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 hexachlorobenzene. 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 hexachlorobenzene 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.

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 (thermal byproducts of which include hexachlorobenzene and octachlorostyrene) 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 (93.4±223.3 ng/mL) than in non-factory workers (16.9±17.1 ng/mL). However, there were no cases of clinical porphyria cutanea tarda in either group, no evidence that preclinical porphyria was more prevalent in the factory workers than in other residents, and no 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.

Sunyer et al. (2002) determined the urinary porphyrin profile among the most highly hexachlorobenzene-exposed residents of Flix (n=241). Concentrations of coproporphyrins I and III decreased with increasing hexachlorobenzene concentration ($p<0.05$) independent of age, alcohol consumption, smoking, or exposure to other organochlorine compounds. No significant association was found between excretion of uroporphyrin I, uroporphyrin III, or heptaporphyrin and blood hexachlorobenzene concentration. Sunyer et al. (2008) determined the urinary porphyrin profile among 68 neonates from Flix and surrounding towns who provided urine samples on the third day of life for porphyrin assessment; urine and blood samples were successfully analyzed from 52 of these children at 4 years of age as well. In the 4-year-old children, quantitative porphyrin excretion was within normal values, but total porphyrins, coproporphyrin I, and coproporphyrin III (adjusted to creatinine excretion) increased with increasing levels of hexachlorobenzene independent of breastfeeding and of organochlorine and porphyrin levels at

birth. The increase of urinary coproporphyrins was considered suggestive of an incipient toxic effect on the hepatic heme pathway.

Ozalla et al. (2002) collected maternal serum samples and cord blood and neonatal urine samples on day 3 after birth from full-term singleton neonates born in Flix and compared them with samples from unexposed mothers and neonates from neighboring villages. Detectable hexachlorobenzene concentrations were found in all samples of fetal cord blood and maternal serum, but the exposed population from Flix was statistically higher ($p<0.05$). There was no positive relationship between urinary porphyrin excretion and hexachlorobenzene levels in maternal serum or cord blood, but heptaporphyrin isomer III was detected significantly ($p<0.012$) more in the exposed group compared with the unexposed group, and neonates in the highest tertile of hexachlorobenzene had lower levels of coproporphyrin I ($p<0.05$).

The NOAEL for liver effects in the Flix residents is 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 inhaled 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.

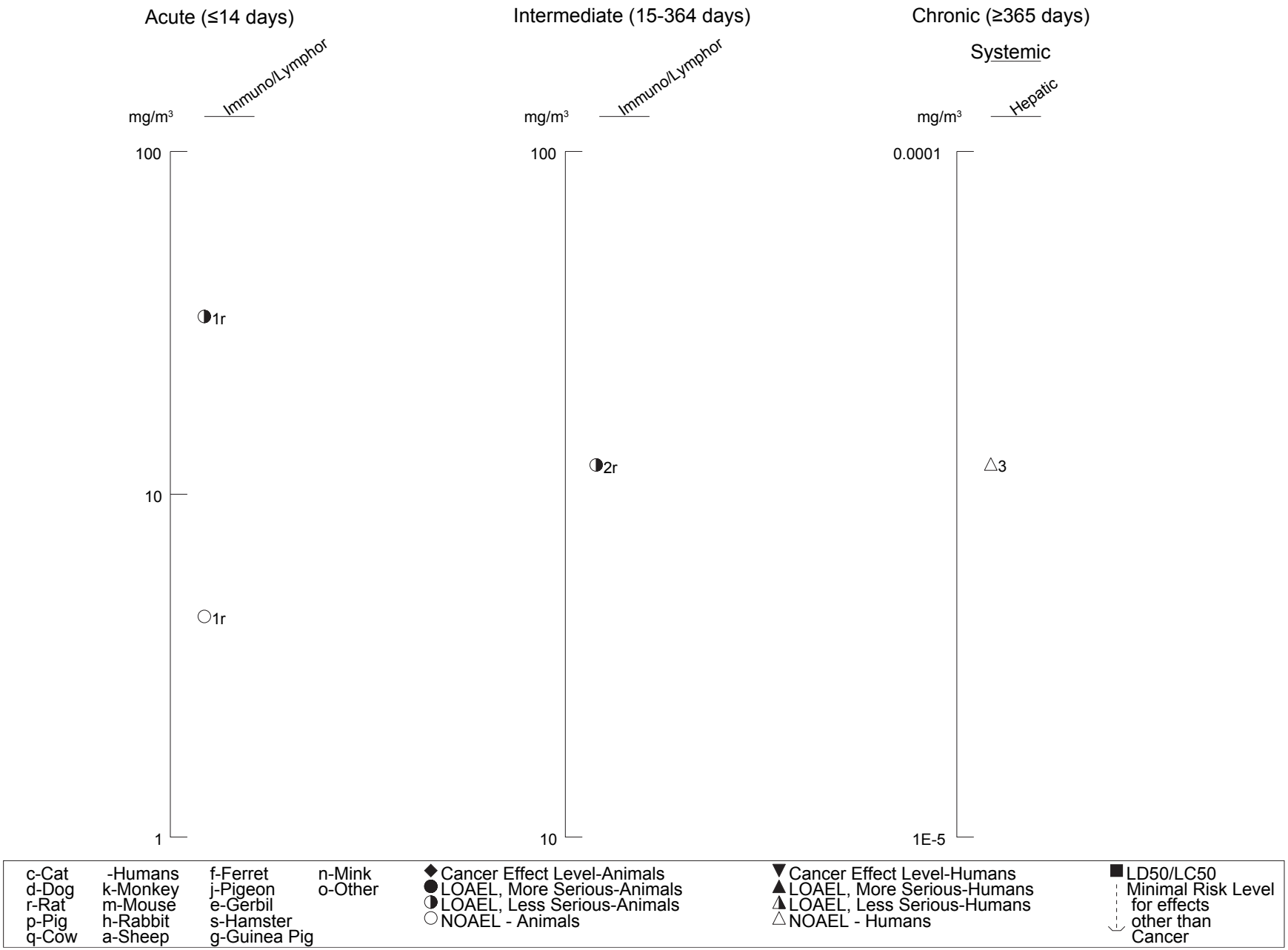
Table 3-1 Levels of Significant Exposure to Hexachlorobenzene - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Immuno/ Lymphoret								
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	
INTERMEDIATE EXPOSURE								
Immuno/ Lymphoret								
2	Rat	4 wk 4 d/wk 4 hr/d			35 M (slight impairment of pulmonary immune defense)		Sherwood et al. 1989	
CHRONIC EXPOSURE								
Systemic								
3	Human	40 yr (Occup)	Hepatic	0.000035			Herrero et al. 1999 HCB	

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

d = day(s); hr = hour(s); Immuno/Lymphoret = immunological/lymphoreticular; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; Occup = occupational; wk = week(s); yr = year(s)

Figure 3-1 Levels of Significant Exposure to Hexachlorobenzene - Inhalation



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, 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). Ribas-Fitó et al. (2003b) assessed possible associations between prenatal exposure to organochlorine compounds, including hexachlorobenzene, and thyroid status in newborns (a total of 98 mother-infant pairs) born during 1997–1999 in the Flix area. Concentrations of TSH were measured in plasma from the newborns 3 days after birth. TSH concentrations were all within the normal range. There was no significant association between blood TSH and hexachlorobenzene levels.

Álvarez-Pedrerol and coworkers assessed possible associations between cord serum organochlorine levels (including hexachlorobenzene) and thyroid status in 387 newborns exposed prenatally (Álvarez-Pedrerol et al. 2008a) and between serum organochlorine levels and thyroid status in 259 children 4 years of age (Álvarez-Pedrerol et al. 2008b). The newborns and 4-year-old children were selected from the general population on the Spanish island of Menorca, which is not in the vicinity of chemical facilities that produce organochlorine compounds. Although measurable hexachlorobenzene levels were observed in the newborns and the 4-year-old children, there were no significant associations between cord blood hexachlorobenzene levels and serum TSH levels in the newborns or serum hexachlorobenzene and serum thyroid hormones (free T4, total triiodothyronine [T3], TSH) in the 4-year-old children.

No studies were located regarding endocrine effects of inhaled hexachlorobenzene in animals.

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, although dermal exposure was possible as well. 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.

No studies were located regarding the neurological effects of inhaled hexachlorobenzene in animals.

Neurodevelopmental effects are discussed in Section 3.2.1.6 (Developmental Effects).

3.2.1.5 Reproductive Effects

No studies were located regarding the reproductive effects of inhaled hexachlorobenzene in humans or animals.

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), a village with unusually high atmospheric levels of hexachlorobenzene), and female residents who had never worked at the factory (n=719–936), despite 5-fold higher blood hexachlorobenzene levels in the workers (Sala et al. 1999b). The small number of women factory workers (n=46–60 for the different end points) is a limitation of this study. A possible association between prenatal exposure to hexachlorobenzene and alterations in anthropometric measures (prematurity, small length for gestational age, crown-heel length) was examined among 70 infants whose

mothers lived in Flix or in neighboring towns (Ribas-Fitó et al. 2002). Cord serum levels of hexachlorobenzene and other polychlorinated hydrocarbons were measured in newborns born between 1997 and 1999. The 50th percentile concentration of hexachlorobenzene was 1.13 ng/mL. Cord serum hexachlorobenzene levels >1.48 ng/mL were significantly ($p<0.05$) correlated with a smaller crown-heel length and small length for gestational age. No significant associations between hexachlorobenzene concentrations and birth weight, head circumference, or prematurity were found. The study was limited by small sample size, as <15 infants were born premature and/or had a small weight or small length for gestational age.

The effect of prenatal exposure to hexachlorobenzene on social behavior was examined in two cohorts of 4-year-old children for whom cord serum organochlorines (including hexachlorobenzene) had been measured at birth (Ribas-Fitó et al. 2007). One cohort (n=70) was from the Ribera d'Ebre area of Spain, which encompasses the village of Flix where unusually high atmospheric levels of hexachlorobenzene (as high as 35 μg/m^3) in the proximity of an electrochemical factory have been measured. The other cohort (n=405) was from the Spanish island of Menorca, which is not in the vicinity of organochlorine-producing facilities. The ranges of hexachlorobenzene concentrations in cord blood were 0.17–5.77 ng/mL (median of 1.13 ng/mL) for the cohort from the Flix, Spain area, and 0.14–9.82 ng/mL (median of 0.68 ng/mL) for the cohort from Menorca. Behavioral assessments were conducted at 4 years of age using several tests of social competence. For both cohorts, subjects with hexachlorobenzene levels ≥1.5 ng/mL at birth had statistically significant increased risk of having poor social competence (adjusted relative risk of 4.04; 95% confidence interval [CI] 1.79, 9.58) and attention deficit hyperactivity disorder (adjusted relative risk of 2.71; 95% CI 1.05, 6.96). Adjustments for exposure to other organochlorine compounds such as polychlorinated biphenyls (PCBs), *p,p'*-DDE, and *p,p'*-DDT did not change the results, and no significant associations were seen between serum levels of these organochlorine compounds at 4 years of age and measures of social behavior.

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.

Álvarez-Pedrerol et al. (2008b) did not find an association between cord serum hexachlorobenzene levels and plasma thyrotropin concentration in 387 newborns (3 days old) born on the Spanish island of Menorca. Several organochlorines in addition to hexachlorobenzene were measured in the cord serum of the newborns, including seven PCB congeners, beta-hexachlorocyclohexane, *p,p'*-DDE, and *p,p'*-DDT. Only five children had serum thyrotropin levels above the detection limit (10 mU/L).

No studies were located regarding the developmental effects of inhaled hexachlorobenzene in animals.

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 hexachlorobenzene 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 hexachlorobenzene 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 hexachlorobenzene 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 3.2.2.7).

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 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 dose of 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 control 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). Sala et al. (1999a) found higher hexachlorobenzene levels in capillary blood of infants in Flix and nearby villages than in cord blood or maternal blood; levels were higher in breastfed infants than bottle-fed infants. A report on an epidemiological study in New South Wales, Australia, which produced 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).

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 205 mg/kg/day in most (the exception is discussed below) intermediate-duration feeding studies (Cantoni et al. 1990; 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; Smith et al. 1987) and from 6 to 16 mg/kg/day in chronic oral studies (Cabral et al. 1977, 1979; Gralla et al. 1977). 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. 1977; Ockner and Schmid 1961).

Aside from duration of exposure, other factors that appear to influence susceptibility to hexachlorobenzene-induced mortality include species, strain, sex, age, pregnancy status, diet, nutritional status (fasting versus 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 526, 976, 385, and 161 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 hexachlorobenzene; 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. 1977; 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 15.7 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 (19–205 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 hexachlorobenzene was added either in corn oil or as crystalline chemical. At 1,000 ppm (estimated dose of 129 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) receiving the same estimated 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 hexachlorobenzene 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 lesions, typically occurring together, were found in six different strains of rats and both sexes, at doses as low as 5.9–46 mg/kg/day in intermediate-duration feeding studies (Goldstein et al. 1978; Kimbrough and Linder 1974; Kitchin et al. 1982; Michielsen et al. 1997). They were seen at doses as low as 0.4 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).

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
ACUTE EXPOSURE								
Death								
1	Mouse (NS)	8-10 d (F)				976 F (death)	de Matteis et al. 1961 HCB	
2	Gn Pig (NS)	8-10 d (F)				385 F (death)	de Matteis et al. 1961 HCB	
Systemic								
3	Rat (Wistar)	1 or 2 wk 5 d/wk 1 x/d (G)	Hepatic		1000 F (increased porphyria, altered hepatic enzyme levels)		Billi de Catabbi et al. 1991	
4	Rat (Wistar)	1, 2, 3, or 4 wk 5 d/wk 1 x/d (GW)	Hepatic		1000 F (increased porphyria, altered hepatic enzyme levels)		Billi de Catabbi et al. 2000a HCB	
5	Rat (Sprague-Dawley)	2 wk 5 d/wk 1 x/d (GO)	Renal		100 M (increased kidney weight, 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 (decreased serum thyroxine)		Foster et al. 1993	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
7	Rat (CD)	1 wk (F)	Hepatic	5 F	16 F (increased hepatic ALA-S activity)		Goldstein et al. 1978	
			Bd Wt	159 F				
8	Rat (Wistar)	1 d (F)	Hepatic		128 F (increased highly carboxylated porphyrins in liver)		Kennedy and Wigfield 1990	
9	Rat (Wistar)	Gd 6-21, 6-16, 6-9 or 10-13 1 x/d (G)	Bd Wt	40 F		80 F (loss of body weight by pregnant rats)	Khera 1974	
10	Rat (Sprague-Dawley)	1 d 2 x/d (G)	Hepatic	700 F	1400 F (increased ornithine decarboxylase activity in the liver)		Kitchin and Brown 1989	
11	Rat (Wistar)	7 d 1 x/d (GW)	Hepatic	250 F	500 F (decreased hepatic URO-D activity)		Kleiman de Pisarev et al. 1990	
			Endocr		250 F (decreased serum T4 levels)			

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
12	Rat (Wistar)	1 wk (GO)	Endocr		1000 F (18% decreased serum T4; 70% decreased serum T3)		Kleiman de Pisarev et al. 1995	
			Bd Wt	1000 F				
13	Rat (Sprague-Dawley)	2-16 d 1 x/d (GO)	Hepatic		25 F (increased urinary porphyrins; increased hepatic porphyrin content)		Krishnan et al. 1991	
14	Rat (Sprague-Dawley)	12 d 1x/d (GO)	Hepatic		50 F (increased hepatic and urinary uroporphyrins)		Krishnan et al. 1992	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
15	Rat (Sprague-Dawley)	once (GO)	Resp	600 F			Lecavalier et al. 1994	
			Cardio	600 F				
			Gastro	600 F				
			Hemato	600 F				
			Musc/skel	600 F				
			Hepatic		400 F (16-18% increased liver weight; cytoplasmic vacuolation, anisokaryosis, and pyknotic hepatocytes)			
			Renal	600 F				
			Endocr		400 F (reduced follicle size and colloidal density, increased epithelial height in the thyroid; reduced cortical and medullary volume in thymus)			
			Dermal	600 F				
			Ocular	600 F				
			Bd Wt	600 F				
16	Rat (Sprague-Dawley)	6 d 1 x/d (GO)	Hepatic		10 M (increased liver weight)		Mehendale et al. 1975	
			Bd Wt	25 M				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
17	Rat (Brown Norway)	7 or 21d (F)	Hepatic		50.8 F (31% increased liver weight)		Michielsen et al. 2001	
			Dermal		50.8 F (skin lesions after 10 days of treatment)			
			Bd Wt	50.8 F				
18	Rat (Brown Norway)	6, 14, or 21 d (F)	Hepatic		50.8 F 33% increased relative liver weight)		Michielsen et al. 2002	
			Dermal		50.8 F (head and neck skin lesions)			
			Bd Wt	50.8 F				
19	Rat (NS)	5 d 1 x/d (GO)	Bd Wt	221 M			Simon et al. 1979	
20	Rat (Wistar)	2 wk 3 d/wk 1 x/d (IP)	Endocr	484 M	740 M (decreased serum total and free T4)		van Raaij et al. 1993a	
			Bd Wt	997 M				
21	Mouse (CD-1)	Gd 7-16 (GO)	Hepatic		100 F (increased dam liver weight)		Courtney et al. 1976	
			Bd Wt	100 F				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
22	Gn Pig (NS)	10 d (F)	Hepatic		385 F (fatty changes in liver)		de Matteis et al. 1961 HCB	
			Renal		385 F (increased blood ammonia levels)			
Immuno/ Lymphoret								
23	Rat (Brown Norway)	7 or 21d (F)			50.8 F (eosinophilic lung inflammation)		Michielsen et al. 2001	
24	Rat (Brown Norway)	6, 14, or 21 d (F)			50.8 F (increases in weights of spleen and lymph nodes, increased in vitro tracheal reactivity to arecoline, eosinophilic lung inflammation)		Michielsen et al. 2002	
Neurological								
25	Rat (Wistar)	Gd 6-21, 6-16, 6-9 or 10-13 1 x/d (G)		40 F		80 F (hyperesthesia, tremors, and convulsions in pregnant rats)	Khera 1974	
26	Rat (Brown Norway)	7 or 21d (F)			50.8 F (tremors starting at treatment day 14)		Michielsen et al. 2001	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
27	Mouse (NS)	8-10 d (F)				976 F (marked weakness, hyperexcitability, tremors, clonic contractions)	de Matteis et al. 1961 HCB	
28	Gn Pig (NS)	8-10 d (F)				385 F (marked weakness, hyperexcitability, tremors, convulsions)	de Matteis et al. 1961 HCB	
Reproductive								
29	Rat (Sprague-Dawley)	5 d 1 x/d (GO)			50 F (increased serum progesterone)		Foster et al. 1993	
30	Rat (NS)	5 d 1 x/d (GO)		70 M	221 M (decreased male impregnation of females)		Simon et al. 1979	
Developmental								
31	Rat (Sprague-Dawley)	4 d 1 x/d (GO)			2.5[b] F hyperactivity in young pups)		Goldey and Taylor 1992	
32	Rat (Wistar)	Gd 6-21, 6-16, 6-9 or 10-13 1 x/d (G)		20 F	40 F (increased incidence of skeletal variations)		Khera 1974	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
33	Mouse (CD-1)	Gd 7-16 (GO)				100 (increased incidence of abnormal fetuses per litter)	Courtney et al. 1976	
INTERMEDIATE EXPOSURE								
Death								
34	Rat (Wistar)	80 d (F)				205 F (16/25 died)	Cantoni et al. 1990	
35	Rat (Wistar)	80 d (F)				205 F (60/90 died)	Cuomo et al. 1991 HCB	
36	Rat (Wistar)	13 wk (F)				19 F (4/9 died)	den Besten et al. 1993	
37	Rat (Sprague-Dawley)	4 gen (F)				15.7 F (1/20 dams died)	Grant et al. 1977	
38	Rat (Wistar)	Up to 56 d (F)				129 F (2/29 died)	Kennedy and Wigfield 1990	
39	Rat (Sherman)	4 mo (F)				50 M (14/20 died) 56.5 F (2/10 died)	Kimbrough and Linder 1974	
40	Rat (Wistar)	15 wk 3-4 d/wk 1 x/d (GO)				50 F (5/19 died)	Koss et al. 1978	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
41	Rat (COBS)	15 wk (F)				32 F (increased mortality in females)	Kuiper-Goodman et al. 1977	
42	Rat (Sprague-Dawley)	56 d (F)				172 M (13/33 died within 1 month)	Ockner and Schmid 1961	
43	Hamster (Golden Syrian)	6, 18, or 28 wk (F)				19 M (2/11 and 8/18 died during 18 and 28 weeks of treatment, respectively)	Smith et al. 1987 HCB	
44	Rabbit (NS)	12 wk (F)				161 F (4/4 died)	de Matteis et al. 1961 HCB	
45	Pig (SPF)	90 d (F)				50 M (5/5 died)	Den Tonkelaar et al. 1978	
Systemic								
46	Monkey (Cynomolgus)	90 d 1 x/d (C)	Hemato	10 F			Foster et al. 1995a HCB	
			Bd Wt	10 F				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
47	Monkey (Rhesus)	60 d 1 x/d (G)	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				
48	Monkey (Cynomolgus)	90 d 1 x/d (C)	Hepatic	0.1 F	1 F (hepatocellular vacuolation, intrahepatic cholestasis)		Jarrell et al. 1993	
			Endocr	1 F	10 F (increased adrenal weight)			
			Bd Wt	10 F				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
49	Monkey (Rhesus)	60 d 1 x/d (GW)	Hemato	128 F			Knauf and Hobson 1979	
			Hepatic	64 F	128 F (increased blood urea nitrogen)			
			Renal	64 F	128 F (increased serum AST)			
			Bd Wt		8 F (unspecified weight loss)			
50	Rat (Wistar)	30 d 1 x/d (GW)	Hepatic		1000 F (increased liver weight)		Alvarez et al. 2000 HCB	
			Bd Wt	1000 F				
51	Rat (Fischer 344)	5 wk 5 d/wk 1 x/day (GO)	Hepatic	0.1 M	1 M (increased liver weight)		Andrews et al. 1988	
			Renal	25 M				
			Endocr		10 M (increased serum 1,25-dihydroxy- vitamin D3 and parathyroid hormone)			
			Bd Wt		0.1 M (9 % depressed body weight)			

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
52	Rat (Fischer 344)	15 wk 5 d/wk 1 x/d (GO)	Musc/skel	0.1 M	1 M (increased femur density)		Andrews et al. 1989	
			Hepatic	1 M	10 M (increased liver weight)			
			Renal	1 M	10 M (increased kidney weight; increased urinary LDH and alkaline phosphatase)			
			Endocr	0.1 M	1 M (increased serum 1,25-dihydroxy vitamin D3)			
			Bd Wt	25 M				
53	Rat (Fischer 344)	15 wk 5 d/wk 1 x/d (GO)	Musc/skel	0.1 M	1 M (increased femur density and cortical area)		Andrews et al. 1990	
			Renal	0.1 M	1 M (increased urinary LDH)			
			Endocr	1 M	10 M (increased serum parathyroid hormone)			

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
54	Rat (Sprague-Dawley)	3 mo prior to mating through weaning of F1 pups (F)	Hemato	3.4 M 3.9 F			Arnold et al. 1985	
			Hepatic	0.14 M 3.9 F	0.69 M (increased liver weight)			
			Renal	3.4 M 3.9 F				
			Bd Wt	3.4 M 3.9 F				
55	Rat (Wistar)	3 or 4 wk 5 d/wk 1 x/d (G)	Hepatic		1000 F (increased porphyria, altered hepatic enzyme levels)		Billi de Catabbi et al. 1991	
56	Rat (Wistar)	1, 2, 3, or 4 wk 5 d/wk 1 x/d	Hepatic		1000 F (increased porphyria, altered hepatic enzyme levels)		Billi de Catabbi et al. 2000a HCB	
57	Rat (Wistar)	1 or 7 wk	Hepatic		1000 F (increased urinary porphyrin excretion)		Billi de Catabbi et al. 2000a HCB	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
58	Rat (Wistar)	1, 2, 4, or 4 wk 5 d/wk 1 x/d (GW)	Hepatic		1000 F (hepatic porphyria changes in hepatic sphingolipid levels)		Billie de Catabbi et al. 2000b	
59	Rat (Chbb)	1, 2, 4, or 4 wk 5 d/wk 1 x/d (GW)	Hepatic		1000 F (hepatic porphyria changes in hepatic sphingolipid levels)		Billie de Catabbi et al. 2000b	
60	Rat (Sprague-Dawley)	7 wk 5 d/wk 1 x/d (GO)	Renal		50 M (increased kidney weight, glucosuria, proteinuria, degenerative and regenerative foci in tubules, accumulation of protein droplets in tubular cells)		Bouthillier et al. 1991	
61	Rat (Wistar)	80 d (F)	Hepatic		205 F (increases in liver weight, hepatic porphyrins, lipid peroxidation; decreased hepatic URO-D)		Cantoni et al. 1990	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
62	Rat (Wistar)	Up to 28 d 1x/d (GO)	Endocr		4 M (decreased free and total T4, increased TSH in serum; increased thyroid weight, histopathological thyroid lesions)		Chalouati et al. 2013 HCB	
			Bd Wt	16 M				
63	Rat (Wistar)	1, 2, 3, 4, or 8 wk 5 d/wk 1 x/d (GW)	Hepatic		1000 F (hepatic porphyria)		Cochon et al. 2001 HCB	
64	Rat (Chbb)	1, 2, 3, 4, or 8 wk 5 d/wk 1 x/d (GW)	Hepatic		1000 F (hepatic porphyria)		Cochon et al. 2001 HCB	
65	Rat (Wistar)	80 d (F)	Hepatic		205 F (porphyria, increased liver weight, fatty degeneration)		Cuomo et al. 1991 HCB	
			Dermal		205 F (photosensitive skin lesions)			
			Bd Wt	205 F				
66	Rat (NS)	NS (F)	Hepatic		526 (porphyria)		de Matteis et al. 1961 HCB	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
67	Rat (Wistar)	13 wk (F)	Hepatic		9.5 F (increased liver weight, increased urinary and liver porphyrins)		den Besten et al. 1993	
			Renal	9.5 F	19 F (increased kidney weight, basophilic renal tubules, protein casts)			
			Endocr	9.5 F	19 F adrenal: increased weight, cortical hypertrophy and hyperplasia; thyroid: decreased serum T4 and T3)			
			Dermal	9.5 F	19 F (skin lesions)			
			Bd Wt	9.5 F		19 F (severe weight loss in 4/9 rats)		
68	Rat (Wistar)	107 d (F)	Hepatic		308 F (marked decrease in hepatic URO-D activity, massive increase in hepatic porphyrin content)		Elder and Urquhart 1986	
69	Rat (Brown Norway)	21 d (F)	Dermal		50.8 F (skin lesions)		Ezendam et al. 2004a	
			Bd Wt	50.8 F				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
70	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 HCB	
71	Rat (Sprague-Dawley)	21 d 1 x/d (GO)	Bd Wt	100 F			Foster et al. 1992b	
72	Rat (Sprague-Dawley)	30 d 1 x/d (GO)	Endocr		1 F (decreased serum corticosterone)		Foster et al. 1995b	
			Bd Wt	100 F				
73	Rat (CD)	4 mo (F)	Resp	4 F	12 F (hypertrophy and proliferation of endothelial cells, increased macrophages)		Goldstein et al. 1978	
			Cardio	36 F				
			Hepatic	4 F	12 F (increased urinary and hepatic porphyrins, enlarged hepatocytes)			
			Endocr	36 F				
			Dermal	12 F	36 F (small sores)			
			Bd Wt	36 F				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
74	Rat (Fischer- 344)	5 wk (GO)	Hepatic		28 M (liver enlargement, porphyria, increased enzyme expression, increased protooncogene expression)		Gustafson et al. 2000 HCB	
75	Rat (Sprague-Dawley)	4 wks 1 x/d (GO)	Endocr	0.16 M	4 M (decreased plasma total T4)		Hadjab et al. 2004 HCB	
76	Rat (Wistar)	56 d (F)	Hepatic		12.9 F (increased highly carboxylated porphyrins in liver)		Kennedy and Wigfield 1990	
			Renal		12.9 F (increased highly carboxylated porphyrins in kidney)			
			Bd Wt	12.9 F		129 F (large decrease in body weight gain)		

