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 HCH. 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 HCH are indicated in Tables 3-2, 3-3, and 3-5 and Figures 3-2 and 3-3. Because cancer effects could occur at lower exposure levels, Figure 3-3 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.

HCH exists as several isomers. The four major isomers discussed in this profile are alpha-HCH (α-HCH), beta-HCH (β-HCH), gamma-HCH (γ-HCH), and delta-HCH (δ-HCH). γ-HCH is also commonly known as lindane. Technical-grade HCH consists of at least five isomers (approximately 60–70% α-HCH, 5–12% β-HCH, 10–15% γ-HCH, 6–10% δ-HCH, and 3–4% ε-HCH). The toxicity of the isomers varies. With respect to acute exposure, γ-HCH is the most toxic, followed by α-, δ-, and β-HCH. With chronic exposure, however, β-HCH is the most toxic followed by α-, γ-, and δ-HCH. With chronic exposures, the increased toxicity of β-HCH is probably due to its longer biological half-life in the body and its accumulation in the body over time.

3.2.1 Inhalation Exposure

Studies examining the inhalation toxicity of HCH in humans are limited. Most of the available information is from case reports of acute poisoning in the home following accidental inhalation of pesticidal powder or from the use of γ-HCH vaporizers, whereby γ-HCH pellets are vaporized by

electrical warming of a ceramic jacket, and from studies of workers engaged in the manufacture and formulation of pesticides and fertilizers. Limitations inherent in these reports or studies include unquantified exposure concentrations and concomitant exposure to HCH mixtures, pyrolysis products from vaporizers, and other pesticides and chemicals. Studies that provide levels of significant exposure for inhalation exposure to γ-HCH are shown in Table 3-1 and Figure 3-1.

3.2.1.1 Death

γ-HCH was once used in vaporizers, resulting in human exposure to unspecified levels via inhalation and dermal routes. Occasional deaths associated with the use of this product for several months or years have been reported, but in no case is it clear that γ-HCH was responsible for the deaths (Loge 1965). Two fatalities resulting from pulmonary edema were reported in toddlers inhaling and ingesting unknown quantities of γ-HCH-containing pesticidal powder (McQueen 1968). No human deaths from inhalation exposure to other isomers have been reported.

An acute study with rats exposed nose-only to γ-HCH aerosol for 4 hours, followed by a 22-day observation period, estimated the acute LC_{50} to be 1,560 mg/m^3 (Ullmann 1986b). Rats inhaling up to 603 mg/m^3 γ-HCH aerosol for 4 hours in whole-body exposure chambers exhibited no mortality throughout the 14-day observation period (Oldiges et al. 1980). In an intermediate-duration study with mice inhaling γ-HCH dust aerosol in whole-body exposure chambers, 16% mortality was observed after 1 week of exposure to 10 mg/m^3, while exposures of up to 14 weeks resulted in 22% mortality at 5 mg/m^3, 2% mortality at 1 mg/m^3, and no mortality at 0.3 mg/m^3 (Klonne and Kintigh 1988).

The lethal concentrations reported by Ullmann (1986b) and Klonne and Kintigh (1988) are presented in Table 3-1 and plotted in Figure 3-1.

3.2.1.2 Systemic Effects

No studies were located regarding gastrointestinal, musculo/skeletal, or dermal effects in humans or animals following inhalation exposure to HCH.

The highest NOAEL values and all NOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

Table 3-1 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
ACUTE EXPOSURE							
Death							
1	Rat (Wistar)	4 hr				1560 (LC50)	Ullmann 1986b lindane
2	Mouse (CD-1)	1 wk 5 d/wk 6 hr/d				10 (16% mortality)	Klonne and Kintigh 1988 lindane
Systemic							
3	Rat (Wistar)	4 hr	Resp	603			Oldiges et al. 1980 lindane
			Hepatic	603			
			Renal	603			
Neurological							
4	Rat (Wistar)	4 hr			101 (sedation)	642 (ataxia)	Ullmann 1986b lindane
INTERMEDIATE EXPOSURE							
Death							
5	Mouse (CD-1)	14 wk 5 d/wk 6 hr/d				1 (2% mortality)	Klonne and Kintigh 1988 lindane
Systemic							
6	Rat (Wistar)	90 d 6 hr/d	Resp	5			Oldiges et al. 1983 lindane
			Hemato	5			
			Hepatic	5			
			Renal	5			
			Bd Wt	5			

Table 3-1 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - Inhalation (continued)

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
Neurological							
7	Mouse (CD-1)	14 wk 5 d/wk 6 hr/d	Other	5			Klonne and Kintigh 1988 lindane

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

Bd Wt = body weight; d = day(s); Hemato = hematological; hr = hour(s); LC50, lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

Figure 3-1 Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Inhalation

Acute (≤14 days)

