachlorobenzene has been estimated to be 8 days at the start of the elimination phase, 10 weeks after 3 months, and 12 months after 1.5 years (Koss et al. 1983), suggesting differential release of hexachlorobenzene from tissue stores, perhaps as a function of lipophilicity. Ingested hexachlorobenzene is excreted predominantly in the feces, mainly as unchanged parent compound, and to a lesser extent in the urine, as its metabolites (PCP, pentachlorothiophenol, pentachlorobenzene) (Mehendale et al. 1975). Approximately 99% of unchanged ingested hexachlorobenzene was excreted in the feces; 50% of urinary excretion was PCP, 25% was pentachlorobenzene, and 25% was unchanged hexachlorobenzene in Rhesus monkeys treated with 0.03 mg/kg/day in the diet for 15 months (Rozman et al. 1977a). Based on animal studies, the urinary excretion of hexachlorobenzene exhibits sex- and age-specific differences; the excretion of pentachlorothiophenol increases with sexual maturity in female rats and slightly decreases in male rats (To-Figueras et al. 1991). Biliary excretion was not an important excretory pathway in rats given a single hexachlorobenzene dose of 10 mg/kg by gavage in peanut oil, accounting for <4% of the administered dose (Ingebrigtsen et al. 1981).

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Limited data show that hexachlorobenzene can be absorbed through the respiratory tract in humans, although no information is available as to the rate and extent of respiratory tract absorption of hexachlorobenzene in either humans or animals.

Spanish researchers have studied a population with long-term exposure to high levels of hexachlorobenzene in air (Grimalt et al. 1994; Herrero et al. 1999; Sala et al. 1999b; To-Figueras et al. 1997). The rural Spanish village of Flix contains an organochlorine factory that has been producing volatile chlorinated solvents for decades, and no other large industrial facilities. Following complaints of odor, approximately 40 air samples were collected in July and November of 1989 and May and October of 1992 at diverse sites in the village. As a control, five air samples were collected in the city of Barcelona. Average air levels of hexachlorobenzene in Flix (35 ng/m^3) were over 100-fold higher than in Barcelona (0.3 ng/m^3), while other organochlorines were found at similar or lower concentrations in Flix than in Barcelona. Corresponding to the high air levels, it was found that residents of Flix had unusually high serum levels of hexachlorobenzene (mean of 39.8 ng/mL based on a total number of 604 tested) in comparison to populations in Barcelona (mean=4.13 ng/mL, n=100), the United States

(mean=0.19 ng/mL, n=370), Croatia (mean=1.00, n=15), and Germany (mean=1.12, n=6). Serum levels of other organochlorines in Flix residents were much lower than hexachlorobenzene levels and did not differ from other populations. Among Flix residents, serum hexachlorobenzene levels were several fold higher in factory workers (mean=93.4 ng/mL, n=185) than other residents (mean=16.9 ng/mL, n=419). Factory workers were presumably exposed to much higher air levels of hexachlorobenzene than other village residents, and some may have had dermal exposure as well.

It is noteworthy that mean serum hexachlorobenzene levels in Flix residents who did not work at the factory (and therefore, can be assumed to have had no direct dermal exposure to hexachlorobenzene) were still 4-fold higher than Barcelona residents. However, the difference was not entirely due to inhalation exposure. In addition to working at the factory, other variables associated with serum hexachlorobenzene levels were age and consumption of local fish. Among women (very few of whom had ever worked at the factory), the geometric mean serum hexachlorobenzene level was 14.9 ng/mL in those that did not eat local fish (176/180) and 18.2 ng/mL in those that did (only 4/180). Therefore, indirect exposure to hexa-chlorobenzene via consumption of contaminated fish may have contributed slightly to serum hexachloro-benzene levels in nonfactory worker Flix residents, but was not a major factor. This study shows that exposure to high levels of hexachlorobenzene in air leads to high levels of hexachlorobenzene in serum, and that a significant portion of hexachlorobenzene uptake in this situation can be attributed to inhalation and absorption across the respiratory tract.

Studies of workers with occupational exposure to hexachlorobenzene, where exposure was probably primarily by inhalation, but may have involved dermal contact as well, also show increased serum levels of hexachlorobenzene in the exposed workers. Selden et al. (1997) found significantly higher serum hexachlorobenzene levels in 29 hazardous waste incineration workers (63 ng/g lipid) than in 60 matched controls (35 ng/g lipid). The exposed workers also had significantly increased serum hexachlorobenzene levels compared with their own historical samples given before the start of employment (0.40 ng/g plasma vs. 0.27 ng/g plasma). Airborne hexachlorobenzene levels in different locations in the plant ranged from 0.066 to 11 ng/m^3. Queiroz et al. (1997, 1998a, 1998b) observed that each of the 51 workers on leave from a closed chemical plant had blood hexachlorobenzene levels >0.1 µg/dL (mean=4.4 µg/dL), while controls chosen from blood donors at the local blood bank to be similar in age and race to the exposed group all had blood hexachlorobenzene levels lower than the limit of detection (0.02 µg/dL). The plant produced carbon tetrachloride and tetrachloroethylene; hexachlorobenzene was generated as a byproduct of the production process as a solid residue. Richter et al. (1994) documented high serum hexachloro-benzene levels in workers exposed to 2.1–10.8 mg/m^3 of hexachlorobenzene in air, which persisted even

after air concentrations of hexachlorobenzene were reduced to 0.012–0.022 mg/m^3. Although dermal exposure cannot be ruled out in these studies, uptake of hexachlorobenzene across the respiratory tract is likely to have contributed significantly to hexachlorobenzene body burden in all of these studies.

No studies were located regarding inhalation exposure to hexachlorobenzene in animals.

3.4.1.2 Oral Exposure

Widespread occurrence of porphyria cutanea tarda in southeast Anatolia in Turkey in the late 1950s was shown to be due to ingestion of bread made from grain that had been treated with hexachlorobenzene (Cam and Nigogosyan 1963). The ingested dose of hexachlorobenzene was estimated to be in the range of 0.05–0.2 g/day, or 0.7–2.9 mg/kg/day for a 70-kg person. The occurrence of systemic health effects following ingestion of hexachlorobenzene demonstrates that this chemical can be absorbed via the gastrointestinal tract in humans, and in amounts sufficient to produce serious health effects. Follow-up studies conducted between 1977 and 1981 found that hexachlorobenzene levels in 56 samples of human milk obtained from porphyric mothers averaged 0.51 ppm (standard deviation [SD]=0.75 ppm, highest value=2.8 ppm), while levels in women from families without porphyria or outside the affected area had an average level of 0.07 ppm (SD=0.07 ppm) (Peters et al. 1982). Therefore, even 20 years after exposure, there was a large difference in breast milk hexachlorobenzene concentrations between people from families that had consumed the contaminated grain and those that did not.

One study was located in which gastrointestinal absorption of hexachlorobenzene in humans was quantified. Schlummer et al. (1998) used a mass-balance approach to estimate absorption of hexachloro-benzene ingested at low concentrations in the diet in seven volunteers (four males and three females) ranging in age from 24 to 81 years. Hexachlorobenzene was measured in the food (uniform meals of varying portion sizes) ingested by volunteers over a 3-day period (using duplicate portions) and in the corresponding feces (first and last meals identified using iron capsules to produce black feces). Similar experiments were then conducted in which the volunteers chose their own foods. Whole blood samples were collected 3 weeks after the last mass balance experiment. Percent net absorption was calculated as the difference between ingested and excreted hexachlorobenzene, divided by the ingested amount. When fed a standardized meal, the percent absorption was a relatively uniform 70–82% in the four young adults tested (one female and three males ranging in age from 24 to 36 years). It decreased to 1% in a 53-year-old male volunteer and further to -56 and -210% in 76- and 81-year-old female volunteers, respectively.

The negative values in the elderly volunteers indicate net excretion, rather than absorption, in these individuals. Similar results were reportedly obtained when volunteers chose their own meals.

Blood levels of hexachlorobenzene (expressed as ng/g blood lipid) also varied with age, ranging from 65 to 82 in the young adult volunteers and increasing to 230, 680, and 1,420 in the 53-, 76-, and 81-year-old volunteers, respectively (Schlummer et al. 1998). The researchers noted a trend for decreasing absorption with increasing blood levels in the volunteers, and a linear regression was fit to these data. The calculated regression equation was:

percent absorption hexachlorobenzene = 0.8538–0.0021 x (ng hexachlorobenzene/g blood lipid)

This equation suggests that 85.4% of ingested hexachlorobenzene will be absorbed when the blood contains no hexachlorobenzene, and that this percentage will be reduced by 0.2% for each ng of hexa-chlorobenzene per g lipid in blood. This regression equation provided an excellent fit to the data (R^2=0.98), leading to the conclusion that concentration of hexachlorobenzene in the blood is a major factor influencing absorption of hexachlorobenzene from the gut. Although absorption of lipophilic compounds such as hexachlorobenzene is thought to occur by simple passive diffusion, analysis of the entire data set in this study (17 polychlorinated dibenzo-*p*-dioxins/furans [PCDD/F] congeners and 10 PCBs in addition to hexachlorobenzene) showed that absorption of these compounds could not be explained by simple gradients between lipid-based food and blood concentrations or between gut contents and gut tissue. The researchers offered a "fat-flush" hypothesis of absorption for hexachlorobenzene and related compounds whereby lipid absorbed into gut tissue would temporarily increase lipid content of the tissue, diluting contaminant concentration in the gut tissue (on a lipid basis) and increasing the diffusion gradient for the contaminant from the gut contents, before being transported from the gut tissue to the lymph and blood.

Gastrointestinal absorption of hexachlorobenzene has been well studied in laboratory animals. Ingebrigtsen and Nafstad (1983) demonstrated that gastrointestinal absorption of hexachlorobenzene in an oil vehicle is rapid. Male Wistar rats were given a single gavage dose of 0.4 mg/kg of radiolabeled hexa-chlorobenzene dissolved in peanut oil and examined by whole body autoradiography at various time intervals starting 2 hours after dosing. A considerable amount of radiolabeled material was absorbed and distributed throughout the body within 2 hours of dosing, and peak levels were reached within 24 hours. Rapid absorption was also shown in beagle dogs that had hexachlorobenzene levels in blood monitored during and after 7 days of daily oral dosing with 10 or 100 mg/kg of hexachlorobenzene in corn oil by

capsule (Sundlof et al. 1982). Peak blood concentrations occurred 3 hours after dosing in low-dose dogs, and after a somewhat longer (unspecified) interval in high-dose dogs. Blood concentrations continued to increase over several days after the last dose was administered in both groups, possibly due to continued absorption from the intestines during that time. This finding suggests that while the bulk of an oral dose of hexachlorobenzene in oil is absorbed rapidly in a few hours, absorption of residual quantities can continue for a period of days.

Hexachlorobenzene administered by gavage in aqueous methylcellulose suspension is also absorbed from the gut within a few hours (Iatropoulos et al. 1975). Sprague-Dawley rats given single gavage doses of 0.15 mg (approximately 0.6 mg/kg) of radiolabeled hexachlorobenzene in 1% methylcellulose solution were sacrificed at intervals between 1 and 48 hours after dosing for determination of tissue radioactivity levels. The ingested material was absorbed by the walls of the stomach and duodenum within 1 hour of dosing, and by the jejunum and ileum within 3 hours. Peak levels in the duodenum and jejuno-ileum occurred 3 hours after dosing. The majority of ingested hexachlorobenzene was absorbed from these regions of the small intestine by the lymphatic system and deposited in the fat, bypassing portal circulation to the liver, systemic circulation, and the excretory organs.

Although absorption of hexachlorobenzene from aqueous suspension occurred in a similar time frame as absorption from an oil vehicle in the studies described above, other studies have shown that the extent of absorption from aqueous suspension is much less. Koss and Koransky (1975) measured absorption of radiolabeled hexachlorobenzene in female Wistar rats following gavage administration of the chemical in olive oil at doses of 20, 60, and 180 mg/kg, and in aqueous suspension (6% gum arabic in water) at doses of 16, 120, and 970 mg/kg. Two days after dosing in olive oil, 73–88% of the administered radioactivity was recovered in the body, while 1% was recovered in the gut contents, 18–26% in the feces, and 0.4–0.6% in the urine in the different dose groups. This finding suggests oral absorption of about 80% of ingested hexachlorobenzene, regardless of dose, when administered in oil. When administered in aqueous suspension, however, absorption was much lower and appeared to depend on dose. At the low dose of 16 mg/kg, roughly 20% of administered radioactivity was recovered in body tissues 3 days after dosing in aqueous suspension, compared with 0.4% in the gut contents, 74% in the feces, and 0.4% in the urine. At the higher doses, only 2–5% of the administered hexachlorobenzene was absorbed from the aqueous suspension.

Other studies determined absorption of hexachlorobenzene from oil vehicles to be similar to that reported by Koss and Koransky (1975). Albro and Thomas (1974) gave male CD rats single gavage doses of 12 or

30 mg/kg of hexachlorobenzene in cottonseed oil. They found that after 96 hours, 72% (high dose) to 82% (low dose) of the dose had not been excreted in the feces. No hexachlorobenzene was detected in the bile or urine, and only about 3% of the dose was present in the intestinal tissue and contents (primarily the former), and an associated *in vitro* experiment showed that fecal bacteria do not metabolize hexachlorobenzene; this suggests that the "removed" 72–82% had been absorbed into the body. Ingebrigtsen et al. (1981) used bile duct cannulated rats to monitor biliary excretion of radiolabeled hexachlorobenzene after gavage dosing with 10 mg/kg in peanut oil. A total of 3.6% of the administered radioactivity was recovered in the bile within 48 hours, while bile flow remained steady, showing that biliary excretion is only a minor pathway for hexachlorobenzene. After 96 hours, approximately 25% of the administered radioactivity was recovered in the feces and 2% in the urine. These data again suggest oral absorption of somewhere near 80% of the ingested dose for hexachlorobenzene administered in oil.

The studies described above showed that absorption of hexachlorobenzene in the gut is much more extensive from oil vehicles than from aqueous vehicles. Zabick and Schemmel (1980) demonstrated that a high fat diet also enhances absorption of hexachlorobenzene, in comparison to a high carbohydrate diet. Groups of 6 female Osbourne-Mendel rats were fed either a high fat diet or one of two high carbohydrate diets (one using corn starch, the other using sucrose) supplemented with 32 mg/kg/day of hexachlorobenzene for 6, 12, or 18 days. The high fat diet resulted in higher carcass fat content (data not shown; cited to Shier and Schemmel 1975) and greatly increased concentrations of hexachlorobenzene in perirenal fat, liver, and gastrocnemius. At the same time, concentrations of hexachlorobenzene in the feces were much lower with the high fat diet, suggesting that the high fat diet facilitated absorption of hexachlorobenzene from the gut, thereby leading to the increase in tissue levels. The data from all of these studies showing enhanced absorption of hexachlorobenzene when administered in oil or a high fat diet are consistent with the "fat-flush" hypothesis for hexachlorobenzene absorption proposed by Schlummer et al. (1998) based on the human data.

3.4.1.3 Dermal Exposure

No studies were located regarding absorption in humans following dermal exposure to hexachlorobenzene.

Evidence from rats suggests that hexachlorobenzene can be absorbed across the skin. Using radiolabelled hexachlorobenzene, Koizumi (1991) conducted a mass-balance study of dermal absorption in male Fisher 344 rats. A dermal dose of approximately 2.5 mg/kg of 14C-hexachlorobenzene dissolved in

tetrachloroethylene was applied to a 4 cm^2 clipped area on the back under occlusion. The rats, 3 per group, were transferred to metabolic cages and sacrificed after 6, 24, or 72 hours. Cumulative absorption of hexachlorobenzene (the sum recovered from the urine, feces, liver, carcass, skin not directly contaminated, and subcutaneous tissue) increased with duration of exposure from 1.05% of the applied dose after 6 hours to 2.67% after 24 hours and 9.71% after 72 hours. A one-compartment linear pharmacokinetic model was used to calculate an absorption constant of 1.40×10^{-3} per hour.

A modeling exercise conducted by Koizumi (1991) suggests that dermal absorption of hexachlorobenzene can contribute significantly to body burden in exposed workers. Assuming the rate constant in rats applies to man and a biological half-life ranging from 100 to 730 days, a three-compartment linear pharmacokinetic model developed based on the rat data and scaled up to a 70-kg man showed that hexa-chlorobenzene blood levels will increase with duration of exposure and that dermal doses as low as 2.56–18.2 mg (which could result from contamination small enough to go unnoticed) could, over a period of years, lead to hexachlorobenzene blood levels in the vicinity of 200 ppb, regarded by some researchers (Currier et al. 1980) as the upper safe limit in humans.

Koizumi (1991) also collected data showing that washing can decrease the absorption of dermally-contacted hexachlorobenzene by a significant degree. In the rats, washing the test area with soap 6 hours after application of hexachlorobenzene removed 34% of the applied dose and reduced the cumulative amount absorbed after 72 hours by 50% (from 9.71 to 4.90%).

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

Two studies have investigated hexachlorobenzene distribution in people exposed via inhalation. The Aral Sea in Uzbekistan is a putative source of airborne exposure to hexachlorobenzene, metals, and other pollutants because its water levels are decreasing, revealing sediments to wind dissemination over the surrounding area. Ataniyazova et al. (2001) analyzed 18 maternal blood samples, 28 umbilical cord blood samples, and 41 milk samples collected from mothers and infants within 200 kilometers of the souther border of the Aral Sea in Uzbekistan. The respective mean concentrations of hexachlorobenzene in maternal and cord blood were 167 ng/L (range 72–9,920 ng/L) and 70 ng/L (range 25–1,300 ng/L) and in 93% of milk samples, with a mean of 28 ng/g fat (range 10–109 ng/L). Sala et al. (2001a) compared two populations; for a group of 31 pairs of mothers and infants from Flix, Spain, who had been highly

exposed to airborne hexachlorobenzene from a chlorinated solvent factory, the respective geometric means of hexachlorobenzene maternal and cord blood levels were 3.98 ng/mL (range 0.50–20.78 ng/mL) and 1.40 ng/mL (range 0.30–5.77 ng/mL). For subjects from villages nearby to Flix, the respective geometric means of hexachlorobenzene maternal and cord blood levels were 2.51 ng/mL (range 0.36–7.46 ng/mL) and 0.85 ng/mL (range 0.13–2.45 ng/mL). A statistically significant correlation between maternal and umbilical cord blood was detected.