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
77	Rat (Sherman)	4 mo (F)	Resp		10 M (increased macrophages, focal areas of fibrosis)	56.5 F (extensive intra-alveolar hemorrhage, inflammation, and edema, accompanied by an increase in lung weight)	Kimbrough and Linder 1974	
					11.3 F (increased macrophages, focal areas of fibrosis)			
			Cardio	10 M		50 M (fibrosis, degeneration)		
				11.3 F		56.5 F (fibrosis, degeneration)		
			Hemato		10 M (decreased hemoglobin and hematocrit)			
					11.3 F (decreased hemoglobin and hematocrit)			
			Hepatic		10 M (increased liver weight, enlarged hepatocytes)	50 M (necrosis, fibrosis)		
						56.5 F (necrosis, fibrosis)		
					11.3 F (increased liver weight, enlarged hepatocytes)			
			Renal	10 M	50 M (increased kidney weight)			
				11.3 F	56.5 F (increased kidney weight)			
			Endocr		10 M (hyperplasia of the adrenal cortex)			
					11.3 F (hyperplasia of the adrenal cortex)			
			Dermal	10 M	50 M (skin eruptions)			
				11.3 F	56.5 F (skin eruptions)			
			Bd Wt	10 M	50 M (decreased body weight gain in males)			
				112.9 F				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
78	Rat (Fischer- 344)	5 wk (F)	Hepatic		4.5 M (increased liver weight, centrilobular hypertrophy, foci of GST-P)		Kishima et al. 2000 HCB	
79	Rat (Sprague-Dawley)	1 gen (F)	Resp		5.9 F (intraalveolar foamy histiocytes, hypertrophy and proliferation of endothelial cells of pulmonary venules in dams)		Kitchin et al. 1982	
			Bd Wt	13.7 F				
80	Rat (Wistar)	4 wk 1 x/d (GW)	Endocr		1000 F (decreased serum T4, increased T4 metabolism, increased serum TSH, increased thyroid iodine uptake)		Kleiman de Pisarev et al. 1989	
			Bd Wt	1000 F				
81	Rat (Wistar)	30 d 1 x/d (GW)	Endocr	125 F	500 F (decreased serum T4 level)		Kleiman de Pisarev et al. 1989	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
82	Rat (Wistar)	8 wk 7 d/wk 1 x/d (GW)	Hepatic		1000 F (increased liver weight, decreased URO-D activity, increased hepatic and urinary porphyrins, increased dehalogenation of T4)		Kleiman de Pisarev et al. 1990	
			Endocr		1000 F (decreased serum T4, increased serum TSH)			
			Bd Wt	1000 F				
83	Rat (Wistar)	4 wk 7 d/wk 1 x/d (GW)	Hepatic		1000 F (increased liver weight)		Kleiman de Pisarev et al. 1995	
			Endocr		1000 F (decreased serum T4; increased serum TSH)			
			Bd Wt	1000 F				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
84	Rat (Wistar)	15 wk 3-4 d/wk 1 x/d (GO)	Resp	50 F			Koss et al. 1978	
			Cardio	50 F				
			Hepatic		50 F (increased liver weight, massive increased liver and urine porphyrins)			
			Renal		50 F (increased kidney weight)			
			Endocr		50 F (increased adrenal weight)			
			Dermal		50 F (rough appearance of fur, hair loss, skin lesions)			
			Bd Wt	50 F				
85	Rat (Sprague-Dawley)	3-6 wk 5 d/wk 1 x/d (GO)	Hepatic		50 F (increased urinary and hepatic porphyrins)		Krishnan et al. 1991	
86	Rat (Sprague-Dawley)	6 wk 5 d/wk 1 x/d (GO)	Hepatic		50 F (increased 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 (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
87	Rat (COBS)	15 wk (F)	Hemato	8 F	32 F (decreased red blood cell count, hematocrit, and hemoglobin; leukocytosis in females)		Kuiper-Goodman et al. 1977	
			Musc/skel	32				
			Hepatic	0.5	2 (slight basophilic clumping without hepatocyte enlargement)			
			Renal	8	32 (increased kidney weight)			
			Dermal	8	32 (alopecia)			
			Bd Wt	8 M	32 M (13% decreased terminal body weight)			
88	Rat (Sprague-Dawley)	13 wk 5 d/wk 1 x/d (GO)	Musc/skel	0.3 F	1 F (minimal incisor degeneration)		Long et al. 2004	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
89	Rat (Wistar)	30 d (F)	Resp		46 F (cuboidal venule endothelial cells, perivascular infiltrate, accumulation of alveolar macrophages)		Michielsen et al. 1997 HCB	
			Hepatic		46 F (increased liver weight, hepatocyte hypertrophy with cytoplasmic inclusions)			
			Renal	92 F				
			Dermal		46 F (gross skin lesions, epidermal hyperplasia, inflammatory infiltrate in the dermis)			
			Bd Wt	92 F				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
90	Rat (Lewis)	29 d (F)	Resp		17 F (cuboidal venule endothelial cells, perivascular infiltrate, accumulation of alveolar macrophages)		Michielsen et al. 1997 HCB	
			Hepatic		17 F (increased liver weight, hepatocyte hypertrophy with cytoplasmic inclusions)			
			Renal	51 F				
			Dermal		17 F (gross skin lesions, epidermal hyperplasia, inflammatory infiltrate in the dermis)			
			Bd Wt	51 F				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
91	Rat (Brown Norway)	28 d (F)	Resp		17 F (cuboidal venule endothelial cells, perivascular infiltrate, accumulation of alveolar macrophages)		Michielsen et al. 1997 HCB	
			Hepatic		17 F (increased liver weight, hepatocyte hypertrophy with cytoplasmic inclusions)			
			Renal	102 F				
			Dermal		17 F (gross skin lesions, epidermal hyperplasia, inflammatory infiltrate in the dermis)			
			Bd Wt	51 F		102 F (sudden weight loss)		
92	Rat (Brown Norway)	4 wk (F)	Resp	50.8 F			Michielsen et al. 2000 HCB	
			Hepatic		50.8 F (increased liver weight)			
			Dermal		50.8 F (dermal lesions-hyperplasia, deep venules with activated endothelium, inflammation)			

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
93	Rat (Brown Norway)	7 or 21d (F)	Hepatic		50.8 F (86% increased relative liver weight)		Michielsen et al. 2001	
			Dermal		50.8 F (skin lesions after 10-14 days of treatment)			
			Bd Wt	50.8 F				
94	Rat (Brown Norway)	6, 14, or 21 d (F)	Hepatic		50.8 F (78% increased relative liver weight)		Michielsen et al. 2002	
			Dermal		50.8 F (head and neck skin lesions)			
			Bd Wt	50.8 F				
95	Rat (Wistar)	60 d (F)	Hepatic		427 M (increased serum ALT, bilirubin, and glutamate dehydrogenase)		Nikolaev et al. 1986	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
96	Rat (Sprague-Dawley)	90d 5d/wk 1x/d (GO)	Resp	1 F	3 F (chronic pulmonary inflammation)		NTP 2002 HCB	
			Hepatic	3 F	10 F (hepatocellular hypertrophy)			
			Dermal	10 F	25 F (skin inflammation and ulceration)			
			Bd Wt	25 F				
97	Rat (Sprague-Dawley)	56 d (F)	Hemato	172 M			Ockner and Schmid 1961	
			Musc/skel		172 M (porphyrin accumulation in cortex of long bones)			
			Hepatic		172 M (porphyria, hepatomegaly, hepatocellular degeneration)			
98	Rat (Wistar)	13 weeks (F)	Dermal		15.4 F (skin lesions)		Schielen et al. 1995a	
			Bd Wt	30.8 F				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
99	Rat (Wistar)	3 wk (F)	Dermal		92 M (skin lesions) 103 F (skin lesions)		Schielen et al. 1995b	
			Bd Wt	92 M 103 F				
100	Rat (Porton-Wistar)	112 d (F)	Hepatic	10 F			Smith et al. 1979	
101	Rat (Agus)	112 d (F)	Hepatic		10 F (increased hepatic porphyrin content, decreased hepatic URO-D activity, increased hepatic ALA-S activity)		Smith et al. 1979	
102	Rat (Fischer 344)	15 wk (F)	Hepatic		20 M (increased liver weight and hepatocellular hypertrophy) 22.6 F (increases in liver weight, hepatocellular hypertrophy, and hepatic porphyrin)		Smith et al. 1985	
			Renal		22.6 F (increased renal porphyrin level)			

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
103	Rat (Wistar)	60 d 1 x/d (GW)	Hepatic		1000 F (decreased URO-D activity in liver)		Sopena de Kracoff et al. 1994	
			Endocr		1000 F decreased serum T4, increased serum TSH			
104	Rat (Fisher CD)	6 wk (F)	Hepatic			226 F (severe porphyria)	Sweeney et al. 1986	
105	Rat (Wistar)	6 wk (F)	Hepatic	13.8 M	41.5 M (increased liver weight)		van Loveren et al. 1990 HCB	
106	Rat (Wistar)	4 wk 3 d/wk 1 x/d (GW)	Endocr		997 M (reduced serum total and free TT4 and FT4 levels; increased serum TSH)		van Raaij et al. 1993a	
			Bd Wt	997 M				
107	Rat (Wistar)	4 wk 3 d/wk 1 x/d (GW)	Hepatic		1000 M (increased liver weight)		van Raaij et al. 1993b	
			Endocr		1000 M (decreased serum T4 level)			

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
108	Rat (Wistar)	gestation + lactation + 2 wks post-weaning (F)	Hepatic	5.1	15.4 (increased liver weight)		Vos et al. 1979a	
			Endocr	5.1	15.4 (increased adrenal gland weight)			
109	Rat (Wistar)	3 wk (F)	Hemato		46 M (increased extramedullary hematopoiesis in spleen; neutrophilia)		Vos et al. 1979b	
			Hepatic		46 M (increased liver weight, liver cell hypertrophy and cytoplasmic hyalinization)			
			Renal	184 M				
			Endocr	184 M				
			Bd Wt	184 M				
110	Rat (BD VI)	5 wk 5 d/wk 1 x/d (GW)	Hepatic		1000 decreased URO-D activity in males and females; increased hepatic and urinary porphyrins in females)		Wainstok de Calmanovici et al. 1991	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
111	Rat (Sprague-Dawley)	2 gen (GO)	Hepatic	2.5	12.5 25-39% increased relative liver weight in males and females, hepatocellular degeneration and fatty changes in males)		Wolfe and Pepperl 2005 HCB	
			Renal	2.5 M 12.5 F	12.5 M (22% increased relative kidney weight)			
			Bd Wt	12.5				
112	Mouse (BALB/c)	6 wk (F)	Resp	30 M			Loose et al. 1977	
			Hepatic		30 M (increased liver weight, hepatocellular hypertrophy)			
			Bd Wt	30 M				
113	Mouse (C57BL/ 10ScSn)	7 wk (F)	Hepatic		36 M (increased hepatic porphyrin levels)		Vincent et al. 1989	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
114	Hamster (Golden Syrian)	6, 18, or 28 wk (F)	Hepatic			19 M (2.5 to 2.7-fold increased liver weight; extensive hepatocellular hypertrophy and necrosis)	Smith et al. 1987 HCB	
			Endocr			19 M (3-fold decreased thyroid weight; 2.5 to 2.7-fold depressed serum T3)		
			Bd Wt		19 M (18-22% lower terminal body weight)			
115	Dog (Beagle)	21 d 1 or 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 Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
116	Rabbit (NS)	12 wk (F)	Hemato	161 F			de Matteis et al. 1961 HCB	
			Musc/skel			161 F (necrosis, degeneration, and focal calcification in muscle, increased porphyrins in bone)		
			Hepatic			161 F (increased urinary and hepatic porphyrins, fatty change, necrosis)		
			Bd Wt			161 F (weight loss)		
117	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 (increased kidney weight)	50 M (degeneration of proximal tubules and loop of Henle)		
			Endocr	0.5 M	5 M (increased thyroid weight)			
			Bd Wt	5 M		50 M (seriously depressed growth)		

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Immuno/ Lymphoret								
118	Monkey (Rhesus)	60 d 1 x/d (G)			8 F (thymic cortical atrophy)		Iatropoulos et al. 1976	
119	Rat (Brown Norway)	21 d (F)			50.8 F (increased weight of spleen and auricular lymph nodes, increased serum IgE and IgM, increased numbers of splenic T-cells and auricular lymph node B-cells, histopathologic lesions in lung and spleen)		Ezendam et al. 2004a	
120	Rat (Wistar)	56 d (F)		12.9 F	129 F (88% increased spleen weight; increased highly carboxylated porphyrins in spleen)		Kennedy and Wigfield 1990	
121	Rat (Sherman)	4 mo (F)		10 M 11.3 F	50 M (increased white blood cell count, increased spleen weight) 56.5 F (increased white blood cell count, increased spleen weight)		Kimbrough and Linder 1974	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
122	Rat (Wistar)	15 wk 3-4 d/wk 1 x/d (GO)			50 F (increased spleen weight)		Koss et al. 1978	
123	Rat (COBS)	15 wk (F)		8	32 (increased spleen weight in males and females, increased lymphocyte count and congestive splenomegaly in females)		Kuiper-Goodman et al. 1977	
124	Rat (Wistar)	30 d (F)			46 F (increased spleen weight, “high endothelial” venules in popliteal lymph node, increased serum IgM)		Michielsen et al. 1997 HCB	
125	Rat (Lewis)	29 d (F)			17 F (“high endothelial” venules in popliteal lymph node)		Michielsen et al. 1997 HCB	
126	Rat (Brown Norway)	28 d (F)			17 F (increased spleen weight, “high endothelial” venules in popliteal lymph node; increased serum IgM autoantibodies)		Michielsen et al. 1997 HCB	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
127	Rat (Brown Norway)	4 wk (F)			50.8 F (increased spleen weight)		Michielsen et al. 2000 HCB	
128	Rat (Brown Norway)	7 or 21d (F)			50.8 F (increased spleen weight)		Michielsen et al. 2001	
129	Rat (Brown Norway)	6, 14, or 21 d (F)			50.8 F (increases in weights of spleen and lymph nodes, increased in vitro tracheal reactivity to arecoline, eosinophilic and granulomatous lung inflammation)		Michielsen et al. 2002	
130	Rat (Sprague-Dawley)	90d 5d/wk 1x/d (GO)		3 F	10 F (splenic lymphoid hyperplasia)		NTP 2002 HCB	
131	Rat (Wistar)	3 wk (F)			46 M (increased IgM autoantibodies)		Schielen et al. 1993	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
132	Rat (Wistar)	13 weeks (F)			15.4 F (increases in spleen weight, total serum IgM, serum IgM autoantibodies)		Schielen et al. 1995a	
133	Rat (Wistar)	3 wk (F)			92 M (increased spleen and lymph node weight, altered size distribution of splenocytes, selective activation of splenic B-1 cells) 103 F (increased spleen and lymph node weight, altered size distribution of splenocytes, selective activation of splenic B-1 cells)		Schielen et al. 1995b	
134	Rat (Wistar)	6 wk (F)			13.8 M (decreased NK cell activity in lung)		van Loveren et al. 1990 HCB	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
135	Rat (Wistar)	3 wk (F)			46 M (increased weight and proliferation of high-endothelial venules in lymph nodes, enlarged white pulp [marginal zones and follicles] in spleen, increased neutrophil count)		Vos et al. 1979b	
136	Mouse (Balb/C, nude)	6 wk (F)				30.1 M (increased susceptibility to hepatitis infection) 32.6 F (increased susceptibility to hepatitis infection)	Carthew et al. 1990	
137	Mouse (BALB/c)	6 wk (F)			30 M (immunosuppression, decreased antibody production)		Loose et al. 1977	
138	Mouse (Balb/c)	3 or 6 wk (F)			30 M (increased susceptibility to bacterial endotoxin and protozoan infection)		Loose et al. 1978	
139	Mouse (BALB/c)	18 wk (F)		30 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 (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
140	Mouse (BALB/c)	18 wk (F)			0.9 M (increased susceptibility to tumor challenge in vivo, decreased killing of tumor cells in vitro, reduced spleen cell cytocidal activity)		Loose et al. 1981	
141	Mouse (C57BL/6)	40 wk (F)			30 M (decreased graft-host activity)		Silkworth and Loose 1981 HCB	
142	Pig (SPF)	90 d (F)		5 M	50 M (atrophy of lymph nodes)		Den Tonkelaar et al. 1978	
Neurological								
143	Monkey (Rhesus)	60 d 1 x/d (G)		128 F			Iatropoulos et al. 1976	
144	Monkey (Rhesus)	60 d 1 x/d (GW)		32 F		64 F (severe tremors, muscular weakness)	Knauf and Hobson 1979	
145	Rat (Wistar)	1, 2, 3, 4, or 8 wk 5 d/wk 1 x/d (GW)			1000 F (altered phospholipid levels in brain)		Cochon et al. 2001 HCB	
146	Rat (Chhb)	1, 2, 3, 4, or 8 wk 5 d/wk 1 x/d (GW)			1000 F (altered phospholipid levels in brain)		Cochon et al. 2001 HCB	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
147	Rat (Wistar)	80 d (F)				205 F (severe neurotoxicity)	Cuomo et al. 1991 HCB	
148	Rat (NS)	NS (F)				526 (tremors, paresis)	de Matteis et al. 1961 HCB	
149	Rat (CD)	4 mo (F)		12 F		36 F (excessive irritability)	Goldstein et al. 1978	
150	Rat (Sprague-Dawley)	4 gen (F)		7.3 F		14.6 F (convulsions in dams)	Grant et al. 1977 HCB	
151	Rat (Sprague-Dawley)	4 wks 1 x/d (GO)		0.16 M	4 M (significantly increased auditory threshold in the 2-16 kHz sound frequency range)		Hadjab et al. 2004 HCB	
152	Rat (Wistar)	56 d (F)		12.9 F		129 F (lethargy, tremor, convulsions)	Kennedy and Wigfield 1990	
153	Rat (Sherman)	4 mo (F)		10 M 11.3 F		50 M (tremor, hyperexcitability) 56.5 F (tremor, hyperexcitability)	Kimbrough and Linder 1974	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
154	Rat (Wistar)	15 wk 3-4 d/wk 1 x/d (GO)				50 F (muscle fasciculations, tremors)	Koss et al. 1978	
155	Rat (COBS)	15 wk (F)		8		32 (tremors, ataxia, hind limb paralysis)	Kuiper-Goodman et al. 1977	
156	Rat (Brown Norway)	7 or 21d (F)			50.8 F (tremors beginning on treatment day 14)		Michielsen et al. 2001	
157	Rat (Wistar)	60 d (F)				427 M (clonic convulsions, tremors, hyper-excitability)	Nikolaev et al. 1986	
158	Rat (Sprague-Dawley)	56 d (F)				172 M (ataxia, tremor, paralysis)	Ockner and Schmid 1961	
159	Rat (Sprague-Dawley)	20 wk (F)			69 M (decreased nerve conduction velocity)		Sufit et al. 1986	
160	Mouse (C57BL/6J)	9, 15, 17 wk (F)				39 F (tremor)	Hahn et al. 1988	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
161	Dog (Beagle)	21 d 1 or 2 x/d (C)				50 F (dysrhythmic electroencephalogram)	Sundlof et al. 1981	
162	Rabbit (NS)	12 wk (F)				161 F (tremors, paresis)	de Matteis et al. 1961 HCB	
163	Pig (SPF)	90 d (F)		5 M		50 M (tremors, unsteady gait)	Den Tonkelaar et al. 1978	
Reproductive								
164	Monkey (Cynomolgus)	90 d 1 x/d (C)			0.1 F (cellular degeneration of ovarian surface epithelium)		Babineau et al. 1991	
165	Monkey (Cynomolgus)	90 d 1 x/d (C)			0.01[c] F (mitochondrial degeneration in developing ovarian follicles)		Bourque et al. 1995 HCB	
166	Monkey (Cynomolgus)	13 wk 1 x/d (C)		0.1 F	1 F (decreased serum progesterone levels during the luteal phase of menstruation)		Foster et al. 1992a	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
167	Monkey (Cynomolgus)	90 d 1 x/d (C)		1 F	10 F (increased length of menstrual cycle, decreased ovulatory levels of estradiol)		Foster et al. 1995a HCB	
168	Monkey (Rhesus)	60 d 1 x/d (G)			8 F (degeneration of the germinal epithelium, reduced number of primary follicles, and multiple follicular cysts in the ovaries)		Iatropoulos et al. 1976	
169	Monkey (Cynomolgus)	90 d 1 x/d (C)			0.1 F (degenerative lesions in oocytes)		Jarrell et al. 1993	
170	Monkey (Rhesus)	24 d 1 x/d (GW)				4 F (blocked ovulation)	Muller et al. 1978 HCB	
171	Monkey (Cynomolgus)	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	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
172	Rat (Wistar)	30 d 1 x/d (GW)			1000 F (increased estrus duration, altered hormone levels, reduced ovulation, degenerative ovarian lesions)		Alvarez et al. 2000 HCB	
173	Rat (Sprague-Dawley)	3 mo prior to mating through weaning of F1 pups (F)		3.4[d] O			Arnold et al. 1985	
174	Rat (Sprague-Dawley)	21 d 1 x/d (GO)			1 F (increased serum progesterone levels)		Foster et al. 1992b	
175	Rat (Sprague-Dawley)	4 gen (F)		13.8[d] O		27.6[d] O (decreased fertility; increased number of stillborns)	Grant et al. 1977 HCB	
176	Rat (COBS)	15 wk (F)		32			Kuiper-Goodman et al. 1977	
177	Rat (Sprague-Dawley)	90d 5d/wk 1x/d (GO)		10 F	25 F (mammary gland hyperplasia)		NTP 2002 HCB	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
178	Rat (Wistar)	3 wk (F)		184 M			Vos et al. 1979b	
179	Rat (Sprague-Dawley)	2 gen (GO)		2.5	12.5 M (8% increased number of F0 males with abnormal sperm; 25% decrease in total sperm/cauda of F0 males)		Wolfe and Pepperl 2005 HCB	
Developmental								
180	Monkey (Rhesus)	60 d (G)				64 F (67% pup mortality; hematoma and bilateral hemorrhagic pneumonia, congested lungs; ataxia)	Bailey et al. 1980	
181	Monkey (Rhesus)	22-60 d 1 x/d (GW)				64 (2/3 infants died after exhibiting lethargy, ataxia, and listlessness)	Iatropoulos et al. 1978 HCB	
182	Rat (Sprague-Dawley)	3 mo prior to mating through weaning of F1 pups (F)				3.4[d] O (decreased postpartum day 4 pup viability)	Arnold et al. 1985	
183	Rat (Sprague-Dawley)	4 gen (F)		3.4	6.9 (decreased pup weight gain)	13.8 (decreased pup viability)	Grant et al. 1977	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
184	Rat (Sprague-Dawley)	1 gen (F)			5.9 (decreased pup weight gain)	7.8 (decreased neonatal survival)	Kitchin et al. 1982	
185	Rat (Wistar)	2 gen (F)		0.6 M	1.3 M (reduced efficiency of pups in operant behavior task)		Lilienthal et al. 1996 HCB	
186	Rat (Wistar)	gestation + lactation + 2 wks post-weaning (F)			5.1 F (decreased resistance to infection, increased IgG response to tetanus toxoid, and proliferation of high endothelial venules in lymph nodes in pups)		Vos et al. 1979a	
187	Rat (Wistar)	gestation + lactation + 2 wks or 7 mo post-weaning (F)			0.4 F (increased IgG and IgM response to tetanus toxoid, increased delayed-type hypersensitivity reaction to ovalbumin, and accumulation of foamy macrophages in the lung in offspring)	10.3 (high pup mortality)	Vos et al. 1983 HCB	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
188	Rat (Sprague-Dawley)	2 gen (GO)		2.5		12.5 (20-25% decreased postpartum F1c pup body weight, 100% F1c pup mortality by PND 9)	Wolfe and Pepperl 2005 HCB	
189	Mouse (BALB/c)	Gd 0-18 (F)			0.5 (depressed delayed-type hypersensitivity response in offspring)		Barnett et al. 1987 HCB	
190	Pig (SPF)	90 d (F)		5 M	50 M (retarded development of the testes)		Den Tonkelaar et al. 1978	
Cancer								
191	Rat (Sprague-Dawley)	90 d (F)				10 (CEL: renal adenomas, hepatocarcinomas, lymphosarcomas)	Ertürk et al. 1986	
CHRONIC EXPOSURE								
Death								
192	Rat (Wistar)	75 wk (F)				8.4 F (1/6 died)	Smith and Cabral 1980	
193	Mouse (Swiss)	120 wk (F)				24 (decreased survival)	Cabral et al. 1979	
194	Hamster (Syrian)	lifespan (F)				16 (decreased lifespan)	Cabral et al. 1977	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
195	Dog (Beagle)	1 yr 1 x/d (C)				11 F (2/6 females died)	Gralla et al. 1977	
Systemic								
196	Rat (Sprague-Dawley)	130 wk and via mothers during gestation and lactation (F)	Hemato	2.8 M 3.2 F			Arnold et al. 1985	
			Hepatic	0.13 F	0.022[e] M (increased peribiliary lymphocytosis and fibrosis in the liver of F1 adults at terminal sacrifice)			
					0.64 F (dose-related increases in the incidence and/or severity of hepatic centrilobular basophilic chromogenesis in F1 females)			
			Renal	0.55 M 3.2 F	2.8 M (increased incidences of severe chronic nephrosis)			
			Bd Wt	2.8 M 3.2 F				
197	Rat (Sprague-Dawley)	1 yr (F)	Hepatic	0.069 M 0.08 F	0.34 M (mitochondrial swelling and elongation)		Mollenhauer et al. 1975	
					0.4 F (mitochondrial swelling and elongation)			