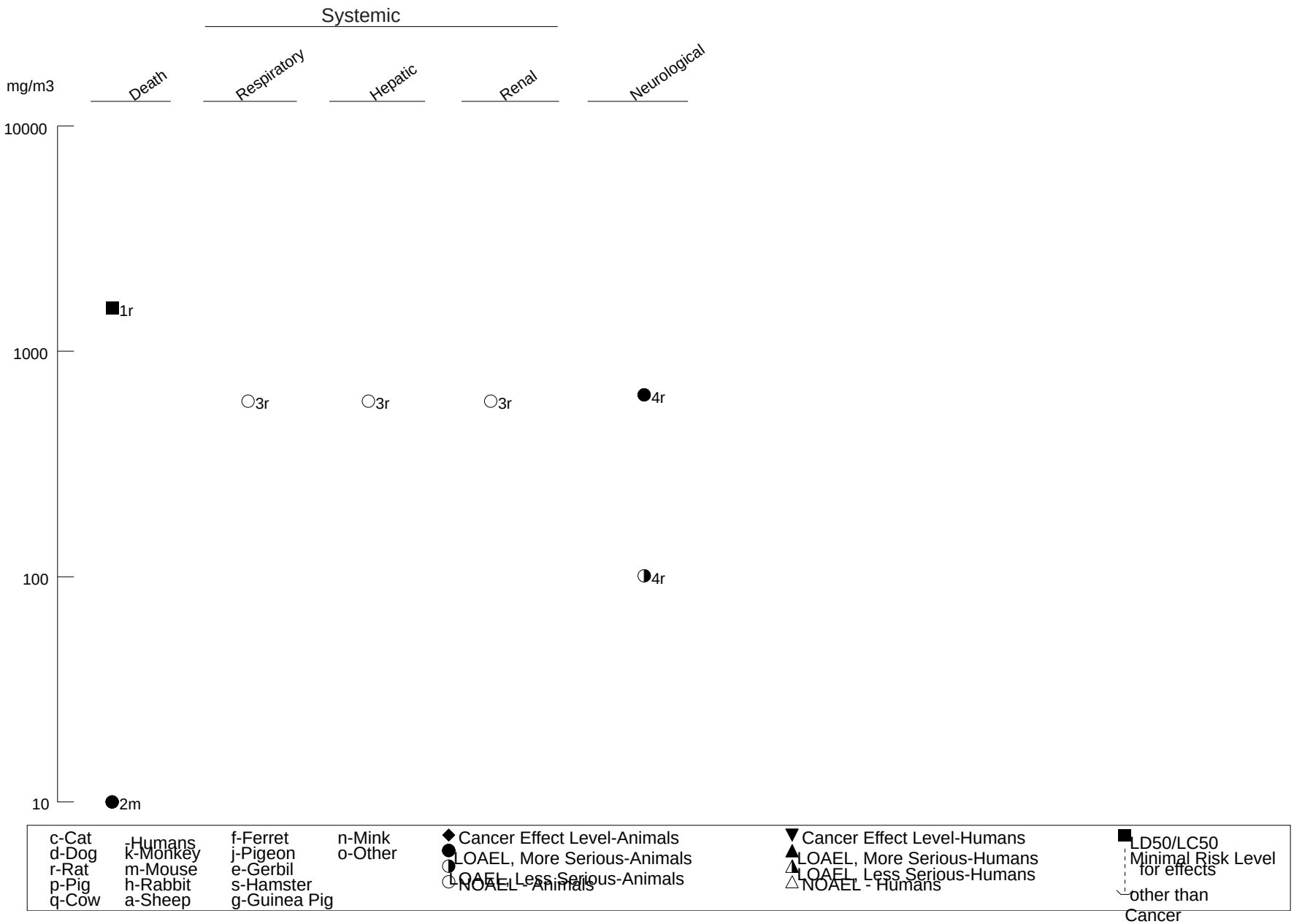
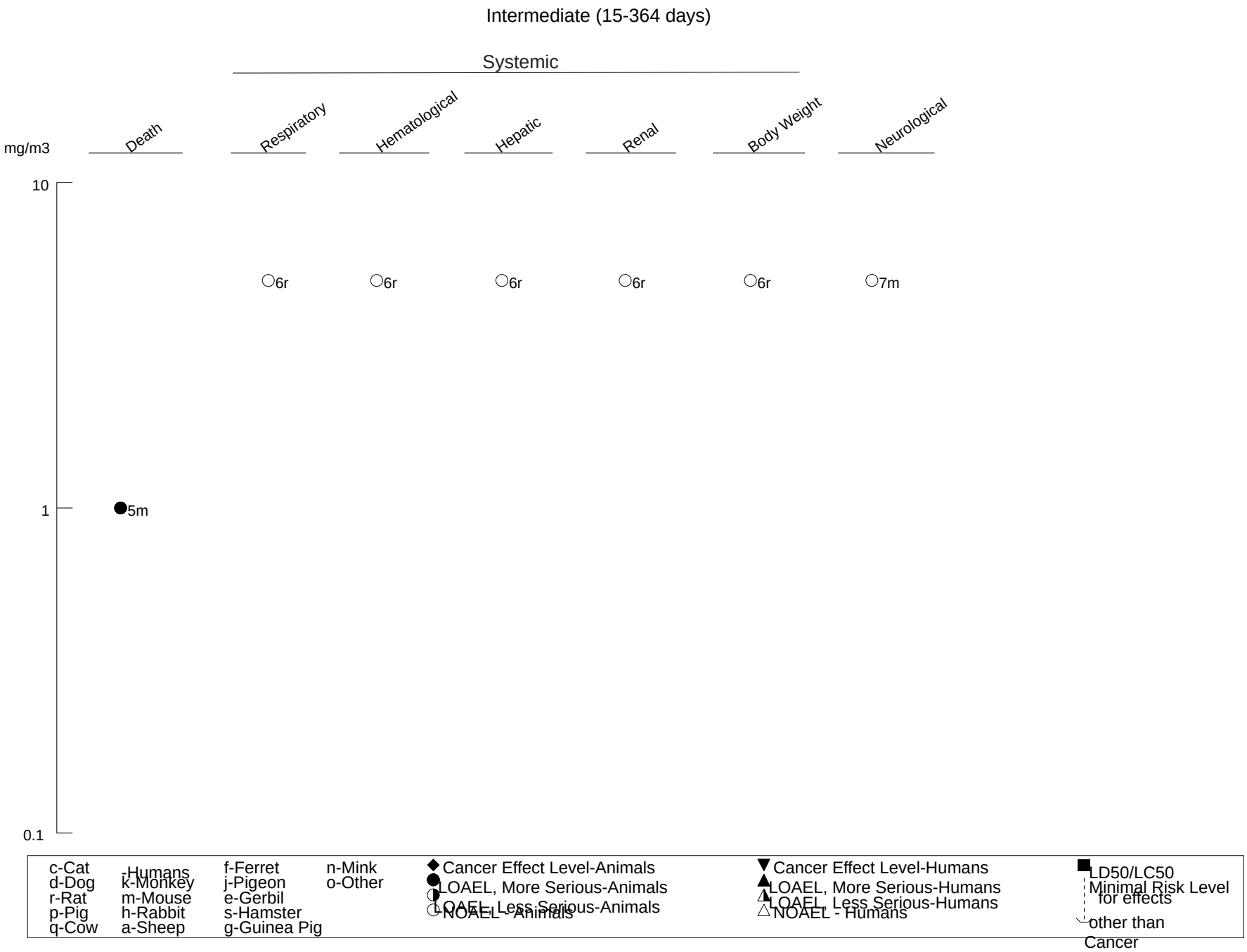