No studies were located regarding distribution of hexachlorobenzene following inhalation in animals.

3.4.2.2 Oral Exposure

Hexachlorobenzene rapidly distributes throughout the body following absorption. A whole-body autoradiography experiment in rats showed that hexachlorobenzene was extensively distributed throughout the body within 2 hours of receiving a single oral dose of 0.4 mg/kg in peanut oil (Ingebrigtsen and Nafstad 1983). In another experiment, radiolabeled hexachlorobenzene was found in multiple internal tissues in rats 3 hours after the rats received a single oral dose of about 0.6 mg/kg in aqueous methylcellulose (Iatropoulos et al. 1975).

Although hexachlorobenzene is widely distributed in the body, it is not evenly distributed. Due to its lipophilic nature, hexachlorobenzene distributes preferentially to fat, and to a lesser extent, other lipid-rich tissues. In the Iatropoulos et al. (1975) study, radiolabel occurred at the highest levels and was most persistent in the mesenteric lymph node and adipose tissue. Levels were much lower and declined rapidly in the lung, liver, and kidney. The researchers interpreted these findings to indicate that a significant fraction of the gastrointestinally absorbed hexachlorobenzene is transported via the lymphatic system to the fat, bypassing portal circulation to the liver and systemic circulation to the excretory organs.

Numerous studies have shown preferential distribution of hexachlorobenzene to adipose tissue. In the rat autoradiography study mentioned above, Ingebrigtsen and Nafstad (1983) found peak levels of radioactivity in the fat to be roughly 60-fold higher than peak levels in the blood and 30-fold higher than peak levels in the brain and liver. Besides the fat, other tissues found to contain relatively high concentrations of radioactivity included the skin, bone marrow, Harderian gland, nasal mucosa, preputial gland, and intestinal tract. Koss and Koransky (1975) found similar results in rats given single gavage doses of 20–180 mg/kg of radiolabeled hexachlorobenzene in oil, with radioactivity in fat two days after administration being about 60-fold higher than in blood, 30-fold higher than in liver, and 5-fold higher

than in skin. Levels in brain and kidney were intermediate between liver and blood, while levels in muscle were lower. Lecavalier et al. (1994) also found approximately 30-fold higher concentrations of hexachlorobenzene in the fat than in the liver 14 days after a single gavage dose of 400 or 600 mg/kg of hexachlorobenzene in corn oil. Brain and kidney concentrations were slightly (within a factor of 2) lower than liver concentrations, while serum levels were an order of magnitude lower than liver concentrations. In a repeated dose study, ovariectomized adult female rats treated by gavage in oil with 1, 10, or 100 mg/kg/day of hexachlorobenzene for 30 days had hexachlorobenzene concentrations in fat that were 30-fold higher than levels in liver, which were in turn 20-fold higher than levels in serum. Levels in the adrenals were roughly 20-fold greater than levels in liver (Foster et al. 1995a). A study in which rats were dosed with 50 mg/kg of hexachlorobenzene by gavage in oil every other day for 15 weeks also showed hexachlorobenzene concentrations in the fat to be 30–60 times greater than concentrations in the liver, brain, kidney, and blood throughout dosing and a subsequent 38-week observation period (Koss et al. 1978).

Preferential distribution to fat and high lipid tissues has also been demonstrated in other animal species. Dogs given seven consecutive daily doses of 10 or 100 mg/kg/day of hexachlorobenzene in corn oil by capsule had peak hexachlorobenzene concentrations in fat that were 30-fold greater than peak liver levels (Sundlof et al. 1982). Other tissues with relatively high levels (5- to 10-fold greater than liver) were the skin, adrenal, and thyroid. Levels in the kidney, heart, brain, spleen, pancreas, and muscle were similar to levels in the liver. Cynomolgus monkeys treated with 0.1, 1, or 10 mg/kg/day of hexachlorobenzene in gelatin capsules for 90 days had hexachlorobenzene concentrations in fat that exceeded liver concentrations by 10- to 15-fold (Jarrell et al. 1993). Kidney and brain hexachlorobenzene levels were lower than in the liver, while serum and follicular fluid levels were much lower still (Foster et al. 1995b; Jarrell et al. 1993). In Rhesus monkeys given oral doses of 8–128 mg/kg/day of hexachlorobenzene by gavage in aqueous methyl cellulose for 60 days, the highest concentrations of hexachlorobenzene were found in the body fat and bone marrow, with considerably lower concentrations in the adrenals, liver, kidney, brain, ovaries, muscle, and serum (Knauf and Hobson 1979).

The animal studies showed that levels of hexachlorobenzene increased in a dose-dependent manner in all tissues, at least at doses up to around 100 mg/kg/day. The study by Foster et al. (1995a) found dose-dependent increases in tissue levels of hexachlorobenzene in rats treated with 1, 10, or 100 mg/kg/day, the study by Sundlof et al. (1982) found dose-dependent tissue levels in dogs treated with 10 or 100 mg/kg/day, and the study by Jarrell et al. (1993) found dose-dependent tissue levels in monkeys treated with 0.1, 1, or 10 mg/kg/day. In the fat, the increase in hexachlorobenzene concentration was

directly proportional to dose at doses up to 100 mg/kg/day in dogs (Sundlof et al. 1982), 10 mg/kg/day in rats (Foster et al. 1995a), and 1 mg/kg/day in monkeys (Jarrell et al. 1993). Lecavalier et al. (1994) found no difference in tissue levels of hexachlorobenzene following dosing with 400 or 600 mg/kg in rats, showing that dose-dependence is lost at high doses. Knauf and Hobson (1979) found no clear relationship between tissue levels of hexachlorobenzene and dose in Rhesus monkeys given between 8 and 128 mg/kg. However, their highly variable results may have been due to very small group sizes (one or two monkeys per dose) and, they speculated, variation in the amount of body fat in the monkeys used. Although Koss and Koransky (1975) tested multiple dose levels, their results were not presented in sufficient detail to assess dose-dependence of tissue levels.

In humans, data regarding tissue concentrations of hexachlorobenzene are limited to autopsy cases and easily sampled tissues and fluids, such as breast milk and blood serum. Two studies were located in which both breast adipose/milk and blood serum were collected from the same individuals and analyzed for hexachlorobenzene. In a study of 36 Connecticut women between 50 and 80 years of age, hexa-chlorobenzene was detected in the breast adipose of all 36 women (median concentration=17.7 ng/g fat), but was not detected in the serum of any of these women (quantitation limit=0.6 ng/g) (Archibeque-Engle et al. 1997). In a study of seven pregnant/lactating New York women (each with a differing interval between collection of blood and milk samples), hexachlorobenzene was detected in the serum (0.03–0.29 ng/g), but at lower concentrations than in the milk (0.21–0.74 ng/g) (Greizerstein et al. 1999). The difference between serum and milk levels can be attributed in part to the differing lipid content of these fluids. Lipid-normalized concentrations of hexachlorobenzene were 8–48.4 ng/g lipid in serum and 11–22.5 ng/g lipid in milk. Two other studies were located that looked at both breast adipose/milk and serum levels of hexachlorobenzene in the same populations, although not necessarily in the same individuals. In Veracruz, Mexico, a group of 65 volunteer mothers in the hospital for delivery had average hexachlorobenzene blood serum concentrations of 1.1 ng/g, and a group of 60 volunteer mothers in the hospital for Cesarian delivery (extent of overlap with blood volunteers unknown) had average hexachlorobenzene concentrations in milk fat and adipose tissue of 25 and 58 ng/g fat, respectively, 30 days after delivery (Waliszewski et al. 1999a, 1999b). Similarly, a group of six women from a city in northern Germany had a mean serum hexachlorobenzene level of 1.0 ng/g, while breast milk samples from seven women from northern Germany in the same year (overlap with blood donors not known) showed a mean hexachlorobenzene concentration of about 70 ng/g fat (Petzold et al. 1999).

Other studies have found hexachlorobenzene in human blood (Rutten et al. 1988; Sala et al. 1999b; Schlummer et al. 1998; Siyali 1972), liver (Dewailly et al. 1999; Weistrand and Noren 1998), bone

marrow (Bucholski et al. 1996; Scheele et al. 1995), brain (Dewailly et al. 1999), fat (Ansari et al. 1986; Dewailly et al. 1999; Lordo et al. 1996; Robinson et al. 1990; Scheele et al. 1995; Siyali 1972; Teufel et al. 1990; Weistrand and Noren 1998), and breast milk (Craan and Haines 1998; Czaja et al. 1997; Gladen et al. 1999; Gocmen et al. 1989; Lunden and Noren 1998; Newsome and Ryan 1999; Scheele et al. 1995; Weisenberg 1986; Wickstrom et al. 1983; and many others). In human plasma, close to half of the hexachlorobenzene present is found in the lipoprotein depleted fraction (containing primarily albumin), while the rest is split between the high density (. 20%), low density (. 20%), and very low density (. 10%) lipoprotein fractions (Noren et al. 1999).

Schlummer et al. (1998) found that blood levels of hexachlorobenzene (expressed as ng/g blood lipid) varied with age in a group of seven volunteers, ranging from 65 to 82 in four young adult volunteers and increasing to 230, 680, and 1,420 in the 53-, 76-, and 81-year-old volunteers, respectively. This result shows that hexachlorobenzene accumulates in people as they age. Tissue build up occurs because people are continually exposed to hexachlorobenzene in the environment and excretion is slow. In their rat experiments, Koss and Koransky (1975) observed an estimated elimination half-time of 8–10 days for hexachlorobenzene in the fat and other tissues following administration of a single gavage dose of 20–180 mg/kg. However, this finding was based on only a 14-day observation period. Koss et al. (1978) monitored tissue hexachlorobenzene levels 14 and 38 weeks after a 15-week dosing period (50 mg/kg every other day) in rats. Although the researchers could not produce an estimate of biological half-life, they found that the rate of elimination decreased over time and speculated that elimination of hexachlorobenzene might continue for years. This issue is discussed in more detail in Section 3.4.4 on Elimination and Excretion.

Human studies have shown that hexachlorobenzene can pass from the mother to the neonate through the placenta. In a study of 160 full-term neonates in Germany, hexachlorobenzene was found at an average concentration of 2.03 μg/L in 1984/1985 and 0.61 μg/L in 1994/1995 in neonatal blood obtained from an unblocked peripheral vein within the first 12 hours of life before the first oral feeding (Lackmann et al. 1996, 1999). Ando et al. (1985) found hexachlorobenzene in maternal blood, placenta, and neonatal cord blood in 36 pregnant Japanese women and their babies. There was a statistically significant correlation between the concentration of hexachlorobenzene in the placenta and in cord blood. Waliszewski et al. (1999c) reported a statistically significant correlation (R=0.87) between levels of hexachlorobenzene in maternal blood serum (mean=1 μg/L, detected in 100% of samples) and umbilical cord serum (mean=0.8 μg/L, detected in 98% of samples) in a group of 65 volunteer mothers in Veracruz, Mexico. Hexachlorobenzene was also found in the cord blood of all 63 births (geometric mean [GM]=1 μg/L)

analyzed in the village of Flix, Spain (Sala et al. 1999a), and in all 656 births (GM=0.04 µg/L) analyzed in Quebec, Canada (Rhainds et al. 1999).

Studies in laboratory animals support the findings in humans that hexachlorobenzene is transferred from pregnant mother to the fetus through the placenta. Hexachlorobenzene was found in the fetus and placenta of pregnant mice treated with 50 mg/kg/day of hexachlorobenzene by gavage in corn oil on days 7–11 of gestation and examined 24 hours after the last dose (Courtney et al. 1976). Follow-up studies by these researchers demonstrated that fetal (whole body) and placental hexachlorobenzene concentrations increased with dose, with the duration of dosing, and with the day of dosing (dosing later in gestation leads to higher levels) in mice, and that results in rats were similar to those in mice (Courtney and Andrews 1985; Courtney et al. 1979). Other studies showing transfer of maternal hexachlorobenzene to the fetus in rats were reported by Nakashima et al. (1997) and Cripps (1990). Goldey et al. (1990) measured maternal and fetal tissue levels of hexachlorobenzene in rats given a total dose of 11 mg/kg over a 3-day period 3 weeks prior to breeding. They found that fetal blood and liver concentrations were slightly lower than maternal blood concentrations, while fetal brain levels were about half of the maternal blood levels. Villeneuve and co-workers measured hexachlorobenzene levels in fetal tissues after administering hexachlorobenzene at a series of dose levels. The fetuses of pregnant rats treated with 5–120 mg/kg/day of hexachlorobenzene by gavage in corn oil on days 6–16 of gestation and sacrificed for cesarian section on day 22 of gestation showed dose-related increases in hexachlorobenzene concentration in whole fetus, fetal liver, and fetal brain (Villeneuve and Hierlihy 1975). The concentration of hexachlorobenzene in fetal liver was about 20–40% of the concentration in maternal liver. The concentration in fetal brain was about half that in fetal liver. A similar study in rabbits also demonstrated dose-dependent placental transfer of hexachlorobenzene, although in this species, fetal liver concentrations of hexachlorobenzene were 2–3-fold higher than maternal liver concentrations (Villeneuve et al. 1974a). Fetal brain levels, however, were much lower than fetal liver levels and were also less than maternal brain concentrations in rabbits.

The occurrence of hexachlorobenzene in breast milk of humans is well documented in many populations, as noted above. Many of the same animal studies that investigated placental transfer of hexachloro-benzene also studied lactational transfer from mothers to their offspring (Courtney and Andrews 1985; Cripps 1990; Goldey et al. 1990; Nakashima et al. 1997). These data are discussed in Section 3.4.4 on Elimination and Excretion.

3.4.2.3 Dermal Exposure

No studies were located regarding distribution of hexachlorobenzene following dermal exposure in humans.

Male Fischer 344 rats that received a single dermal dose of approximately 2.5 mg/kg ^{14}C-hexachloro-benzene dissolved in tetrachloroethylene applied to a 4 cm^2 clipped area on the back absorbed only 9.7% of the dose; 90.3% of the applied dose remained unabsorbed on the skin after 72 hours. The concentration of hexachlorobenzene in the liver and blood increased steadily after dermal application. Washing decreased mean hexachlorobenzene concentrations in blood from 263 to 0.128 μg/g and in the liver from 0.68 to 0.556 μg/g liver at 72 hours. The authors developed a compartment model based on the data collected, for application to a 70-kg worker (Koizumi 1991).

3.4.3 Metabolism

3.4.3.1 Inhalation Exposure

No studies were located regarding metabolism in humans or animals after inhalation exposure to hexa-chlorobenzene.

3.4.3.2 Oral Exposure

Hexachlorobenzene is slowly metabolized to pentachlorophenol by the hepatic cytochrome P-450 system (CYP3A1, CYP3A2, CYP3A4 isoforms) (Den Besten et al. 1994; Mehmood et al. 1996; Schielen et al. 1995a), conjugated with glutathione to yield pentachlorothiophenol, or reductively dechlorinated to form pentachlorobenzene (Ingebrigtsen et al. 1986; Koss et al. 1979; Renner 1988). Other metabolites include less chlorinated benzenes, chlorophenols, S-conjugated phenols, and benzenes (Den Besten et al. 1994; Koss et al. 1986). Pentachlorophenol is subsequently converted to tetrachlorohydroquinone (Mehmood et al. 1996; van Ommen et al. 1985).

Pentachlorobenzene and pentachlorophenol were identified as the major metabolites of 14C-labeled hexa-chlorobenzene (0.03 mg/kg/day) administered in the diet to Rhesus monkeys for 15 months (Rozman et al. 1977). In the urine, approximately 50% of the excreted radiolabel was pentachlorophenol, 25% was pentachlorobenzene, and the remaining 25% consisted of unidentified metabolites and unchanged hexa-

chlorobenzene. Of the excreted radioactivity in the feces, 99% was unchanged hexachlorobenzene, with
<1% pentachlorobenzene and trace amounts of pentachlorophenol. A subsequent report of a similar study
in Rhesus monkeys found that fecal excretion consisted of 99% unchanged parent compound, about 1%
pentachlorobenzene, and traces of pentachlorophenol (Rozman et al. 1978). Urinary metabolites
consisted of 50–75% pentachlorophenol. The remainder of radioactivity (25–50%) was composed of
pentachlorobenzene, hexachlorobenzene, and tetrachlorobenzene. Only unchanged parent compound was
found in the plasma, and the red blood cells contained 95% unchanged parent compound and 5%
pentachlorophenol.

A similar metabolic pattern was observed in the rat. Extraction and analysis of fecal radioactivity, which
accounted for 16% of the administered dose, 7 days after gavage administration of 5 mg/kg of
14C-labeled hexachlorobenzene in arachis oil to rats did not reveal the presence of metabolites. Urine,
which contained 0.85% of the administered radiolabel, contained 2,4,5-trichlorophenol,
pentachlorophenol, and several unidentified chlorinated benzenes (Mehendale et al. 1975). Gas/liquid
chromatography-mass spectrometry identified 20% of the radioactivity as parental hexachlorobenzene
together with the metabolites pentachlorothiophenol and pentachlorophenol in isolated perfused rat (male
Wistar) liver treated with 14C-hexachlorobenzene diluted with unlabeled hexachlorobenzene to yield a
concentration of 0.1 mg hexachlorobenzene/mL perfusate. Most of the radioactivity was found in the
perfusate and in the liver; unchanged hexachlorobenzene was responsible for most of the radioactivity.
Traces of pentachlorothiophenol and pentachlorophenol were identified in the perfusate and the liver,
respectively (Ingebrigtsen et al. 1981, 1986). A study reported that 98% of biliary radioactivity, which
constituted 3.6% of the administered dose 48 hours after administration, was in the form of metabolites;
25% of this radioactivity was glutathione-conjugated pentachlorophenol (Ingebrigtsen et al. 1981). No
sulfur-containing metabolites of hexachlorobenzene were found in the bile. However, a study of the
metabolic fate of hexachlorobenzene (particularly as it relates to transformation of hexachlorobenzene
into any methylthio- and methylsulfonyl-metabolites) in male Wistar rats identified methylthiopenta-
chlorobenzene and 1,4-bis-(methylthio)-2,3,5,6-tetrachlorobenzene as metabolites of hexachlorobenzene
(Jansson and Bergman 1978). These compounds were excreted to a greater extent than the corresponding
monosubstituted metabolites. One methylthiotetrachlorobenzene was also found. Pentachlorophenol was
the only detectable metabolite in blood, liver, urine, or feces of female Wistar rats 38 weeks after the end
of 15-week gavage exposure to 50 mg/kg/day of hexachlorobenzene (Koss et al. 1978).