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
198	Rat (Agus)	90 wk (F)	Hepatic		7 F (porphyria)		Smith and Cabral 1980	
			Dermal		7 F (alopecia)			
			Bd Wt		7 F (decreased body weight gain)			

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
199	Rat (Fischer 344)	90 wk (F)	Hepatic		15.8 M (decreased URO-D activity, increased hepatic porphyrin levels, hepatocyte hypertrophy, fatty degeneration, bile duct hyperplasia)		Smith et al. 1985	
					18.3 F (decreased URO-D activity, increased hepatic porphyrin levels, hepatocyte hypertrophy, fatty degeneration, bile duct hyperplasia)			
			Renal		15.8 M (increased kidney weight and renal porphyrin levels, nephrosis)			
					18.3 F			
			Endocr	15.8 M				
				18.3 F				
			Bd Wt		15.8 M (decreased body weight)			
					18.3 F (decreased body weight)			
200	Rat (Fischer- 344)	65 wk (F)	Hepatic		18.3 F (increased liver weight; biliary hyperplasia; increased liver porphyrins; induction of microsomal enzymes and glutathione S-transferase)		Smith et al. 1993	
			Bd Wt	18.3 F				

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
201	Mouse (C57BL/ 10ScSn)	18 mo (F)	Hepatic		17.2 M (hepatocyte hypertrophy)		Smith et al. 1989	
			Bd Wt		17.2 M (decreased body weight)			
202	Hamster (Syrian)	lifespan (F)	Bd Wt			16 M (marked decrease in body weight gain)	Cabral et al. 1977	
203	Dog (Beagle)	1 yr 1 x/d (C)	Cardio	11		110 (arteriopathy)	Gralla et al. 1977	
			Gastro	1		11 (diarrhea)		
			Hemato	1	11 (neutrophilia)	110 (anemia)		
			Hepatic	1		11 (hepatomegaly, bile duct hyperplasia, pericholangitis, periportal fibrosis, increased serum alkaline phosphatase and AST)		
			Bd Wt	1		11 (body weight loss)		
Immuno/ Lymphoret								
204	Rat (Sprague-Dawley)	130 wk and via mothers during gestation and lactation (F)			0.022 M (peribiliary lymphocytosis)		Arnold et al. 1985	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
205	Dog (Beagle)	1 yr 1 x/d (C)			0.1 (increased severity of nodular hyperplasia of the gastric lymphoid tissue)		Gralla et al. 1977	
Neurological								
206	Rat (Agus)	90 wk (F)		7 F			Smith and Cabral 1980	
207	Rat	2 yr (F)			9.1 (slight decrease in nerve conduction velocity)		Sufit et al. 1986	
208	Mouse (Swiss)	120 wk (F)				24 (tremors, convulsions)	Cabral et al. 1979	
Reproductive								
209	Dog (Beagle)	1 yr 1 x/d (C)			110 M (slight testicular degeneration)		Gralla et al. 1977	
Developmental								
210	Rat (Fischer 344)	90 wk (F)			15.8 M (40% increased testicular weight)		Smith et al. 1985	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
Cancer								
211	Rat (Sprague-Dawley)	130 wk and via mothers during gestation and lactation (F)				2.8 M (CEL: parathyroid adenoma, adrenal pheochromocytoma)	Arnold et al. 1985	
						3.2 F (CEL: neoplastic liver nodules, adrenal pheochromocytoma)		
212	Rat (Sprague-Dawley)	104 wk (F)				5.2 M (CEL: hepatoma, bile-duct adenoma, hepatocarcinoma, renal adenoma)	Ertürk et al. 1986	
						6 F (CEL: hepatoma, bile-duct adenoma, hepatocarcinoma, renal adenoma)		
213	Rat (Wistar)	75 wk (F)				4.5 F (CEL: liver cell tumors)	Smith and Cabral 1980	
214	Rat (Agus)	90 wk (F)				7 F (CEL: liver-cell tumors)	Smith and Cabral 1980	
215	Rat (Fischer 344)	90 wk (F)				15.8 M (CEL: hepatocarcinoma)	Smith et al. 1985	
						18.3 F (CEL: hepatocarcinoma)		
216	Rat (Fischer- 344)	65 wk (F)				18.3 F (CEL: liver tumors)	Smith et al. 1993	

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

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form	Comments
217	Mouse (Swiss)	120 wk (F)				12 (CEL: liver tumors)	Cabral et al. 1979	
218	Mouse (C57BL/ 10ScSn)	18 mo (F)				17.2 M (CEL: hepatocellular carcinoma in iron-pretreated mice)	Smith et al. 1989	
219	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 an 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 This value represents the most sensitive gender-independent dose because both male and female animals were exposed to hexachlorobenzene, and the observed LOAEL or NOAEL for the effect could not be attributed to a particular gender.

e Used to derive a chronic oral MRL of 0.00007 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; ALT = alanine aminotransferase; AST = aspartate aminotransferase; Bd Wt = body weight; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); Endocr = endocrine; (F) = feed; F = Female; FT4 = free thyroxine; (G) = gavage; Gastro = gastrointestinal; Gd = gestational day; Gen = generation(s); Gn pig = guinea pig; (GO) = gavage in oil; GST-P = glutathione S-transferase; (GW) = gavage in water; Hemato = hematological; IgE = immunoglobulin E; IgG = immunoglobulin G; IgM = immunoglobulin M; Immuno/Lymphoret = immunological/lymphoreticular; LDH = lactose dehydrogenase; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; NS = not specified; PND = post-natal day; Resp = respiratory; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone; TT4 = total thyroxine; URO-D = uroporphyrinogen decarboxylase; wk = week(s); x = time(s); yr = year(s)

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

Acute (≤14 days)

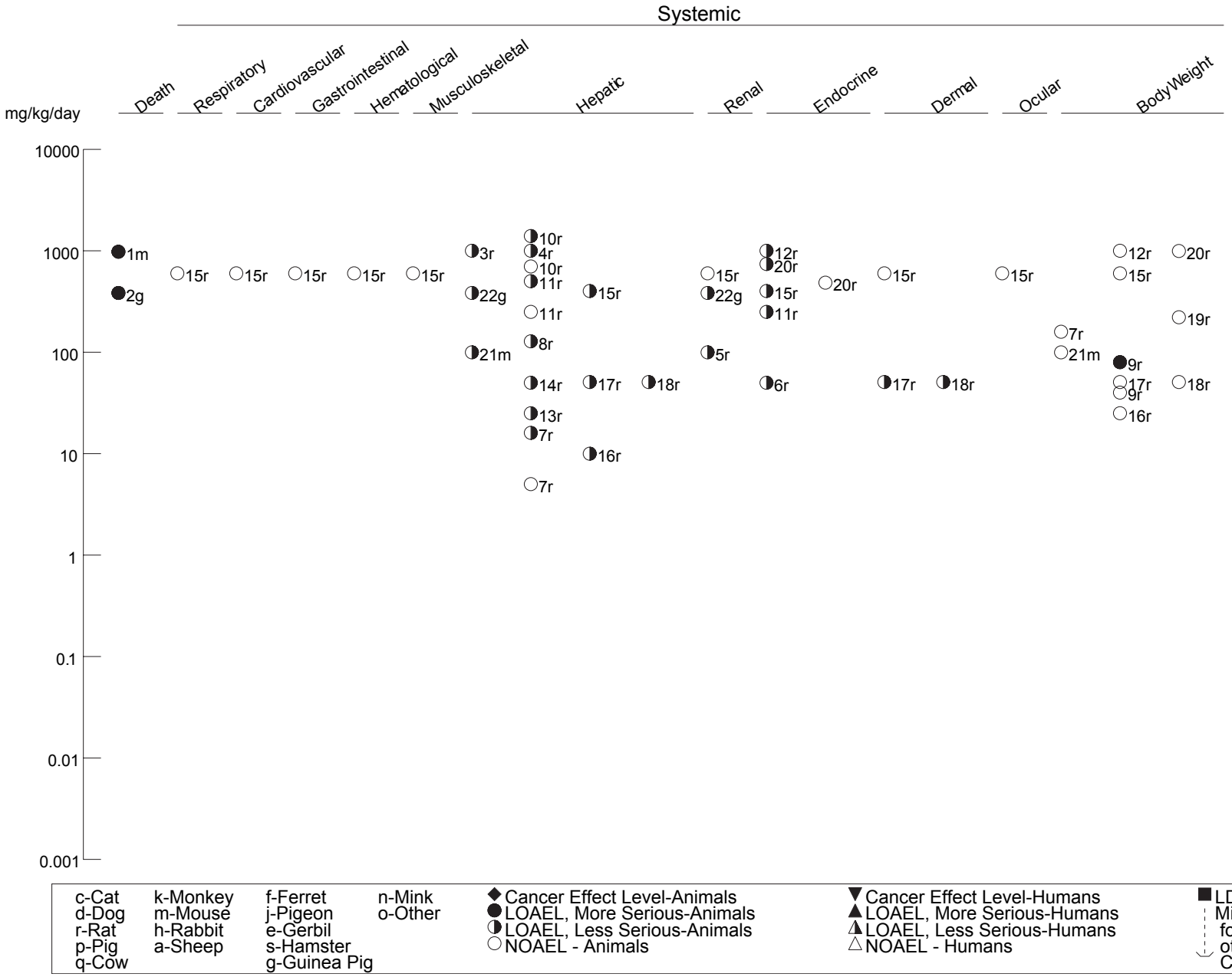


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Acute (≤14 days)