Figure 3-1 Levels of Significant Exposure to Gamma-Hexachlorocyclohexane (Lindane) - Inhalation (*Continued*)



Respiratory Effects. In humans, mucous membrane irritation of the nose and throat was observed after acute exposure to the HCH products dispensed by an overheated γ-HCH vaporizer (Conley 1952). Exposure levels were not reported and dermal exposure may also have occurred, although the observed irritation was probably due to direct action upon the mucous membranes.

No respiratory effects were observed in rats exposed to up to 603 mg/m^3 γ-HCH aerosol for 4 hours (Oldiges et al. 1980). No respiratory effects were observed in rats exposed to γ-HCH aerosol (up to 5 mg/m^3) 6 hours/day for 90 days (Oldiges et al. 1983) or in mice similarly exposed for 14 weeks (Klonne and Kintigh 1988).

Cardiovascular Effects. Cardiovascular effects of HCH have been reported in humans exposed to HCH. Kashyap (1986) reported electrocardiogram (ECG) abnormalities in 15% of 45 factory workers involved in the production of technical-grade HCH; exposure concentrations were not reported and dermal exposure may have occurred.

No studies were located regarding cardiovascular effects in animals following inhalation exposure to HCH.

Hematological Effects. Hematological effects have been reported in humans following acute or chronic inhalation exposure to γ-HCH; however, a causal relationship between exposure to γ-HCH and hematological effects in humans has not been established. Hypochromic anemia was reported in a 2.5-year-old boy who was exposed to γ-HCH in a home in which a pesticide vaporizer was operated. Air γ-HCH concentrations measured in the basement and living room of the house were 2.4–5.5 μg/m^3; however, the actual concentration the child was exposed to and the duration of exposure were not determined (Morgan et al. 1980). Aplastic anemia was reported in a boy exposed to γ-HCH used as an insecticide in his home and in a man exposed at work (Rugman and Cosstick 1990). In both cases, the anemia was reversible and was not present in other family members. The levels and routes of exposure are not known, although they are presumed to be inhalation and dermal. Other hematological abnormalities, including isolated instances of leukopenia, leukocytosis, granulocytopenia, granulocytosis, eosinophilia, monocytosis, and thrombocytopenia, have been reported following chronic human occupational exposure to γ-HCH (Brassow et al. 1981; Jedlicka et al. 1958). Exposure concentrations were not specified in these studies and concomitant dermal exposure probably occurred. Although Brassow et al. (1981) reported slight changes in clinical chemistry tests in 60 human workers exposed to