In other rat studies, N-acetyl-S(pentachlorophenyl)cysteine (PCTP-NAC) was the most abundant urinary
product in female Wistar rats administered dietary hexachlorobenzene in 4% corn oil for 13 weeks

resulting in doses of 7.5 or 15 mg/kg/day (Den Besten et al. 1994) or treated twice a week for 35 weeks by gavage with 50 mg/kg in olive oil (Rietjens et al. 1995). Other rats in the Den Besten et al. (1994) study were similarly administered dietary levels of 0.03 or 0.13% pentachlorobenzene to provide a basis for comparison. Pentachlorophenol and tetrachlorohydroquinone were common urinary metabolites of both hexachlorobenzene and pentachlorobenzene. Mercaptotetrachlorothioanisole (MTCTA), which was excreted as a glucuronide, was also detected in the urine of rats given hexachlorobenzene. Pentachloro- phenol, pentachlorothiophenol, 2,3,4,6- and 2,3,5,6-tetrachlorophenol, and pentachlorobenzene were identified as metabolites of hexachlorobenzene in another study (Richter et al. 1981). Significant sex-related differences were observed, with higher amounts of pentachlorothiophenol observed in the livers of female rats. This was accompanied by a slower decrease in hepatic levels of hexachlorobenzene in the female rat liver compared to the male liver. Sex differences in the metabolism of hexachloro- benzene in the adult rat have also been observed. After 10 weeks of treatment, the urinary excretion of pentachlorophenol, 2,3,5,6-tetrachlorobenzene-1,4-diol, and pentachlorothiophenol was greater in females than in males in this study (Rizzardini and Smith 1982). Sex-related differences in biotransformation of hexachlorobenzene could account for differences observed in porphyrinogenic activity of hexachloro- benzene in male and female rats (D'Amour and Charbonneau 1992; Richter et al. 1981; Rizzardini and Smith 1982).

Two alternatives have been proposed for the mechanism of conversion of hexachlorobenzene to pentachlorophenol: formation of an epoxide or free radical attack on the carbon-chlorine bond. Substitution of adjacent carbons with chlorine argues against a mechanism involving an epoxide; either cleavage of the bond by free radical attack followed by hydroxylation, or conjugation with glutathione seems more plausible. However, if the presence of o- and p-hydroxy derivatives of pentachlorophenol could be confirmed, there would be a strong possibility that epoxides are intermediates in the dechlorination or hydroxylation of hexachlorobenzene or both (Lui et al. 1976). *In vitro* studies with perfused rat livers demonstrated that 14C-labeled hexachlorobenzene was converted to acidic conjugates (45%), while 5% was converted to sulfate or glucuronic acid conjugates (Ingebrigtsen et al. 1986). There is evidence that mammalian metabolism of hexachlorobenzene to pentachlorophenol is mediated by the hepatic cytochrome P-450 system (CYP3A1, CYP3A2, CYP3A4 isoforms; others) (Den Besten et al. 1994; Mehmood et al. 1996; Schielen et al. 1995a).

Several other studies in laboratory animals also identified the following mammalian biotransformation products of hexachlorobenzene: pentachlorophenol (Ingebrigtsen et al. 1981; Koss et al. 1976, 1979; Lui and Sweeney 1975; van Ommen et al. 1985; Yang et al. 1978); pentachlorothiophenol (D'Amour and

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Charbonneau 1992; To-Figueras et al. 1992); less chlorinated benzenes, chlorophenols, S-conjugated phenols and benzenes (Engst et al. 1976; Koss et al. 1979; Renner 1988; Stewart and Smith 1986); and tetrachlorohydroquinone and tetrachlorocatechol (Koss et al. 1976, 1979; Lui et al. 1976; Mehmood et al. 1996; van Ommen et al. 1985, 1989). The various pathways and metabolites of hexachlorobenzene are depicted in Figure 3-3.

There is some evidence to indicate that metabolism of hexachlorobenzene to pentachlorophenol is accelerated in rats fed fish oil, in comparison to lard or soybean oil (Umegaki and Ikegami 1998). Rats fed fish oil had significant increases in liver cytochrome P-450, blood pentachlorophenol and

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Figure 3-3. Metabolism and Urinary Metabolites of Hexachlorobenzene

MAJOR METABOLITES
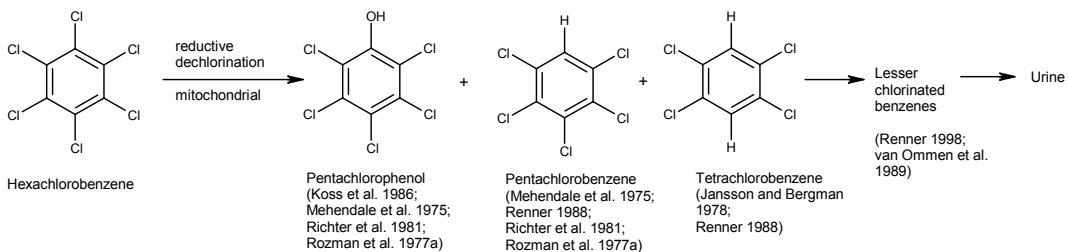
MINOR METABOLITES
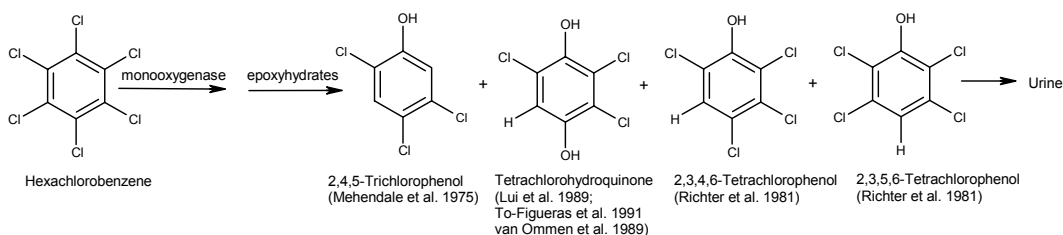
GLUTATHIONE CONJUGATION: FORMATION OF SULFUR DERIVATIVES
(Adapted from Ingebrigtsen et al. 1981; Jansson and Bergman 1978; Renner 1988; To-Figueras et al. 1992)
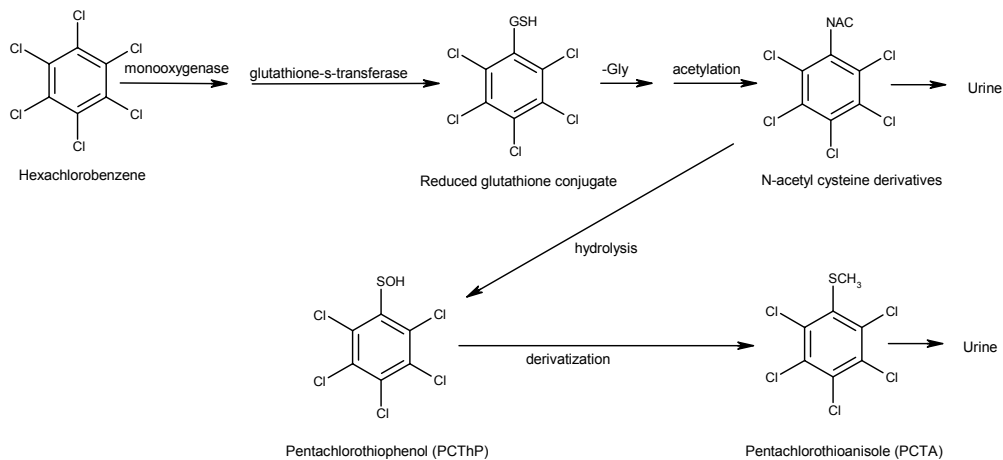
pentachlorophenol:hexachlorobenzene ratio, and urinary excretion of pentachlorophenol, while levels of hexachlorobenzene in the feces were unchanged (indicating no difference in absorption between groups).

Limited human data are consistent with results from animal studies. Portions (0.5 g) of abdominal subcutaneous adipose tissue obtained as histological samples during surgery of patients and urine from these patients in Germany were extracted with benzene for hexachlorobenzene and its metabolites (Koss et al. 1986). Hexachlorobenzene was detected in the adipose tissue of the patients, while only pentachlorophenol was detected in the urine. A correlation was found between levels of hexachloro-benzene found in adipose tissue and urinary pentachlorophenol. However, it is possible that the urinary pentachlorophenol originated from other chlorinated hydrocarbons such as pentachlorobenzene, gamma-hexachlorocyclohexane, or pentachloronitrobenzene. Human cytochrome P-450 3A4 expressed in the yeast *Saccharomyces cerevisiae* metabolized hexachlorobenzene to pentachlorophenol, which was further transformed to tetrachlorohydroquinone, in both *in vitro* and *in vivo* experiments (Mehmood et al. 1996).

3.4.3.3 Dermal Exposure

No studies were located regarding metabolism in humans or animals after dermal exposure to hexachloro-benzene.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

To-Figueras et al. (2000) observed a high correlation between fecal and blood levels of hexachloro-benzene in a group of 25 men and 28 women from Flix, Spain. This population was highly exposed to airborne hexachlorobenzene from a nearby chlorinated solvent factory. The geometric mean of hexa-chlorobenzene in blood was 163 µg/5.4 L. Estimated fecal excretion of unchanged hexachlorobenzene was 10.4 µg/day, 4–6.4% of the estimated total blood level. No unchanged hexachlorobenzene was detected in urine; urinary excretion of metabolites was 5.1 µg/day, 1.8–3.1% of the estimated total blood level.

No studies were located regarding excretion of hexachlorobenzene in animals following inhalation exposure.

3.4.4.2 Oral Exposure

Elimination of absorbed hexachlorobenzene is slow and occurs primarily via the feces, with smaller amounts being excreted in the urine. Hexachlorobenzene eliminated in the feces is predominantly unchanged parent compound, although small amounts of various metabolites have also been found. Conversely, hexachlorobenzene in the urine is almost all in the form of metabolites. Following a single intraperitoneal injection of radiolabeled hexachlorobenzene (4 mg/kg) in rats, approximately 34% of the administered radioactivity was recovered in the feces over the following 14 days, 80% of which was unchanged parent compound (Koss and Koransky 1975). By contrast, only 5% of the administered radioactivity was recovered in the urine, and only 4% of that was unchanged parent compound. A 14-day recovery period was used due to the slow elimination of hexachlorobenzene from the body. Yang et al. (1978) conducted an experiment in which rats were injected intravenously with radiolabeled hexachloro-benzene and excretia collected over the following 48 hours. Only 1% of the administered dose appeared in the feces, and 0.2% in the urine, within 48 hours of dosing. These researchers also conducted a long-term experiment in monkeys (Yang et al. 1978). One year after intravenous injection, only 28.2% of the administered dose had been excreted in the feces (90% unchanged hexachlorobenzene) and 1.6% in the urine (all metabolites). The researchers attributed slow elimination of hexachlorobenzene to long-term storage of the chemical in the fat. Neither Koss and Koransky (1975) nor Yang et al. (1978) detected radioactivity in the expired air from treated animals.

Both biliary and intestinal excretion contribute to fecal excretion of hexachlorobenzene. Hexachloro-benzene and/or its metabolites were found in the bile after intravenous injection of hexachlorobenzene in monkeys, suggesting the possibility of biliary excretion (Yang et al. 1978). Bile duct cannulated rats given hexachlorobenzene by gavage excreted 3.6% of the administered dose in the bile within 48 hours (Ingebrigtsen et al. 1981). Although one report suggested that biliary excretion was more important than intestinal excretion (Sundlof et al. 1982), other studies have shown that biliary excretion is a minor contributor to fecal excretion. Rozman et al. (1981) estimated biliary excretion to account for about 10% of fecal excretion in rats and monkeys treated with oral hexachlorobenzene. Intestinal excretion was responsible for the bulk of the fecal excretion in this study. Feeding with aliphatic hydrocarbons (mineral oil, hexadecane) enhanced fecal excretion in both rats and monkeys, with a corresponding decrease in blood and adipose hexachlorobenzene concentrations, primarily due to increased elimination of hexa-chlorobenzene in the large intestine (Rozman et al. 1981). In contrast to aliphatic hydrocarbons, cholestyramine, which interferes with enterohepatic recycling, had no effect on fecal excretion of hexa-chlorobenzene (confirming the minor role of biliary excretion for this chemical), and sesame oil produced

only a very small increase in fecal hexachlorobenzene excretion (possibly by increasing gastrointestinal absorption). Richter and Schafer (1981) showed that addition of hydrocarbons (light liquid paraffin and squalane) to the perfusion medium enhanced elimination of unchanged hexachlorobenzene in perfused intestine into the lumen of the jejunum, ileum, and colon. The researches hypothesized that the hydrocarbons, which are not significantly absorbed, act as a lipophilic compartment in the gut lumen, shifting the equilibrium between gut wall and lumen in favor of the lumen for hydrophilic substances such as hexachlorobenzene. This is consistent with the fat-flush hypothesis of gastrointestinal absorption proposed by Schlummer et al. (1998). After fat-flush enhanced lipid absorption in the duodenum and jejunum is complete, the diffusion gradient is reversed in subsequent portions of the intestines.

In lactating mothers, breast milk is also an important route of excretion for hexachlorobenzene. Hexachlorobenzene has been detected in human breast milk in many studies (Craan and Haines 1998; Czaja et al. 1997; Gladen et al. 1999; Gocmen et al. 1989; Lunden and Noren 1998; Newsome and Ryan 1999; Scheele et al. 1995; Weisenberg 1986; Wickstrom et al. 1983; and many others). Several animal studies have quantified lactational transfer of hexachlorobenzene from mothers to their offspring (Courtney and Andrews 1985; Cripps 1990; Goldey et al. 1990; Nakashima et al. 1997). These studies confirm the importance of breast milk as a route of elimination in the mother and as a source of exposure in neonates.

Studies that monitored elimination of hexachlorobenzene for an extended period of time noted that the rate of elimination decreases over time (Koss et al. 1978, 1983; Sundlof et al. 1982; Yang et al. 1978). In rats treated with hexachlorobenzene every other day for 6 weeks, the elimination half-time was estimated as a relatively rapid 8 days soon after exposure stopped, a much slower 10 weeks 3 months later, and a very slow 12 months after 1.5 years, suggesting that elimination of hexachlorobenzene could continue for years (Koss et al. 1978, 1983). Yang et al. (1978) and Sundlof et al. (1982) both applied 3-compartment pharmacokinetic models to their data on dogs and monkeys, respectively. Sundlof et al. (1982) obtained elimination half-time estimates ranging from 6 weeks to 3 years in the three dogs modeled. Yang et al. (1978) calculated elimination rate constants corresponding to half-times of 91–114 days in two monkeys, but also performed additional modeling exercises that suggested elimination half times as long as 250 years. Freeman et al. (1989) used a physiologically based pharmacokinetic (PBPK) model of hexachlorobenzene in the rat to show that approximately 75% of the decline in fat concentrations over time is due to growth (i.e., dilution), with only 25% due to excretion. Thus, the growth of animals during experiments may affect the apparent half-life of hexachlorobenzene. A PBPK model developed by Yesair et al. (1986) predicted a half-life of 215 days for hexachlorobenzene in a growing human female exposed to doses of 0.5–32 mg/kg/day for 15 weeks at 15 years of age.

3.4.4.3 Dermal Exposure

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

3.4.4.4 Other Routes of Exposure

No studies were located regarding excretion in humans after other routes of exposure to hexachlorobenzene.

The major portion of injected hexachlorobenzene is eliminated unchanged in feces, while a smaller fraction, composed of metabolites, is eliminated in urine. Yang et al. (1978) administered a single intravenous dose of hexachlorobenzene to monkeys; 1 year after intravenous injection, only 28.2% of the administered dose had been excreted in the feces, 90% unchanged, and in urine, 1.6% of the total dose was excreted as metabolites (no unchanged compound detected). The researchers attributed slow elimination of hexachlorobenzene to long-term storage of the chemical in the fat. Unchanged hexachlorobenzene and metabolites were detected in bile. Yang et al. (1978) administered a single intravenous dose of hexachlorobenzene to rats; within 48 hours, only 1% appeared in the feces, and 0.2% in the urine. Following a single intraperitoneal injection of hexachlorobenzene, rats excreted 34% of the total dose in feces over 14 days, 80% of which was unchanged parent compound; only 5% was excreted in urine over 14 days and only 4% was unchanged parent compound (Koss and Koransky 1975). Neither Koss and Koransky (1975) nor Yang et al. (1978) detected hexachlorobenzene elimination in air expired by treated animals. Yang et al. (1978) calculated an initial half-life of 91–114 days, and subsequent half-lives as long as 250 years.

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

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

pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste

sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-4 shows a conceptualized representation of a PBPK model.

3.4.5.1 Summary of PBPK Models

PBPK models for hexachlorobenzene have been developed by Yesair et al. (1986) and Freeman et al. (1989). The Yesair et al. (1986) model describes the absorption, distribution, and elimination of ingested hexachlorobenzene in growing rats and humans. The Freeman et al. (1989) model describes distribution and excretion of intravenously injected hexachlorobenzene in growing rats.

3.4.5.2 Hexachlorobenzene PBPK Model Comparison

The Yesair et al. (1986) and Freeman et al. (1989) models are similar attempts to describe distribution and clearance of hexachlorobenzene. Both models included numerous tissue compartments and allowed for growth over time. The Yesair et al. (1986) model went further than the Freeman et al. (1989) model by including oral exposure, fetal and breast milk compartments, and metabolism and tissue sequestration of hexachlorobenzene, and by modeling humans as well as rats. Both rat models were validated using experimental data.