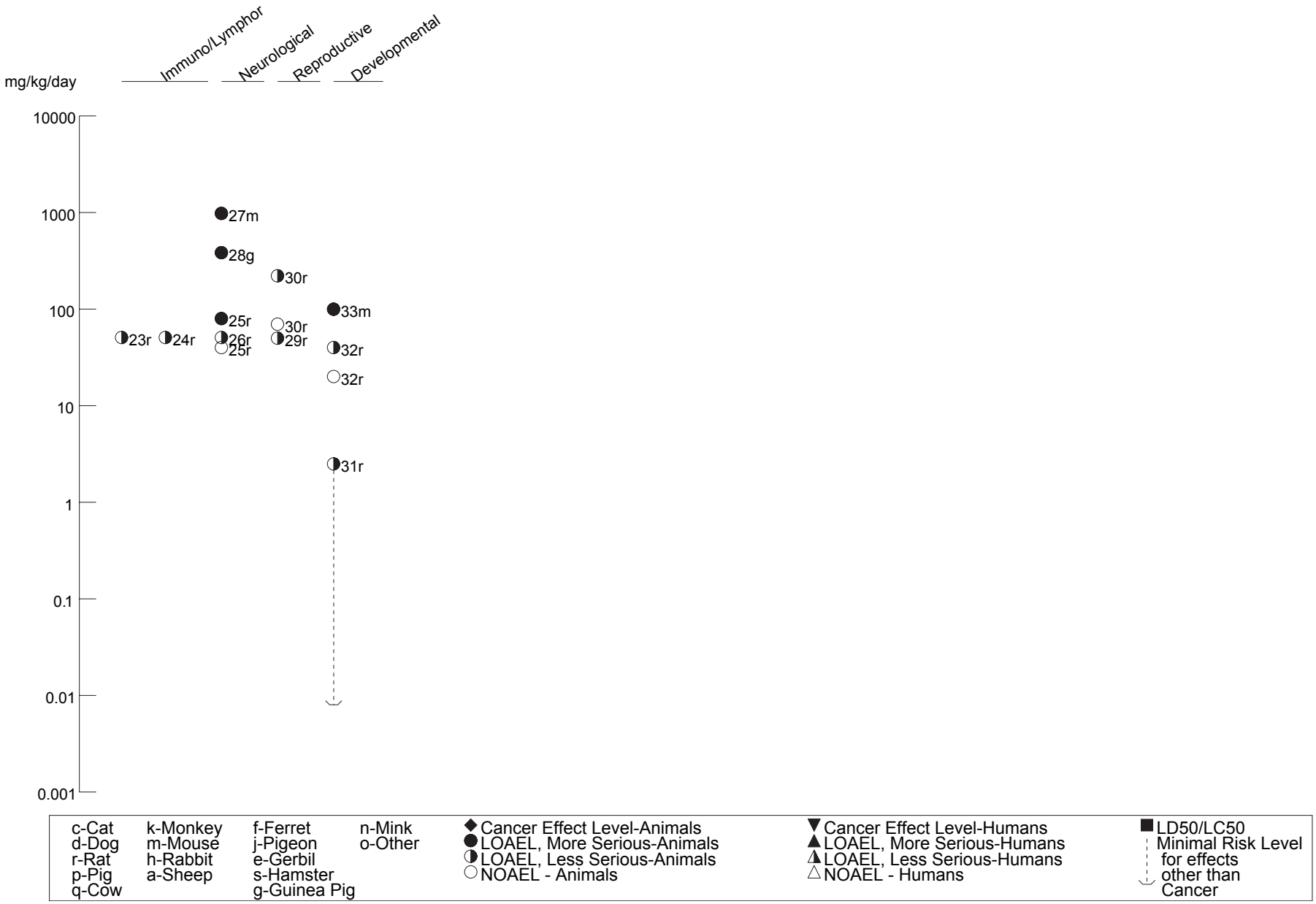


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

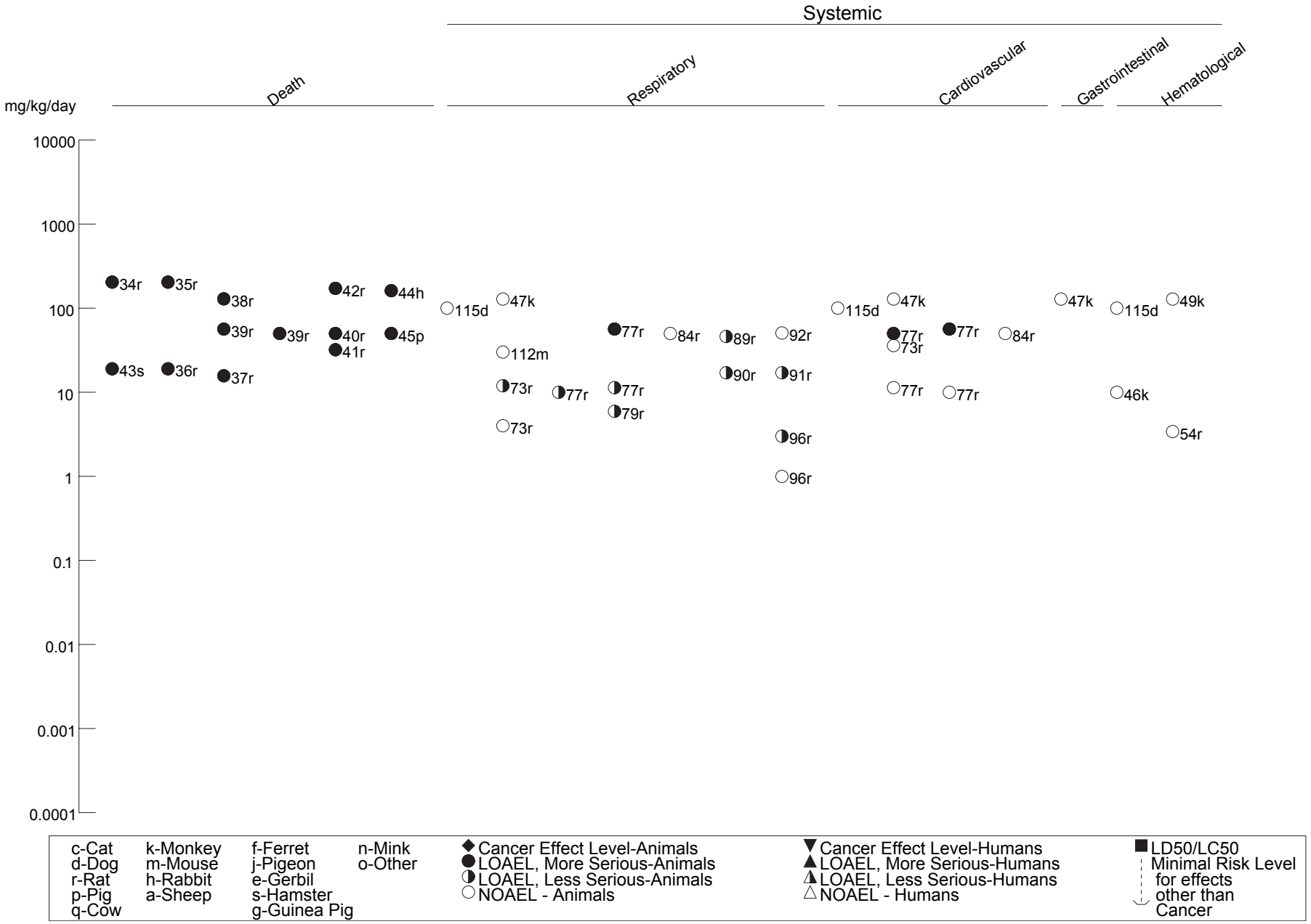


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

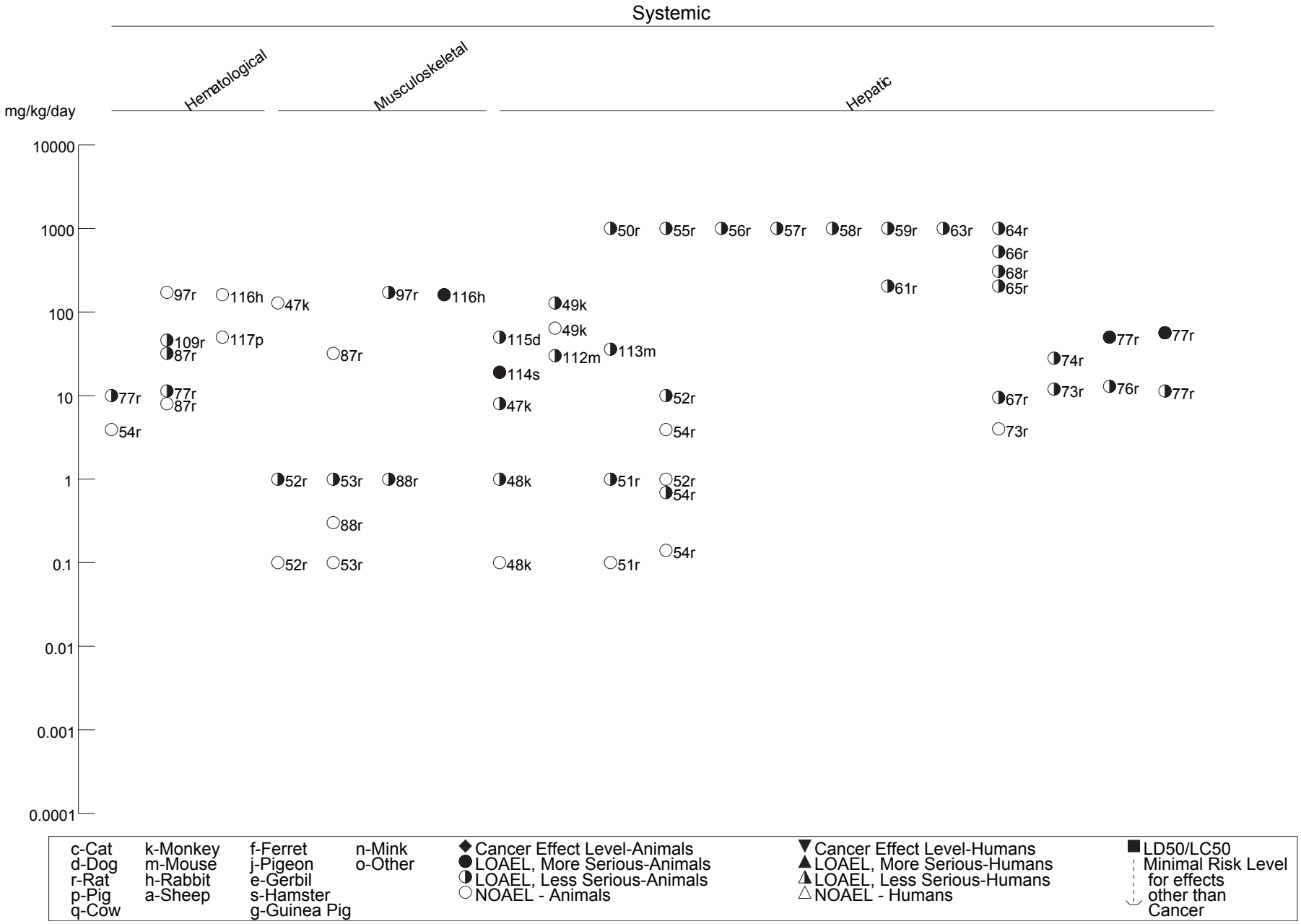


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

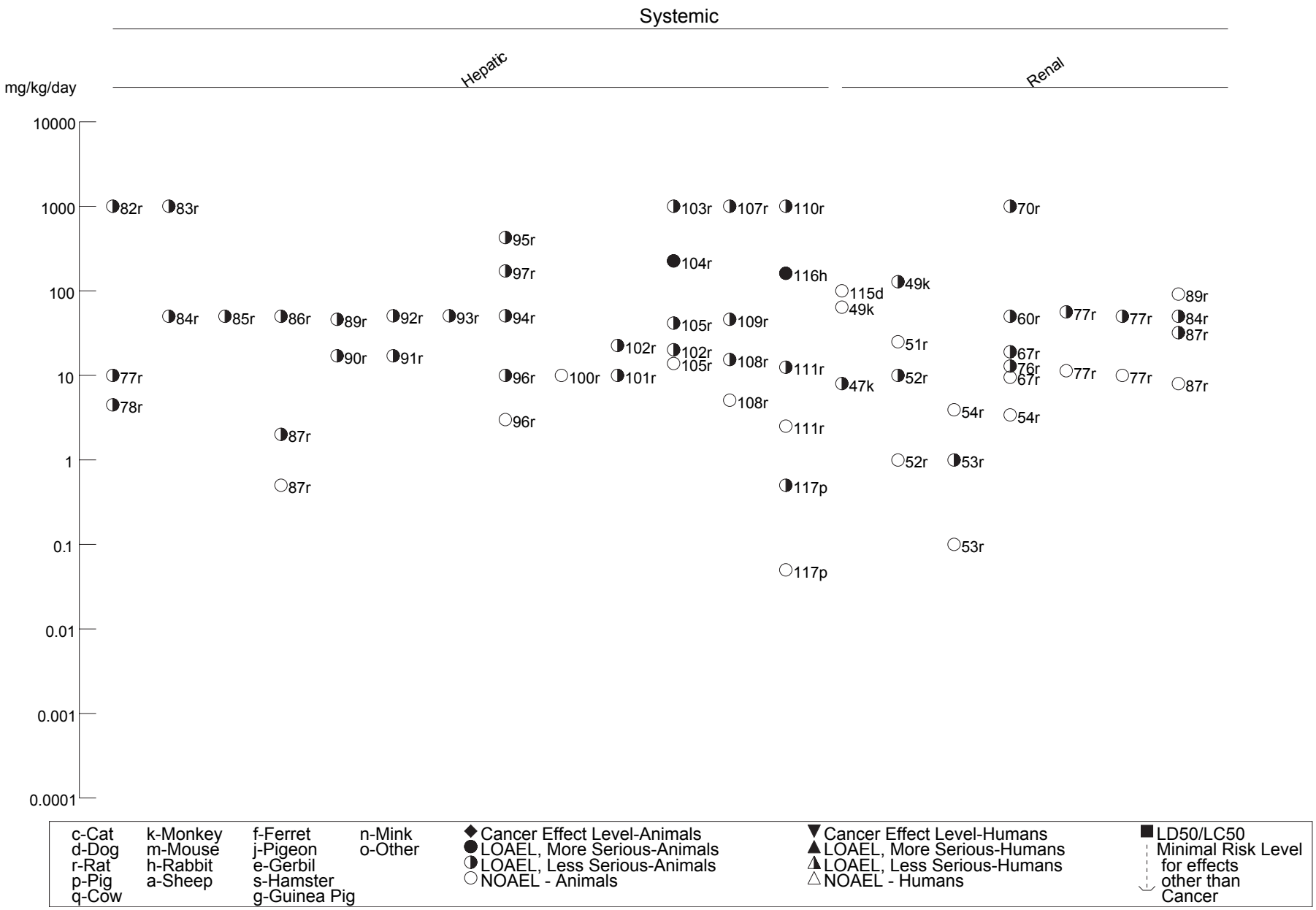


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

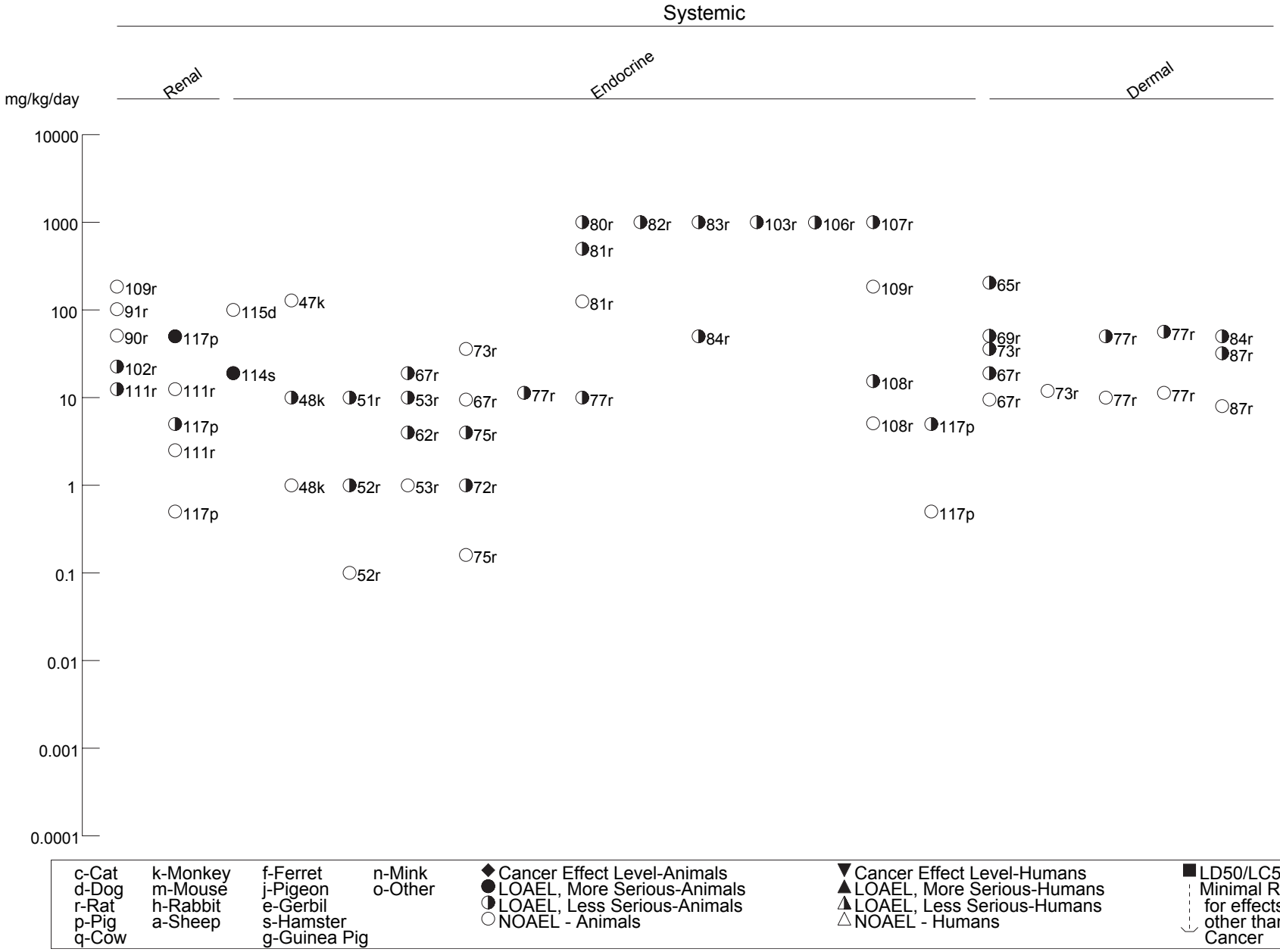


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

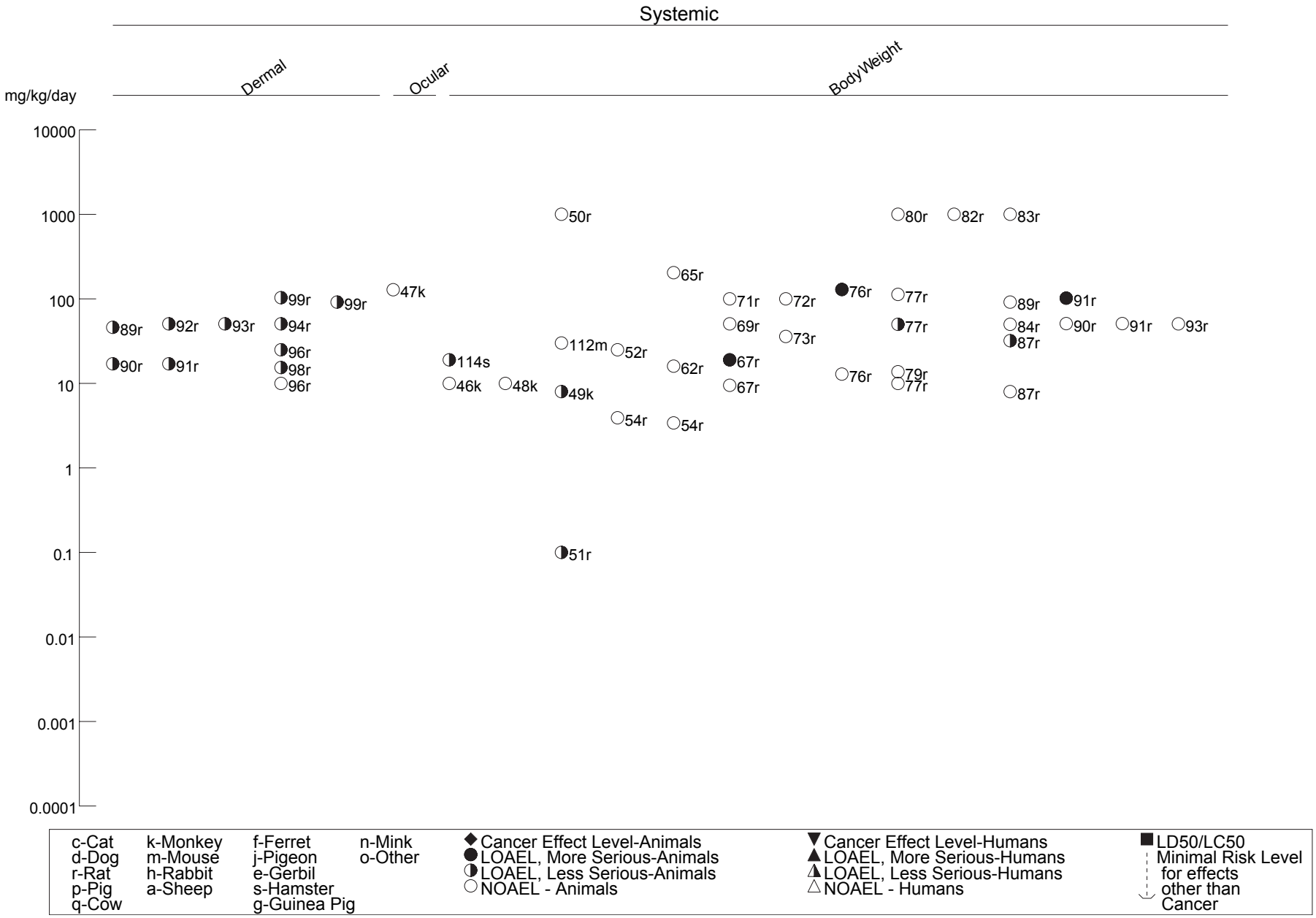


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

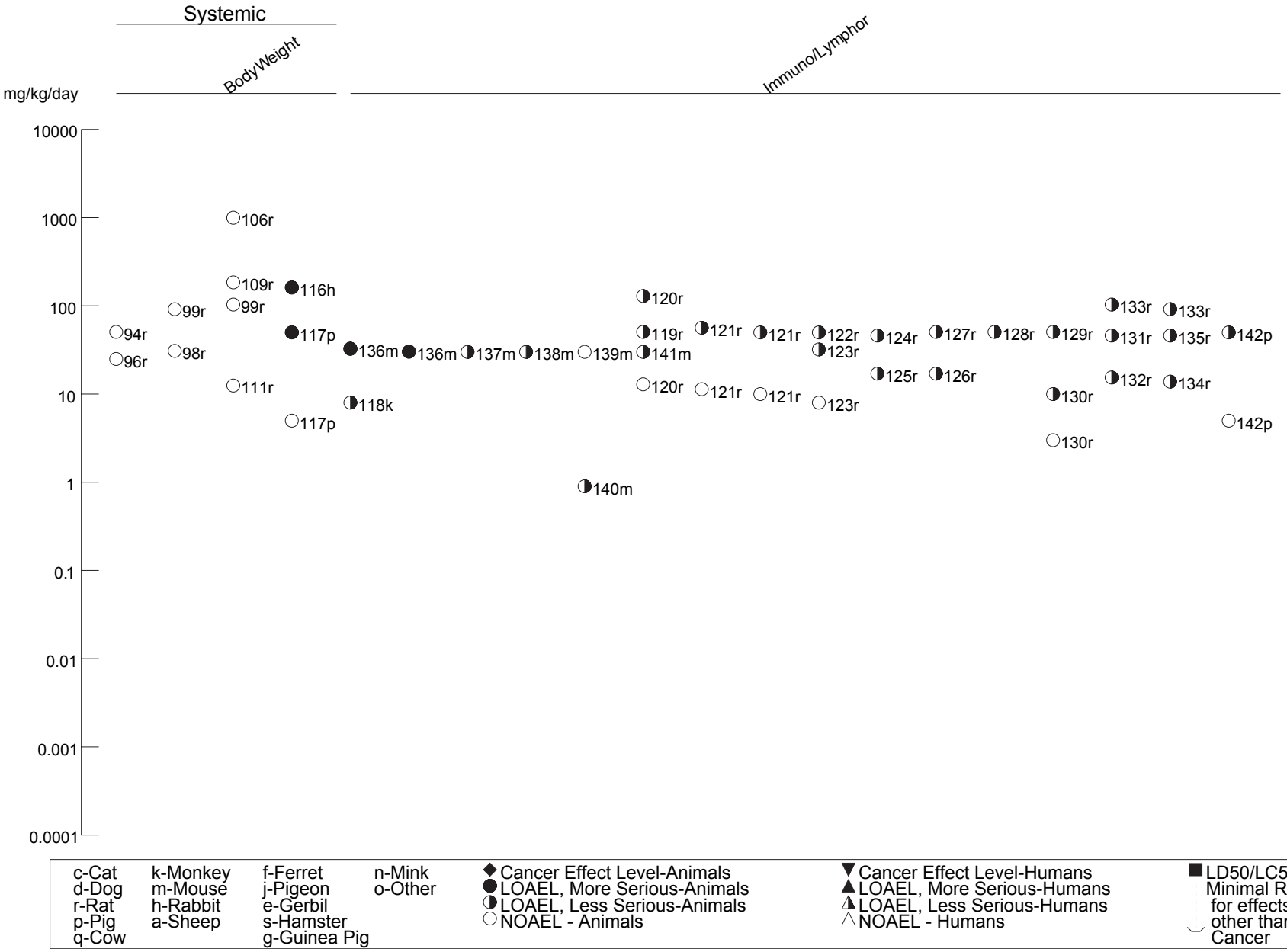


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)

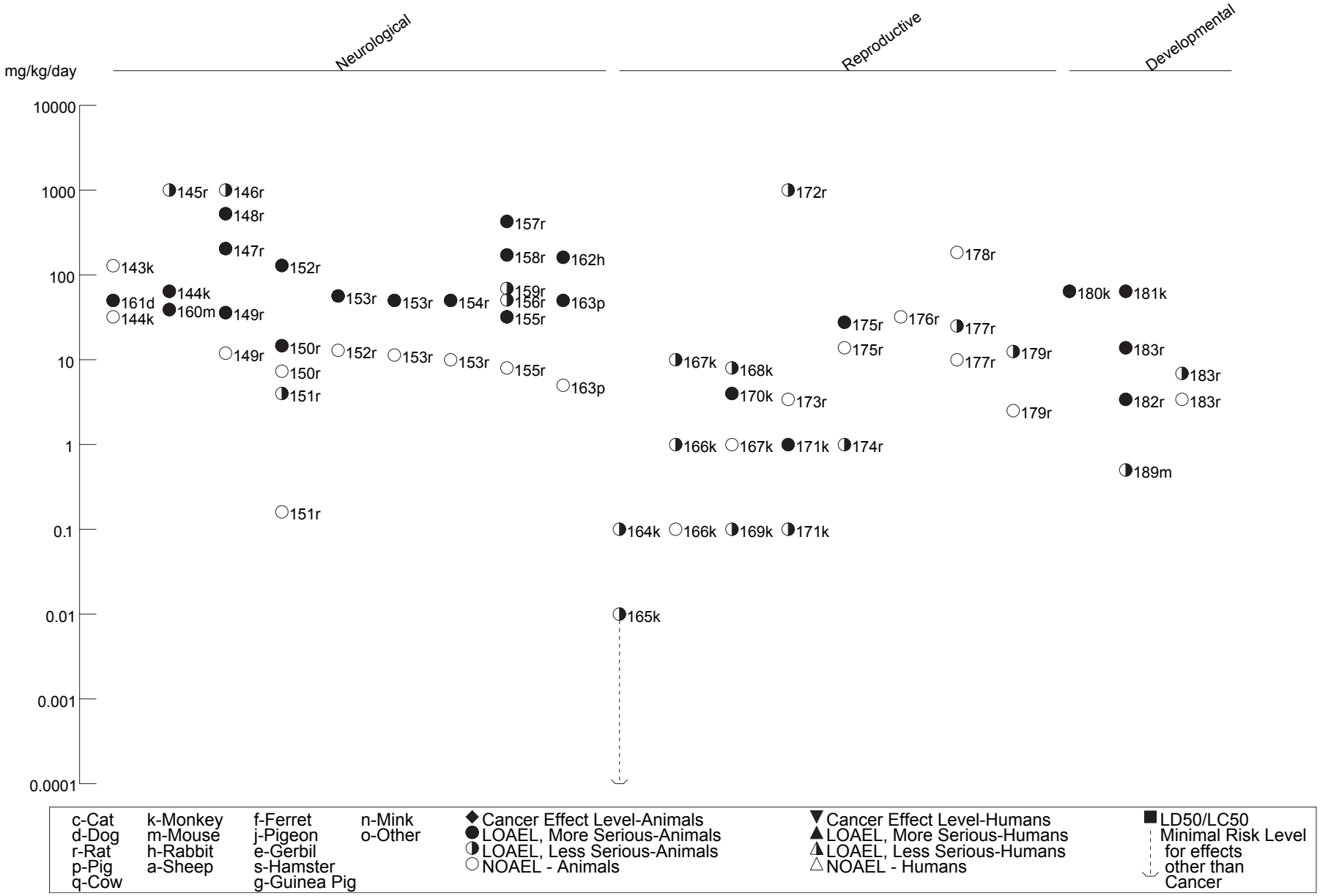
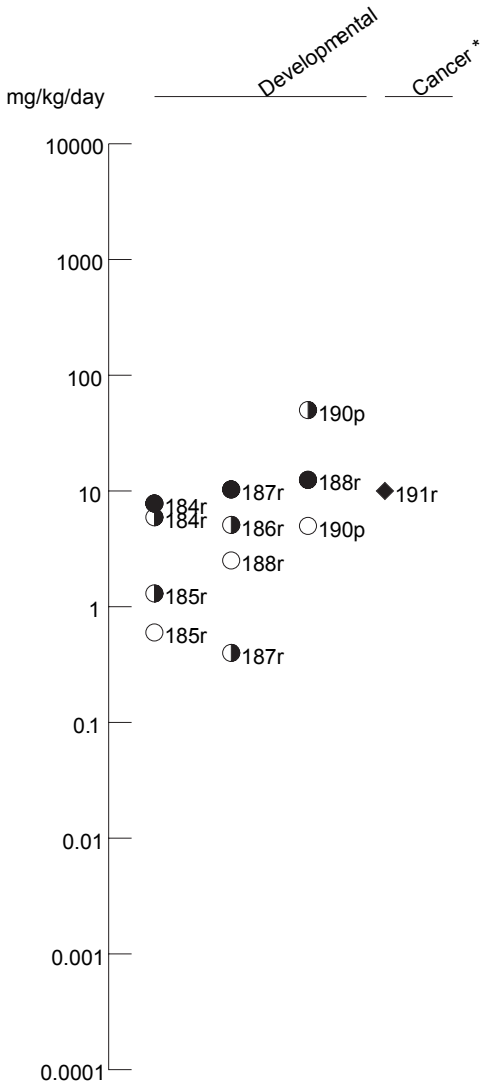


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Intermediate (15-364 days)



*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 endpoint.

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

◆ 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)

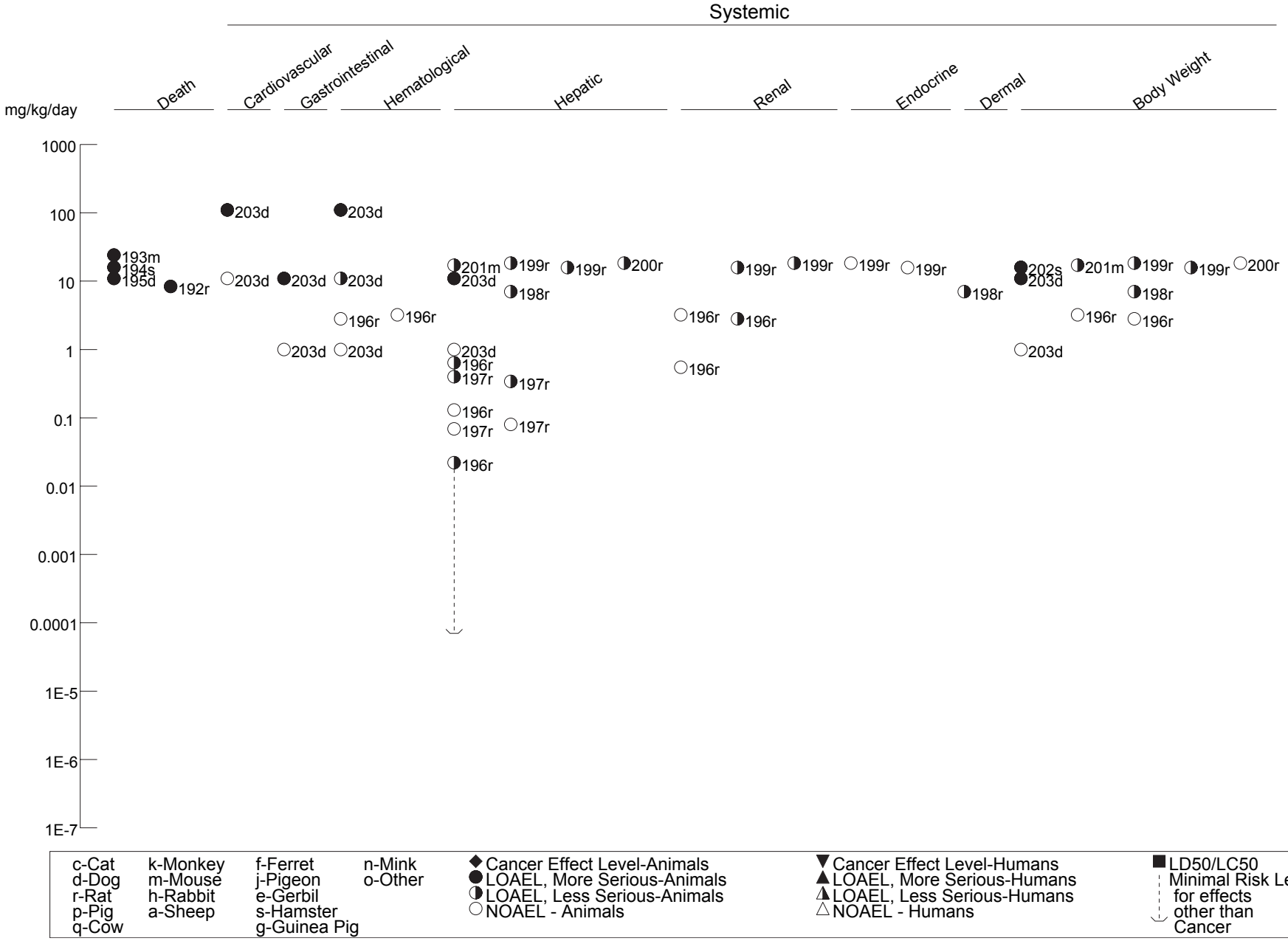
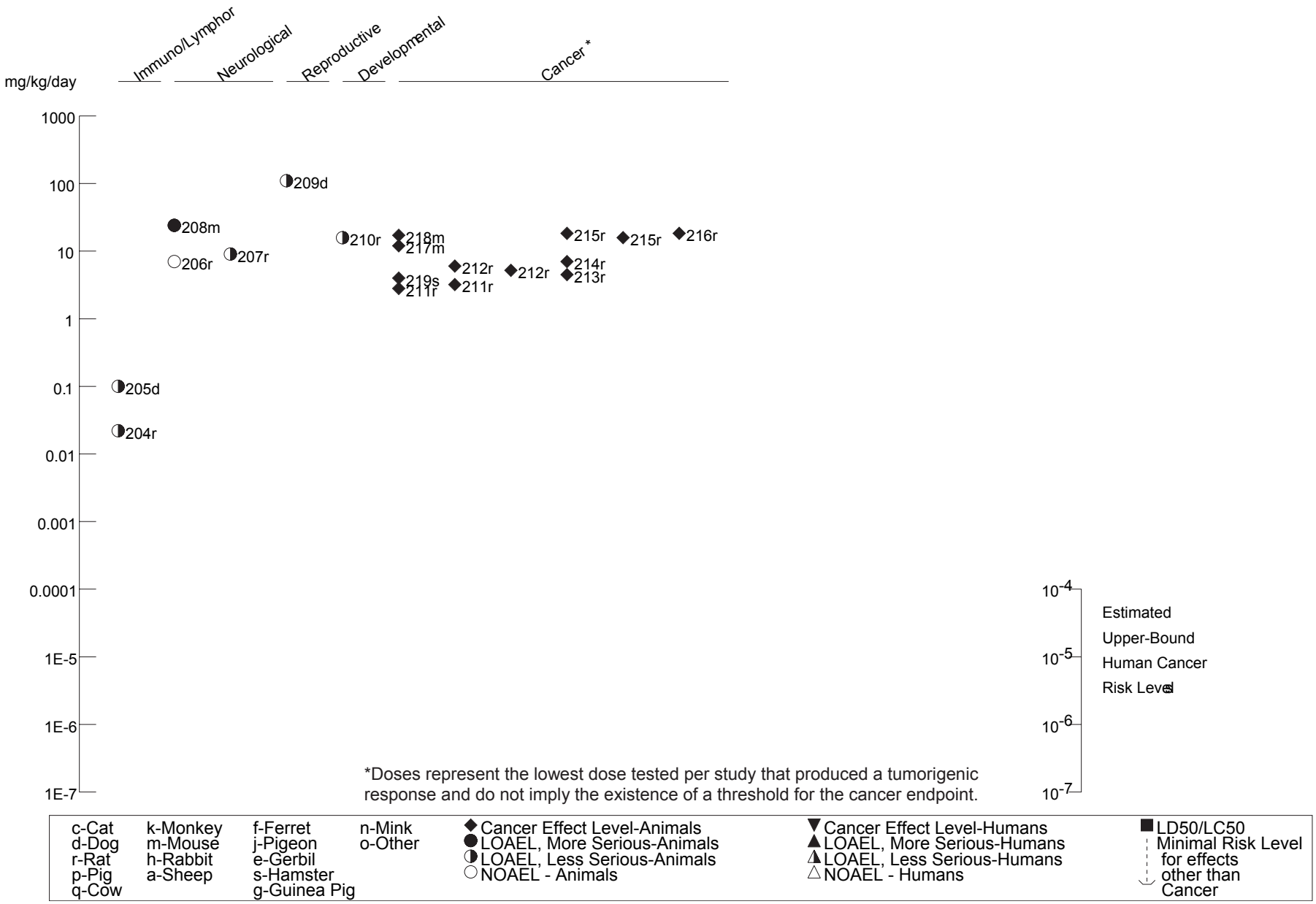


Figure 3-2 Levels of Significant Exposure to Hexachlorobenzene - Oral *(Continued)*

Chronic (≥365 days)



*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 endpoint.

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

NTP (2002) administered hexachlorobenzene by gavage (in corn oil with 1% acetone) to female Sprague-Dawley rats (10/group) once/day, 5 days/week for 90 days at doses ranging from 0 to 25 mg/kg/day. Significantly ($p<0.05$) increased incidences of chronic pulmonary inflammation were noted at hexachlorobenzene doses ≥3 mg/kg/day (incidences of 3/10, 1/10, 1/10, 2/10, 7/10, 8/10, 10/10, and 10/10 for dose groups of 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10, and 25 mg/kg/day, respectively). 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 ingesting ≥10–11 mg hexachlorobenzene/kg/day from 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 at doses ≥56.5 mg/kg/day. Adverse effects on respiratory function, granulomatous lung inflammation, and airway hyperresponsiveness have been associated with exposure of rats to hexachlorobenzene via the diet at concentrations resulting in estimated daily doses of approximately 50 mg/kg/day (Ezendam et al. 2004a; Michielsen et al. 2001, 2002); however, these effects are most likely attributable to hexachlorobenzene-induced effects on the immune system (see Section 3.2.2.3). 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 hexachlorobenzene exposure in humans.

There have been a few reports of cardiovascular lesions in animals exposed to hexachlorobenzene. Gralla et al. (1977) 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 receiving hexachlorobenzene from the diet for 4 months at doses ≥50 mg/kg/day. 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. 1977). 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.