γ-HCH, there were no cases of severe impairment of health. Granulocytopenia, aplastic anemia, and pancytopenia have been reported in a number of case reports of individuals following exposure to γ-HCH and other pesticides such as DDT in the home, during the handling of the pesticide, or from a nearby formulating plant (Danopoulos et al. 1953; Friberg and Martensson 1953; Gewin 1939; Loge 1965; Mendeloff and Smith 1955). Exposure concentrations were not reported, dermal exposure was likely, and in many cases, there was concomitant exposure to other pesticides; therefore, determination of a causal relationship between exposure and hematological effects cannot be made.

No hematological effects were seen in rats exposed to γ-HCH aerosol (up to 5 mg/m^3) for 90 days (Oldiges et al. 1983).

Hepatic Effects. In humans, statistically significant increases in the blood levels of the enzymes lactate dehydrogenase (33%), leucine aminopeptidase (45%), and γ-glutamyl transpeptidase (174%) were reported in 19 individuals occupationally exposed to technical-grade HCH for over 10 years in an HCH-formulating plant (Kashyap 1986); the HCH isomer concentrations in serum showed a 10-fold increase compared to the control group of workers. Both inhalation and dermal exposure probably occurred. The large standard deviation (SD) from the mean reported for γ-glutamyl transpeptidase in exposed workers (mean±SD =22.2±40.31 IU/mL) suggests the increased activity of this enzyme may not be related to HCH exposure or that individual responses may vary.

No hepatic effects were observed in rats after acute exposure to 603 mg/m^3 γ-HCH (Oldiges et al. 1980). Rats exposed to γ-HCH aerosol (5 mg/m^3, 6 hours/day) exhibited increased hepatic cytochrome P-450 concentration after 90 days, but this level returned to control values after a 4-week recovery period (Oldiges et al. 1983).

Renal Effects. No studies were located regarding renal effects in humans following inhalation exposure to HCH.

No renal effects were seen in rats exposed to up to 603 mg/m^3 γ-HCH aerosol for 4 hours (Oldiges et al. 1980) or up to 5 mg/m^3 γ-HCH aerosol 6 hours/day for 90 days (Oldiges et al. 1983).

Endocrine Effects. Serum luteinizing hormone levels were significantly increased in 54 men occupationally exposed to γ-HCH for approximately 8 years in a γ-HCH producing factory compared to a group of 20 control individuals (Tomczak et al. 1981). The mean serum concentration of follicle

stimulating hormone was increased and testosterone was decreased, but the differences relative to controls were not statistically significant (Tomczak et al. 1981).

No studies were located regarding endocrine effects in animals following inhalation exposure to HCH.

Ocular Effects. No studies were located regarding ocular effects in humans following inhalation exposure to HCH.

Mice exposed to γ-HCH aerosol (up to 5 mg/m^3) 6 hours/day for 14 weeks exhibited no ophthalmic effects (Klonne and Kintigh 1988).

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

No body weight effects were seen in rats exposed to up to 5 mg/m^3 γ-HCH aerosol 6 hours/day for 90 days (Oldiges et al. 1983).

3.2.1.3 Immunological and Lymphoreticular Effects

A statistically significant increase (approximately 18%) in the level of immunoglobulin M (IgM) was noted in 19 workers occupationally exposed to technical-grade HCH during pesticide formulation as compared to 14 nonexposed workers (Kashyap 1986). The HCH isomer concentrations in serum showed a 10-fold increase when compared to the control group. Both inhalation and dermal exposure probably occurred, and the measurement of IgM alone is not a reliable measure of immune function in adults.

No studies were located regarding immunological or lymphoreticular effects in animals following inhalation exposure to HCH.

3.2.1.4 Neurological Effects

Paresthesia of the face and extremities, headache, and vertigo have been reported in a group of 45 workers occupationally exposed during manufacture and formulation of technical-grade HCH for several years (Kashyap 1986); exposure concentrations were not reported. Both inhalation and dermal

exposure probably occurred. Abnormal electroencephalographic (EEG) patterns (increased variation in the frequency and amplitude of wave pattern or more serious changes without specific EEG signs) have been reported in 16 of 37 workers following exposure to γ-HCH for 0.5–2 years in a fertilizer plant (Czegledi-Janko and Avar 1970). Exposure concentrations were not reported; however, these EEG changes were found to correlate with blood levels of γ-HCH. Weakness of the left and right limbs, dysarthria, and dysphagia were seen in an agricultural worker exposed by inhalation and dermal contact to unspecified levels of several organochlorine pesticides, including γ-HCH (Fonseca et al. 1993).