**Figure 3-4. Conceptual Representation of a Physiologically
Based Pharmacokinetic (PBPK) Model for a
Hypothetical Chemical Substance**
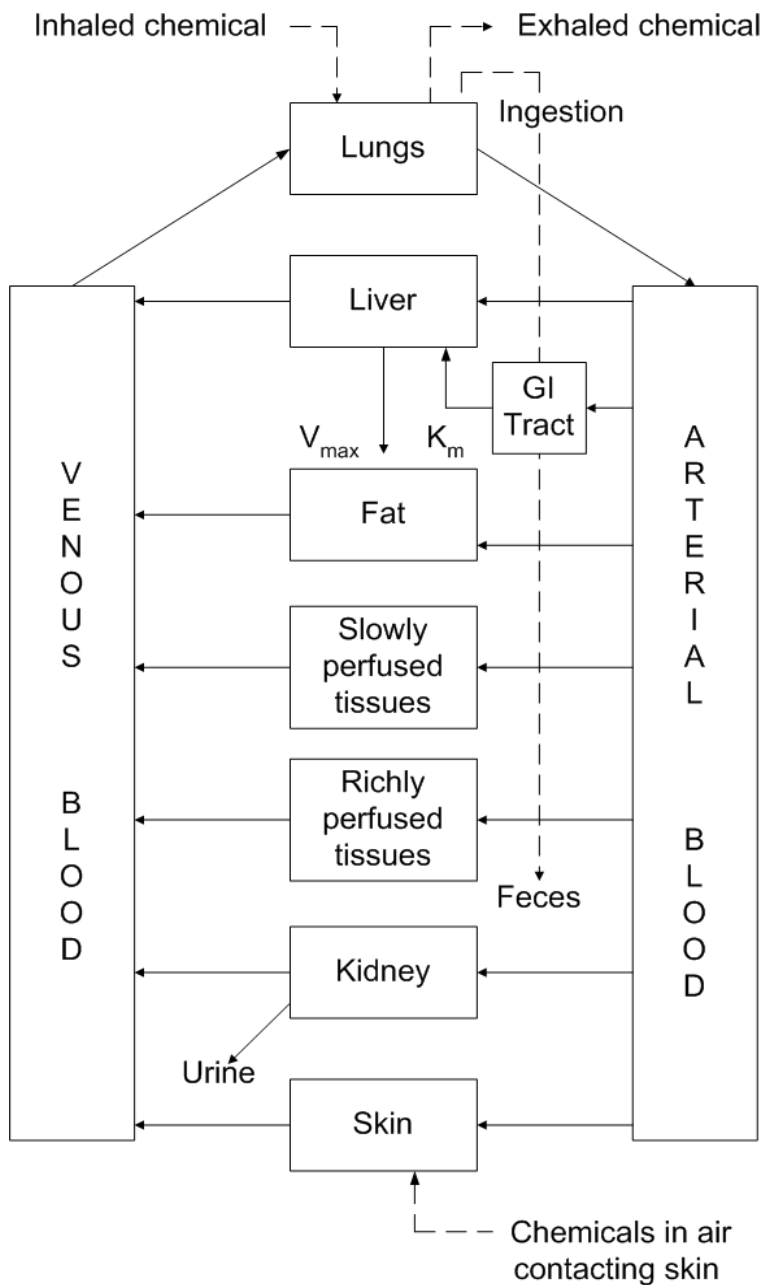
Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

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3.4.5.3 Discussion of Models

The Yesair Model.

Risk assessment. The Yesair model was not used for risk assessment.

Description of the model. The Yesair model was initially developed to simulate oral exposure to hexachlorobenzene in growing male and female rats, and was then expanded to include pregnancy and offspring in the female model. A human model was produced by using the same model structure with human physiological parameter values. The model includes compartments for intestinal lumen, systemic circulation, feces, liver, metabolites, kidney, urine, brain, richly perfused tissues, poorly perfused tissues, breast milk, fetus, and lactating offspring. Parameters used in the model included body and organ weights and growth rates, blood-flow rates, empirical clearance factors, reaction-rate constants, distribution ratios, and capacity limits. The forms of the growth characteristics and the parameter values were obtained from the literature, experimental data from Kuiper-Goodman et al. (1977), and empirical considerations. Both free and sequestered forms of hexachlorobenzene were estimated in each compartment, and only freely available material was allowed to leave the compartment (except for the systemic circulation and breast milk compartments). When the rat models were adapted for humans, the appropriate human physiological values were substituted for the rat values.

Validation of the model. The rat model was compared to data from Courtney et al. (1979), Iatropoulos et al. (1975), Koss and Koransky (1975), Koss et al. (1978), and Kuiper-Goodman et al. (1977). In general, the model approximated the observed results reasonably well in all tissues. The human model was not validated.

Target tissues. Using the rat model, correlations were obtained between predicted yield of metabolites and experimentally observed liver toxicity (porphyria and increased liver weight reported by Koss et al. 1978 and Kuiper-Goodman et al. 1977), between estimated concentrations of hexachloro-benzene in the brain and observed central nervous system effects (tremors and other signs reported by Koss et al. 1978 and Kuiper-Goodman et al. 1977), and between estimated extent of lactational transfer of hexachlorobenzene and observed offspring mortality (data from Kitchen et al. 1982). The model predicts little transfer of hexachlorobenzene to the fetus during gestation and extensive mobilization of hexa-chlorobenzene to the offspring during lactation. The data are consistent with this model. A half-life of 215 days was predicted for hexachlorobenzene in a growing human female exposed to doses of

0.5–32 mg/kg/day for 15 weeks at 15 years of age, suggesting that approximately 4 years (7 half-lives) are required to establish equilibrium between intake and excretion. Further simulations showed that doubling or halving the administered dose resulted in doubling or halving, respectively, of the tissue concentrations after 3–5 years.

Species extrapolation.   Species extrapolation was not attempted in this model.

Interroute extrapolation.   Interroute extrapolation was not attempted in this model.

The Freeman Model.

Risk assessment.   The Freeman model was not used for risk assessment.

Description of the model.   The Freeman model was developed to simulate intravenous injection of hexachlorobenzene in growing rats. The model includes compartments for plasma, gastrointestinal tract, colon, feces, liver, lung, kidney, urine, brain, heart, spleen, skin, muscle, and fat. Parameters used in the model include organ weights and blood flow fractions obtained from the literature and tissue:serum partition coefficients derived from experimental studies by Scheufler and Rozman (1984a, 1984b). The model was designed to accommodate differential growth of tissue/organ weights as a function of total body weight. Metabolism was assumed to be zero based on experimental data (attributed to Rozman and colleagues) suggesting little metabolism of hexachlorobenzene in the rat.

Validation of the model.   The model predictions were compared to data from Scheufler and Rozman (1984a, 1984b). In general, the model approximated the observed results reasonably well in the compartments examined: blood, liver, fat, urine, and feces.

Target tissues.   Levels in liver and fat were well-predicted by this model. An interesting prediction is that approximately 75% of the decline in fat concentrations over time is due to growth (i.e., dilution), with only 25% due to excretion. Thus, the growth of animals during experiments may affect the apparent half-life of hexachlorobenzene.

Species extrapolation.   Species extrapolation was not attempted in this model.

Interroute extrapolation.   Interroute extrapolation was not attempted in this model.

3.5   MECHANISMS OF ACTION

3.5.1   Pharmacokinetic Mechanisms

Absorption.   Schlummer et al. (1998) found that absorption following oral exposure to hexachloro-
benzene decreased with increasing hexachlorobenzene blood levels in volunteers. Data was fit to a linear
regression. The calculated regression equation was:

  percent absorption hexachlorobenzene = 0.8538–0.0021 x (ng hexachlorobenzene/g blood lipid)

This equation indicates that 85.4% of ingested hexachlorobenzene will be absorbed when the blood
contains no hexachlorobenzene, and that this percentage will be reduced by 0.2% for each ng of hexa-
chlorobenzene per g lipid in blood. This regression equation provided an excellent fit to the data
(R^2=0.98), leading to the conclusion that concentration of hexachlorobenzene in the blood is a major
factor influencing absorption of hexachlorobenzene from the gut. Although absorption of lipophilic
compounds such as hexachlorobenzene is thought to occur by simple passive diffusion, analysis of the
entire data set in this study, which included 17 polychlorinated dibenzo-*p*-dioxins/furan congeners and
10 PCBs in addition to hexachlorobenzene, showed that absorption of these compounds could not be
explained by simple gradients between lipid-based food and blood concentrations or between gut contents
and gut tissue. The researchers offered a "fat-flush" hypothesis of absorption for hexachlorobenzene and
related compounds whereby lipid absorbed into gut tissue would temporarily increase lipid content of the
tissue, diluting contaminant concentration in the gut tissue (on a lipid basis) and increasing the diffusion
gradient for the contaminant from the gut contents, before being transported from the gut tissue to the
lymph and blood.

Animal studies have demonstrated that hexachlorobenzene is absorbed more rapidly from oil than from
aqueous suspension (Albro and Thomas 1974; Iatropoulos et al. 1975; Ingebrigtsen and Nafstad 1983;
Ingebrigtsen et al. 1981; Koss and Koransky1975; Sundlof et al. 1982; Zabick and Schemmel 1980; and
others). Nakashima et al. (1999) observed that lactational transfer of hexachlorobenzene was increased
by feeding nursing rats high fat diets. Zabick and Schemmel (1980) demonstrated that a high fat diet also

enhances absorption of hexachlorobenzene, in comparison to a high carbohydrate diet. Groups of six female Osbourne-Mendel rats were fed either a high fat diet or one of two high carbohydrate diets supplemented with 32 mg/kg/day of hexachlorobenzene for 6–18 days. The high fat diet resulted in higher carcass fat content and greatly increased concentrations of hexachlorobenzene in perirenal fat, liver, and gastrocnemius. At the same time, concentrations of hexachlorobenzene in the feces were much lower with the high fat diet, suggesting that the high fat diet facilitated absorption of hexachlorobenzene from the gut, thereby leading to increased tissue levels. These data showing enhanced absorption of hexachlorobenzene when administered in oil or a high fat diet are consistent with the "fat-flush" hypothesis for hexachlorobenzene absorption proposed by Schlummer et al. (1998).

Iatropoulos et al. (1975) measured absorption in Sprague-Dawley rats following oral gavage of hexa-chlorobenzene in aqueous methylcellulose suspension, and observed that hexachlorobenzene was absorbed through the walls of the stomach, duodenum, jejunum, and ileum. Based on their data, the authors concluded that the majority of ingested hexachlorobenzene was absorbed from the small intestine by the lymphatic system and deposited in the fat, bypassing portal circulation (to the liver), systemic circulation, and the excretory organs.

Distribution.   Hexachlorobenzene has been detected in human and animal blood, and circulation is the primary mechanism of inter-tissue distribution. Due to its lipophilic nature, hexachlorobenzene distributes preferentially to fat, and to a lesser extent, other lipid-rich tissues (Foster et al. 1995a, 1995b; Iatropoulos et al. 1975; Ingebrigtsen and Nafstad 1983; Jarrell et al. 1993; Knauf and Hobson 1979; Koss and Koransky 1975; Koss et al. 1978; Lecavalier et al. 1994; Sundlof et al. 1982). Although distribution studies have produced somewhat conflicting results, a rough approximation of hexachlorobenzene distribution is: fat > lymph nodes, adrenal and thyroid glands > skin > bone marrow, Harderian gland, nasal mucosa, preputial gland, and intestinal tract > liver, lung > kidney, brain, blood, heart, muscle, spleen, pancreas, ovary.

Human and animal studies suggest that breast milk is enriched with hexachlorobenzene, relative to blood, and that blood levels actually drop in lactating mothers (Greizerstein et al. 1999; Petzold et al. 1999; Nakashima et al. 1997, 1999; Nakashima and Ikegari 2000; Waliszewski et al. 1999a, 1999b). This is probably due to the lipophilicity of hexachlorobenzene.

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Metabolism.   Hexachlorobenzene is slowly metabolized by hepatic cytochrome P-450 system (CYP3A1, CYP3A2, CYP3A4 isoforms) (Den Besten et al. 1994; Mehmood et al. 1996; Schielen et al. 1995a); by conjugation with glutathione, glucuonide, and sulfate; and by reductive dechlorination (Ingebrigtsen et al. 1986; Koss et al. 1979; Renner 1988).

Two alternatives have been proposed for the mechanism of conversion of hexachlorobenzene to PCP: formation of an epoxide or free radical attack on the carbon-chlorine bond. Substitution of adjacent carbons with chlorine argues against a mechanism involving an epoxide; either cleavage of the bond by free radical attack followed by hydroxylation, or conjugation with glutathione seems more plausible. However, if the presence of o- and p-hydroxy derivatives of PCP could be confirmed, there would be a strong possibility that epoxides are intermediates in the dechlorination or hydroxylation of hexachloro-benzene or both (Lui et al. 1976).

Excretion.   Following metabolism to more polar metabolites, hexachlorobenzene is excreted in urine (Ingebrigtsen et al. 1981, 1986; Koss and Koransky 1975; Koss et al. 1986; Lui and Sweeney 1975; Rozman et al. 1977a; Scheufler and Rozman 1984a, 1984b; To-Figueras et al. 1992; van Ommen et al. 1985; Yang et al. 1978).

Hexachlorobenzene eliminated in the feces is predominantly unchanged parent compound, although small amounts of various metabolites have also been found (Koss and Koransky 1975; Yang et al. 1978). Fecal elimination is primarily the product of fecal excretion, although biliary excretion (from the liver) is also important (Ingebrigtsen et al. 1981; Richter and Schafer 1981; Rozman et al. 1981; Yang et al. 1978).

For nursing mothers, excretion of unchanged hexachlorobenzene into milk may represent a significant, and even the primary, route of excretion (Courtney and Andrews 1985; Craan and Haines 1998; Cripps 1990; Czaja et al. 1997; Gladen et al. 1999; Gocmen et al. 1989; Goldey et al. 1990; Lunden and Noren 1998; Nakashima et al. 1997; Newsome and Ryan 1999; Scheele et al. 1995; Weisenberg 1986; Wickstrom et al. 1983; and many others).

3.5.2 Mechanisms of Toxicity

Hexachlorobenzene induces porphyria characterized by increased d-ALA synthase (the enzyme that controls the rate of porphyrin production) activity and decreased uroporphyrinogen decarboxylase (the enzyme that converts uroporphyrinogen III to coproporphyrinogen III) activity (Dowdele et al. 1967; Rajamanickam et al. 1972; Smith and de Matteis 1990; Strand et al. 1971). Uroporphyrinogen III is the first cyclic tetrapyrrole in the pathway of heme biosynthesis. This is the reduced colorless precursor of uroporphyrin III (hexahydro-uroporphyrin) and will give rise to the corresponding porphyrin on reoxidation. Uroporphyrinogen decarboxylase (a cytosolic enzyme) converts uroporphyrinogen III to coproporphyrinogen III by the stepwise decarboxylation of the four acetic acid side chains to leave methyl residues, but the corresponding porphyrin (uroporphyrin III) cannot be decarboxylated and will not be metabolized further. Thus, the accumulation of uroporphyrins in the liver may be due to a deficiency of the decarboxylation of uroporphyrinogen III catalyzed by uroporphyrinogen decarboxylase. This hypothesis led to the proposal that in certain porphyria where uroporphyrin accumulates (uroporphyrias), the mechanism responsible may be an accelerated oxidation of uroporphyrinogen, causing an "oxidative escape" of this intermediate from the pathway of heme biosynthesis (Meola and Lim 1993; Smith and de Matteis 1990). A marked inhibition of uroporphyrinogen decarboxylase has been widely reported to occur prior to the manifestation of the typical porphyrinogenic effects of hexa-chlorobenzene (Blekkenherst et al. 1976; Elder et al. 1976; Smith and Francis 1987; Smith et al. 1986a). A study conducted with Osteogenic Disorder Shionogi (ODS) rats provided evidence that chemically-induced porphyria seems to be mediated by inhibition of CYP1A2-catalyzed uroporphyrinogen oxidation (Sinclair et al. 1995).

In vitro studies with hexachlorobenzene have demonstrated that this chemical does not exert a direct action on uroporphyrinogen decarboxylase (Rios de Molina et al. 1980). The major hexachlorobenzene metabolites (TCHQ, PCP, pentachlorothiophenol, pentachlorothioanisole) had no influence on the porphyrin pathway as indicated by alteration in total hepatic porphyrin levels and urinary levels of d-ALA, porphobilinogen, or uroporphyrins (Goldstein et al. 1977, 1978; Kimbrough and Linder 1978; Lui et al. 1976; Smith and Francis 1987; Wainstok de Calmanovici and San Martin de Viale 1980). However, PCP and TCHQ appear to be capable of altering porphyrin metabolism in *in vitro* systems containing d-ALA (Goerz et al. 1978; Goldstein et al. 1977). Furthermore, co-administration of PCP and TCHQ with hexachlorobenzene increased the severity of the resultant porphyria (Debets et al. 1980a), indicating a probable role for these metabolites in porphyria induction. It has been suggested that changes in K^+ permeability mediated by lipid peroxidation and mitochondrial dysfunction may be contributing

factors in hexachlorobenzene-induced hepatotoxicity. This was based on the results of a study in rats in which mitochondrial lipid peroxidation was found to have increased proportionally with a 100-fold increase in hepatic porphyrin content (Feldman and Bacon 1989; Masini et el. 1988). Porphyrin uptake in the mitochondria of iron-supplemented rats was inhibited by PCP (hexachlorobenzene metabolite), indicating that peroxidative reactions in the mitochondrial membranes may be responsible for changes in membrane permeability (Masini et al. 1988).

It is unlikely, however, that the hexachlorobenzene metabolites, pentachlorobenzene and PCP, are by themselves porphyrinogenic agents, and P-450 induction may not correlate with porphyria development. In one study, rats fed diets containing hexachlorobenzene or its metabolites (pentachlorobenzene and PCP) exhibited increases in hepatic cytochrome P-450, but the metabolites had no effect on urinary porphyrin excretion, while hexachlorobenzene produced a high level of urinary porphyrins (Vos et al. 1988). In other studies, the major hexachlorobenzene metabolites (TCHQ, PCP, pentachlorothiophenol, pentachlorothioanisole) had no influence on the porphyrin pathway as indicated by alteration in total hepatic porphyrin levels and urinary levels of d-ALA, porphobilinogen, or uroporphyrins (Goldstein et al. 1977, 1978; Kimbrough and Linder 1978; Smith and Francis 1987; Wainstok de Calmanovici and San Martin de Viale 1980). However, PCP and TCHQ appear to be capable of altering porphyrin metabolism in *in vitro* systems containing d-ALA (Goerz et al. 1978; Goldstein et al. 1977). Furthermore, co-administration of PCP and TCHQ with hexachlorobenzene increased the severity of hexachlorobenzene-induced porphyria (Debets et al. 1980a).