Limited animal data suggest that hexachlorobenzene can produce anemia and leukocytosis. Decreases in hemoglobin, hematocrit, and/or red blood cell count were reported in rats administered hexachlorobenzene. in the diet for approximately 4 months at concentrations resulting in estimated doses of 10–32 mg/kg/day (Kimbrough and Linder 1974; Kuiper-Goodman et al. 1977). 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 46 mg/kg/day after dietary exposure for 3 weeks (Vos et al. 1979b), and reduced medullary area in the femur, which was found at doses ≥10 mg/kg/day 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. 1977), but not in dogs exposed to 100 mg/kg/day for only 3 weeks (Sundlof et al. 1981), in rabbits exposed to 161 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 (≥32 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. 1977). 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 ingesting 172 mg hexachlorobenzene/kg/day from the diet for 56 days (Ockner and Schmid 1961) and rabbits ingesting 161 mg/kg/day from 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 ingesting 161 mg hexachlorobenzene/kg/day from the diet 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 receiving up to 32 mg/kg/day from the diet for 15 weeks (Kuiper-Goodman et al. 1977).

Degenerative lesions of maxillary incisors were noted in female Sprague-Dawley rats administered hexachlorobenzene at doses ranging from 1 to 25 mg/kg/day, but not at doses ≤0.3 mg/kg/day (Long et al. 2004). Incidences and severity of the degenerative lesions increased with increasing dose.

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, hepatocellular hypertrophy, 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 synthetase 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 ingesting ≥16 mg/kg/day of hexachlorobenzene from 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 [Billi de Catabbi et al. 2000a; Krishnan et al. 1991; Mylchreest and Charbonneau 1997], 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 synthetase 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 hexachlorobenzene 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 synthetase 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 porphyria lasting at least as long as the 20-week observation period.

Hexachlorobenzene doses as low as 5–51 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 synthetase activity, in female rats exposed for intermediate durations (Den Besten et al. 1993; Goldstein et al. 1978; Kennedy and Wigfield 1990; Kuiper-Goodman et al. 1977; Michielsen et al. 2001, 2002; Smith et al. 1979, 1985; Wolfe and Pepperl 2005). 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 172 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 hexachlorobenzene 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 receiving up to 50 mg/kg/day from 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 7–18 mg/kg/day from 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; Mollenhauer et al. 1975). Male mice ingesting slightly higher doses of hexachlorobenzene (17 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. 1977).

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, 2001, 2002; 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. 1977; 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. NTP (2002) administered hexachlorobenzene by gavage (in corn oil with 1% acetone) to female Sprague-Dawley rats (10/group) once/day, 5 days/week for 90 days at doses ranging from 0 to 25 mg/kg/day. Significantly ($p<0.001$) increased incidences of hepatocellular hypertrophy were noted at hexachlorobenzene doses ≥10 mg/kg/day (incidences of 0/10, 0/10, 0/10, 0/10, 0/10, 0/10, 9/10, and 10/10 for dose groups of 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10, and 25 mg/kg/day, respectively). 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 hexachlorobenzene via their mothers during gestation and lactation followed by direct ingestion of hexachlorobenzene at 0.022 mg/kg/day from the diet for their postweaning lifetime (Arnold et al. 1985; listed as a developmental effect in Table 3-2 and the basis for the chronic-duration oral MRL of 0.00007 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; Wolfe and Pepperl 2005). 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). However, Bouthillier et al. (1991) observed increased kidney weight in male Sprague-Dawley rats administered hexachlorobenzene by gavage at 100 mg/kg/day, 5 days/week for 2 weeks. Multiple-dose feeding studies of 12–16 weeks identified LOAEL values of 19–56.5 mg/kg/day and NOAEL values of 5–11.3 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). Increased kidney weight was observed at doses much lower than 100 mg/kg/day in pigs treated for 90 days (Den Tonkelaar et al. 1978), rats administered hexachlorobenzene by gavage in oil rather than in the diet (Andrews et al. 1989, 1990), and rats receiving hexachlorobenzene from the diet for 1 year instead of 12–16 weeks (Smith et al. 1985). The lowest LOAEL and NOAEL for increased kidney weight in any study were 5 and 0.5 mg/kg/day, respectively, for hexachlorobenzene-treated pigs (Den Tonkelaar et al. 1978). 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 gender-specific nephrotoxic effect in rats includes findings of mild to severe nephrosis in 12/12 surviving male rats ingesting 15.8 mg hexachlorobenzene/kg/day from the diet for 90 weeks, but 0/10 surviving male controls and only 1/10 surviving treated females (Smith et al. 1985), and significantly increased incidences of severe chronic nephrosis in male (but not female) rats exposed to hexachlorobenzene via their mothers during gestation and lactation followed by direct ingestion of hexachlorobenzene from their diet for a lifetime at a concentration resulting in an estimated dose of 2.8 mg/kg/day (Arnold et al. 1985).

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 receiving hexachlorobenzene from the diet at doses as low as 12.9 mg/kg/day for 56 days (Kennedy and Wigfield 1990). 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 blood urea nitrogen (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 receiving hexachlorobenzene from the diet at 385 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 hexachlorobenzene in the diet for 90 days at doses as low as 17.2 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 doses as low as 13–23 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 receiving hexachlorobenzene from the diet or by gavage in oil at doses up to 25 mg/kg/day for 15 weeks (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 50–100 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; 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 or report on hexachlorobenzene levels in fat, which would likely have been a more relevant measure of long-term exposure.

Several more recent studies focused on possible associations between serum hexachlorobenzene and circulating thyroid hormone levels. It should be noted that other organochlorine compounds and residues were detected in test samples as well. Among 342 adult men who were partners of, and/or patients at, an infertility clinic in Boston, Massachusetts, a statistically significant ($p<0.05$) decline in total T3 levels was associated with an interquartile range increase in serum hexachlorobenzene levels (Meeker et al. 2007).

In a population-based survey that included 193 children (mean age 6.5 years) living in an area of Brazil where high levels of organochlorine pesticides were present in soil, water, and local food, a significant trend ($p<0.01$) was observed for increasing serum T3 with increasing serum hexachlorobenzene levels; no significant association was found between serum T4 or TSH and serum hexachlorobenzene (Freire et al. 2012).

In a population-based survey of 303 men and 305 women living in the same areas of Brazil, a significant ($p<0.05$) positive association was noted between serum hexachlorobenzene levels and free T4 among the women (but not the men); no significant association was observed between serum total T3, TSH, or anti-thyroperoxidase and serum hexachlorobenzene level among men or women (Freire et al. 2013).

Within a group of 105 pregnant women in Korea who supplied serum samples at delivery, a slight, but significant ($p<0.05$) negative association was found between serum free T4 and serum hexachlorobenzene levels; there was no significant association between serum hexachlorobenzene and serum free T3, total T3, total T4, or TSH (Kim et al. 2013).

Significant negative associations were reported between plasma hexachlorobenzene concentrations and total free T4 in groups of 623 Inuit adults (Dallaire et al. 2009a) and 149 pregnant women in a region of southwest Quebec, Canada (Takser et al. 2005), and cord blood hexachlorobenzene and free T3 and T4

from 198 births among subjects in the Netherlands (Maervoet et al. 2007). Croes et al. (2014a, 2014b) reported a significant (p=0.02) positive association between serum hexachlorobenzene and serum TSH (OR 1.03; 95% CI 1.01, 1.05) in a group of 606 adolescents in the Flemish Environment and Health Study (FLEHS II).

Within a group of 334 pregnant women from Salinas Valley, California, serum hexachlorobenzene was not significantly associated with T4 levels after adjusting for serum PCBs (Chevrier et al. 2008). In studies of Akwesasne Mohawk subjects, no significant associations were found between serum hexachlorobenzene and T4 levels among children <17 years of age (Schell et al. 2004, 2008) or levels of anti-thyroid peroxidase antibodies in adults aged 17–20 years (Schell et al. 2009). Among 410 neonates from remote coastal regions in Quebec, Canada, cord blood hexachlorobenzene levels did not adversely affect cord blood thyroid hormone levels (Dallaire et al. 2008). No significant associations were found between hexachlorobenzene and thyroid hormone levels in serum from 204 pregnant Inuit women, cord blood from 108 newborns, or serum from 175 infants 7 months of age (Dallaire et al. 2009b).

In a large-scale study of 2,046 male and female adults (age 20–75 years) from a polluted town in East Slovakia located near a former PCB-producing chemical factory, and two control populations located in adjacent towns, statistically significant positive correlations were found between elevated hexachlorobenzene levels and thyroid volume ($p<0.01$), TSH levels ($p<0.001$), and thyroperoxidase antibody levels ($p<0.01$) (Langer et al. 2007). An earlier study using smaller numbers of subjects reported similar results (Langer et al. 2003). However, elevated levels of PCBs were also found in the subjects living in the polluted area and a determination could not be made as to whether the thyroid effects were due to hexachlorobenzene exposure, PCB exposure, or both.

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 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). Hadjab et al. (2004) reported significantly decreased plasma total T4 levels in male Sprague-Dawley rats (as compared to pre-exposure levels) exposed to hexachlorobenzene at gavage doses of 4 or 16 mg/kg/day for 4 weeks; no alterations in plasma T4 were observed at a dose level of 0.16 mg/kg/day.

Smith et al. (1987) reported significantly enlarged thyroid glands and depressed serum T3 (but not T4) in hamsters receiving hexachlorobenzene from the diet at 47.4 mg/kg/day for 6 weeks or 19.0 mg/kg/day for 18 weeks or 28 weeks; serum T3 levels were as much as 3-fold lower than that of controls. Thyroid weight increased significantly, and correlated with histopathological observations of large and irregularly shaped follicles in the thyroid. Decreased T3 and T4 levels were observed in female Wistar rats ingesting hexachlorobenzene from the feed at doses up to 19.0 mg/kg/day for 13 weeks (Den Besten et al. (1993), and significantly increased thyroid weight was noted in male Landvarken pigs fed 5 mg/kg/day of hexachlorobenzene for 12 weeks (Den Tonkelaar et al. 1978); these effects were observed in the absence of histopathological signs of thyroid lesions.

Chalouati et al. (2013) reported significantly decreased plasma free and total T4 levels and significantly increased plasma TSH in male Wistar rats administered hexachlorobenzene by gavage (in olive oil) at 4 or 16 mg/kg/day for 28 days; in addition, the high-dose group exhibited significantly decreased plasma free T3 and significantly increased mean relative thyroid gland weight (40% greater than that of controls). The study authors reported histological changes in the thyroid glands from hexachlorobenzene-treated rats (closed follicles and increased number of follicles at 4 mg/kg/day and hyperplasia and hypertrophy of follicular cells and appreciable loss of colloid mass at 16 mg/kg/day); however, incidence data were not presented in the study report. In a time-course portion of the study, hexachlorobenzene-treated rats exhibited significantly decreased plasma free and total T4 and free T3 and significantly increased plasma TSH at days 21 and 28, but no significant differences from controls were observed at day 14. In a recovery portion of the study, decreased plasma free and total T4 and T3 levels, increased plasma TSH,

and increased mean relative thyroid weight that were observed following 28 days of hexachlorobenzene dosing at 16 mg/kg/day persisted after 28 days of vehicle-only treatment.

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-glucuronyltransferase (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) 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 show that hexachlorobenzene affects the adrenal gland (weight, histopathology, and hormone levels). Hyperplasia of the adrenal cortex was observed in male and female Sherman rats receiving hexachlorobenzene from the diet at concentrations resulting in estimated doses ≥10 mg/kg/day; adrenal gland weight was significantly increased at doses ≥50 mg/kg/day for 4 months (Kimbrough and Linder 1974). Adrenal gland weight was also significantly increased in Wistar rat pups exposed to hexachlorobenzene via their mothers at a maternal dietary concentration resulting in an estimated dose of 25.6 mg/kg/day during gestation and lactation and for 2 weeks postweaning directly from the diet (Vos et al. 1979a). In female Wistar rats receiving hexachlorobenzene from the diet at 9.5 or 19.0 mg/kg/day 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) revealed 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/day 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 T4 to free T3 (Skaare et al. 2001). In female Wistar rats receiving hexachlorobenzene from the diet at 19 (but not 9.5) mg/kg/day 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 vesiculo-bullous 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 causal relationship (Koss et al. 1978; Michielsen et al. 1997, 2000; NTP 2002; 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 hexachlorobenzene 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 estimated doses of 17 or 51 mg/kg/day from the diet, while Wistar rats received estimated doses of 46 or 92 mg/kg/day from the diet. Dose-related increased incidence of macroscopic skin lesions were noted in all dose groups of each mouse strain compared to strain-specific 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, skin lesions and immune effects were considered 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 (Michielsen et al. 2000) verified that a dose of 50.8 mg/kg/day from 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, female Wistar rats receiving hexachlorobenzene from the diet at 15 or 30 mg/kg/day for 13 weeks exhibited increased incidence of wounds appearing by weeks 5–7 on the face, neck, shoulders, and behind the ears (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. Hexachlorobenzene-induced skin lesions of the head and neck have been reported in other rat studies as well (Ezendam et al. 2004a; Michielsen et al. 2001, 2002). NTP (2002) administered hexachlorobenzene by gavage (in corn oil with 1% acetone) to female Sprague-Dawley rats (10/group) once/day, 5 days/week for 90 days at doses ranging from 0 to 25 mg/kg/day. Significantly ($p<0.001$) increased incidences of chronic pulmonary inflammation were noted at the highest dose level (incidences of 0/10, 0/10, 0/9, 0/10, 0/10, 0/9, 0/10, and 9/10 for dose groups of 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10, and 25 mg/kg/day, respectively). Ulcerated skin was observed only among 7 of the 10 high-dose rats. 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 hexachlorobenzene ingestion at 50 mg/kg/day (Goerz et al. 1978, 1994). No dermal lesions were observed in female Agus or Wistar rats ingesting 7 or 5 mg/kg/day of hexachlorobenzene, respectively, from the diet 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 128 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.

Body weight was not affected in rats or mice orally exposed to hexachlorobenzene for acute durations at doses ranging from 25 to 1,000 mg/kg/day (Courtney et al. 1976; Goldstein et al. 1978; Kleiman de Pisarev et al. 1995; Lecavalier et al. 1994; Mehendale et al. 1975; Michielsen et al. 2001, 2002; Simon et al. 1979; van Raaji et al. 1993a). Similarly, many intermediate-duration experiments did not detect significant changes in body weight in Wistar rats receiving hexachlorobenzene by gavage or from the diet at up to 1,000 mg/kg/day 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 receiving approximately 51 mg/kg/day for 3–4 weeks (Ezendam et al. 2004a; Michielsen et al. 1997, 2000), in ovariectomized adult female Sprague-Dawley rats gavaged with doses up to 100 mg/kg/day of hexachlorobenzene in corn oil for 30 days (Foster et al. 1995b), in female CD rats receiving up to 36 mg/kg/day from the diet for 4 months (Goldstein et al. 1978), in male BALB/c mice receiving 30 mg/kg/day of hexachlorobenzene from the diet for 6 weeks (Loose et al. 1977), or in male or female Sprague-Dawley rats administered hexachlorobenzene by daily gavage for 90 days at doses up to 25 mg/kg/day (NTP 2002). 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. Although one study reported statistically significant decreases in body weight (ca. 9% less than controls) 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 revealed no signs of 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). Smith et al. (1987) reported 18–22% depressed terminal body weight in male Syrian golden hamsters ingesting hexachlorobenzene from the diet at 19 mg/kg/day for 18 or 28 weeks. Among Syrian golden hamsters ingesting 16 mg/kg/day (but not lower doses up to 8 mg/kg/day) hexachlorobenzene from the diet for life, males exhibited “marked weight reduction” and

significantly increased mortality (Cabral et al. 1977). Similar mortality was observed in treated female hamsters in the absence of corresponding weight loss.

Similar findings have also been demonstrated in dogs, monkeys, and pigs. Weight loss was observed in 4 of 12 beagle dogs fed capsules of hexachlorobenzene at 11 mg/kg/day for up to 12 months (Gralla et al. 1977). Similarly, 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 contrast, experiments in which female Cynomolgus monkeys were dosed with hexachlorobenzene 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 fetal and immature pups, hexachlorobenzene-induced changes in 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 approximately 2–3 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 1974); 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 6.9 mg/kg/day (Grant et al. 1977). Maternal signs reported by these two studies (Grant et al. 1977; Khera 1974) included mortality, convulsions, and reduced fertility.

Metabolic Effects. Several groups of investigators evaluated possible associations between serum organochlorine pesticide levels (including hexachlorobenzene) and risk or prevalence of diabetes (Burns et al. 2014; Codru et al. 2007; Cox et al. 2007; Gasull et al. 2012; Glynn et al. 2003; Lee et al. 2010; 2011; Son et al. 2010; Wu et al. 2013). Study limitations include the use of mostly small group sizes, lack of quantification of exposure to hexachlorobenzene, and/or presence of other organochlorine compounds in the serum.

Within a group of 205 women in areas of Sweden where organochlorine-contaminated fish were readily available, Glynn et al. (2003) reported a significant difference (p=0.008) in mean hexachlorobenzene serum levels among 7 women with reported diabetes (85 ng/g lipid; 95% CI 66, 109) and 198 women without diabetes (60 ng/g lipid; 95% CI 58, 63).

Codru et al. (2007) reported a significant association between serum hexachlorobenzene and evidence of diabetes (serum fasting glucose values >125 mg/dL and/or use of antidiabetic medication) (OR 4.8: 95% CI 1.7, 13.9) in a cross-sectional study of 352 adults from a Native American (Mohawk) population.

Wu et al. (2013) reported a significant positive association between plasma hexachlorobenzene level and incident type 2 diabetes (OR 3.59; 95% CI 1.49, 8.64; third tertile versus first tertile) in a group of 1,095 women who were free of diabetes at blood draw in 1989–1990 and who participated in two case-control studies in the Nurses' Health Study. The study included 48 cases of diabetes and 1,047 controls.

In a cohort study of 318 boys with serum organochlorine pesticide measurements at entry into the Russian Children's Study (8–9 years of prepubertal age at baseline; 10–13 years of pubertal age at follow up), a significant association was reported between baseline serum hexachlorobenzene (third tertile versus first tertile) and risk of insulin resistance at follow up (OR 4.37; 95% CI 1.44, 13.28) (Burns et al. (2014).

In a community-based health interview survey of 886 subjects in Catalonia, Spain (Gasull et al. 2012), prevalence of prediabetes (202 cases) and diabetes(143 cases) increased across quartiles of serum hexachlorobenzene levels; comparing the highest quartile with the lowest quartile, ORs were 2.1 (95% CI 1.0, 4.4) for prediabetes and 3.2 (95% CI 1.3, 8.1) for diabetes. Both groups exhibited significant trends for increasing incidence with increasing quartile of serum hexachlorobenzene.

In another community-based health survey of 40 cases of type 2 diabetes and 40 controls in Uljin County, South Korea (Son et al. 2010), comparison of the highest tertile of serum hexachlorobenzene with the lowest tertile resulted in an OR of 6.1 (95% CI 1.0, 36.6) for prevalence of type 2 diabetes; the trend for increasing prevalence with increasing serum hexachlorobenzene was statistically significant (p=0.03).

In a sample of 1,303 Mexican-Americans from the Hispanic Health and Nutrition Examination Survey who were 20–74 years of age and resided in the southwestern United States from 1982 to 1984, no significant association was found between serum hexachlorobenzene and prevalence of self-reported diabetes (Cox et al. 2007). There were no significant associations between serum hexachlorobenzene and

prevalence of type 2 diabetes in a nested case-control study of 90 diabetes cases and 90 controls (Lee et al. 2010) or in a cross-sectional study of 725 subjects living in Uppsala, Sweden (70 years of age and free of diabetes at baseline; 36 cases of diabetes diagnosed during 5-year follow up) (Lee et al. 2011).

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 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). 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).

In a birth cohort of 965 women in Denmark, Hansen et al. (2014) evaluated possible associations between maternal serum persistent organochlorine pollutants (POPs), including hexachlorobenzene, and risk of asthma in offspring followed through 20 years of age. After dividing the study group by tertiles of serum hexachlorobenzene levels (0.07–0.45, >0.45–0.63, >0.63–2.45 ng/mL), comparison of the first and third tertile suggested a significant association between maternal serum hexachlorobenzene level and reported offspring asthma medication use (hazard ratio 1.92; 95% CI 1.15, 3.21).

Gascon et al. (2014) evaluated possible associations between maternal serum DDE, hexachlorobenzene, and PCB levels in 405 participants of the INMA-Menorca birth cohort (Spain) and reported increased prevalence of wheeze, chest infections, atopy, and asthma among the offspring from birth to 14 years of age. At 4 years of age, 275 children provided serum samples for measurement of cytokines and

biomarkers of inflammation (IL2, IL8, IL10, and TNFα). Increasing maternal serum hexachlorobenzene was significantly associated with increasing postnatal serum IL10 (β 0.22; 95 CI 0.02, 0.41), but not with IL6, IL8, or TNFα. Maternal hexachlorobenzene serum level was also significantly associated with wheeze (relative risk [RR] 1.58; 95% CI 1.04, 2.41) and chest infections (RR 1.89; 95% CI 1.10, 3.25) at 10 years of age.

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. 1977). 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. The lowest dose to cause an immune response was 0.022 mg/kg/day, which induced peribiliary lymphocytosis in F_1 Sprague-Dawley rats exposed via the diet for life after having been exposed via their mothers during gestation and lactation (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. Hexachlorobenzene doses as low as 0.9 mg/kg/day, received from the diet, resulted in significantly reduced spleen cell cytocidal activity after 6 weeks of exposure and increased susceptibility to tumor challenge *in vivo* and decreased killing of tumor cells *in vitro* after exposure for 18 weeks (Loose et al. 1981). Ingestion of 18 mg/kg/day decreased survival time by as much as 46% following ascites tumor cell challenge (Loose et al. 1981), and ingestion of 30 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 (30 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 30 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 46 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 and Brown Norway rats ingesting doses of approximately 50–129 mg/kg/day for 3 weeks, immunological effects observed included increased spleen and lymph node (auricular, popliteal, inguinal, and/or mediastinal, but not parathymic) weight (Ezendam et al. 2004a, 2004b; Kennedy and Wigfield 1990; Koss et al. 1978; Michielsen et al. 2001, 2002; Schielen et al. 1995b; Vos et al. 1979a, 1979b); 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 numbers of splenic T-cells and auricular lymph node B-cells (Ezendam et al. 2004a); increased splenic and/or serum levels of total IgM and/or IgE (Ezendam et al. 2004a, 2004b; Schielen et al. 1993, 1995b); 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); increased CD25 expression in mesenteric lymph nodes (Ezendam et al. 2004b); hyperplasia of the B-cell compartments and increased extramedullary hemopoiesis in the spleen (Ezendam et al. 2004b); and histopathologic evidence of inflammatory lesions in the lung (Ezendam et al. 2004a, 2004b; Michielsen et al. 2002). The 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 184 mg/kg/day for 3 weeks (Vos et al. 1979b). Feeding of male Wistar rats with doses as low as 13.8 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 ingesting 184 mg/kg/day for 3 weeks (Vos et al. 1979b) and in female Wistar rats ingesting 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 ingested estimated 15.4 or 25.6 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 25.6 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 *L. monocytogenes*. However, the follow-up study found that hexachlorobenzene stimulated both humoral and cell-mediated immunity in the Wistar rat pups whose mothers ingested an estimated 0.4, 2.1, or 10.3 mg/kg/day of hexachlorobenzene from early pregnancy through lactation; weaning pups were exposed to the same dietary concentrations as their mothers for 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 0.4 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 2.1 mg/kg/day and above, increased popliteal lymph node weight with a corresponding increase in lymph node cellular proliferation were observed, and cell proliferation was also detected in the endothelial cells lining pulmonary capillaries and venules. At 10.3 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 fed diets containing hexachlorobenzene (Michielsen et al. 1997). For 28 days, Wistar rats consumed approximately 46 or 92 mg hexachlorobenzene/kg/day; Lewis rats ingested approximately 17 or 51 mg hexachlorobenzene/kg/day; and Brown Norway rats ingested approximately 17, 51, or 102 mg/kg/day. 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.

NTP (2002) administered hexachlorobenzene by gavage (in corn oil with 1% acetone) to female Sprague-Dawley rats (10/group) once/day, 5 days/week for 90 days at doses ranging from 0 to 25 mg/kg/day. Significantly ($p<0.01$) increased incidences of splenic lymphoid hyperplasia were noted at hexachlorobenzene doses ≥10 mg/kg/day (incidences of 0/10, 0/10, 0/10, 0/10, 0/10, 0/10, 6/10, and 8/10 for dose groups of 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10, and 25 mg/kg/day, respectively).

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 (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 hexachlorobenzene. 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. 1977).

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 1974; 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 14.6 mg/kg/day in Sprague-Dawley rats as part of a multigeneration study, with convulsions preceding death (Grant et al. 1977). Mice receiving 39 mg/kg/day of hexachlorobenzene from the diet for up to 17 weeks exhibited severe tremors prior to death (Hahn et al. 1988). Male SPF pigs fed diets containing hexachlorobenzene at concentrations resulting in ingestion of approximately 50 mg/kg/day 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).

Other studies have investigated less overt neurological effects resulting from oral exposure to hexachlorobenzene. 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 ingesting hexachlorobenzene at 69 mg/kg/day of hexachlorobenzene for 20 weeks or at least 9.1 mg/kg/day for 2 years (Sufit et al. 1986). In male Wistar rats gavaged with 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). Hadjab et al. (2004) reported significantly increased auditory threshold in the 2–16 kHz frequency range in male Sprague-Dawley rats administered hexachlorobenzene by gavage for 4 weeks at 4 or 16 mg/kg/day. 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 hexachlorobenzene 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 hyperactivity 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 in the high-dose group 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 for increased hyperactivity in the offspring was used to calculate an acute-duration oral MRL as described in Table 3-2 and in Appendix A.