Rats exposed to various concentrations of 99.6% γ-HCH aerosol via nose-only inhalation for 4 hours exhibited concentration-related neurological effects when observed for up to 22 days after exposure (Ullmann 1986b). Slight-to-moderate sedation was observed after exposure to 101 mg/m^3; slight-to-severe sedation was noted after exposure to 378 mg/m^3; restlessness, excitation, and ataxia were seen after exposure to 642 and 2,104 mg/m^3; and spasms were also noted at the highest concentration (2,104 mg/m^3). Rats exposed to 0.02–5 mg/m^3 γ-HCH aerosol for 90 days exhibited a "slightly disturbed general condition" beginning at day 15 (Oldiges et al. 1983). Mice were similarly exposed for 14 weeks and exhibited no clinical signs of neurotoxicity (Klonne and Kintigh 1988).

NOAELs and LOAELs for neurological effects in animals following inhalation exposure are listed in Table 3-1 and plotted in Figure 3-1.

3.2.1.5 Reproductive Effects

Statistically significant increases in the levels of serum luteinizing hormone were reported in a group of 54 men occupationally exposed to unspecified concentrations of γ-HCH for approximately 8 years in a γ-HCH-producing factory (Tomczak et al. 1981). Although the mean serum concentration of follicle stimulating hormone was increased and testosterone was decreased, these differences were not statistically significant compared to mean values determined in a control group. These hormonal changes may have resulted in diminished reproductive capability.

No studies were located regarding reproductive effects in animals following inhalation exposure to HCH.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals following inhalation exposure to HCH.

3.2.1.7 Cancer

There is no clear evidence of increased risk of non-Hodgkin's lymphoma among farmers from Kansas, Nebraska, Iowa, and Minnesota who used γ-HCH (Blair et al. 1998). Results of four case control studies conducted in the 1980s were pooled for analysis of a combined data set of 987 men with non-Hodgkin's lymphoma and 2,895 population-based controls. Odds ratios (ORs) indicated that reported use of γ-HCH significantly increased the odds of developing non-Hodgkin's lymphoma by 50% (OR=1.5, 95% confidence interval (CI) 1.1–2.0). Some use characteristics suggested a dose-response relationship, although differences between cases and controls were not statistically significant. For example, ORs were greater among individuals who first used γ-HCH ≥20 years before diagnosis (OR=1.7, 95% CI 1.1–2.5) compared to those with <20 years of use (OR=1.3, 95% CI 0.7–2.3), and among persons who reported ≥5 days per year of γ-HCH use (OR=2.0, 95% CI 0.6–6.4) compared with those with <5 days per year of use (OR=1.6, 95% CI 0.6–4.0). Other factors reduced apparent risk, including adjustment for potential confounding by use of other pesticides such as 2,4-D and diazinon, which reduced the OR associated with γ-HCH use from 1.5 (95% CI 1.1–2.0) to 1.2 (95% CI 0.5–2.2) and 1.3 (95% CI 0.9–1.9), respectively. The authors concluded that γ-HCH is not a major factor in the development of non-Hodgkin's lymphoma but may play some role. There was also no clear evidence of an increased risk of non-Hodgkin's lymphoma in a population-based study of Canadian men of varying occupations. There was a significantly increased risk of non-Hodgkin's lymphoma with exposure to γ-HCH; however, after additional multivariate analysis to factor in exposure to other chemicals, history of cancer among first-degree relatives, and personal history of measles and allergy sensitization, γ-HCH was not considered a significant independent predictor (McDuffie et al. 2001).

No studies were located regarding carcinogenic effects in animals following inhalation exposure to HCH.

3.2.2 Oral Exposure

The Levels of Significant Exposure for oral exposure to γ-HCH are presented in Table 3-2 and Figure 3-2. Levels of Significant Exposure for α-, β-, δ-, and technical-grade HCH are presented in Table 3-3 and Figure 3-3.

3.2.2.1 Death

Case reports have described deaths in humans (usually children, some suicidal adults) following ingestion of γ-HCH, often from the tablets intended for γ-HCH vaporizers (Storen 1955; Sunder Ram Rao et al. 1988). The amounts of γ-HCH associated with these deaths are not known.

γ-HCH has been shown to be lethal to animals following single gavage administration (Gaines 1960; Liu and Morgan 1986; Tusell et al. 1987). The LD_{50} value for female rats is 91 mg/kg, and the LD_{50} value for male rats is 88 mg/kg (Gaines 1960). One of seven male Wistar rats died following a single oral administration of 60 mg/kg γ-HCH (Martinez et al. 1991). DBA/2 strain mice, recognized as being "unresponsive" to microsomal enzyme induction, are more sensitive to the acute lethal effects of γ-HCH than C57BL/6 strain mice when exposed to 20 mg/kg/day for 10 days (Liu and Morgan 1986). In a 15-week study, 2 of 12 F-344 rats treated with 20 mg/kg/day died (Chadwick et al. 1988). A 2-year study in rats fed γ-HCH in their diets (32 mg/kg/day) also found a significantly increased mortality rate compared with controls (Amyes 1990). The oral LD_{50} for technical-grade HCH in CFT-Wistar rats treated once by gavage was 2,428 mg/kg (Joseph et al. 1992a). Exposure to 5 mg/kg/day of technical-grade HCH for 90 days resulted in the deaths of 6/12 male rats and 4/12 female rats (Dikshith et al. 1991b). Exposure to low levels (0.4 mg/kg/day) of technical-grade HCH in the diet for 360 days resulted in deaths of 4/20 rats (Dikshith et al. 1991a). However, the deaths occurred late in the study and were accompanied by other changes, indicating that they were due to pathogenic infection rather than HCH exposure. The LD_{50} for rats and the LOAEL values from the intermediate-duration studies are recorded in Tables 3-2 and 3-3 and plotted in Figures 3-2 and 3-3.