In a study with rats, it was proposed that the involvement of the histidine residue of the enzyme in substrate (hexachlorobenzene) binding may be the mechanism by which hexachlorobenzene exerts its porphyrinogenic action *in vivo* (Billi de Catabbi et al. 1991). Another study in rats and mice concluded that hexachlorobenzene induces chronic porphyria by modifying sulfhydryl groups in porphyrinogen decarboxylase, the action restricted to the catalytic or substrate-binding sites (Elder and Urquhart 1986).

Iron (as iron dextran) alone has been shown to induce porphyria; therefore, iron may have a role in the pathogenesis of hexachlorobenzene-induced porphyria (Siersema et al. 1991; Smith and Francis 1983; Smith et al. 1986a) and hepatocarcinogenesis (Smith 1989), although co-administration of carbonyl iron did not have a significant effect on elevated hepatic and mitochondrial fraction porphyrin contents in rats following hexachlorobenzene treatment (Masini et al. 1988), but in other animal studies, exposure of mice to hexachlorobenzene and iron produced a dramatic increase (nearly 1,000-fold) in hepatic uroporphyrin levels (Vincent et al. 1989). The small number of animals used in this study limits the reliability of the

conclusions of this study. Another investigator concluded that liver mitochondrial porphyrin uptake may involve the K^+ transmembrane gradient and further suggested that peroxidative reactions in the mitochondrial membranes may be responsible for changes in membrane permeability that affects K^+ permeability (Masini et al. 1988). Liver malonaldehyde levels increased while glucose-6-phosphate activity decreased in rats administered intraperitoneal injections of 50 mg of hexachlorobenzene per day (total dose=300 mg) during a 42-day test period followed by treatment with intraperitoneal doses of iron (as ferrihydroxide-dextran complex), suggesting a close relationship between accumulation of porphyrins, iron overload, and free radical formation or lipid peroxidation (Visser et al. 1989).

Experiments with rats and iron-loaded mice indicate that there may also be an association between the induction of uroporphyria and the development of liver tumors after the administration of poly-halogenated aromatic chemicals (Smith and De Matteis 1990). Hyperplastic nodules were observed in the liver lobes of 80% of female Fischer 344 rats pretreated with Imferon (iron-dextran) and then given dietary hexachlorobenzene in arachis oil for 65 weeks resulting in a dose of 5 or 10 mg/kg/day. There was a high incidence of fibrin throughout the liver with sinusoidal telangiectasis, and nodular peliosis hepatitis and hepatocellular necrosis. The study proposed a nongenotoxic mechanism for tumor induction by hexachlorobenzene, concluding that the formation of hepatomas and hemangiomas with elements of peliosis could be explained by the compensatory hyperplastic responses to hepatocellular injury or necrosis and by the simultaneous loss of hepatocellular cords. The study further concluded that the accumulation of iron in the liver would strongly potentiate the development of hepatic tumors (Carthew and Smith 1994).

Iron overload also greatly sensitized mice to the development of liver tumors. Mice given oral hexa-chlorobenzene doses preceded by subcutaneous administration of iron developed iron-excluded hyperplastic nodules (all treated animals) and hepatocellular carcinoma (most animals). Based on the results of this investigation, an alternate mechanism has been suggested for the hepatic toxicity of hexa-chlorobenzene that may involve the uncoupling of an induced cytochrome P-450 system releasing active oxygen species. Iron is seen as catalyzing the formation of the hydroxyl radical or perhaps forming reactive iron-oxygen complexes (Smith 1989).

Data from a study in male Long Evans rats suggested that the metabolism of hexachlorobenzene to penta-chlorobenzene and other more polar metabolites proceed either through a free-radical mechanism or by initial formation of an arene oxide. These reactive intermediates may form covalent bonds with cellular constituents (such as protein amino acids or DNA nucleic acids) leading to irreversible cell damage (Lui

and Sweeney 1975). Several other studies have also found evidence of binding to cellular proteins by reactive electrophilic metabolites of hexachlorobenzene formed by cytochrome P-450 system (Gopalaswamy and Nair 1992; Rajamanickam and Padmanaban 1974; van Ommen et al. 1985).

3.5.3 Animal-to-Human Extrapolations

Studies have investigated the adverse effects of hexachlorobenzene in rats, mice, hamsters, dogs, pigs, and monkeys following subchronic exposure and in rats, mice, and hamsters following chronic exposure. Substantial bodies of both of human data and animal data are available that demonstrate qualitative similarities between animals and humans for such end points as porphyria and dermal lesions. Overall, data in animal studies do not suggest species variations in the toxicokinetics of hexachlorobenzene except in carcinogenic responses. The cancer toxicity data suggest that species differences exist, as demonstrated by multi-tumor-type responses evident in hamsters and single-tumor-type responses observed in mice (Cabral et al. 1977, 1979; EPA 1980b; Ertürk et al. 1986; Lambrecht et al. 1983a; Smith 1989; Smith et al. 1985).

3.6   TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Clement (1992), was also used in 1996 when Congress mandated the Environmental Protection Agency (EPA) to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), which in 1998 completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of

natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997c). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

Hexachlorobenzene mediates multiple adverse effects through the neuroendocrine axis. Hormonal changes associated with exposure to hexachlorobenzene include decreased serum thyroxine (hypothyroidism), increased serum parathyroid hormone (hyperparathyroidism), decreased corticosterone released from the adrenal gland, and changes in estradiol and progesterone levels in females at certain times in the menstrual cycle. The female hormone changes are coincident with ovarian lesions and changes in female reproductive cycles in the same studies. Reduced fertility in breeding trials with hexa-chlorobenzene may be secondary to the ovarian effects. These alterations are described in more detail in Section 3.2. The effect of hexachlorobenzene on throxine appears to involve stimulation of dehalogenation of the hormone in the liver, rather than an effect on synthesis of the hormone in the thyroid (Kleiman de Pisarev et al. 1989, 1990). There is also some evidence that hexachlorobenzene may competitively inhibit binding of thyroxine to serum carrier proteins, further depressing circulating levels of the hormone (Foster et al. 1993; Van Den Berg 1990). The effects on ovarian and adrenal hormones have been hypothesized to reflect alterations in steroidogenesis in these tissues, possibly as a consequence of lipid peroxidation of mitochondrial membranes (Foster et al. 1995a, 1995b). Ultrastructural lesions consistent with lipid peroxidation have been observed in mitochondria from the ovaries of monkeys treated with hexachlorobenzene (Bourque et al. 1995); similar lesions have also been observed in rats (Alvarez et al. 2000). Breast cancer is another end point believed to be influenced through the neuroendocrine axis. Studies available to date have found little or no evidence for an association between hexachlorobenzene and breast cancer in humans (Dorgan et al. 1999; Guttes et al. 1998; Liljegren et al. 1998; Mendonca et al. 1999; Moysich et al. 1998; Zheng et al. 1999). Adipose hexachlorobenzene levels were increased in males with undescended testis compared to controls (Hosie et al. 2000); this adverse effect may be related to *in utero* changes in hormone levels.

3.7   CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6 Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient

tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Infants and young children appeared to be especially sensitive to the effects of hexachlorobenzene in the Turkish grain poisoning epidemic. During the epidemic, there was an extremely high rate of mortality (close to 100% in some villages) in breast fed infants (under 2 years of age) of mothers known to have ingested the contaminated bread (Gocmen et al. 1989; Peters et al. 1982). This is in contrast to a 10% rate of mortality in exposed adults (Peters et al. 1982, 1987). Poisoned infants displayed a condition known as *pembe yara* or "pink sore" because of the associated skin lesions (blistering and epidermolysis and annular erythema) (Cripps et al. 1984; Peters et al. 1982, 1987). The infant deaths were caused by respiratory and cardiovascular failure resulting from the disease, and sometimes followed tremors and convulsions (Peters et al. 1982). Pink sore was not seen in exposed adults. Infants in this study were likely exposed *in utero* via transplacental transfer and postnatally by lactational transfer (Cripps et al. 1984; Peters et al. 1982, 1987). No quantitation of exposure (dose and duration) was presented in any of these clinical reports. However, an estimated dose of 0.05–0.2 g/day (0.7–2.9 mg/kg/day for a 70-kg person) is considered to be reliable by the original investigators of the Turkey epidemic (Cam and Nigogosyan 1963). Analysis of human milk from exposed women and unexposed controls in this epidemic showed hexachlorobenzene concentrations of 0.51 and 0.07 ppm, respectively (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987).

Based on 20–30-year follow-up studies (Cripps et al. 1984; Peters et al. 1982), patients who were young children (average age 7 years) during this exposure later developed numerous dermatologic, neurologic, and orthopedic abnormalities associated with the developmental toxicity of hexachlorobenzene. The reproductive histories of 42 females exposed to hexachlorobenzene as children or young adults were also studied. Of the 188 pregnancies in the 42 women that occurred in a 4-year period (1977–1981), there were 15 fetal deaths (13 miscarriages and 2 stillbirths) and 173 live births (Peters et al. 1982, 1987);

however, the relevance of this study is limited because the numbers of expected miscarriages and stillbirths were not provided. These mothers had 0.51 ppm hexachlorobenzene in their breast milk compared to 0.07 ppm in unexposed controls (Gocmen et al. 1989). The fetal mortalities may be related to the mobilization of hexachlorobenzene from the maternal fat pool and its subsequent exposure to the fetus through the placenta.

Based on partial evaluation of 63 of a planned 100 cases, Sala et al. (1999b) published a preliminary study, reporting that a significant association between prenatal hexachlorobenzene exposure and impaired development of locomotor skills had been detected in newborn babies in Flix, compared with those of nearby villages. A study of a less-exposed population in New York was unable to correlate hexachlorobenzene levels in umbilical blood or breast milk with infant intelligence test results (Darvill et al. 2000).

A German case-control study found that adipose hexachlorobenzene levels in 18 male patients who underwent orchidopexy to correct unilateral or bilateral undescended testis (mean age 4.2 years) were 3-fold higher compared to a group of 30 male control patients (mean age 3.5 years); this difference was highly significant (Hosie et al. 2000). A similar correlation was also observed for heptachloroepoxide (HCE), but not for other organochlorines. The weaknesses of this study are the small study size, the lack of age-adjustment between groups, and the potentially confounding effect of HCE.

Although there is only limited direct evidence that hexachlorobenzene crosses the placenta in humans (Ando et al. 1985), animal studies have shown that hexachlorobenzene crosses the placenta readily and accumulates in fetal tissues (Courtney and Andrews 1985; Courtney et al. 1979; Cripps 1990; Villeneuve and Hierlihy 1975; Villeneuve et al. 1974a). While numerous human studies have demonstrated the presence of hexachlorobenzene in breast milk, animal studies have shown in addition that hexachlorobenzene concentrates in the breast milk and is transferred to the suckling neonate in considerable amounts (Bailey et al. 1980; Cripps 1990; Goldey et al. 1990).

Animal studies have also confirmed that the developing organism is an especially sensitive target for hexachlorobenzene. Findings from laboratory animal single- and multi-generation reproductive toxicity studies conducted in rats exposed to hexachlorobenzene indicate that fertility, gestational viability, and lactational indices may be affected by hexachlorobenzene exposure (Grant et al. 1977; Kitchin et al. 1982). Studies on prenatally exposed animals have shown immune and neurological effects at lower doses in the young developing animals than in adults (Goldey and Taylor 1992; Vos et al. 1979a). The

most sensitive end point in any study, and the basis for the chronic MRL, was liver lesions that developed during adulthood in rats treated with combined pre- and postnatal lifetime exposure (Arnold et al. 1985).

Animal studies have also shown that hexachlorobenzene mediates toxicity through the neuroendocrine axis, with multiple effects on the thyroid gland (hypothyroidism), parathyroid gland (hyperparathyroidism), adrenal gland, and ovaries (Alvarez et al. 2000; Andrews et al. 1988, 1990; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Foster et al. 1992b, 1993, 1995a, 1995b; Kimbrough and Linder 1974; Kleiman de Pisarev et al. 1989, 1990). Because the hormones produced by these endocrine organs play a crucial role in growth and development of the organism, it is not surprising that hexachloro-benzene interferes with these processes. Neuroendocrine end points have not been studied in developing organisms.

3.8   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/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to hexachlorobenzene are discussed in Section 3.8.1.

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 hexachlorobenzene are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10 "Populations That Are Unusually Susceptible".

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Hexachlorobenzene

Human tissues and body fluids that have been analyzed for hexachlorobenzene to identify and quantify exposure include blood and serum (Ataniyazova et al. 2001; Glynn et al. 2000; Hagmar et al. 2001; Karmaus et al. 2001; Rutten et al. 1988; Sala et al. 1999b, 2001b; Schlummer et al. 1998; Siyali 1972; Waliszewski et al. 2001; Weiderpass et al. 2000; and many others), liver (Dewailly et al. 1999; Weistrand and Noren 1998), bone marrow (Bucholski et al. 1996; Scheele et al. 1995), brain (Dewailly et al. 1999), fat (Ansari et al. 1986; Dewailly et al. 1999; Lordo et al. 1996; Robinson et al. 1990; Scheele et al. 1995; Siyali 1972; Szymczynski and Waliszewski 1981; Teufel et al. 1990; Weistrand and Noren 1998), semen (Szymczynski and Waliszewski 1981), the placenta (Poli et al. 1999), the umbilical cord (Burse et al. 2000; Darvill et al. 2000), and breast milk (Ataniyazova et al. 2001; Craan and Haines 1998; Czaja et al. 1997; Darvill et al. 2000; Dewailly et al. 2000; Fitzgerald et al. 2001; Gladen et al. 1999; Gocmen et al. 1989; Huang et al. 1989; Lunden and Noren 1998; Newsome and Ryan 1999; Scheele et al. 1995; Weisenberg 1986; Wickstrom et al. 1983; and others).

Reliable methods are also available to measure hexachlorobenzene in feces (Albro and Thomas 1974; Koss and Koransky 1975; Schlummer et al. 1998) and urine. Trace amounts of unchanged hexachloro-benzene have been detected in urine; however, urinary metabolites are more easily detected and quantified (Ingebrigtsen et al. 1981, 1986; Koss et al. 1976; Lui and Sweeney 1975; Rozman et al. 1977a;

To-Figueras et al. 1992; van Ommen et al. 1985; Yang et al. 1978) as biomarkers for hexachlorobenzene exposure. Although urinary PCP and tissue hexachlorobenzene correlated in 60 patients studied, it is possible that the urinary PCP originated from other chlorinated hydrocarbons such as pentachloro-benzene, alpha-hexachlorocyclohexane, or pentachloronitrobenzene (Burton and Bennett 1987; Currier et al. 1980; Koss et al. 1986; To-Figueras et al. 1992).

Indirect biomarkers of hexachlorobenzene exposure include measurement of *gamma*-glutamyl transferase in blood, uroporphyrin and d-ALA in urine, and coproporphyrin in feces (Koss et al. 1986; To-Figueras et al. 1992). Because these biomarkers are not specific for hexachlorobenzene, their usefulness in monitoring exposed populations is limited.

Several studies have correlated hexachlorobenzene levels with different end points. In humans, hexa-chlorobenzene levels are correlated between feces and serum (To-Figueras et al. 2000), maternal and umbilical cord blood levels (Ataniyazova et al. 2001; Sala et al. 2001a; Waliszewski et al. 2000), length of breast-feeding and infant serum levels (Abraham et al. 2000), and the presence of other organochlorines in serum (Burse et al. 2000; Glynn et al. 2000; Hoppin et al. 2000; and others).

Sufficient data of air levels of hexachlorobenzene have not been available to determine quantitative biomarkers of inhalation exposure. However, hexachlorobenzene levels have been assayed in people of Flix, Spain, who were exposed to hexachlorobenzene from an nearby electrochemical plant that produced organochlorines (Ballester et al. 2000; Grimalt et al. 1994; Herrero et al. 1999; Sala et al. 1999a, 1999b; To-Figueras et al. 1997). These studies found higher serum levels in factory workers compared to nonworkers, in male workers compared to females (presumably due to increased work-related exposure), in nonworkers who lived with factory workers compared to nonworkers who did not live with factory workers, and in people living near the factory compared to people living further away.

3.8.2 Biomarkers Used to Characterize Effects Caused by Hexachlorobenzene

Although not specific to hexachlorobenzene, porphyria is the primary biomarker of effect from human exposure to hexachlorobenzene. Disturbance of the heme biosynthesis pathway of the body's porphyrin metabolism in the liver is the major action of hexachlorobenzene in short- or long-term exposure. Due to this disturbance, abnormal levels of porphyrin precursors are found in exposed individuals. In some cases, porphyria cutanea tarda, displayed as scarring or cutaneous annular erythema (a condition termed *pembe yara*, or pink sore), is present. Such exposed people also exhibited painless arthritis, osteoporosis,

and small distinctive hands (Cripps et al. 1984; Peters et al. 1982, 1987). Increases in serum *gamma*-glutamyl transferase, uroporphyrin (red-tinged urine), and d-ALA in the urine, and uroporphyrin and coproporphyrin in the stool are also indicative of the effect of hexachlorobenzene. While low levels of hexachlorobenzene have been found in human tissues and body fluids, such reported low levels have not generally been associated with adverse health effects (Booth and McDowell 1975). Recently, associations have been found between increased hexachlorobenzene levels and decreased interferon-γ (Daniel et al. 2001), decreased lymphocyte IL-10 secretion (Belles-Isles et al. 2000), ear infections in infants (Dewailly et al. 2000), undescended testis (Hosie et al. 2000), and locomotor skill impairment in newborns (Sala et al. 1999b).

For more information on biomarkers for renal and hepatic effects of chemicals see Agency for Toxic Substances and Disease Registry/Centers for Disease Control and Prevention Subcommittee Report on Biological Indicators of Organ Damage (1990) and for information on biomarkers for neurological effects see OTA (1990).