In the other developmental study, groups of female Wistar rats received hexachlorobenzene from the diet at estimated doses of 0.3, 0.6, or 1.3 mg/kg/day for 90 days prior to mating and through gestation and lactation; offspring were maintained on the same diet until postnatal day 150 (Lilienthal et al. 1996). In pups from the high-dose group (1.3 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 developmental 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).

Possible associations between serum hexachlorobenzene and selected reproductive outcomes have been evaluated by a number of investigators. However, these studies provide no clear evidence of hexachlorobenzene-related effects on reproductive outcomes in the study groups. Akkina et al. (2004) found no significant association between serum hexachlorobenzene and age at onset of menopause in a group of 219 Hispanic women residing in the United States. The authors noted that organochlorine serum levels measured at the time of sampling may not be representative of exposure levels at the time of menopause. Cooney et al. (2010) reported an odds ratio (OR) of 6.6 (95% CI 1.0, 42.8) for endometriosis in a group of women with hexachlorobenzene levels >0.04 ng/g serum (adjusted for total serum lipids, smoking, and other pesticides) compared to a referent group with hexachlorobenzene levels <0.02 ng/g serum (adjusted similarly). Thirty-two of the 84 women evaluated in this study were diagnosed with endometriosis. There were 14 endometriosis cases among 27 women with aromatic fungicide (including hexachlorobenzene) levels >0.04 mg/g serum and 6 cases among 26 women in the referent group with aromatic fungicide (including hexachlorobenzene) levels <0.02 mg/g serum. Upon grouping the serum organochlorine pesticides tertiles by chemical structure and odds of an endometriosis diagnosis and adjusting for total serum lipids and smoking, the aromatic fungicides group (which included hexachlorobenzene) with serum levels >0.04 ng/g serum exhibited an OR of 5.3 (95% CI 1.2, 23.6). Significant ORs were not found within the groupings of chlorinated insecticides (included β-benzene hexachloride and dichloro-diphenyl-dichloroethylene) or cyclodiene insecticides (included aldrin, mirex, and trans-nonachlor). The study is limited by small numbers of subjects and lack of quantification of hexachlorobenzene exposure levels. In another case-control study of 80 cases of endometriosis and 78 controls in Rome, Italy, no significant association was found between risk of endometriosis and serum hexachlorobenzene levels (Porpora et al. 2009).

No significant associations were observed between serum hexachlorobenzene and serum testosterone levels among 257 adult male and 436 adult female Native Americans (Mohawks) (Goncharov et al. 2009) or among 341 adult men from a fertility clinic (Ferguson et al. 2012). In a cross-sectional study, Freire et al. (2014) evaluated possible associations between serum organochlorine levels (including hexachlorobenzene) and serum hormone levels among 304 men and 300 women in a rural area of Brazil heavily contaminated with organochlorine pesticides. There were no significant associations between serum hexachlorobenzene and serum testosterone levels in the men or serum estradiol, progesterone,

prolactin, luteinizing hormone (LH), or FSH in premenopausal women; however, a slight but significant ($p<0.05$) negative association was found for serum LH among 77 peri- and postmenopausal women.

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). Simon et al. (1979) reported decreased reproductive performance, as indicated by decreased impregnation of female rats by male rats administered hexachlorobenzene by gavage at 221 mg/kg/day for 5 days prior to mating; there was no effect on fertility index (number of pregnant females/number of females impregnated). 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).

In a 90-day assay in adult female Cynomolgus monkeys, ovarian toxicity was noted at the lowest oral dose tested, 0.01 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-duration oral MRL as described in 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.

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 hexachlorobenzene induced alterations in steroidogenesis of cells of the inner zone of the adrenal cortex.

NTP (2002) administered hexachlorobenzene by gavage (in corn oil with 1% acetone) to female Sprague-Dawley rats (10/group) once/day, 5 days/week for 90 days at doses ranging from 0 to 25 mg/kg/day. Significantly ($p<0.001$) increased incidences of mammary gland hyperplasia were noted at the highest dose level (incidences of 2/10, 2/10, 1/10, 0/10, 2/10, 2/10, 3/10, and 10/10 for dose groups of 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10, and 25 mg/kg/day, respectively).

Some intermediate-duration experiments have demonstrated that hexachlorobenzene adversely affects reproductive performance. In a multigenerational study in which male and female Sprague-Dawley rats received hexachlorobenzene from the diet at doses ranging from approximately 0.9 to 63 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 approximately 28 mg/kg/day (parental males) and 31 mg/kg/day (parental females) and average litter size was decreased at doses ≥14 mg/kg/day (parental males) and ≥16 mg/kg/day (parental females) (Grant et al. 1977). No reproductive toxicity was observed, either in female Sprague-Dawley rats receiving hexachlorobenzene from the diet at doses up to 13.7 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). No reproductive effects were observed in a study in which both male and female Sprague-Dawley rats received hexachlorobenzene from the diet at estimated doses up to 3.4 mg/kg/day (males) and 3.9 mg/kg/day (females) from 3 months prior to mating through weaning of the F_1 offspring (Arnold et al. 1985).

In male Fischer 344/N rats ingesting hexachlorobenzene in arachis oil at approximately 16 mg/kg/day 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. 1977). 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 accumulation 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 paresthesia (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 more recent report by Jarrell and coworkers (Jarrell et al. 2002) found a significantly lower lifetime proportion of male offspring from women reporting hexachlorobenzene exposure at the peak of the Turkish epidemic (1955–1957) compared to women exposed at a later date.

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 in these populations. 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).

Several groups of investigators have evaluated possible associations between levels of hexachlorobenzene in maternal blood or breast milk, cord blood, or children's blood and developmental end points such as birth size (weight and/or length) or preterm birth (Basterrechea et al. 2014; Eggesbø et al. 2009; Fenster et al. 2006; Gladen et al. 2003; Guo et al. 2014; Lopez-Espinosa et al. 2011; Sagiv et al. 2007; Szyrwińska and Lulek 2007; Torres-Arreola et al. 2003; Vafeiadi et al. 2014), recurrent miscarriage (Sugiura-Ogasawara et al. 2003), postnatal growth (Burns et al. 2012; Cupul-Uicab et al. 2013; Mendez et al. 2011; Smink et al. 2008; Valvi et al. 2014), postnatal neurodevelopment (Cheslack-Postava et al. 2013; Darvill

et al. 2000; Forns et al. 2012; Sioen et al. 2013; Strøm et al. 2014), sexual maturation (Croes et al. 2014a, 2014b; Denham et al. 2005; Lam et al. 2014; Schell and Gallo 2010), cryptorchidism (Pierik et al. 2007), hypospadias (Giordano et al. 2010; Rignell-Hydbom et al. 2012), and indicators of postnatal thyroid function (Freire et al. 2011; Julvez et al. 2011). Limitations of these studies include lack of quantifiable exposure to hexachlorobenzene, small numbers of subjects, and/or the presence of measureable levels of other organochlorine compounds. Although most studies found little evidence of significant associations between hexachlorobenzene levels and these developmental end points, significant associations were reported in several studies as discussed below.

Vafeiadi et al. (2014) reported that birth weight was negatively associated with increasing levels of maternal serum hexachlorobenzene (β -154.3 g; 95% CI -300.8, -7.9) in a population-based survey of 1,117 pregnant women and their children in Heraklion, Crete, Greece.

Lopez-Espinosa et al. (2011) reported a marginally significant (p=0.047) decrease of 0.39 cm in birth length for each 10-fold increase in umbilical cord hexachlorobenzene level in a birth cohort of 494 mothers and their newborns in Valencia, Spain (born 2003–2006). In another Spanish birth cohort study of 1,285 infants, Valvi et al. (2014) reported significant positive associations between maternal serum hexachlorobenzene level and rapid postnatal growth during the first 6 months (RR 1.44; 95% CI 1.04, 1.99) and overweight at 14 months of age (RR 1.45; 95% CI 1.10, 1.92) when comparing the highest tertile (maternal serum hexachlorobenzene >73 ng/g lipid) with the lowest tertile (≤22.6 ng/g lipid).

A case-control study of 80 hypospadia cases and 80 controls from two hospitals in Rome, Italy, reported significantly ($p<0.05$) increased risk of hypospadias with each increase of 10 pg/g hexachlorobenzene in the maternal serum (OR 1.26; 95% CI 1.04, 1.52) (Giordano et al. 2010). Another case-control study of 237 hypospadia cases and 237 controls in Sweden reported increased risk of hypospadias among those mothers with serum hexachlorobenzene levels >0.26 ng/mL compared to those mothers with lower serum hexachlorobenzene levels (OR 1.65; 95%CI 1.02, 2.69) (Rignell-Hydbom et al. (2012).

Croes et al. (2014a, 2014b) reported a significant (p=0.02) negative association between serum hexachlorobenzene and reaching menarche at 14–15 years of age (OR 0.35; 95% CI 0.15, 0.84) in a group of 282 girls in FLEHS II; among 324 boys in the same study, serum hexachlorobenzene was significantly positively associated with total serum testosterone (OR 1.04; 95% CI 1.01, 1.07; p=0.004), the ratio of testosterone to estradiol (OR 1.05; 95% CI 1.01, 1.08; p=0.007), and reaching of the adult stage of

testosterone (OR 1.29; 95% CI 1.01, 1.65; p=0.04), and borderline significantly positively correlated with age at pubic hair development (OR 1.77; 95% CI 1.00, 3.14; p=0.052).

In a prospective cohort study of 350 prepubertal Russian boys (8–9 years of age) who were monitored yearly for serum organochlorine levels (including hexachlorobenzene), higher serum hexachlorobenzene levels were associated with later mean age of reaching puberty as determined by testicular volume pubic hair growth; however, there were no significant associations after adjusting for baseline body mass index (BMI) categories and height (Lam et al. 2014).

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 1974). Intermediate-duration developmental studies in rats include reports of 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 approximately 6–14 mg/kg/day (Grant et al. 1977; Kitchin et al. 1982). 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). In a multigenerational reproductive study (chronic-duration exposure) in Sprague-Dawley rats, hexachlorobenzene induced decreased viability of neonatal pups of parental rats administered hexachlorobenzene in the diet for 3 months prior to mating and throughout mating and gestation at estimated doses of 3.4 mg/kg/day (males) and 3.9 mg/kg/day (females) (Arnold et al. 1985).

The most sensitive acute-duration 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-duration 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 14th rib formation at ≥40 mg/kg/day (Khera 1974). 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. 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, doses ≥0.4 mg/kg/day resulted in increased immune responses (IgG and IgM responses to tetanus toxin, delayed-type reaction to ovalbumin, and pulmonary accumulation of foamy macrophages) (Vos et al. 1983). Doses ≥2.1 mg/kg/day caused lymph node endothelial proliferation and increased weights of liver and popliteal lymph nodes. Effects in the high-dose group (10.3 mg/kg/day) included increased pup mortality, increased weights of spleen, lung, adrenals, and mesenteric lymph nodes, increased basophils (without an overall increase in leukocytes) and serum IgM (but not IgG), and histopathologic evidence of hepatocellular hypertrophy and necrosis. In a similarly-designed study (Vos et al. 1979a), F_1 rats from the group receiving 25.6 mg/kg/day of hexachlorobenzene from the diet exhibited significantly increases in liver and adrenal weights, blood levels of eosinophils and basophils, serum IgM and IgG levels, slight cytoplasmic hyalinization of parenchymal cells in the liver, accumulation of foamy macrophages in the lung, increased extramedullary hematopoiesis in the spleen, and increased IgG response to *Trichinella*. In 15.4 and 25.6 mg/kg/day dose groups, effects included proliferation of high-endothelial venules in the paracortex of the lymph nodes, decreased resistance to *Listeria* and *Trichinella* infection, and increased IgG response to tetanus toxoid. Similar developmental effects were observed in an intermediate-duration reproductive study in which female Sprague-Dawley rats were fed hexachlorobenzene for 96 days prior to mating through two rounds of breeding (Kitchin et al. 1982); in both F_{1a} and F_{1b} pups, decreased body weight and decreased survival were observed at dietary concentrations resulting in doses ≥5.9 and 7.8 mg/kg/day, respectively.

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.

A four-generation assay found increased liver weight and hepatic aniline hydroxylase activity at hexachlorobenzene dietary concentrations resulting in doses of approximately 3–4 mg/kg/day (F_{1a} and F_{3a} animals), consistently decreased pup weight at ≥6.9 mg/kg/day (all pup generations), and decreased pup viability at doses ≥13.8 mg/kg/day (F_{1a} and F_{1b} animals) (Grant et al. 1977). Arnold et al. (1985) fed hexachlorobenzene to male and female Sprague-Dawley rats from 3 months prior to mating through weaning; pups were continued on the same diet for their entire lifetime. The high-dose group (estimated doses of 2.8 and 3.2 mg/kg/day for F_0 parental males and females, respectively) exhibited decreased pup survival. When examined as adults (week 130), treatment-related effects in F_1 males included peribiliary lymphocytosis and fibrosis at an estimated dose of 0.022 mg/kg/day and hepatic basophilic chromogenesis at ≥0.55 mg/kg/day. The LOAEL of 0.022 mg/kg/day served as the basis for a chronic-duration oral MRL of 0.00007 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 2014). A cancer assessment for hexachlorobenzene is available on Integrated Risk Information System (IRIS 2003) 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 hexachlorobenzene has been shown to induce tumors in the liver, thyroid, and kidney in three rodent species. IRIS (2003) presents an oral slope factor of 1.6 per (mg/kg)/day and an 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).

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. These 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 could possibly be causal (Axelson 1986; Salata et al. 1985; Topi et al. 1980; Waddington 1972).

Other available epidemiology studies that have assessed possible associations between hexachloro-benzene and cancer end points collectively do not support an association between hexachlorobenzene exposure and increased cancer incidence. However, limitations of these studies (including small study sizes, similar tissue hexachlorobenzene levels between cancer and control groups, and potentially confounding effects of other organochlorines) preclude definitive conclusions regarding the carcinogenicity hazard of hexachlorobenzene in humans.

Most case-control studies (ranging in size from 20 to >300 subjects per group) investigating organochlorine levels in serum or breast tissue samples surgically removed from groups of patients with breast cancer or benign breast tumors and serum or adipose tissue samples from subjects without diagnosed breast tumors were unable to detect statistically significant associations between hexachloro-benzene levels and breast cancer (Dorgan et al. 1999; Falck et al. 1992; Guttes et al. 1998; Høyer et al. 2001; Itoh et al. 2009; Iwasaki et al. 2008; Liljegren et al. 1998; López-Carillo et al. 2002; McCready et al. 2004; Mendonca et al. 1999; Moysich et al. 1998; Mussalo-Rauhammaa et al. 1990; Pavuk et al. 2003; Waliszewski et al. 2003; Zheng et al. 1999). Evidence of a possible association between serum hexachlorobenzene and breast cancer is provided by reports of significantly higher mean serum

hexachlorobenzene levels in breast cancer patients relative to controls with benign breast tumors or without evidence of breast tumors (Charlier et al. 2003, 2004; Dewailly et al. 1994). However, the numbers of breast cancer cases were small (n=≤50 for each study), which limits interpretation of the results.

No significant association was found between serum hexachlorobenzene and risk of testicular germ cell carcinoma in a population-based, case-control study of 18–44-year-old male residents of three Washington State counties (246 cancer cases and 630 controls) (Biggs et al. 2008). In a study of testicular germ cell tumor cases identified in the Norwegian Cancer Registry, no significant association was found between prediagnostic serum hexachlorobenzene (from blood samples taken between 1972 and 1978) and risk of testicular germ cell tumors among 49 cases diagnosed after blood samples had been taken; 51 control subjects were matched by region of residence, blood draw year, and age at blood draw (Purdue et al. 2009). Hardell et al. (2003, 2006b) found no significant association between serum hexachlorobenzene and testicular cancer among 58 cases and 61 age-matched controls in Stockholm, Sweden. The study authors noted that among case and control mothers who gave blood samples, serum hexachlorobenzene was significantly higher in the mothers of the men with testicular cancer compared to the mothers of the controls. Two case-control studies found no significant association between plasma hexachlorobenzene and prostate cancer (Aronson et al. 2010; Sawada et al. 2010). Hardell et al. (2006a) reported a significant association between hexachlorobenzene adipose tissue concentrations and risk of prostate cancer (OR=9.84; 95% CI 1.99, 48.5) among 26 prostate cancer cases with prostate-specific antigen (PSA) >16 ng/mL compared to 10 control subjects.

No significant associations were observed between blood hexachlorobenzene levels and risk of pancreatic cancer among 108 cases in the San Francisco Bay area (Hoppin et al. 2000), risk of endometrial cancer among 154 cases in Sweden (Weiderpass et al. 2000), risk of colorectal cancer among 132 cases in Barcelona, Spain (Howsam et al. 2004), or risk of non-Hodgkin's lymphoma (NHL) among 74 cases in Washington County, Maryland (Cantor et al. 2003). No significant association was observed between hexachlorobenzene bone marrow levels and risk of leukemia among 13 German leukemia patients (Scheele et al. 1996), hexachlorobenzene adipose tissue levels and risk of Ewing's sarcoma of the bone among 4 male Swedish patients (Hardell et al. 1997), or hexachlorobenzene adipose tissue levels and risk of NHL among 175 cases in the U.S. EPA National Human Adipose Tissue Survey (Quintana et al. 2004) or 256 cases enrolled in the Danish Cancer Registry (Bräuner et al. 2012).

In a population-based, case-control study in British Columbia, Canada, Spinelli et al. (2007) reported a significant association between plasma hexachlorobenzene and risk of NHL among 138 cases with hexachlorobenzene levels >22.78 ng/g compared to 83 cases with hexachlorobenzene levels ≤11.45 ng/g (OR=1.94; 95% CI 1.25, 3.03). Björnforth et al. (2007) reported a significant association between hexachlorobenzene adipose tissue levels and risk of pancreatic cancer among 21 cases (OR=53.0; 95% CI 4.64, 605) compared with controls.

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 is unclear.

Chronic oral exposure to hexachlorobenzene induces liver tumors in rats, with female rats appearing more susceptible than males. Ertürk et al. (1986) fed hexachlorobenzene to Sprague-Dawley rats at dietary concentrations resulting in estimated doses of 5.2 or 10.3 mg/kg/day (males) and 6.0 or 12.0 mg/kg/day (females) 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 concentrations resulting in estimated doses of 7 and 4.5 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 hexachlorobenzene at dietary concentrations resulting in estimated doses of 15.8 mg/kg/day (males) and 18.3 mg/kg/day (females) 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 female (but not male) F_1 Sprague-Dawley rats with lifetime dietary exposure to hexachlorobenzene at a concentration resulting in an estimated dose of 3.2 mg/kg/day, 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, or 16 mg/kg/day of hexachlorobenzene in the diet for life (Cabral et al. 1977). All treatment groups exhibited statistically significant increases in incidences of hepatomas and hemangioendotheliomas (liver and spleen), 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 increased incidence of liver cell tumors was observed at 12 and 25 mg/kg/day (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 exposure to hexachlorobenzene in the diet at 0, 100, or 200 ppm (mice) and 0, 200, or 400 ppm (rats and hamsters). 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).

Three studies in rats provide limited evidence that hexachlorobenzene is a promoter but not an initiator of liver cancer. Treatment of intact Sprague-Dawley rats with 100 ppm of hexachlorobenzene in the food for 45 days (estimated dose of 9.1 mg/kg/day) did not induce hepatic foci positive for gamma-glutamyl

transpeptidase, but foci were induced following liver initiation by partial hepatectomy with and without diethylnitrosoamine (a known liver tumor promoter) (Pereira et al. 1982). This finding suggests that hexachlorobenzene may act as a promoter at doses insufficient to initiate tumors. Abdo et al. (2013) administered diethylnitrosamine to male F344 rats by intraperitoneal injection at 200 mg/kg, followed by a 2-week period without treatment and subsequent administration of hexachlorobenzene in the diet for 6 weeks at 0, 70, or 350 ppm; a partial hepatectomy was performed at week 3. The low- and high-dose groups of hexachlorobenzene-treated rats exhibited significant increases in numbers of hepatic GST-P positive foci (approximately 2- and 5-fold, respectively, greater than that of controls) and areas of hepatic GST-P positive foci (approximately 2.3- and 4.2-fold, respectively, greater than that of controls). These results support the findings of Pereira et al. (1982). 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 hexachlorobenzene. 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 hexachlorobenzene via their mothers during gestation and lactation (estimated maternal dose of 3.9 mg/kg/day) and directly from the feed for their lifetime (approximately 130 weeks postweaning; estimated dose 2.8 mg/kg/day for males and 3.2 mg/kg/day for females), 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 10 mg/kg/day for 4 months (Kimbrough and Linder 1974). No other studies have reported any parathyroid histopathology caused by oral exposure to hexachlorobenzene, 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 administered

hexachlorobenzene in the food have not been corroborated (Ertürk et al. 1986). Renal damage with metaplastic regenerative changes was observed 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 more frequently in female mice than in male mice.

Randi and coworkers (García et al. 2010; Peña et al. 2012; Pontillo et al. 2011; Randi et al. 2006) performed a series of experiments designed to elucidate possible mechanisms of hexachlorobenzene mammary tumor co-carcinogenicity observed in rats (Randi et al. 2006). The investigators found that hexachlorobenzene: (1) induced cell proliferation in the MCF-7 breast cancer cell line in an estrogen receptor (ER) alpha-dependent manner; (2) induced migration in the MDA-MB-231 breast cancer cell line; and (3) increased cellular sarcoma/human growth factor receptor1 (cSrc/HER1) and ER alpha signaling pathways. The results suggest that alterations in the estrogenic microenvironment may influence the biological behavior of mammary gland or breast tumors.

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

Collectively, the results of available studies do not indicate that hexachlorobenzene acts as a genotoxic agent, although the database of information is limited.

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 perchloroethylene (da Silva Augusto et al. 1997). The usefulness of this study is limited by the confounding effect of exposure to multiple chemicals.

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 in which rats were exposed orally at doses ranging from 60 to 221 mg/kg (Khera 1974; 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, hexachlorobenzene was observed to be bound to DNA (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. However, there is evidence that phenobarbital is mutagenic *in vitro* in several test systems (Jackson et al. 1993). 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).

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 pentachlorobenzene 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.

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

End point	Species (test system)	Exposure route	Results	Reference
Mammalian systems:				
Dominant lethals	Rat	Oral	–	Khera 1974
	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		Reference
		With activation	Without activation	
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

3.4 TOXICOKINETICS

In humans, inhalation accounts for an unknown, but probably low, amount of exposure due to the low vapor pressure of hexachlorobenzene ($1.1x10^{-5}$ mmHg at 25 °C; see Table 4-2). 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 (Arnot et al. 2010; 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), people living in the area of Flix, Spain, who have been exposed to airborne hexachlorobenzene from an organochlorine factory (Carrizo et al. 2008; Grimalt et al. 1994; Herrero et al. 1999; Ozalla et al. 2002; Ribas-Fitó et al. 2003a, 2003b, 2005, 2007; Sala et al. 1999b; Sunyer et al. 2002, 2008; To-Figueras et al. 1997), and people living in the area of Menorca, Spain, a rural county with no known source of high levels of atmospheric hexachlorobenzene (Carrizo et al. 2008). 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 (Arnot et al. 2010; 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; 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 $1.4x10^{-3}$ per hour (Koizumi 1991).

Information on distribution in people following inhalation exposure to hexachlorobenzene is limited (Ataniyazova et al. 2001; Sala et al. 1999c, 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 hexachlorobenzene 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). Pentachlorophenol has been identified

in human liver preparations incubated with hexachlorobenzene (Koss et al. 1986). In animals, hexachlorobenzene is slowly metabolized to pentachlorophenol by the hepatic cytochrome P-450 system, conjugated with glutathione to yield glutathione conjugates excreted in the bile, or reductively dechlorinated to form pentachlorobenzene. Other metabolites include less chlorinated benzenes, pentachlorothiophenol, chlorophenols, S-conjugated phenols, and S-conjugated benzenes. Pentachlorophenol is subsequently converted to tetrachlorohydroquinone (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% pentachlorobenzene and traces of pentachlorophenol after oral hexachlorobenzene exposure in mammals, while urinary excretion consists of mostly the metabolites, pentachlorobenzene, 2,4,5-trichlorophenol, *N*-acetyl-*S*(pentachlorophenyl)cysteine (a mercapturic acid), 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, pentachlorophenol, 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. 1976; 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 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).

To-Figueras et al. (2000) observed a high correlation between fecal and blood levels of hexachlorobenzene 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, pentachlorophenol and pentachlorothiophenol (To-Figueras et al. 1992). In animals, the excretion of hexachlorobenzene 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 (pentachlorophenol, pentachlorothiophenol, pentachloro-

benzene) (Mehendale et al. 1975). Approximately 99% of unchanged ingested hexachlorobenzene was excreted in the feces; 50% of urinary excretion was pentachlorophenol, 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; Ozalla et al. 2002; Sala et al. 1999b; Ribas-Fitó et al. 2003a, 2003b, 2005, 2007; Sunyer et al. 2002, 2008; 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 hexachlorobenzene via consumption of contaminated fish may have contributed slightly to serum hexachlorobenzene levels in nonfactory worker Flix residents, but was not a major factor. Studies of the rural Spanish village of Flix show 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 hexachlorobenzene 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 hexachlorobenzene 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). A trend for decreasing net absorption ([ingested – excreted in feces]/ingested) with increasing blood levels was observed in the volunteers, and a linear

regression was fit to these data. The calculated regression equation was (R^2=0.98):net absorption hexachlorobenzene = 0.8538–0.0021 x (ng hexachlorobenzene/g blood lipid). This equation predicts nearly complete absorption of ingested hexachlorobenzene (approximately 85%) at low blood concentrations and that net absorption decreases by approximately 0.2% for each ng increase in hexachlorobenzene per g lipid in 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 hexachlorobenzene 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 (as would be expected based on a water solubility of

0.006 mg/L at 20–25 °C; see Table 4-2). 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 hexachlorobenzene 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

Information regarding the distribution of hexachlorobenzene in humans exposed via inhalation derives mainly from studies of people employed by, or living near, an organochlorine-producing electrochemical factory in the area of Flix, Spain, where high levels of airborne hexachlorobenzene were detected. Mean serum hexachlorobenzene levels as high as 26–37 ng/mL have been reported in groups of local inhabitants (Grimalt et al. 1994; Sala et al. 1999c). A mean serum hexachlorobenzene level of 54.6 ng/mL was noted for a group of males who worked at the factory (Sala et al. 1999b). Among inhabitants of the area, relatively high levels of hexachlorobenzene have been measured in maternal blood, umbilical cord blood, and breast milk samples of people living nearby. Sala et al. (2001a) compared two populations; for a group of 31 pairs of mothers and infants from Flix, 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. Several other reports include measurements of elevated hexachlorobenzene levels in serum and/or cord blood from inhabitants of the area surrounding Flix (Ribas-Fitó et al. 2003a, 2003b, 2007; Sunyer et al. 2002, 2008; To-Figueras et al. 2000). Ribas-Fitó et al. (2005) reported a statistically significant ($p>0.05$) correlation between breastfeeding and serum hexachlorobenzene levels in 1-year-old children living in Flix. Hexachlorobenzene and other organochlorines (*p,p'*-DDE, PCBs) were measured in the colostrum of mothers and cord blood serum of infants in 92 infant-mother pairs within the first 3 days of delivery. Hexachlorobenzene levels were measured in breast milk at 3 weeks, and serum blood hexachlorobenzene concentration was measured again at 13 months after delivery. Hexachlorobenzene concentrations in breastfed and formula-fed children at 13 months post-delivery were 4.26 and 2.13 ng/mL, respectively. However, the correlation between breastfeeding and hexachlorobenzene levels in children did not remain statistically significant after adjustment for parity, maternal age, maternal body mass index, and residence time in Flix.