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
ACUTE EXPOSURE							
Death							
1	Rat (Sherman)	once (GO)				88 M (LD50) 91 F (LD50)	Gaines 1960 lindane
2	Rat (Wistar)	once (GO)				60 M (1/7 deaths)	Martinez et al. 1991 lindane
Systemic							
3	Rat (Wistar)	1 x/d 5-21 d (G)	Hepatic	2.5 M	5 M (increased EROD, PROD, and NDMA-d enzyme levels)		Parmar et al. 2003 lindane
4	Rat (Sprague-Dawley)	48h 1x/d (F)	Hepatic		30 (Reduced number of cells per field; increased cell, nucleus, and nucleolus size; slight cellular disorganization)		Shahid Ali and Rauf Shakoori 1998 lindane
5	Rat	2wks (F)	Hepatic		72 (Altered activities of serum aminotransferases, alkaline phosphatase, altered soluble enzymes and altered carbohydrate metabolism)		Srinivasan and Radhakrishnamurty 1988 lindane

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
6	Rat (Wistar)	14 d ad libitum (F)	Renal			72 M (10% increase in kidney weight, altered excretion patterns, distention of glomeruli, swelling of tubular epithelia)	Srinivasan et al. 1984 lindane
7	Mouse (B6C3F1)	10 d 1 x/d (GO)	Resp	20 M			Hong and Boorman 1993 lindane
			Cardio	20 M			
			Gastro	20 M			
			Hemato		10 M (Transient decrease in marrow progenitor cell numbers)		
			Hepatic	20 M			
			Renal	20 M			
			Bd Wt	20 M			

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
8	Mouse (B6C3F1)	3 d 1x/d (GO)	Resp	40 M			Hong and Boorman 1993 lindane
			Cardio	40 M			
			Gastro	40 M			
			Hemato		20 M (Transient reduction in marrow progenitor cell number)		
			Hepatic	40 M			
			Renal	40 M			
			Endocr	40 M			
			Bd Wt	40 M			
Immuno/ Lymphoret							
9	Mouse (B6C3F1)	3 d 1x/d (GO)		10 M	20 M (decreased thymus weights)	40 M (Atrophy of thymus cortex)	Hong and Boorman 1993 lindane
10	Mouse (B6C3F1)	10 d 1x/d (GO)			10 M (Dose-related decrease in relative thymus and spleen weights)		Hong and Boorman 1993 lindane
Neurological							
11	Rat (Sprague-Dawley)	6 d 1x/d (GO)			3 M (increased pineal N-acetyltransferase, decreased serotonin levels)		Attia et al. 1991 lindane

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
12	Rat (Long- Evans)	once (GO)			5 M (myoclonic jerks and single clonic seizure in kindled animals)	10 M (myoclonic jerks and single clonic seizures in naive animals)	Gilbert and Mack 1995 lindane
13	Rat	once (G)		6	20 (Decreased motor activity and grooming behavior, increased forelimb grip strength)	60 (Clinical signs of neurotoxicity including tremors and convulsions)	Hughes 1999a lindane
14	Rat (Sprague-Dawley)	4 d 1x/d (GO)			3 M (increased kindling acquisition)	10 M (seizures)	Joy et al. 1982 lindane
15	Rat (Wistar)	once (GO)				60 (convulsions)	Martinez and Martinez-Conde 1995 lindane
16	Rat (Wistar)	once (GO)				60 M (tonic-clonic seizures)	Martinez et al. 1991 lindane
17	Rat (Wistar)	1 x/d 5 d (G)		5 M	10 M (increased EROD, PROD, and NDMA-d enzyme levels in the brain)		Parmar et al. 2003 lindane

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
18	Rat (Wistar)	1 d (G)				35 M (convulsions in 4/10 animals)	Parmar et al. 2003 lindane
19	Rat	once (G)			20 (altered acquisition of a passive avoidance task in 15-day-old pups)		Rivera et al. 1998 lindane
20	Rat (Wistar)	3 d 1x/d (GO)			5 (decreased myelin and 2',3'-cyclic nucleotide 3'-phosphodiesterase activity in brains)		Serrano et al. 1990a lindane
21	Rat (Wistar)	once (GO)		15 M		20 M (convulsions)	Vendrell et al. 1992a lindane
22	Rat (Sprague-Dawley)	once (GO)				30 M (seizures)	Wooley and Griffith 1989 lindane