3.9 INTERACTIONS WITH OTHER CHEMICALS

Certain chemicals may interfere with the toxicity of hexachlorobenzene indirectly by influencing its metabolism through their actions on drug metabolizing enzymes. The duration and intensity of action of hexachlorobenzene are largely determined by the speed at which it is metabolized in the body by the liver microsomal cytochrome P-450 system. More than 200 drugs, insecticides, and other chemicals are known to induce the activity of liver microsomal drug-metabolizing enzymes. The characteristic biological actions of these chemicals are highly varied. Although there is no clear relationship between their actions and structures and their ability to induce enzymes, most of the inducers are lipid soluble at physiological pH. These inducers of the P-450 system include the following classes of drugs: hypnotics and sedatives (barbiturates, ethanol); anesthetic gases (methoxyflurane, halothane); central nervous system stimulants (amphetamine); anticonvulsants (diphenylhydantoin); tranquilizers (meprobamate); antipsychotics (triflupromazine); hypoglycemic agents (carbutamide); anti-inflammatory agents (phenylbutazone); muscle relaxants (orphenadrine); analgesics (aspirin, morphine); antihistaminics (diphenhydramine); alkaloids (nicotine); insecticides (chlordane, DDT, BHC, aldrin, dieldrin, heptachlorepoxide, pyrethrins); steroid hormones (testosterone, progesterone, cortisone); and carcinogenic polycyclic aromatic hydrocarbons (3-methylcholanthrene, 3,4-benzpyrene) (Klaassen et al. 1995; Williams and Burson 1985).

Hexachlorobenzene has been reported to increase the activity of aryl hydrocarbon hydroxylase and other enzymes associated with both the 3-MC and phenobarbital-inducible isozymes of cytochrome P-450 in the rat (Goldstein et al. 1986). Thus, exposure to any of these enzyme inducers prior to, concurrent with, or after exposure to hexachlorobenzene may result in accelerated biotransformation to the more toxic PCP. The extent of toxicity mediated by this phenomenon is dependent on how rapidly the PCP is hydrolyzed or conjugated to the less chlorinated benzenes which are much less toxic. In animal studies, pretreatment of rats with 3-methylcholanthrene or phenobarbital increased the metabolism and toxicity of hexachlorobenzene (Gopalaswamy and Nair 1992; Rajamanickam and Padmanaban 1974; van Ommen et al. 1985; Vos et al. 1988). PCP (500 ppm) accelerated the onset of hexachlorobenzene-induced porphyria, indicated by an increase in urinary excretion of uroporphyrin and a decrease of porphyrin with 2 and 3 carboxylic groups in female rats fed diets containing 1,000 ppm hexachlorobenzene (Debets et al. 1980a). This increase occurred 3 weeks earlier in the hexachlorobenzene plus PCP-treated animals than in animals treated with hexachlorobenzene alone. Intraperitoneal pretreatment with diethylstilbestrol followed by oral administration of hexachlorobenzene to male and female rats stimulated the excretion of hexachlorobenzene metabolites via urine and feces (Rizzardini and Smith 1982). A 2-fold increase in the accumulation of hexachlorobenzene in tissues also occurred (Villeneuve et al. 1977). An exacerbation in the increase of hepatic accumulation and urinary excretion of uroporphyrin occurred in rats given doses of hexachlorobenzene (25 mg/kg/day for 12 consecutive days) when hexachlorobenzene was co-administered with methyl isobutyl ketone. The authors speculated on the involvement of hepatic isozyme inhibition and porphyria induction by methyl isobutyl ketone in hexachlorobenzene porphyrinogenic action (Krishnan et al. 1992).

Similarly, prior or concurrent exposure to hexachlorobenzene and MFO enzyme-inhibiting substances (e.g., carbon monoxide; ethylisocyanide; SKF 525A, halogenated alkanes, such as CCl_4; alkenes, such as vinyl chloride; and allelic and acetylenic derivatives) may decrease the toxicity of hexachlorobenzene by decreasing the rate of the hydrolytic dealkylation and hydrolysis of both parent hexachlorobenzene (Williams and Burson 1985). Rats treated with hexachlorobenzene in combination with the cytochrome P450IIIA1 (CYP3A1) inhibitor, TAO, showed a marked reduction in hexachlorobenzene-induced immunomodulatory effects. These results suggest that the oxidative metabolites, PCP and TCHQ, are not likely to be implicated in the immunostimulatory effects of hexachlorobenzene (Schielen et al. 1995a). Similar conclusions were reached by the investigators of a 13-week rat study to assess the role of oxidative metabolism in hexachlorobenzene-induced porphyria and thyroid hormone homeostasis (Den Besten et al. 1993) as well as in other animal studies in which pretreatment of rats with TAO decreased

the metabolism of hexachlorobenzene (Gopalaswamy and Nair 1992; Rajamanickam and Padmanaban 1974; van Ommen et al. 1985; Vos et al. 1988).

Food deprivation has also been shown to increase susceptibility of animals to the toxicity of hexachloro-benzene. During 4 weeks of exposure to hexachlorobenzene at 40 and 200 mg/kg, food deprivation (50%) increased the ability of hexachlorobenzene to cause liver hypertrophy and induce microsomal enzyme activity. A 2-fold increase in the accumulation of hexachlorobenzene in tissues also occurred (Villeneuve et al. 1977). Since absorption was not measured, it is not clear whether these observations are due to metabolic changes in fat metabolism and release of hexachlorobenzene to target organs or to increased fractional absorption of hexachlorobenzene. These findings were validated by Kishima et al. (2000); 50 ppm hexachlorobenzene did not induce liver toxicity in liver-initiated Wistar rats when administered in a normal diet, but caused liver damage (decreased weight, foci of altered enzyme expression, hypertrophy) when administered in an energy-restricted diet which provided only 50% of the calories in the normal diet.

Results from other studies indicate that hexachlorobenzene has the potential to alter the toxicity of other chemicals. Co-treatment of hexachlorobenzene (400 µmol/kg) and 2,3,7,8-TCDD (10 or 30 µg/kg) in rats exacerbated both body weight loss and thymic atrophy caused by 2,3,7,8-TCDD, while hexachloro-benzene administered at doses as high as 3,000 µmol/kg did not cause any significant effects on these parameters (Li et al. 1989). Exposure to 4 mg/kg/day of hexachlorobenzene from 2 weeks prior to mating through lactation and partially through the placenta increased the LD_{50} value for malathion in 17–18-day-old Wistar rat pups by more than a factor of 2. In general, the inhibitory effect of malathion on cholinesterase activities was decreased by pretreatment with hexachlorobenzene. The authors attributed the increased resistance to intoxication by malathion and reduction of esterase inhibition in the pups to an increase in tissue carboxylesterase activity, presumably malathionase, and a decrease in malaxon formation (Mendoza and Shields 1976). In a study with female Sprague-Dawley rats administered single gavage doses of 400 or 600 mg/kg hexachlorobenzene in corn oil, or 10 or 12.5 mg/kg mercuric chloride, or a combination of 400 or 600 mg/kg hexachlorobenzene and 10 or 12.5 mg/kg mercuric chloride, hexachlorobenzene and mercuric chloride interacted additively with respect to lethality and endocrine, kidney, and liver toxicity. Although no deaths were reported in the 400 mg/kg hexachlorobenzene or 10 mg/kg mercuric chloride dose group animals, one death each was reported in the combined 400 mg/kg hexachlorobenzene plus 10 mg/kg mercuric chloride, and 600 mg/kg hexachlorobenzene plus 10 mg/kg mercuric chloride dose group animals; and two animals died in the 600 mg/kg hexachlorobenzene plus 12.5 mg/kg mercuric chloride dose group animals. Similarly, mild to

moderate morphological changes observed in the liver, thyroid, thymus, and bone marrow of rats exposed to hexachlorobenzene or hexachlorobenzene plus mercuric chloride; and in the kidneys of mercuric chloride- or mercuric chloride plus hexachlorobenzene-exposed rats were more severe in animals that received a combination of hexachlorobenzene and mercuric chloride (Lecavalier et al. 1994). The mechanism of these interactive effects are not known.

Iron overload aggravates hexachlorobenzene-induced porphyria and related hepatopathology. There was increased porphyrin excretion in female C57BL/6J mice (strains B6-Ah[b] and B6-Ah[d]) pretreated with iron and then fed diets containing 26 mg/kg/day of hexachlorobenzene for 9, 15, or 17 weeks as compared to rats given hexachlorobenzene alone. This is consistent with the proposition that the sustained induction of either P3-450 (the mouse CYP1A2 ortholog), or P1-450 (CYP1A1), or both may be a causative factor in the development of this disease. Furthermore, differential induction of the P3-450 (the mouse CYP1A2 ortholog) and P1-450 (CYP1A1) isozymes in B6-Ah[b] responsive versus B6-Ah[d] nonresponsive mice suggests that hexachlorobenzene may act through the Ah receptor (Hahn et al. 1988). Iron overload also caused a significantly depressed EROD (an estimate of CYP1A1 activity), in the livers of hexa-chlorobenzene-fed rats for 5 or 15 weeks while PROD (an estimate of CYP2B1 activity) and BROD (an estimate of CYP2B1 and other P-450 isozymes activity) were depressed in female Fischer 344 rats that received iron-dextran solution (50 mg/mL) by subcutaneous injection for 1 week and then were administered dietary hexachlorobenzene at a dose of 10 mg/kg/day in corn oil for 65 weeks (Smith et al. 1993).

The interactive effect of hexachlorobenzene with other substances in cancer induction has also been studied in animals. The toxicological effects of hexachlorobenzene exposure as a consequence of varying the dietary levels of vitamin A were evaluated in a single-generation lifetime study. There were no significant differences in hematological and pathological lesions in rats fed basal diets with either 0.1 or 10 times the vitamin A content of the control diet and animals fed similar diets which also included hexa-chlorobenzene (Arnold et al. 1985). Hyperplastic nodules were observed in the liver lobes of 80% of female Fischer 344 rats pretreated with Imferon (iron-dextran) then given dietary hexachlorobenzene at doses of 5 or 10 mg/kg/day in arachis oil for 65 weeks. There was a high incidence of fibrin throughout the liver with sinusoidal telangiectasis, and nodular peliosis hepatitis and hepatocellular necrosis. The study proposed a nongenotoxic mechanism for tumor induction by hexachlorobenzene, concluding that the formation of hepatomas and hemangiomas with elements of peliosis could be explained by the compensatory hyperplastic responses to hepatocellular injury or necrosis and by the simultaneous loss of

hepatocellular cords. The study further concluded that the accumulation of iron in the liver would strongly potentiate the development of hepatic tumors (Carthew and Smith 1994).

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to hexachlorobenzene than will most persons exposed to the same level of hexachlorobenzene in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of hexachlorobenzene, or compromised function of organs affected by hexachlorobenzene. Populations who are at greater risk due to their unusually high exposure to hexachlorobenzene are discussed in Section 6.7, Populations With Potentially High Exposures.

Hexachlorobenzene has been shown to elevate porphyrin levels in humans following inhalation exposure (Herrero et al. 1999; Sala et al. 1999b; Seldon et al. 1999) and to cause porphyria cutanea tarda (a specific disease resulting from elevated porphyrin levels) following oral exposure (Cam and Nigogosyan 1963; Cripps et al. 1984; Dogramaci 1964; Gocmen et al. 1989; Peters et al. 1982, 1987). Studies unrelated to hexachlorobenzene-exposure have associated the diagnosis of porphyria cutanea tarda with infections of HIV and hepatitis C virus (Drobacheff et al. 1998; Egger et al. 2002; Meola and Lim 1993). It is not known if, or the degree to which, these diseases contribute to or exacerbate one another; however, HIV and hepatitis C-infected individuals may have increased susceptibility to porphyria cutanea tarda following hexachlorobenzene exposure.

Case studies of hexachlorobenzene poisoning in humans indicate that young children are more sensitive to hexachlorobenzene intoxication. Children (average age, 7 years) who had ingested hexachlorobenzene-contaminated bread during the epidemic of hexachlorobenzene poisoning in Turkey between 1955 and 1959 developed short stature, pinched faces, osteoporosis of bones of the hand, and painless arthritic changes. Some of the young children in this study were presumed to have been exposed *in utero* via transplacental transfer and postnatally by lactational transfer. The children who died were between the ages of 1 and 2 years, and died from a disease known as *pembe yara* or "pink sore" (Cripps et al. 1984; Peters et al. 1982, 1987). Analysis of human milk from exposed women and unexposed controls in this epidemic showed hexachlorobenzene concentrations of 0.51 and 0.07 ppm, respectively (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987).

In laboratory animals, reduced survival of suckling offspring of lactating mothers and fetuses of mothers exposed to hexachlorobenzene was also reported in several studies (Arnold et al. 1985; Grant et al. 1977; Kitchin et al. 1982). There is evidence that hexachlorobenzene is concentrated in milk of lactating monkeys exposed to hexachlorobenzene, suggesting that the risk of exposure to nursing infants may be greater than the risk to their mothers. Blood and tissue levels in the infants were higher than in mothers, and infants exhibited clinical symptoms of toxicity sooner than their mothers (Bailey et al. 1980).

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to hexachlorobenzene. 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 hexa-chlorobenzene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to hexachlorobenzene:

Tintinalli JE, Ruiz E, and Krone RL, eds. 1996. Emergency medicine. A comprehensive study.
American College of Emergency Physicians. 4th ed. The McGraw-Hill Companies, Inc.

Goldfrank RL, et al. 1994. Goldfrank's toxicologic emergencies. 5th ed. Appleton and Lange 5th Edition.

Ellenhorn MJ and Barceloux DG. 1988. Medical toxicology: Diagnosis and treatment of human poisoning. New York, NY: Elsevier Science Publishing Company, Inc.

3.11.1   Reducing Peak Absorption Following Exposure

For inhalation exposure, management commonly includes moving the exposed individual to fresh air, and then monitoring for respiratory distress. Injuries to the lungs are more likely when there is severe respiratory irritation and persistent cough. Emergency airway support and 100% humidified supplemental oxygen with assisted ventilation may be needed (Dreisbach 1983; Ellenhorn and Barceloux 1988; Haddad and Winchester 1990; Rumack and Lovejoy 1991).

Emesis may be indicated in acute ingestion unless the patient is obtunded, comatose, or convulsing, has lost the gag reflex, or if hexachlorobenzene was suspended in petroleum distillates, so that there is danger that emesis would result in aspiration. It is most effective if initiated within 30 minutes of exposure. Administration of mineral oil or castor oil-based cathartics are contraindicated because they tend to

increase intestinal absorption of lipophilic toxicants like hexachlorobenzene. Adrenergic amines (decongestants, bronchodilators, or caffeine) and sympathomimetics (e.g., epinephrine) are not recommended because they may increase myocardial irritability and produce refractory ventricular arrhythmias or dysrhythmias. Cholestyramine or other agents that may bind to hexachlorobenzene may be useful in limiting absorption (Bryson 1989; Dreisbach 1983; Ellenhorn and Barceloux 1988; Haddad and Winchester 1990; Rumack and Lovejoy 1991). Gastric lavage with subsequent administration of activated charcoal and sorbitol cathartic have been recommended in acute management to reduce gastrointestinal absorption. Repeated dosing with activated charcoal or cholestyramine resin may be administered to enhance elimination by interrupting enterohepatic circulation, as has been demonstrated for chlordane and kepone (Cohn et al. 1978; Ellenhorn and Barceloux 1988).

Decontamination is the first step in reducing dermal absorption. It is recommended that decontamination begin immediately after the exposure; that contaminated clothing be removed; and that the skin, hair, and nails be washed copiously with soap and water. Leather clothing absorbs hexachlorobenzene and should be discarded. The use of oils could facilitate dermal absorption and their use is not recommended. If the material enters the eyes, thorough rinsing with sterile physiological saline is suggested (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990; Rumack and Lovejoy 1991). If irritation, lacrimation, or especially pain, swelling, and photophobia persist after 15 minutes of irrigation, then ophthalmologic consultation to evaluate potential ocular damage is recommended (Haddad and Winchester 1990).

3.11.2 Reducing Body Burden

Little can be done to reduce the body burden of absorbed hexachlorobenzene (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990; Rumack and Lovejoy 1991). Diuresis is not likely to be effective because of the high lipophilic nature of hexachlorobenzene. Exchange transfusion, peritoneal dialysis, hemodialysis, and hemoperfusion are not likely to be beneficial because of the rapidity with which hexa-chlorobenzene leaves the blood and locates in peripheral compartments, since this substance has an initial large volume of distribution. However, continued treatment with charcoal and cholestyramine may be useful to reduce enterohepatic recirculation (Cohn et al. 1978; Ellenhorn and Barceloux 1988), although no differences in fecal excretion of hexachlorobenzene were observed when cholestyramine or sesame oil was administered orally to rats and Rhesus monkeys following ingestion of hexachlorobenzene. When mineral oil was added to the diet of Rhesus monkeys treated with hexachlorobenzene, a 6–9-fold increase in fecal excretion of hexachlorobenzene and its metabolites was observed. Continuous administration of mineral oil led to increased depletion of hexachlorobenzene from both the blood and adipose tissues.

Administration of n-hexadecane enhanced fecal excretion of hexachlorobenzene about 5-fold in rats (Rozman et al. 1981). The barbiturates, which have been used to control poison-induced convulsions, may hasten metabolism and elimination of hexachlorobenzene (Smith 1991).

Hexachlorobenzene is known to be toxic to developing perinatal animals. The particularly potent effects seen in nursing infants following maternal exposure to hexachlorobenzene must be recognized when counseling hexachlorobenzene-exposed women of childbearing age. It is recommended that plans for pregnancy and contraception be included in the physician's clinical assessment, with consideration to specialty evaluation of residual hexachlorobenzene contamination.