In a study on serum hexachlorobenzene levels in 4-year-old children in Ribera d'Ebra county, Spain, a location exposed to high levels of atmospheric hexachlorobenzene and Menorca, Spain, a rural county

with no known source of hexachlorobenzene (Carrizo et al. 2008), breastfed children had statistically higher concentrations of hexachlorobenzene than formula-fed children ($p<0.01$ in the high-exposure population and $p<0.0001$ in the low-exposure population). Mean concentrations of hexachlorobenzene in breastfed versus nonbreastfed children from Ribera d'Ebra were 1.5 ng/mL (range 0.30–0.58 ng/L) and 0.99 ng/mL (range 0.17–2.05 ng/L), respectively, and mean concentrations in breastfed versus nonbreastfed children from Menorca were 0.47 ng/mL (range 0.067–2.1 ng/L) and 0.23 ng/mL (range 0.11–0.46 ng/L), respectively. In the Menorca population, there was a statistically significant correlation ($p<0.05$) between serum hexachlorobenzene concentration in children and maternal body mass index. Maternal feeding was the primary route of exposure of children in both populations.

The Aral Sea in Uzbekistan is a putative source of airborne exposure to hexachlorobenzene, metals, and other pollutants because its water levels are decreasing, resulting in sediment-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 southern 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).

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 10–15-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.

The effect of repetitive weight gain and weight loss on the metabolism of hexachlorobenzene in mice was investigated by Jandacek et al. (2005). Radiolabeled hexachlorobenzene ([^{14}C]-hexachlorobenzene) was administered 1 time via gavage in groups of eight C57BL/6 mice at a dose of 0.7 µCi/day. The groups were then given alternating diets high in fat or of reduced calorie over 5-day intervals to simulate a “yo-yo diet”. Higher hexachlorobenzene concentrations in the brain, kidney, and adipose tissue were associated with loss of body fat, as was an increase in plasma hexachlorobenzene with prolonged caloric restriction. Hexachlorobenzene levels in the epididymal fat pad remained constant during caloric restriction. Weight regains resulted in a statistically significant (significance level not reported) increase in liver lipids and hexachlorobenzene concentrations.

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, hexachlorobenzene 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. Three 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). Ntow et al. (2008) evaluated organochlorine levels (including hexachlorobenzene) in serum samples from 115 subjects (56 males and 59 women) and breast milk samples from 109 women (45 of whom also provided serum) among vegetable farmers in Ghana. Mean levels of hexachlorobenzene in pooled serum samples and pooled milk samples were 5.3 and 4.9 ng/g lipid, respectively. Collectively, these results indicate that hexachlorobenzene is readily stored in fat, which may result in long-term health implications.

In studies of 199 full-term healthy neonates in Fulda and Dusseldorf, Germany, the median hexachlorobenzene cord blood concentration from the 95th percentile by rank of hexachlorobenzene concentration in both locations was 0.5 µg/L in 1998 (Lackmann 2002). This concentration was approximately 90% less than levels measured in cord blood of neonates from the same localities sampled in 1994 and 1995 (Lackmann et al. 1996).

An association between breastfeeding and serum hexachlorobenzene levels in 6-week-old infants was examined by Lackmann (2004). Blood samples from 25 breast- and bottle-fed infants born in Germany were analyzed for hexachlorobenzenes, PCBs, and *p,p'*-DDE. Mean hexachlorobenzene levels were significantly different ($p<0.0001$) for breastfed and bottle-fed infants (0.13 and 0.04 µg/L, respectively).

Confounding factors such as gestational age, age of the mother, and smoking behavior of the parents did not alter significance levels. Lackmann (2006) reported significantly ($p<0.0001$) different mean serum hexachlorobenzene levels among breast- and bottle-fed infants 6 months of age as well (0.43 and 0.073 µg/L, respectively).

Link et al. (2005) found that 10-year-old children in Baden-Wuerttemberg, Germany, who had been breastfed as babies had statistically significantly ($p<0.0001$) higher concentrations of hexachlorobenzene and other organochlorines (*p,p'*-DDE, PCBs, gamma-hexachlorocyclohexane, dioxins, furans) than formula-fed children. Blood serum levels of hexachlorobenzene and other constituents were measured in fourth grade primary school children between 1996 and 2003; 1,614 blood samples were analyzed individually and 2,372 blood samples were pooled (30–70 individual samples were typically pooled per analysis) and analyzed during this time period. Mean concentrations of serum hexachlorobenzene levels in the individual blood samples (computed on a yearly basis) were 0.21, 0.14, 0.12, and 0.08 µg/L in breastfed children and 0.017, 0.12, 0.09, and 0.07 µg/L in nonbreastfed children in 1996/1997, 1998/1999, 2000/2001, and 2002/2003, respectively. In individually analyzed samples, boys had a statistically significantly ($p<0.0001$) higher blood concentration of hexachlorobenzene than girls after adjusting for influencing factors such as age, weight, concentration of triglycerides and cholesterol, duration of breastfeeding, investigation area, and year. There was also a positive association ($p=0.0004$) between serum triglycerides and hexachlorobenzene concentration in individual samples after adjusting for influencing factors. Hexachlorobenzene showed a statistically significant ($p<0.0001$) decrease in concentration during the time period investigated.

Hexachlorobenzene concentrations in breast milk of women from Tromsø and Oslo, Norway collected between 2000 and 2002 were low, averaging 18 ng/g (Polder et al. 2008). Breast milk samples were collected between 2000 and 2002, as part of the third World Health Organization (WHO)-coordinated exposure study, from 29 women living in Norway and analyzed for hexachlorobenzene, hexachlorocyclohexane, chlordanes, trans-nonachlor, PCBs, *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE, mirex, toxaphenes, polybrominated diphenylethers, and hexabromocyclodecanes. There were no significant differences in concentrations between the locations studied. Compared to data published by Johansen et al. (1994), hexachlorobenzene levels in breast milk in Norway have declined 56% since 1991. Declining hexachlorobenzene levels in breast milk have been observed in other regions as well. For example, Klinčić et al. (2014) reported a nearly 9-fold decrease in hexachlorobenzene levels in breast milk from 2006 to 2009/2010 in Zagreb, Croatia. Zietz et al. (2008) reported a 43% reduction in breast milk hexachlorobenzene levels in northern Germany in 2006 compared to levels measured in 1999. Mikeš et

al. (2012) observed >10-fold decrease in hexachlorobenzene concentrations in breast milk samples collected in the Czech Republic between 1994 and 2009.

Levels of hexachlorobenzene and other organochlorines in follicular fluid were measured by De Felip et al. (2004). Follicular fluid specimens from 12 women undergoing *in vitro* fertilization at a fertility clinic in Rome, Italy were obtained, and the specimens were pooled into two samples (six specimens each) for analysis of hexachlorobenzene, PCBs, dioxins, furans, and *p,p'*-DDT and metabolites. Hexachlorobenzene concentrations in the follicular fluid of the two pooled samples were 0.021 and 0.022 ng/g wet weight (69 and 73 ng/g lipid basis).

An association between hexachlorobenzene in breast milk and age of mother and number of children was investigated by Ennaceur et al. (2007). Breast milk samples were obtained from 87 lactating mothers in Tunisia between September 2002 and February 2003 and analyzed for hexachlorobenzene, hexachlorocyclohexane (beta and gamma), dieldrin, DDT, DDD, and DDE (*o,p'*- and *p,p'*). All subjects tested contained hexachlorobenzene residues in their breast milk; the mean concentration was 0.260 mg/kg milk fat (range 0.003–3.127 mg/kg milk fat). No significant relationship between number of childbirths and concentration of hexachlorobenzene was found, and although a relationship between increasing hexachlorobenzene levels and mothers' age was observed, there was no statistical significance ($p>0.05$). Ntow et al. (2008) evaluated relationships between maternal age and levels of DDTs, hexachlorocyclohexanes, hexachlorobenzene, and dieldrin in breast milk samples from 51 farmers in Ghana. For hexachlorobenzene, breast milk levels increased nonsignificantly ($p=0.067$) with increasing age (range 20–40 years).

Other studies have found hexachlorobenzene in human blood (e.g., Alvarado-Hernandez et al. 2013; Arrebola et al. 2012; Becker et al. 2008; Chovancová et al. 2004; Croes et al. 2014a, 2014b; Porta et al. 2012; 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 (e.g., Ansari et al. 1986; Arrebola et al. 2012; Dewailly et al. 1999; Lordo et al. 1996; Malarvannan et al. 2013; Robinson et al. 1990; Scheele et al. 1995; Siyali 1972; Teufel et al. 1990; Weistrand and Noren 1998), and breast milk (e.g., Behrooz et al. 2009; Çok et al. 2011; Colles et al. 2008; Craan and Haines 1998; Czaja et al. 1997; Devanathan et al. 2009; Ennaceur and Driss 2013; Ennaceur et al. 2007, 2008; Erdoğrul et al. 2004; Fujii et al. 2012; Gladen et al. 1999; Gocmen et al. 1989; Greizerstein et al. 1999; Guerranti et al. 2011; Haraguchi et al. 2009; Johansen et al. 1994; Johnson-Restrepo et al. 2007; Klinčić et al. 2014; Lunden and Noren 1998; Malarvannan et al. 2009;

Mannetje et al. 2013; Mikeš et al. 2012; Nasir et al. 1998; Newsome and Ryan 1999; Ntow et al. 2008; Petzold et al. 1999; Polder et al. 2008; Scheele et al. 1995; Shen et al. 2007; Szyrwińska and Lulek 2007; Tsydenova et al. 2006; Waliszewski et al. 1999a, 1999b; Weisenberg 1986; Wickstrom et al. 1983; Zhou et al. 2011, 2012; Zietz et al. 2008).

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). Mean serum hexachlorobenzene levels have been declining in recent years. For example, the FLEHS reported a mean serum hexachlorobenzene level of 21 ng/g lipid for the years 2003–2004 (FLEHS I), whereas a mean serum hexachlorobenzene level of 8.34 ng/g lipid was noted for the years 2008–2009 (FLEHS II) (Croes et al. 2014a, 2014b).

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. For example, 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), in all 656 births (GM=0.04 μg/L) analyzed in Quebec, Canada (Rhainds et al. 1999), and at a mean concentration of 0.54 μg/L among 92.8% of 1,438 cord blood samples from deliveries in Shanghai, China (Cao et al. 2011). Alvarado-Hernandez et al. (2013) reported higher concentrations of hexachlorobenzene in umbilical cord plasma than in maternal plasma (median concentrations of 137 and 58 ng/g lipid, respectively) among mother infant pairs living in a rural agricultural area of Mexico.

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). Nakashima et al. (1997) observed that lactational transfer of hexachlorobenzene was increased by feeding nursing rats high fat diets. 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 hexachlorobenzene 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-hexachlorobenzene 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

Pentachlorophenol may be a metabolite of hexachlorobenzene, as measured in 4-year-old children exposed to high levels of atmospheric hexachlorobenzene (Carrizo et al. 2008). Serum hexachlorobenzene, pentachlorobenzene, and pentachlorophenol were measured in populations of preschoolers (age 4 years) from the town of Flix, Spain, where there are high atmospheric concentrations of hexachlorobenzene, and from Menorca, in the Balearic Islands, a rural area not exposed to any known source of hexachlorobenzene. Neither area has any known source of pentachlorophenol exposure. Both hexachlorobenzene and pentachlorophenol levels in Flix children were higher than the Menorca population. The correlation between hexachlorobenzene and pentachlorophenol levels in Flix children

suggests a precursor-metabolite relationship. No other studies were located regarding metabolism in humans or animals after inhalation exposure to hexachlorobenzene.

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 a glutathione conjugate and ultimately 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 hexachlorobenzene (0.03 mg/kg/day) administered in the diet to Rhesus monkeys for 15 months (Rozman et al. 1977a). In the urine, approximately 50% of the excreted radiolabel was pentachlorophenol, 25% was pentachlorobenzene, and the remaining 25% consisted of unidentified metabolites and unchanged hexachlorobenzene. 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 ^{14}C-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 ^{14}C-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 methylthiopentachlorobenzene 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. Pentachlorophenol, 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 hexachlorobenzene 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 hexachlorobenzene in male and female rats (D'Amour and Charbonneau 1992; Richter et al. 1981; Rizzardini and Smith 1982).

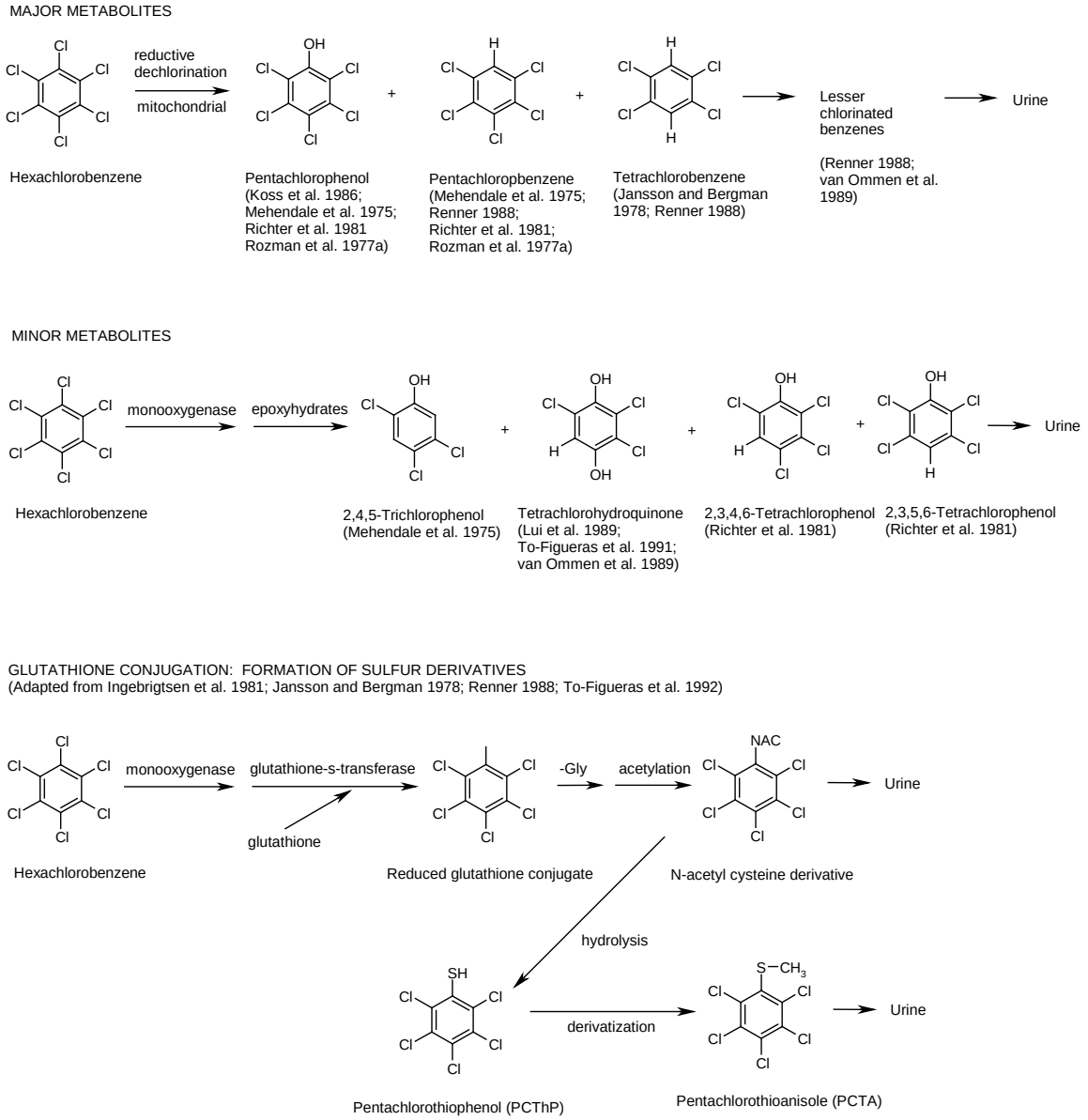
Two alternatives have been proposed for the mechanism of conversion of hexachlorobenzene to pentachlorophenol: formation of an epoxide or free radical attack on the carbon-chlorine bond. Substitution of adjacent carbons with chlorine argues against a mechanism involving an epoxide; either cleavage of 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 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 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 hexachlorobenzene

Figure 3-3. Metabolism and Urinary Metabolites of Hexachlorobenzene



found in adipose tissue and urinary pentachlorophenol. However, it is possible that the urinary pentachlorophenol originated from other chlorinated hydrocarbons such as pentachlorobenzene 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 hexachlorobenzene.

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 hexachlorobenzene 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 hexachlorobenzene in blood was 163 µg/5.4 L (30.2 µg/L). Estimated fecal excretion of unchanged hexachlorobenzene was 10.4 µg/day, 4–6.4% of the estimated total blood level. No unchanged hexachloro benzene 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.
Both biliary and intestinal excretion contribute to fecal excretion of hexachlorobenzene. 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 hexachlorobenzene 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 hexachlorobenzene (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 numerous studies spanning virtually all regions of the world (e.g., Behrooz et al. 2009; Çok et al. 2011; Colles et al. 2008; Craan and Haines 1998; Czaja et al. 1997; Devanathan et al. 2009; Ennaceur and Driss 2013; Ennaceur et al. 2007, 2008; Erdoğrul et al. 2004; Fujii et al. 2012; Gladen et al. 1999; Gocmen et al. 1989; Greizerstein et al. 1999; Guerranti et al. 2011; Haraguchi et al. 2009; Johansen et al. 1994; Johnson-Restrepo et al. 2007; Klinčić et al. 2014; Lunden and Noren 1998; Malarvannan et al. 2009; Mannetje et al. 2013; Mikeš et al. 2012; Nasir et al. 1998; Newsome and Ryan 1999; Ntow et al. 2008; Petzold et al. 1999; Polder et al. 2008; Scheele et al. 1995; Shen et al. 2007; Szyrwińska and Lulek 2007; Tsydenova et al. 2006; Waliszewski et al. 1999a, 1999b; Weisenberg 1986; Wickstrom et al. 1983; Zhou et al. 2011, 2012; Zietz et al. 2008). 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 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. 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. Hexachlorobenzene 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).

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 parameterization, (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) are 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.

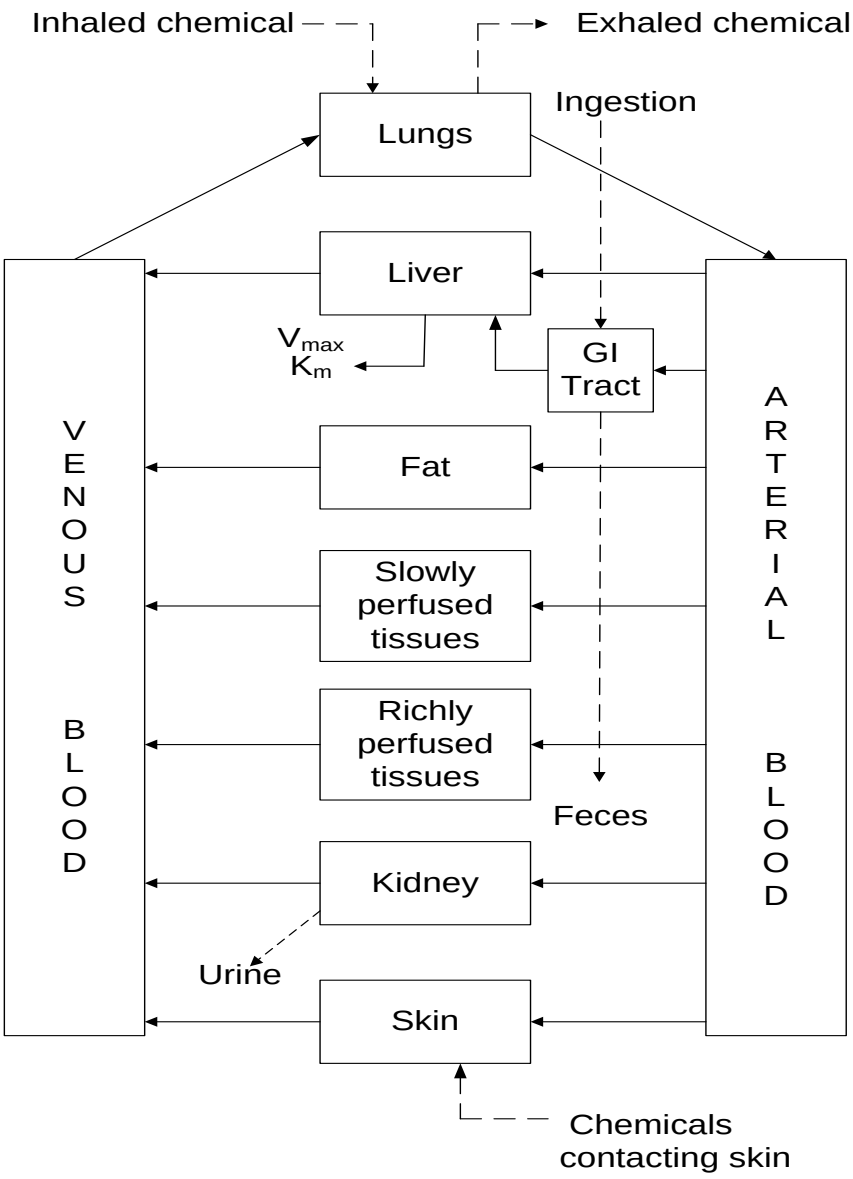
PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in 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.

If PBPK models for hexachlorobenzene exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

3.4.5.1. Summary of PBPK Models

PBPK models for hexachlorobenzene have been developed by Yesair et al. (1986), Freeman et al. (1989), and Lu et al. (2006). 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. The Lu

Figure 3-4. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



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.

Source: adapted from Krishnan and Andersen 1994

et al. (2006) model describes the distribution and excretion of intravenously injected and orally administered hexachlorobenzene in rats, and the effect of partial hepatectomy treatment on hexachlorobenzene distribution in the liver, muscle, and blood.

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. Compared to the Yesair et al. (1986) and Freeman et al. (1989) models, Lu et al. (2006) updates the PBPK model in rats by including erythrocyte binding and exsorption (passive diffusion from blood to digestive tract) processes. Additionally, the Lu et al. (2006) model simulates the effect of a partial hepatectomy on hexachlorobenzene distribution.

3.4.5.3 Discussion of Models

The Yesair Model

Risk assessment. The Yesair model is not adequate for use in risk assessment. Although both rat and human models were developed and the rat model was validated with experimental data, the human model was not validated. Interspecies and interroute extrapolations were not attempted with this model.

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 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 model predicts little transfer of hexachlorobenzene to the fetus during gestation and extensive mobilization of hexachlorobenzene 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. Although Yesair et al. (1986) developed both rat and human models, the human model was not validated and species extrapolation was not attempted.

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

The Freeman Model

Risk assessment. The Freeman model is not adequate for use in risk assessment. The model was developed to simulate intravenous injection of hexachlorobenzene in growing rats. Interspecies and interroute extrapolations were not attempted.

Description of the model. 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.

The Lu Model

Risk Assessment. The Lu model is not adequate for use in risk assessment. Interspecies and interroute extrapolations were not attempted.

Description of the model. Lu et al. (2006) developed a PBPK model for both intravenous injection and gavage (single and repeated dose) of hexachlorobenzene in the rat that incorporated erythrocyte binding, exsorption processes, and pathophysiological conditions following partial hepatectomy. The model includes compartments for liver, blood (plasma and erythrocytes), fat, rapidly and slowly perfused muscle tissues, and upper and lower gastrointestinal lumen. Parameters used in the model included body weights, growth rates, tissue and organ volumes, blood-flow and plasma-flow rates, and tissue:plasma coefficients derived from Koss et al. (1978), and rate constants of metabolism, exsorption, reabsorption,

and fecal excretion. The forms of the growth characteristics and the parameter values were obtained from the literature and from a time-course pharmacokinetics bioassay.

Validation of the model. The model (single oral dose only) was compared to data from Koss and Koransky (1975). Model predictions agreed with the data when the exsorption rate constant (an adjustable parameter) was set at 0.02 L/hour, but underpredicted the second and third time points when the value was 0.045 L/hour.

Sensitivity analysis. Sensitivity analysis was determined for liver concentration, fat and liver volume fractions, tissue partition coefficients, and the adjustable parameters (metabolism, exsorption, resorption, fecal excretion) following a single oral dose. The liver partition coefficient had the largest effect on the liver concentration. Fat volume fraction and partition coefficients had moderate effects. Other parameters had little or no effect.