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference Chemical Form
Reproductive							
23	Rat	Ld 9-14 1x/d (GO)			1 M[b] (Reduced relative testicular and epididymis weight (~10%), spermatid and sperm counts (~10%), and testosterone levels (30-50%) at maturity with no effect on fertility)		Dalsenter et al. 1997b lindane
24	Rat	Ld 9 or 14 once (GO)			6 M (Reduced relative testical and epidymis weight (~10%), spermatid and sperm counts (~8-10%), testosterone levels (~30-50%), Leydig cell numbers and spermatogenisis at maturity with no effect on fertility)		Dalsenter et al. 1997b lindane
25	Rat (Long- Evans)	7 d 1x/d (GO)		40 F			Laws et al. 1994 lindane
26	Rat (CDF-F344)	once			25 (increased length of estrous cycle)		Uphouse and Williams 1989 lindane

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
27	Mouse (CD-1)	3 d 1 x/d (GO)		15 F	25 F (increase in degenerating two-cell embryos following preovulatory exposure)		Scascitelli and Pacchierotti 2003 lindane
28	Mouse (CD-1)	7 d Gd 9-16 1 x/d (GO)			15 M (Reduced testicular sperm head count and concentration and other effects on spermatogenesis in adult F1 males exposed during gestation)		Traina et al. 2003 lindane
Developmental							
29	Rat (Wistar)	Pc 15 1x (GO)			30 (reduction of serum testosterone concentration in adult offspring)		Dalsenter et al. 1997a lindane
30	Rat (Wistar)	Gd 6-15 1x/d (GO)		25 F			Khera et al. 1979 lindane
31	Rat (CFY)	Gd 6-15 1x/d (GW)		20 F			Palmer et al. 1978a lindane

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
32	Rat (Wistar)	once (GO)			20 (regional changes in brain noradrenaline and serotonin levels in suckling rats)		Rivera et al. 1991 lindane
33	Mouse C57BL/6J	Single oral dose on day 12 of gestation (GI)			30 (decrease in fetal weight, fetal thymus weight)		Hassoun and Stohs 1996a lindane
34	Mouse DBA/2J	Single oral dose on day 12 of gestation (GI)			45 (decrease in fetal and placental weight)		Hassoun and Stohs 1996a lindane
35	Rabbit (New Zealand)	Gd 6-18 1x/d (GW)		20 F			Palmer et al. 1978a lindane
INTERMEDIATE EXPOSURE							
Death							
36	Rat (Fischer- 344)	15 wk 1x/d (GO)				20 F (2/12 deaths)	Chadwick et al. 1988 lindane

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
Systemic							
37	Rat (albino)	1x d 6 wks (G)	Cardio		3 M (tachycardia, increase in blood pressure, plasma calcium levels, myocardial calcium influx. Decreased Ca,K-ATPase activity. ECG changes: increase in ST segment, T-wave amplitude; reduced R-R interval, P-wave		Anand et al. 1995 gamma
38	Rat (Wistar)	15 d ad libitum (F)	Hepatic		1.8 M (Increases in lipid peroxidation, level of cytochrome P-450, and activities of superoxide dismutase)		Barros et al. 1991 lindane
39	Rat (Wistar)	30 d ad libitum (F)	Hepatic		1.8 M (Increases in lipid peroxidation, level of cytochrome P-450, and activities of superoxide dismutase)		Barros et al. 1991 lindane
40	Rat (Wistar)	40d (F)	Hepatic	50			Desi 1974 lindane
			Renal		5 (increased kidney weight)		

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
41	Rat (Wistar)	1 x/d 5-21 d (G)	Hepatic		2.5 M (increased liver weight;increased P-450 content and P-450 dependent enzymes)		Parmar et al. 2003 lindane
42	Rat (Sprague-Dawley)	15d 1x/d (F)	Hepatic		18 (Reduced number of cells per field; incresed cell, nucleus, and nucleolus size; vacuoles in the cytoplasm and granulation; apparent fatty degeneration)		Shahid Ali and Rauf Shakoori 1998 lindane
43	Rat (Wistar)	12 wk ad libitum (F)	Hemato	10			Suter 1983 lindane
			Hepatic	0.4	2 (centrilobular hypertrophy)		
			Renal	0.4	2 (tubular distension, basophilic tubules)		
44	Mouse (dd)	24 wk ad libitum (F)	Hepatic		90 M (centrilobular hypertrophy)		Ito et al. 1973 lindane