3.11.3   Interfering with the Mechanism of Action for Toxic Effects

The most serious acute toxicological effect of exposure to chlorinated hydrocarbon pesticides appears to be central nervous system toxicity. These organochlorine compounds interfere with the normal flux of sodium and potassium ions across the neuronal membrane and disrupting the normal repolarization of neurons, resulting in hyperexcitation and seizures. Organochlorines also inhibit the ability of calmodulin, a calcium mediator in the nerves, to transport calcium ions essential for the intraneuronal release of neurotransmitters, thereby further inhibiting repolarization and increasing neuronal sensitivity to subsequent stimuli resulting in hyperexcitation and seizures (Klaassen et al. 1995). Hexachlorobenzene appears to produce little central nervous system toxicity at low doses. At high doses, central nervous system depression can dominate the clinical profile (de Matteis et al. 1961). Thus, management of seizures with anticonvulsants is much less likely to be needed except in acute intoxication management. The number of acute hexachlorobenzene-intoxication patients with seizures, however, was negligible (de Matteis et al. 1961). Diazepam or phenobarbital is recommended for use to reduce seizures, if seizures occur (Bryson 1989).

In Turkey, in 1956, prolonged oral exposure to hexachlorobenzene was associated with an outbreak of acquired porphyria cutanea tarda characterized by neurological, visceral, arthritic, cutaneous, and hepatic symptoms. The victims also exhibited bulbous, erythematous skin lesions, which progressed to atrophy, hyperpigmentation, hypertricosis (increased body hair), and ulcerations. Treatment for these conditions was primarily supportive. Anecdotal reports from Turkey indicated that chelating agents (disodium ethylenediaminetetraacetic acid [EDTA] and dimercaptopropanol [BAL]) administration over 3 months (1.5 g daily for 5 days intravenously followed by daily oral doses of 1–2 g) successfully reduced the symptoms of patients with hexachlorobenzene-induced porphyria (Ellenhorn and Barceloux 1988; Peters

1956, 1993; Peters and Cripps 1985; Peters et al. 1957, 1966, 1986b). However, this method of treating acute porphyria has not been validated.

The role of iron overload in the pathogenesis of hexachlorobenzene-induced porphyria has also been examined based on observations that 80% of patients with porphyria cutanea tarda have increased liver stores of iron and increased levels of uroporphyrin 1 (Smith and de Matteis 1990; Wainstok de Calmanovici et al. 1991). In these patients, phlebotomy often induces disease remission and a decrease in urinary porphyrin 1 excretion. It remains to be seen whether iron overload plays a permissive or etiologic role in patients exposed to porphyria-producing toxins, and whether phlebotomy has any role in the treatment of these patients.

In other reports, co-administration of S-adenosyl-L-methionine (SAM) via subcutaneous injection for the last 15 days of treatment with an oral dose of hexachlorobenzene (100 mg/kg/day) reversed some of the effects of hexachlorobenzene exposure (elevation of liver porphyrin content and liver weight). It has been suggested that the beneficial effects of SAM on hexachlorobenzene-induced toxicity may be related to effects on adenosine triphosphate availability (Cantoni et al. 1990; Cuomo et al. 1991).

A study conducted with Osteogenic Disorder Shionogi (ODS) rats provided evidence that large doses of ascorbic acid may inhibit chemically-induced uroporphyria in humans. This effect seems to be mediated by inhibition of CYP1A2-catalyzed uroporphyrinogen oxidation. However, a significant depletion of hepatic ascorbic acid may be required for any beneficial effect of ascorbic acid to be observed (Sinclair et al. 1995).

3.12 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 hexachlorobenzene 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 hexachlorobenzene.

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.

3.12.1   Existing Information on Health Effects of Hexachlorobenzene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to hexachlorobenzene are summarized in Figure 3-5. The purpose of this figure is to illustrate the existing information concerning the health effects of hexachlorobenzene. 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* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As shown in Figure 3-5, there are data available regarding a number of toxic end points in humans exposed to hexachlorobenzene by inhalation and ingestion, including death, chronic systemic toxicity, immunological, neurological, reproductive and developmental effects, and cancer. However, the extent of the data available is limited, particularly for inhalation exposure. No data were located regarding the toxicity of hexachlorobenzene by dermal exposure in humans. Hexachlorobenzene has been well studied in animals following oral exposure; the full range of end points have been assessed. However, only immunological effects have been studied in animals following inhalation exposure and no studies at all were located regarding toxicity of hexachlorobenzene in animals following dermal exposure.

3.12.2   Identification of Data Needs

Acute-Duration Exposure.   No information was located regarding health effects in humans following acute-duration exposure to hexachlorobenzene by any route. The available animal data were sufficient to identify target organs of acute oral exposure. Extensive study of animals exposed by the oral route for acute durations has identified doses producing a wide range of health effects, including porphyria and other hepatic effects, renal tubular lesions, changes in thyroid and reproductive hormone levels, impaired male reproductive performance, developmental effects ranging from subtle

neurobehavioral effects in neonates to fetotoxic and teratogenic effects, overt neurological effects, and

lethality (Bouthillier et al. 1991; Courtney et al. 1976; De Matteis et al. 1961; Foster et al. 1993; Goldey

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Figure 3-5. Existing Information on Health Effects of Hexachlorobenzene

Human

Animal

● Existing Studies

and Taylor 1992; Goldstein et al. 1978; Kennedy and Wigfield 1990; Khera 1974a; Mehendale et al. 1975; Simon et al. 1979). Most of the existing acute studies were conducted in rats, but mice and guinea pigs were also studied. Pharmacokinetic models for oral exposure in rats and humans are available (Freeman et al. 1989; Yesair et al. 1986), and provided good correlations between predicted yield of metabolites and experimentally observed liver toxicity (porphyria and increased liver weight reported by Koss et al. 1978 and Kuiper-Goodman et al. 1977), between estimated concentrations of hexachloro-benzene in the brain and observed central nervous system effects (tremors and other signs reported by Koss et al. 1978 and Kuiper-Goodman et al. 1977), and between estimated extent of lactational transfer of hexachlorobenzene and observed offspring mortality (data from Kitchen et al. 1982). The database was sufficient to support derivation of an acute oral MRL for hexachlorobenzene. Acute studies by inhalation exposure in animals were limited to a single study of immunological effects (Sherwood et al. 1989). No acute dermal studies in animals were located. Although an acute study in rats suggested that hexachloro-benzene can be absorbed across the skin (Koizumi 1991), pharmacokinetic models for inter-route extrapolation are not available. Additional acute studies by the inhalation and dermal routes would identify acute effect levels for these routes of exposure.

Intermediate-Duration Exposure. A few case-control studies have found evidence of developmental toxicity in newborn humans (Belles-Isles et al. 2000; Hosie et al. 2000); no information was located regarding health effects in humans following intermediate-duration exposure to hexachloro-benzene by any route. Extensive study of animals exposed by the oral route for intermediate durations has identified doses producing a broad spectrum of health effects, including porphyria and other hepatic effects, renal tubular lesions, pulmonary lesions, cardiac lesions, anemia and leukocytosis, osteosclerotic changes in bone, necrotic lesions in muscle, skin lesions, thymic atrophy, splenomegaly and altered spleen morphology, lymph node histopathology, altered immunoglobulin levels, suppression of immune function, changes in the thyroid, parathyroid, and adrenal glands and associated hormone levels, ovarian and testicular lesions, alterations in female menstrual cycling and reproductive hormone levels, reduced fertility, developmental effects ranging from subtle neurobehavioral effects in neonates to fetotoxic effects and pup death, overt neurological effects, and lethality (Andrews et al. 1988, 1989, 1990; Babineau et al. 1991; Bourque et al. 1995; Bouthillier et al. 1991; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Foster et al. 1992a, 1992b; Iatropoulos et al. 1976; Jarrell et al. 1993; Kennedy and Wigfield 1990; Kimbrough and Linder 1974; Knauf and Hobson 1979; Koss et al. 1978; Kuiper-Goodman et al. 1977; Lilienthal et al. 1996; Loose et al. 1978, 1981; Ockner and Schmid 1961; Schielen et al. 1995a, 1995b; Smith et al. 1985; Vos et al. 1979a, 1979b, 1983; others). Most of the existing intermediate-duration studies were conducted in rats, but monkeys, mice, rabbits, dogs, and pigs were also studied.

Pharmacokinetic models for oral exposure in rats and humans are available (Freeman et al. 1989; Yesair et al. 1986), and provided good correlations between predicted yield of metabolites and experimentally observed liver toxicity (porphyria and increased liver weight reported by Koss et al. 1978 and Kuiper-Goodman et al. 1977), between estimated concentrations of hexachlorobenzene in the brain and observed central nervous system effects (tremors and other signs reported by Koss et al. 1978 and Kuiper-Goodman et al. 1977), and between estimated extent of lactational transfer of hexachlorobenzene and observed offspring mortality (data from Kitchen et al. 1982). The database was sufficient to support derivation of an intermediate-duration oral MRL for hexachlorobenzene. Intermediate-duration studies by inhalation exposure in animals were limited to a single study of immunological effects (Sherwood et al. 1989). No intermediate-duration dermal studies in animals were located. Pharmacokinetic models for inter-route extrapolation are not available. Additional intermediate-duration studies by the inhalation and dermal routes would identify intermediate-duration effect levels for these routes of exposure.

Chronic-Duration Exposure and Cancer.   There are data available on humans chronically exposed to hexachlorobenzene by the inhalation and oral routes, but no quantitative exposure information. The inhalation data are very limited, but tentatively found effects on the liver and immune system of exposed individuals (Queiroz et al. 1997, 1998a, 1998b; Richter et al. 1994; Selden et al. 1999). The oral data much more clearly identified the liver, skin, bone, thyroid, and central nervous system as target tissues for hexachlorobenzene in chronically exposed people (Cam and Nigogosyan 1963; Cripps et al. 1984; Peters et al. 1982, 1987). Based on very limited data, the original investigators of the Turkey epidemic estimated that the daily average oral dose was 0.05–0.2 g/day (0.7–2.9 mg/kg/day for a 70-kg person) (Cam and Nigogosyan 1963). No information regarding chronic dermal exposure in humans was located. Chronic oral animal studies identified dose levels associated with systemic (cardiovascular, gastrointestinal, hematological, hepatic, renal, and dermal), immunological, overt neurological, and developmental effects, as well as death (Arnold et al. 1985; Cabral et al. 1977, 1979; Gralla et al. 1977a; Mollenhauer et al. 1975; Smith and Cabral 1980; Smith et al. 1985, 1989, 1993; others). The database was sufficient to support derivation of a chronic oral MRL for hexachlorobenzene. No chronic animal studies were located using inhalation or dermal exposure. Pharmacokinetic models for inter-route extrapolation are not available. Additional chronic studies by the inhalation and dermal routes would identify chronic effect levels for these routes of exposure.

Data from people exposed to hexachlorobenzene by inhalation provide weak evidence for an association between exposure to hexachlorobenzene and cancer of the thyroid, brain, and liver (Grimalt et al. 1994; Selden et al. 1989), while very limited data from orally exposed people showed no increase in cancer risk

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(Cripps et al. 1984; Peters et al. 1982). One case-control study associated elevated adipose levels of hexachlorobenzene with increased risk of breast cancer (Dewailly et al. 1994), but other case-control studies have not found any relationship between body burdens of hexachlorobenzene and breast cancer (Dorgan et al. 1999; Falck et al. 1992; Guttes et al. 1998; Liljegren et al. 1998; Mendonca et al. 1999; Moysich et al. 1998; Mussalo-Rauhamaa et al. 1990; Zheng et al. 1999), bone sarcoma, or leukemia (Hardell et al. 1997; Scheele et al. 1996). The available epidemiology reports taken together do not support an association between hexachlorobenzene exposure and increased cancer incidence, but their limitations (including small study sizes and potentially confounding effects of other organochlorines) preclude considering them evidence of noncarcinogenicity. Because hexachlorobenzene produces porphyria, it is noteworthy that several human studies have associated porphyria with increased incidence of liver cancer (Axelson 1986; Fracanzani et al. 2001).

Several animal studies have demonstrated that oral exposure to hexachlorobenzene increases the incidence of tumor formation. The evidence of carcinogenicity is strongest in the liver; hexachlorobenzene has been shown to induce hyperplasia (in rats, mice, pigs, dogs, and monkeys), metaplasia (in rats), benign tumors (hepatoma in mice and rats; hemagniohepatoma and bile duct adenoma in rats), and malignant tumors (hepatocarcinoma in rats, mice, and hamsters; bile duct adenocarcinoma in rats) (Arnold et al. 1985; Cabral et al. 1979; Ertürk et al. 1986; Pereira et al. 1982; Smith and Cabral 1980; Smith et al. 1985). Additionally, exposure to hexachlorobenzene has been shown to induce renal metaplasia, adenomas, and renal cell carcinomas (in rats, mice, and hamsters); lymphosarcomas (in rats, mice, and hamsters); adrenal hyperplasia and pheochromocytoma (in rats); parathyroid adenomas (in rats); and hemangioendothelioma and thyroid tumors (in hamsters) (Arnold et al. 1985; Den Besten et al. 1993; Ertürk et al. 1986; Kimbrough and Linder 1974). No animal cancer bioassays by inhalation or dermal exposure were located. Pharmacokinetic models for inter-route extrapolation are not available. Based on these findings in animals, hexachlorobenzene is considered a probable human carcinogen. Additional epidemiological studies of people with known hexachloro-benzene exposure would enable better assessment of the carcinogenic risk of this chemical to humans.

Genotoxicity.   Human genotoxicity data for hexachlorobenzene are limited to a case study (route of exposure unknown) and *in vitro* studies in human cell lines. The frequency of micronuclei in peripheral lymphocyte was increased in 41 workers exposed to a mixture of chlorinated solvents that included hexa-chlorobenzene (da Silva Augusto et al. 1997). Hexachlorobenzene did not produce chromosomal aberrations in human peripheral lymphocytes *in vitro* (Siekel et al. 1991), but did produce weak positive results in assays for DNA fragmentation and micronuclei formation in primary cultures of human

hepatocytes (Canonero et al. 1997) and minimal formation of DNA adducts in cultured human Hep G2 hepatoma cells (Dubois et al. 1997). No information on the genotoxicity of hexachlorobenzene in animals by inhalation or dermal exposure was located. Hexachlorobenzene did not cause gene mutations or unscheduled DNA repair in microbial assays (Gopalaswamy and Nair 1992; Haworth et al. 1983; Siekel et al. 1991), and did not produce dominant lethal mutations in orally-exposed rats (Khera 1974a; Simon et al. 1979), or bind strongly to rat DNA *in vitro* or *in vivo* (Gopalaswamy and Nair 1992). However, hexachlorobenzene did produce DNA fragmentation in cultured rat hepatocytes (Canonero et al. 1997) and DNA adducts in fetal hepatocytes from rats and quail (Dubois et al. 1997). Additional studies employing other *in vivo* and *in vitro* assays would be useful to determine the genotoxic potential of hexachlorobenzene.

Reproductive Toxicity.   No data were located regarding reproductive effects in humans or animals with inhalation or dermal exposure. Several miscarriages and stillbirths were reported among people with previous oral exposure to hexachlorobenzene (Peters et al. 1982, 1987), but it is not clear that the rate of miscarriages was significantly higher than normal for this population. One study associated elevated hexachlorobenzene blood levels with increased risk for spontaneous abortion (Jarrell et al. 1998), but other studies did not (Gerhard et al. 1998; Leoni et al. 1986, 1989). The cessation of agricultural uses of hexachlorobenzene in Xixin did not affect reproductive outcomes there (Huang et al. 1989). Animal studies using oral exposure have identified doses associated with reproductive effects, including ovarian lesions and hormonal and menstrual changes, in female rats and monkeys (Alvarez et al. 2000; Babineau et al. 1991; Bourque et al. 1995; Foster et al. 1992a, 1992b, 1993, 1995a; Iatropoulos et al. 1976; Jarrell et al. 1993; Muller et al. 1978; Sims et al. 1991), reduced fertility in rats (Grant et al. 1977), reduced mating index in male rats (Simon et al. 1979), and testicular effects in rats and pigs (including increased weight, degenerative lesions, and retarded maturation) (Den Tonkelaar et al. 1978; Gralla et al. 1977a; Smith et al. 1985). The intermediate-duration MRL for oral exposure is based on ovarian effects in monkeys. Reproductive effects have not been studied in animals by inhalation or dermal exposure, and pharmacokinetic models for inter-route extrapolation are not available. Epidemiological studies of people with known hexachlorobenzene exposure would be useful to establish whether these effects are also seen in people. Additional mechanistic studies to better understand the ovarian and hormonal changes might also help establish the relevance of these findings to humans.

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Developmental Toxicity.   No studies on the developmental effects of hexachlorobenzene in humans following dermal exposure or in laboratory animals following inhalation or dermal exposure were identified, and pharmacokinetic models for inter-route extrapolation are not available. Dramatic developmental toxicity (high mortality, skin lesions) was seen in infants whose mothers consumed bread contaminated with hexachlorobenzene (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982); this study clearly established hexachlorobenzene as a developmental toxicant. Other human studies have found suggestive evidence linking hexachlorobenzene exposure with developmental toxicity, e.g., locomotor skill impairment associated with inhalation exposure (Sala et al. 1999b) and increased risk of undescended testis (route of exposure unknown) (Hosie et al. 2000), although an additional study found no correlation between hexachlorobenzene levels (in blood and milk) and infant intelligence test results (Darvill et al. 2000). Studies in orally exposed rats have demonstrated neurodevelopmental (Goldey and Taylor 1992; Lilienthal et al. 1996) and immunodevelopmental effects (Barnett et al. 1987; Vos et al. 1979a, 1983), reduced neonatal viability and growth (Grant et al. 1977; Kitchin et al. 1982; Vos et al. 1979a, 1983), and some evidence of teratogenic abnormalities (Courtney et al. 1976; Khera 1974a). Hexachlorobenzene caused neurological, hepatic, and cardiovascular effects, as well as death, in lactionally exposed Rhesus monkey infants (Bailey et al. 1980; Iatropoulos et al. 1978). Additional studies that included an assessment of more sensitive end points, such as endocrine changes and neurological or immunological effects, and an investigation of different periods of developmental sensitivity (such as prenatal versus postnatal exposures) would contribute to a clearer understanding of the developmental toxicity of hexachlorobenzene.