Target tissues. The intravenous injection model traced liver and plasma concentrations well, and simulations matched data better than previous models (Freeman et al. 1989; Roth et al. 1993; Yesair et al. 1986) that did not consider erythrocyte binding. Single and repeated gavage model simulations for fat, liver, and blood hexachlorobenzene concentrations were in good agreement with experimental data reported by Yamaguchi et al. (1986) and Koss et al. (1978) and with the time-course pharmacokinetics bioassay. Estimates of hexachlorobenzene metabolism and excretion via feces were generally in agreement with experimental data. Simulations with partial hepatectomy and hexachlorobenzene treatment were good for late time points, but overpredicted early time points.

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

Results of a study of human subjects indicate that absorption of ingested hexachlorobenzene decreases with increasing blood hexachlorobenzene levels and that absorption from the gut likely includes mechanisms in addition to passive diffusion (Schlummer et al. 1998). Circulation is the primary mechanism for inter-tissue distribution; hexachlorobenzene distributes preferentially to fat due to its

lipophilic nature. Refer to Section 3.4 (Toxicokinetics) for a detailed discussion of absorption, distribution, metabolism, and elimination and excretion following exposure to hexachlorobenzene. 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.

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 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).

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).

Refer to Section 3.4 (Toxicokinetics) for detailed information regarding absorption (Section 3.4.1), distribution (Section 3.4.2), metabolism (Section 3.4.3), and excretion (Section 3.4.4) following exposure to hexachlorobenzene.

3.5.2 Mechanisms of Toxicity

Mechanistic data for hexachlorobenzene focus mainly on hexachlorobenzene-induced porphyria and associated effects as detailed below. Limited data are available regarding mechanisms of hexachlorobenzene-induced endocrine and immunological effects. Limited information was located regarding possible mechanisms of hexachlorobenzene-induced neurotoxic effects.

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 (Dowdle et al. 1967; Rajamanickam et al. 1972; Smith and de Matteis 1990). 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 porphyrias 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 hexachlorobenzene (Blekkenhorst et al. 1976; Elder et al. 1976; Smith and Francis 1987; Smith et al. 1986a; Sopena et al. 2008). 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 (tetrachlorohydroquinone, pentachlorophenol, 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, pentachlorophenol and tetrachlorohydroquinone 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 pentachlorophenol and tetrachlorohydroquinone 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 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 pentachlorophenol (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 pentachlorophenol, 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 pentachlorophenol) 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 (tetrachlorohydroquinone, pentachlorophenol, 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, pentachlorophenol and tetrachlorohydroquinone 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 pentachlorophenol and tetrachlorohydroquinone 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).

Hexachlorobenzene intake has been associated with an initial increase of coproporphyrinogen and subsequent increase in highly carboxylated porphyrins such as uroporphyrin and heptacarboxylic porphyrin in the urine and presence of isocoproporphyrin and smaller amounts of coproporphyrin in the feces. Fecal isocoproporphyrin results from increased pentacarboxylic porphyrinogen III, which is formed in the cytosol and competes with coproporphyrinogen III for coproporphyrinogen oxidase decarboxylation. Results from a series of *in vivo* and *in vitro* (liver assays) in rats indicate that hexachlorobenzene-induced porphyria involves an uncoupling of the enzyme coproporphyrinogen oxidase from the outer surface of the inner mitochondrial inner membrane in the liver, which may allow pentacarboxylic porphyrinogen III to compete with coproporphyrinogen III for the coproporphyrinogen oxidase catalytic site to produce isocoproporphyrin (Sopena et al. 2008).

Lelli et al. (2007) assessed the effects of hexachlorobenzene on adrenal synthesis and stimulation of plasma glucocorticoids, as well as kinetic parameters of its hepatic receptors in orally-treated rats. Hexachlorobenzene caused decreases in plasma corticosterone, number of hepatic glucocorticoid receptors (without modifying affinity), gluconeogenic enzyme phospoenolpyruvate-carboxylase activity, and adrenal corticosterone production. These results suggest that hexachlorobenzene may exert a hormonal effect by disrupting glucocorticoids, their hepatic receptors, and glucose synthesis via gluconeogenic enzyme phospoenolpyruvate-carboxylase regulation, thus modulating porphyria.

Iron (as iron dextran) 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), 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).

Multiple studies indicate that non-heme iron potentiates the hepatocarcinogenic effects of hexachlorobenzene (Adjarov 1990; Elder and Urquhart 1986; Hahn et al. 1988; Smith and Francis 1983; Smith et al. 1989, 1993; Vincent 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 polyhalogenated 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 hexachlorobenzene 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 hexachlorobenzene 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 pentachlorobenzene and other more polar metabolites proceeds 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(Gopalaswamy and Nair 1992; Rajamanickam and Padmanaban 1974; van Ommen et al. 1985).

Hexachlorobenzene has been shown to affect the thyroid (see Section 3.2.2.2 [Endocrine Effects]). The effect of hexachlorobenzene on thyroxine 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). Results from *in vitro* assays of hexachlorobenzene-treated rat thyroid cells led Chiappini and coworkers (Chiapini et al. 2009, 2013, 2014) to conclude that hexachlorobenzene induces apoptosis in thyroid cells via mechanisms that include involvement of transforming growth factor-beta (TGF-β1) in hexachlorobenzene-induced alterations of cytosolic and nuclear p27 protein and cyclin D1 protein levels.

Hexachlorobenzene 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).

Mundy et al. (2010) demonstrated that hexachlorobenzene induced ethoxyresorufin O-deethylase (EROD) activity, CYP1A4 mRNA and CYP1A5 mRNA in chicken embryo hepatocyte primary cultures similar to induction elicited by 2,3,3',4,4'-pentachlorobiphenyl (PCB-105) and 2,3',4,4',5-pentachlorobiphenyl (PCB-118). A dioxin (tetrachlorodibenzo-*p*-dioxin [TCDD]) equivalent factor was determined to be 0.0001. These results suggest that hexachlorobenzene may act through pathways similar to those of TCDD.

Ezendam et al. (2004a) designed a study to assess possible mechanisms for hexachlorobenzene-induced immunopathology. Brown Norway rats were exposed to a control diet or a diet containing hexachlorobenzene at 450 mg/kg food for 21 days. Treatment with hexachlorobenzene resulted in skin lesions, increases in spleen and auricular lymph node weights, increased serum IgE and IgM against ssDNA levels, macrophage infiltration into spleen and lung, and infiltration of eosinophilic granulocytes into the lung. Cotreatment with cyclosporin A, known to decrease peripheral T-cell number and inhibit antigen-induced T-cell activation, greatly reduced or eliminated the hexachlorobenzene-induced immuno-

pathological effects, with the exception of macrophage infiltrations into the spleen and lung. Restimulation of spleen cells with the T-cell mitogen ConA and the macrophage activator, LPS, demonstrated that cyclosporin A inhibited T-cell activation, but not macrophage activation. These results indicate that T-cells and macrophages are involved in hexachlorobenzene immunotoxicity.

Results from *in vitro* exposure of mouse embryonic stem cells to hexachlorobenzene suggest that hexachlorobenzene interferes with neurite outgrowth of GABAergic, but not glutamatergic neuronal cells, presumably via induction of reactive oxygen species (ROS) production since the effect on GABAergic neuronal cells was repressed in the presence of an ROS scavenger (Addae et al. 2013). *In vitro* exposure of human peripheral blood lymphocytes to hexachlorobenzene resulted in increases in ROS formation, numbers of lymphocytes with reduced transmembrane mitochondrial potential, and caspase 3 activity, which were likely related to increased numbers of apoptotic lymphocytes (Michalowicz et al. (2013). Pontillo et al. (2013) demonstrated that *in vitro* exposure of a mouse breast cancer cell line to hexachlorobenzene resulted in enhanced MMP2 (metalloprotease 2) expression and cell invasion and that aryl hydrocarbon receptor (AhR), proto oncogene c-Src, and epidermal growth factor receptor 1 (HER1) pathways were likely involved in these effects.

The toxicogenomics in the Brown Norway rat resulting from subchronic exposure to hexachlorobenzene was investigated by Ezendam et al. (2004b). The rats were administered hexachlorobenzene up to 450 mg/kg in the diet for 4 weeks, and DNA microarray assays, blood and serum analysis, and pathology experiments were performed. Hexachlorobenzene induced expression of genes involved in systemic inflammatory response, oxidative and acute phase response, drug metabolism, hepatic porphyria, and the reproductive system. The study confirmed stimulatory effects of hexachlorobenzene on the immune system and induction of enzymes involved in drug metabolism, porphyria, and the reproductive system. Hexachlorobenzene induced gene expression of proinflammatory cytokines, antioxidants, acute phase responses, complement and mast cell markers, chemokines, and cell adhesion molecules.

Systemic inflammatory responses included increases in gene expression related to tumor necrosis, mast cell enzymes, chemokines, cell adhesion molecules, complement component, cytokine production, antioxidants, and pleiotropic cytokine. Acute-phase gene expression responses included heat shock proteins in spleen and mesenteric lymph node, matrix metalloproteinases and inhibitors in spleen, liver, and mesenteric lymph nodes, and transcript levels of haptoglobin, lipopolysaccharide-binding protein, orosomucoid, metallothionein, and ceruloplasmin. Gene expression increased for autoantibodies in the spleen, thymus, liver, and kidney, expression of T and B cells and major histocompatibility complex II,

and certain drug-metabolizing enzymes associated with estrogen metabolism. Gene expression of CYP1A1 was strongly upregulated in the liver, an effect associated with certain dioxins. Other effects observed included significantly increased body weights in both dose groups, increases in liver and spleen weights in both dose groups, and histopathological changes in the liver and spleen (described in detail in Michielsen et al. 1997). In the high-dose group, kidney weights were significantly increased and thymus weight was significantly decreased.

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 1980a; Ertürk et al. 1986; Lambrecht et al. 1983; 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 Thomas and Colborn (1992), was also used in 1996 when Congress mandated the 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), and in 1998, the EDSTAC 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 1997b). 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 at dose levels ≥1 mg/kg/day include decreased serum thyroxine (hypothyroidism), increased serum parathyroid hormone (hyperparathyroidism), decreased corticosterone released from the adrenal gland, changes in estradiol and progesterone levels in females at certain times in the menstrual cycle, and hirsutism. 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 hexachlorobenzene may be secondary to the ovarian effects. These alterations are described in more detail in Section 3.2. The effect of hexachlorobenzene on thyroxine 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.

The ability of hexachlorobenzene to interact with α-estrogen receptor, androgen receptor, progesterone receptor, and estrogen-related receptor was examined *in vitro* in yeast strains expressing β-galactosidase (Li et al. 2008). Hexachlorobenzene was found to be an antagonist for androgen and estrogen-related receptor; no response was found for the other receptors.

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 fetus/infant has an immature (developing) blood-brain

barrier that past literature has often described as being leaky and poorly intact (Costa et al. 2004). However, current evidence suggests that the blood-brain barrier is anatomically and physically intact at this stage of development, and the restrictive intracellular junctions that exist at the blood-CNS interface are fully formed, intact, and functionally effective (Saunders et al. 2008, 2012).

However, during development of the blood-brain barrier, there are differences between fetuses/infants and adults which are toxicologically important. These differences mainly involve variations in physiological transport systems that form during development (Ek et al. 2012). These transport mechanisms (influx and efflux) play an important role in the movement of amino acids and other vital substances across the blood-brain barrier in the developing brain; these transport mechanisms are far more active in the developing brain than in the adult. Because many drugs or potential toxins may be transported into the brain using these same transport mechanisms—the developing brain may be rendered more vulnerable than the adult. Thus, concern regarding possible involvement of the blood-brain barrier with enhanced susceptibility of the developing brain to toxins is valid. It is important to note however, that this potential selective vulnerability of the developing brain is associated with essential normal physiological mechanisms; and not because of an absence or deficiency of anatomical/physical barrier mechanisms.

The presence of these unique transport systems in the developing brain of the fetus/infant is intriguing; as it raises a very important toxicological question as to whether these mechanisms provide protection for the developing brain or do they render it more vulnerable to toxic injury. Each case of chemical exposure should be assessed on a case-by-case basis. Research continues into the function and structure of the blood-brain barrier in early life (Kearns et al. 2003; Saunders et al. 2012; Scheuplein et al. 2002).

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). In the study of Goldey and Taylor (1992), maternal dosing with hexachlorobenzene occurred prior to conception. 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, mammary 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; Peña et al. 2012). Because the hormones produced by these endocrine organs play a crucial role in growth and development of the organism, it is not surprising that hexachlorobenzene 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).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of the U.S. population to environmental chemicals using biomonitoring. This report is available at http://www.cdc.gov/exposurereport/. The biomonitoring data for hexachlorobenzene from this report is discussed in Section 6.5. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. 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; Cooney et al. 2010; Den Hond et al. 2011; Glynn et al. 2000; Hagmar et al. 2001; Karmaus et al. 2001; Rutten et al. 1988; Sala et al. 1999b, 2001b; Schettgen et al. 2011; 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), follicular fluid (De Felip et al. 2004); the placenta (Poli et al. 1999), the umbilical cord (Burse et al. 2000; Darvill et al. 2000; Lackmann et al. 2002), and breast milk (Ataniyazova et al. 2001; Craan and Haines 1998; Czaja et al. 1997; Darvill et al. 2000; Dewailly et al. 2000; Ennaceur et al. 2007; Fitzgerald et al. 2001; Gladen et al. 1999; Gocmen et al. 1989; Huang et al. 1989; Lunden and Noren 1998; Newsome and Ryan 1999; Polder et al. 2008; 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 pentachlorophenol and tissue hexachlorobenzene correlated in 60 patients studied, it is possible that the urinary pentachlorophenol originated from other chlorinated hydrocarbons such as pentachlorobenzene, 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, hexachlorobenzene 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. 2000b), breast-feeding and serum levels in infants or small children (Abraham et al. 2000; Lackmann 2004; Ribas-Fitó et al. 2005), 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 a nearby electrochemical plant that produced organochlorines (Ballester et al. 2000; Carrizo et al. 2008; Grimalt et al. 1994; Herrero et al. 1999; Ribas-Fitó et al. 2003a, 2003b, 2005, 2007; Sala et al. 1999a, 1999b, 2001a; Sunyer et al. 2002, 2008; To-Figueras et al. 1997, 2000). 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 (see Section 3.5.2 [Mechanisms of Toxicity] for additional information regarding hexachlorobenzene-induced porphyria). 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). 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).

3.9 INTERACTIONS WITH OTHER CHEMICALS

Certain chemicals may modify the toxicity of hexachlorobenzene, which itself may modify the toxicity of other chemicals. Selected 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); this could lead to accelerated biotransformation to the more toxic pentachlorophenol. The extent of toxicity mediated by this phenomenon is dependent on how rapidly the pentachlorophenol 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). Pentachlorophenol (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 pentachlorophenol-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 mixed function oxidase (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, pentachlorophenol and tetrachlorohydroquinone, 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 hexachloro-

benzene (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 hexachlorobenzene. 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 hexachlorobenzene 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 hexachlorobenzene-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 hexachlorobenzene (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; Selden 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. Although no information was located regarding a possible role for certain genetic polymorphisms (e.g., polymorphisms for metabolic enzymes such as CYPs or enzymes involved in the porphyrin cascade) in hexachlorobenzene toxicity, such a role is theoretically possible.

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). Refer to Section 3.7 (Children's Susceptibility) for more detailed information regarding age-related susceptibility to hexachlorobenzene toxicity.

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 hexachlorobenzene. 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:

Bebarta VS, Phillips SD. 2004. Fungicides. In: Dart RC, ed. Medical toxicology. 3rd ed. Philadelphia, PA: Lippincott Williams & Wilkins, 1529-1532.

Craig SA. 1998. Herbicides and fungicides. In: Viccellio P, ed. Emergency toxicology. 2nd ed. Philadelphia, PA: Lippincott-Raven Publishers, 415-423.

Leikin JB, Paloucek FP, eds. 2002. Poisoning and toxicology handbook. 3rd ed. Hudson, OH: Lexi-Comp, Inc., 647-648.

3.11.1 Reducing Peak Absorption Following Exposure

Decontamination should be initiated immediately following overexposure to hexachlorobenzene. For inhalation exposure, management commonly includes moving the exposed individual to fresh air, and then monitoring for respiratory distress. Supplemental oxygen should be applied if needed (Bebarta and Phillips 2004). For acute oral exposure to hexachlorobenzene, gastric lavage is most likely beneficial if administered within 1 hour or so of ingestion using activated charcoal (Bebarta and Phillips 2004; Leikin and Paloucek 2002). Following dermal exposure, contaminated clothing should be removed and exposed

skin should be washed copiously with soap and water. Please consult a physician or clinical toxicologist if you have been exposed to hexachlorobenzene.

3.11.2 Reducing Body Burden

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 hexachlorobenzene leaves the blood and locates in peripheral compartments, since this substance has an initial large volume of distribution. However, continued treatment with multi-dose activated charcoal or cholestyramine may be useful to enhance elimination (Cohn et al. 1978; Leikin and Paloucek 2002), 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.

Olestra fed to mice that were gavaged with radio labeled hexachlorobenzene (0.7 µCi/day) resulted in a 30-fold increase in the excretion of hexachlorobenzene and decreased hexachlorobenzene levels in the epididymal fat pad, brains, and liver, compared with mice undergoing caloric restriction without olestra (Jandacek et al. 2005). The authors suggest that the increased excretion was caused by olestra providing a lipophilic sink that interfered with the enterohepatic circulation of hexachlorobenzene, or else that olestra enhanced transport of hexachlorobenzene into the lumen.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

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 not likely to be needed except in high-dose acute intoxication management. Benzodiazepenes should be used for seizure control. Contact dermatitis can be treated symptomatically with antihistamines and topical or systemic steroids (Bebarta and Phillips 2004).

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 (Peters 1956, 1993; Peters and Cripps 1985; Peters et al. 1957, 1966, 1986). 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).

Injections of 20 mg/kg/day Cyclosporin A (CsA) reduced immunopathological response in Brown Norway rats when administered concurrently with 450 mg/kg hexachlorobenzene via the diet for 21 days (Ezendam et al. 2004b). T-cells were predominantly affected by CsA treatment.

3.12 ADEQUACY OF THE DATABASE

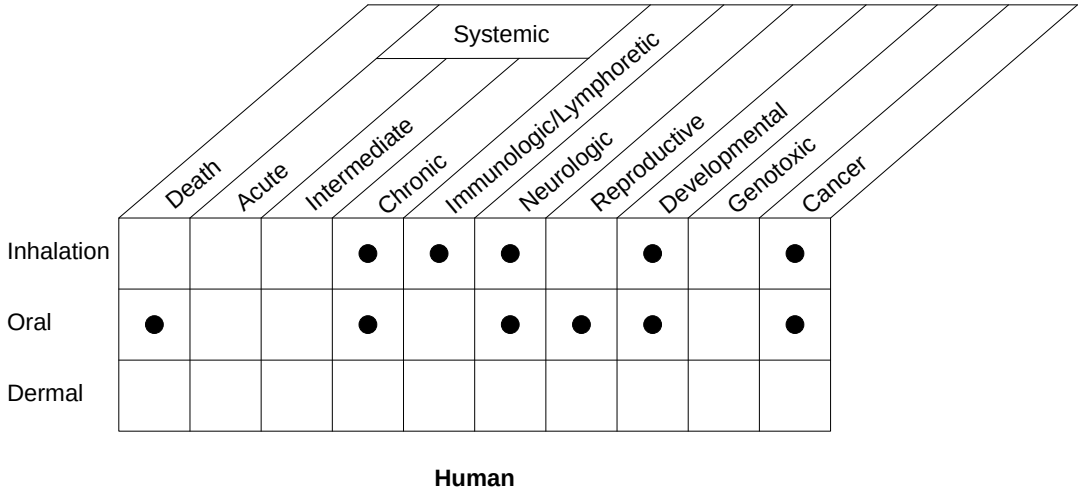
Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of hexachlorobenzene is available. Where adequate information 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.

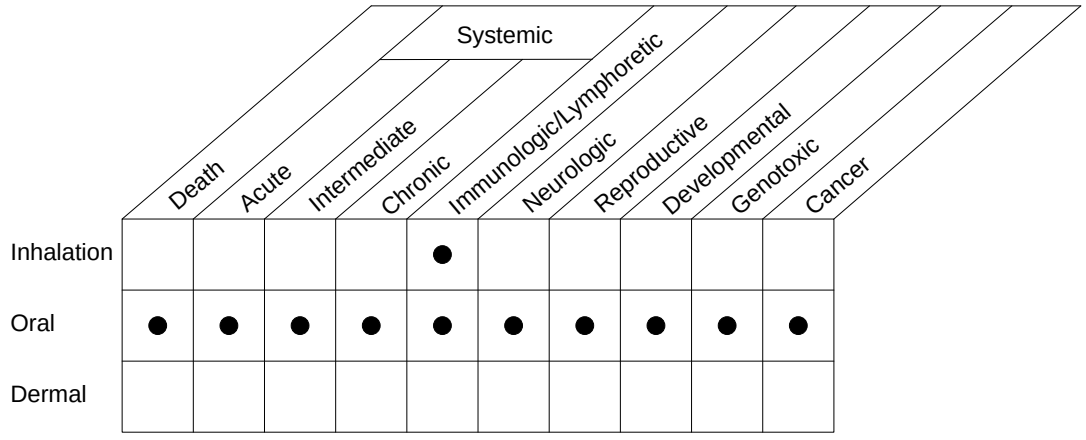
3.12.1 Existing Information on Health Effects of Hexachlorobenzene

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

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



Human



Animal

● Existing Studies

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 has 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 and Taylor 1992; Goldstein et al. 1978; Kennedy and Wigfield 1990; Khera 1974; 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 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 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 hexachlorobenzene 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 hexachlorobenzene 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; Chalouati et al. 2013; 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; NTP 2002; 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. 1977; 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 (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 hexachlorobenzene exposure would enable better assessment of the carcinogenic risk of this chemical to humans. Additional studies that focus on possible mechanisms of hexachlorobenzene toxicity and carcinogenicity are needed.

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 hexachlorobenzene (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 1974; 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). No associations were found between serum reproductive hormone levels and serum hexachlorobenzene levels in men (Freire et al. 2014; Goncharov et al. 2009) or premenopausal women (Freire et al. 2014), although a slight, but significant, negative association between serum hexachlorobenzene and serum LH was noted among peri- and postmenopausal women (Freire et al. 2014). 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), testicular effects in rats and pigs (including increased weight, degenerative lesions, and retarded maturation) (Den Tonkelaar et al. 1978; Gralla et al. 1977; Smith et al. 1985), and mammary gland lesions in rats (NTP 2002). 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.

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). Some studies evaluated possible associations between maternal serum hexachlorobenzene levels and developmental end points such as birth size (weight and/or length) or preterm birth (Basterrechea et al. 2014; Eggesbø et al. 2009; Fenster et al. 2006; Gladen et al. 2003; Guo et al. 2014; Lopez-Espinosa et al. 2011; Sagiv et al. 2007; Szyrwińska and Lulek 2007; Torres-Arreola et al. 2003; Vafeiadi et al. 2014), recurrent miscarriage (Sugiura-Ogasawara et al. 2003), postnatal growth (Burns et al. 2012; Cupul-Uicab et al. 2013; Mendez et al. 2011; Smink et al. 2008; Valvi et al. 2014), postnatal neurodevelopment (Cheslack-Postava et al. 2013; Darvill et al. 2000; Forns et al. 2012; Sioen et al. 2013; Strøm et al. 2014), sexual maturation (Croes et al. 2014a, 2014b; Denham et al. 2005; Lam et al. 2014; Schell and Gallo 2010), cryptorchidism (Pierik et al. 2007), hypospadias (Giordano et al. 2010; Rignell-Hydbom et al. 2012), and indicators of postnatal thyroid function (Julvez et al. 2011). Although most studies found no significant association between maternal serum hexachlorobenzene levels and risk of developmental effects, there were reports of significant associations between maternal or cord blood hexachlorobenzene and birth weight (Lopez-Espinosa et al. 2011; Vafeiadi et al. 2014), postnatal growth (Valvi et al. 2014), and hypospadias (Giordano et al. 2010; Rignell-Hydbom et al. 2012). Some studies that assessed serum hexachlorobenzene levels in young boys and girls reported significant effects on markers of sexual development (Croes et al. 2014a, 2014b; Lam et al. 2014).

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 1974). 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). Two studies reported significant associations between maternal serum hexachlorobenzene and risk of asthma in offspring (Gascon et al. 2014; Hansen et al. 2014).

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. 1977; 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. NTP (2002) reported significantly increased incidences of splenic lymphoid hyperplasia in rats administered hexachlorobenzene by gavage for 90 days. 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 1974; 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.

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 hexachlorobenzene 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. Information regarding biomarkers of exposure to hexachlorobenzene appears adequate; it is uncertain whether additional biomarkers that are specific for hexachlorobenzene exposure could be developed. If so, they might be useful in the monitoring of people living near hazardous waste sites at which hexachlorobenzene has been detected.

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 hexachlorobenzene 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. 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.

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). These results were reported from studies conducted in Turkey 25 to 30 years after the epidemic where people were exposed to very high levels of hexachlorobenzene that was added as a fungicide to wheat seedlings. 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 1974; 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.

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

3.12.3 Ongoing Studies

The following ongoing study pertaining to hexachlorobenzene was identified in the National Institutes of Health (NIH) Research Portfolio Online Reporting Tools (RePORTER 2015):

Lawrence M. Schell, State University of New York at Albany, is assessing the effect of exposure to PCBs, other persistent organic pollutants (presumably including hexachlorobenzene), and lead on characteristics of the menstrual cycle among Mohawk women between 20 and 35 years of age and living in Akwesasne, which is adjacent to a federal and two state Superfund sites. A total of 180 women will be followed through one menstrual cycle with collection of blood, urine, and daily saliva samples to investigate the relationship of PCB congeners and other toxicants to gonadal function, pituitary function, and other characteristics of the menstrual cycle. The study is sponsored by the National Institute on Minority Health and Health Disparities.