Immunotoxicity.   Studies on the immunotoxicity of hexachlorobenzene in humans following oral or dermal exposure are lacking. Occupational studies have associated inhalation exposure to hexachlorobenzene with effects on immunological parameters (neutrophil chemotaxis and cytolytic activity, serum immunoglobulin and IFN-γ levels) (Daniel et al. 2001; Queiroz et al. 1997, 1998a, 1998b; Richter et al. 1994). Case-control studies have associated increased body burdens of hexachlorobenzene (putatively resulting from consumption of contaminated food) with alterations in markers of immune function and susceptibility to infection (Belles-Isles et al. 2000; Dewailly et al. 2000). No studies on the immunotoxicity of hexachlorobenzene in animals after dermal exposure were located. An intermediate-duration study found slight decreases in humoral and pulmonary cellular defenses of rats exposed to hexachlorobenzene via inhalation (Sherwood et al. 1989). Following oral exposure, immunosuppression has been observed in rats, mice, monkeys, and bears (Bernhoft et al. 2000; Carthew et al. 1990; Iatropoulos et al. 1976; Loose et al. 1977, 1981; Michielsen et al. 1997; Silkworth and Loose 1981; Van Loveren et al. 1990) and at least a partial stimulation of the immune system in rats and dogs (Gralla et al.

1977a; Kennedy and Wigfield 1990; Koss et al. 1978; Schielen et al. 1993, 1995b; Vos et al. 1979a, 1979b). Additionally, a number of animal studies have observed inflammation and immune cell infiltration in tissues such as the liver (Arnold et al. 1985; Ertürk et al. 1986), respiratory tract (Goldstein et al. 1978; Kimbrough and Linder 1974; Kitchin et al. 1982; Michielsen et al. 1997, 1999, 2001; Vos et al. 1979a, 1983), and skin (Koss et al. 1978; Michielsen et al. 1997, 2000; Schielen et al. 1993, 1995b; Torinuki et al. 1981) following oral exposure to hexachlorobenzene. Additional chronic-duration studies in humans in the workplace would identify effect levels for immunotoxicity.

Neurotoxicity.   No information regarding neurotoxicity in humans following dermal exposure was located. Following ingestion of bread contaminated with hexachlorobenzene, observed neurological effects included profound weakness, loss of muscle control (inability to handle utensils, myotonia [delayed muscle relaxation after an initial contraction], and cogwheeling [irregular jerkiness of movement due to increased muscle tone as seen in Parkinson's disease]), paresthesia (spontaneous tingling or burning sensations), and sensory shading (graded sensory loss indicative of polyneuropathy) (Cam and Nigogosyan 1963; Gocmen et al. 1989; Peters et al. 1982, 1987). Inhalation data are limited to suggestive evidence linking inhalation exposure to hexachlorobenzene with impaired development of locomotor skills in newborn babies (Sala et al. 1999a). A case-control study did not associate umbilical blood or breast milk hexachlorobenzene levels with infant intelligence test results (Darvill et al. 2000). No studies on the neurotoxicity of hexachlorobenzene in animals after inhalation or dermal exposure were located. Oral studies, primarily in rats but also in mice, rabbits, pigs, monkeys, and quail, have demonstrated serious neurological effects such as convulsions, tremors (intermittent and constant), lethargy, and progressive weakness (Cabral et al. 1979; Cripps 1990; De Matteis et al. 1961; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Grant et al. 1977; Hahn et al. 1988; Kennedy and Wigfield 1990; Kimbrough and Linder 1974; Khera 1974a; Knauf and Hobson 1979; Ockner and Schmid 1961; others) as well as neurobehavioral effects in rats following developmental exposure (Goldey and Taylor 1992; Lilienthal et al. 1996; Taylor and Goldey 1990), changes in adult rat brain chemistry (Billi de Catabbi et al. 2000b; Cochon et al. 2001), and electrophysiological changes in the brains of adult dogs (Sufit et al. 1986; Sundlof et al. 1981). The acute-duration MRL for oral exposure is based on neurobehavioral changes in rats following developmental exposure (Goldey and Taylor 1992). Additional inhalation and dermal studies would identify the potential neurotoxicity of hexachlorobenzene by these routes of exposure.

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Epidemiological and Human Dosimetry Studies.   No information regarding the adverse effects

of hexachlorobenzene in humans following dermal exposure is available, and no human dosimetry data

are available. Health effects (death, systemic [e.g. liver, skin, bone, and thyroid], neurological,

developmental, and endocrine) were identified in cohorts from a group of approximately 4,000 people

orally exposed in Turkey to hexachlorobenzene (in contaminated bread) between 1955 and 1959 (Booth

and McDowell 1975; Cam and Nigogosyan 1963; Cripps et al. 1984; Gocmen et al. 1989; Peters et al.

1982, 1987; Selden et al. 1989). Related to inhalation exposure, health effects (systemic [hepatic, renal,

and endocrine] and neurological) have been identified in residents of a rural town (Flix, Spain) with

airborne hexachlorobenzene pollution attributed to a nearby organochlorine factory, workers from that

factory, and other people with occupational exposure (Grimalt et al. 1994; Herrero et al. 1999; Queiroz et

al. 1997, 1998a, 1998b; Richter et al. 1994; Sala et al. 1999a, 1999b, 2001a; To-Figueras et al. 1997).

Multiple case-studies have investigated possible associations between body burdens of hexachloro-

benzene levels (in blood, fat, urine, and feces) with multiple health effects (Belles-Isles et al. 2000;

Darvill et al. 2000; Dewailly et al. 1994, 2000; Dorgan et al. 1999; Falck et al. 1992; Gerhard et al. 1998;

Guttes et al. 1998; Hagmar et al. 2001; Hosie et al. 2000; Leoni et al. 1986, 1989; Liljegren et al. 1998;

Mendonca et al. 1999; Moysich et al. 1998; Mussalo-Rauhamaa et al. 1990; Zheng et al. 1999; others).

None of these human studies have provided reliable direct exposure data (dose and duration); therefore,

no evidence of an exposure-response relationship has been possible. Further studies of populations with

elevated exposures to hexachlorobenzene (e.g., occupational, consumption of fish from contaminated

areas) would provide additional information useful in assessing dosimetry and health effects such as

reproductive and developmental toxicity.

Biomarkers of Exposure and Effect.

Exposure. Hexachlorobenzene has been measured in human blood and serum, liver, bone marrow, brain,

fat, semen, placenta, umbilical cord (and cord blood), breast milk, feces, and urine (Ataniyazova et al.

2001; Bucholski et al. 1996; Burse et al. 2000; Dewailly et al. 1999; Poli et al. 1999; Schlummer et al.

1998; Szymczynski and Waliszewski 1981; many others). Metabolites of hexachlorobenzene (including

chlorophenols, pentachlorobenzene, alpha-hexachlorocyclohexane, or pentachloronitrobenzene) have

been measured in blood, urine, and feces (Ingebrigtsen et al. 1981, 1986; Koss et al. 1976; Lui and

Sweeney 1975; Rozman et al. 1977a; To-Figueras et al. 1992; van Ommen et al. 1985; Yang et al. 1978).

Indirect biomarkers used to detect intermediate- and chronic-exposure to hexachlorobenzene exposure

include measurement of *gamma*-glutamyl transferase in blood, uroporphyrin and d-ALA in urine, and

coproporphyrin in feces (Koss et al. 1986; To-Figueras et al. 1992); because of their lack of specificity,

the usefulness of these biomarkers is limited. Further studies to develop additional, more sensitive biomarkers of exposure that are specific for hexachlorobenzene exposure would be especially useful in the monitoring of people living near hazardous waste sites.

Effect. Porphyria is the primary biomarker of effect from human acute, intermediate, and chronic exposure to hexachlorobenzene. Studies of an orally exposed population have diagnosed several unusual disease states of porphyria cutanea tarda, including dermal lesions (*pembe yara* or "pink sore" and *kara yara* or "black sore," associated with photosensitivity, dermal fragility and scarring, hyperpigmentation and hirsutism) and small distinctive hands (shortened and spindled fingers with painless swelling and osteoporosis) (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987). Increases in serum *gamma*-glutamyl transferase, uroporphyrin and d-ALA in the urine (red-tinged urine), and uroporphyrin and coproporphyrin in the stool are also indicative of the effect of hexachlorobenzene (Booth and McDowell 1975; others). Additional studies that identified alternative biomarkers would complement these existing biomarkers. Moreover, direct assessments of exposure would facilitate the identification of effect levels.

Absorption, Distribution, Metabolism, and Excretion.   One study was located in which gastrointestinal absorption of hexachlorobenzene in humans was quantified (Schlummer et al. 1998). Information regarding absorption in humans following inhalation exposure is based on observations of toxicity (Grimalt et al. 1994; Herrero et al. 1999; Queiroz et al. 1997, 1998a, 1998b; Richter et al. 1994; Sala et al. 1999b; Selden et al. 1997; To-Figueras et al. 1997). No experimental information regarding absorption in humans by dermal exposures was located; dermal data from a rat study were used to develop a compartment model for application to a 70-kg worker (Koizumi 1991). Only one dermal absorption study (Koizumi 1991) and no inhalation absorption studies in animals are available. Animal data suggest that oral absorption is rapid if dissolved in a lipid, but absorption from aqueous solution is low (Ingebrigtsen and Nafstad 1983; Koss and Koransky 1975). Additional information on the absorption of hexachlorobenzene, especially by the inhalation route, as well as data regarding enterohepatic circulation and gastrointestinal reabsorption would allow more accurate estimations of exposure and evaluation of route-specific differences in hexachlorobenzene toxicity.

No data on distribution in humans following inhalation or dermal exposure or in animals following inhalation exposure were available, but limited information on the distribution of hexachlorobenzene following oral exposure was located. Available data suggest that hexachlorobenzene is preferentially and rapidly distributed to tissues with high lipid content (Cripps 1990; Ingebrigtsen and Nafstad 1983; Jarrell

et al. 1993; Mehendale et al. 1975; Verschueren 1983). Additional distribution studies would provide information regarding the tissue doses associated with adverse effects.

The metabolism of hexachlorobenzene has not been studied in humans. Studies in monkeys and rats indicate that hexachlorobenzene is metabolized to less chlorinated benzenes, chlorinated phenols, other minor metabolites, and glucuronide and glutathione conjugates (Ingebrigtsen and Nafstad 1983; Ingebrigtsen et al. 1981, 1986; Jansson and Bergman 1978; Koss et al. 1986; Rozman et al. 1977a). Because differences in metabolism may occur with differences in the route of exposure, it would be useful to have more data on inhalation and dermal metabolic studies as a comparison with the available oral studies.

No studies were located regarding excretion of hexachlorobenzene in animals or humans following inhalation or dermal exposure. Oral studies in animals indicate that the parent hexachlorobenzene is excreted primarily in feces, while metabolites were detected in urine (Albro and Thomas 1974; Ingebrigtsen et al. 1981; Mehendale et al. 1975; Rozman et al. 1977a, 1981; To-Figueras et al. 1991). Studies on excretion following inhalation and dermal exposure to hexachlorobenznene would be useful to determine if excretion patterns vary with different routes.

PBPK models for hexachlorobenzene have been developed by Yesair et al. (1986) and Freeman et al. (1989). The Yesair et al. (1986) model describes the absorption, distribution, and elimination of ingested hexachlorobenzene in growing rats and humans. The Freeman et al. (1989) model describes distribution and excretion of intravenously injected hexachlorobenzene in growing rats. Additional PBPK models to extrapolate from high- to low-exposure and between routes of exposure would aid in risk analysis.

Comparative Toxicokinetics.   Although no toxicokinetic information is available for humans following dermal or inhalation exposure, data from a rat study were used to develop a compartment model for application to a 70-kg worker (Koizumi 1991).

Overall, data in animal studies do not indicate the toxicokinetics of hexachlorobenzene are similar among species (Albro and Thomas 1974; Cripps 1990; Goldey et al. 1990; Iatropoulos et al. 1975; Koizumi 1991; Koss et al. 1986; Rozman et al. 1977a; others). Differences observed in absorption may be related to vehicle and route of administration (Iatropoulos et al. 1975; Ingebrigtsen and Nafstad 1983; Koss and Koransky 1975; Lecavalier et al. 1994; Sundlof et al. 1982; others), and no remarkable differences have

been seen for distribution, metabolism, or excretion (Cripps 1990; Ingebrigtsen and Nafstad 1983; Jarrell et al. 1993; Mehendale et al. 1975; Verschueren 1983; others).

PBPK models for hexachlorobenzene have been developed for rats to describe the absorption, distribution, and elimination of ingested hexachlorobenzene, but were not considered appropriate for inter-species extrapolations (Freeman et al. 1989; Yesair et al. 1986). One of the models was adapted for human modeling by using the same model structure with human physiological parameter values (Yesair et al. 1986). Although no empirical data are available on the dermal absorption of hexachlorobenzene in humans, dermal data from a rat study were used to develop a compartment model for application to a 70-kg worker (Koizumi 1991).

Further development of a human toxicokinetic model would be valuable in assessing risks to human health from hexachlorobenzene exposure.

Methods for Reducing Toxic Effects.   Although available poison-treatment recommendations provide some guidance for reducing the toxic effects of absorbed hexachlorobenzene through inhalation, oral, or dermal exposures, these recommendations are not specific to hexachlorobenzene. Recommendations to reduce peak absorption following exposure include such general procedures as moving the individual to fresh air following inhalation exposure, emesis and gastric lavage with activated charcoal following oral exposure, and removal of contaminated clothing and washing of the skin following dermal exposure. Little can be done to reduce body burden of hexachlorobenzene. Treatments that interfere with the mechanism of action for toxicity or repair tissue damage have not been developed specifically for hexachlorobenzene. However, some general recommendations are available, such as use of diazepam or phenobarbital to control convulsions related to hexachlorobenzene exposure. Development of methods for reducing toxic effects targeted specifically to hexachlorobenzene would be useful, and additional studies into the mechanisms of action would support this goal.

Children's Susceptibility.   No data regarding children's susceptibility following dermal or inhalation exposure to hexachlorobenzene were identified. The available human data suggest that infants and young children are at increased risk from exposure to hexachlorobenzene compared to adults (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982, 1987). Studies of an orally exposed population reported 95% mortality in exposed infants (under 2 years of age) associated with dermal lesions; adolescents (between the ages of 6 and 15 years) exhibited health effects (including 10% mortality and dermal lesions) more frequently than adults (Cripps et al. 1984; Gocmen et al. 1989; Peters et al. 1982,

1987). Other studies focused on children's health found suggestive evidence of neurological and immunological effects, but did not assess exposure (Belles-Isles et al. 2000; Darvill et al. 2000; Dewailly et al. 2000; Hosie et al. 2000; Sala et al. 1999). Although immunological effects have been seen in humans exposed as adults (Richter et al. 1994; Queiroz et al. 1997, 1998a, 1998b), neurological effects have not; therefore, children may be more susceptible than adults to the neurotoxicity of hexachlorobenzene.

No animal studies relevant to children's susceptibility following dermal or inhalation exposure were identified. Animal studies have confirmed that hexachlorobenzene is transferred to the developing organism through the placenta *in utero* and via lactation after birth, and that the developing animals exhibited signs of toxicity (such as reduced survival and anatomical abnormalities) not seen in parental animals at the same exposure levels (Arnold et al. 1985; Bailey et al. 1980; Courtney et al. 1979; Grant et al. 1977; Iatropoulos et al. 1978; Khera 1974a; Kitchin et al. 1982; others). These experiments suggest that the ability of hexachlorobenzene to sensitively affect the developing organism may be related to its demonstrated capabilities to mediate toxicity through the neuroendocrine axis (Alvarez et al. 2000; Andrews et al. 1988, 1990; Den Besten et al. 1993; Den Tonkelaar et al. 1978; Foster et al. 1992b, 1993, 1995a, 1995b; Kimbrough and Linder 1974; Kleiman de Pisarev et al. 1989, 1990). However, endocrine end points have not been monitored in developing organisms. A developmental study that included assessment of endocrine end points along with other sensitive end points such as neurobehavioral and immune function would be useful to determine the role of endocrine changes with regard to these effects, and would identify critical levels of effect.

No data were located concerning whether pharmacokinetics of hexachlorobenzene in children are different from adults. A PBPK model (Yesair et al. 1986) has modeled fetal and breast milk compartments, in humans as well as rats, for oral exposure to hexachlorobenzene. Two PBPK models (Freeman et al. 1989; Yesair et al. 1986) have characterized the pharmacokinetics of hexachlorobenzene in growing rats, and have been validated using experimental data. No information regarding biomarkers of exposure and effect or potential interactions of hexachlorobenzene with other chemicals pertinent to children's susceptibility were identified. There are no pediatric-specific methods to reduce peak absorption of hexachlorobenzene following exposure, or to reduce body burden, or to interfere with mechanisms of action for hexachlorobenzene.

Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

Child health data needs relating to exposure are discussed in 6.8.1 Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Ongoing studies pertaining to hexachlorobenzene were located in FEDRIP 2001.

The Toxic Substances Research Initiative of the Government of Canada is sponsoring a study titled *Risk assessment for hexachlorobenzene: mechanism of gender related tumor promotion* for fiscal years 1999–2002 by researchers Michel Charbonneau and Daniel Cyr at the Universite du Quebec.

National Institute of Environmental Science (NIEHS) is sponsoring a study for fiscal years 1996–2002 by researcher Gayle Windham at the Public Health Institute in Berkeley, California to investigate whether there is a relationship between exposure to chlorinated chemicals, which mediate toxicity through the neuroendocrine axis in animals, and menstrual cycle disturbances in women in a Laotian immigrant population in the San Francisco Bay area.

NIEHS is sponsoring a study for fiscal years 1996–2002 by researcher Stephen Schwartz at the Fred Hutchinson Cancer Research Center in Seattle, Washington to examine the potential relationship between exposure to organochlorine compounds and development of uterine fibroids in a case-control study.

NIEHS is also sponsoring a study for fiscal years 2001–2005 by researcher Stephen Schwartz to investigate the potential relationship between exposure to organochlorine compounds and the development of testicular germ cell carcinoma in a case-control study.

NIEHS is sponsoring a program for fiscal year 2001 by researcher Maria Schymura at the State University of New York in Albany to monitor organochlorine exposure in a Native American population in the state of New York.

NIEHS is sponsoring a study for fiscal years 2000–2005 by researcher Lawrence Schell at the State University of New York at Albany in Albany, New York, to investigate whether there is a relationship among exposure to environmental pollutants, thyroid function, and psychosocial outcomes in a Native American population in the state of New York.