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
Immuno/ Lymphoret							
45	Rat	8 wk ad libitum (F)			3.6 (reduced serum antibody response to SRBC)		Koner et al. 1998 lindane
46	Mouse (Swiss albino)	24 wk ad libitum (F)			0.012[c] F (changes in cell- and humoral-mediated immune system)	1.2 F (necrosis of thymus)	Meera et al. 1992 lindane
Neurological							
47	Rat (Wistar)	90 d ad libitum (F)				90 M (tonic convulsions)	Arisi et al. 1994 lindane
48	Rat (Long- Evans)	30 d 1x/d (GO)				10 M (myoclonic jerks and clonic seizures)	Gilbert 1995 lindane
49	Rat (Long- Evans)	10 wk 3 d/wk (GO)				10 M (myoclonic jerks and clonic seizures)	Gilbert 1995 lindane
50	Rat (CD)	13 wk ad libitum (F)		7.9 F	30.2 F		Hughes 1999b lindane
51	Rat (Wistar)	30 d (GO)			2 (decreased dopamine levels)		Martinez and Martinez-Conde 1995 lindane

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
52	Rat (Wistar)	30 d ad libitum (F)		12.3 M	25.4 M (reduced tail nerve conduction velocity)		Muller et al. 1981 lindane
53	Rat (Wistar)	25 d GD 6 - LD 10 ad libitum (F)		1.2 F	5.6 F (increased motor activity and decreased motor activity habituation in pups at postnatal days 11 and 65)		Myers 1999 lindane
54	Rat (Wistar)	1 x/d 15 d (G)			2.5 M (increased EROD, PROD, and NDMA-d enzyme levels in the brain)		Parmar et al. 2003 lindane
Reproductive							
55	Rat (Fischer- 344)	15 wk 1x/d (GO)		5 F	10 F (disrupted ovarian cycling, antiestrogenic effects)		Chadwick et al. 1988 lindane
56	Rabbit (hybrid)	12 wk 3 d/wk (GO)			0.8 F (reduced ovulation rate)		Lindenau et al. 1994 lindane
57	Rabbit (New Zealand)	12-15 wk 3 d/wk (GO)		0.8 F			Seiler et al. 1994 lindane

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
58	Mink (NS)	3 generations (F)			1 (reduced litter size in F2 females, reduced testis size in F3 males)		Beard and Rawlings 1998 lindane
59	Mink (NS)	12 wk 3 wk premating 8 wk postpartum (F)			1 F (reduced mating receptivity and whelping rate)		Beard et al. 1997 lindane
60	Mink (NS)	17 wk 6 wk premating 10 wk postpartum (F)			1 F (reduced whelping rate and increased post-implantation embryo loss)		Beard et al. 1997 lindane
Developmental							
61	Rat (Wistar)	Gd 0-21 Ld 1-28 (F)			25 (Increased liver weight and decreased kidney weight in pups exposed during gestatino and lactation)		Srinivasan et al. 1991a lindane
62	Rabbit (New Zealand)	12-15 wk 3 d/wk (GO)		0.8 F			Seiler et al. 1994 lindane
CHRONIC EXPOSURE							
Death							
63	Rat (Wistar)	up to 52 weeks ad libitum (F)				32 F (increased mortality rate)	Amyes 1990 lindane

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
Systemic							
64	Rat (Wistar)	up to 2 yr ad libitum (F)	Hepatic		7 M (periacinar hepatocytic hypertrophy)		Amyes 1990 lindane
			Renal	32 F			
65	Rat (Wistar)	107 weeks (F)	Hepatic	4 F	7 M (Very slight microscopic liver damage in the absence of gross liver damage)	112 M (Moderate microscopic damage [hepatic cell atrophy, fatty degeneration, and focal necrosis] in the presence of slight-to-moderate gross liver damage)	Fitzhugh et al. 1950 lindane
			Renal	4 F	7 M (focal nephritis)		
			Bd Wt	64 F	112 M (17% decrease in body weight gain)		
66	Rat (Sprague-Dawley)	18mo 1x/d (F)	Hepatic		9 (Increased cell, nucleus, and nucleolus size; extensive cytoplasmolysis; slight cytoplasmic degeneration; incresing nuclear distortion)		Shahid Ali and Rauf Shakoori 1998 lindane
Cancer							
67	Mouse (B6C3F1)	80 wk ad libitum (F)				13.6 M (CEL: hepatocellular carcinoma)	NCI 1977 lindane

Table 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - 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
68	Mouse (F-1 hybrid)	24 mo ad libitum (F)				27.2 F (CEL: hepatocellular carcinoma, lung tumors)	Wolff et al. 1987 lindane

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

b Used to derive an acute-duration oral minimal risk level (MRL) of 0.003 mg/kg/day for gamma-HCH; based on a minimal LOAEL of 1 mg/kg/day divided by an uncertainty factor of 300 (3 for use of a LOAEL, 10 for extrapolation from animals to humans, 10 for human variability)

c Used to derive an intermediate-duration minimal risk level (MRL) of 0.00001 mg/kg/day for gamma-HCH; 0.012 mg/kg/day divided by an uncertainty factor of 1000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, 10 for human variability)

Bd Wt = body weight; cardio = cardiovascular; CEL = cancer effect level; d = day(s); endocr = endocrine; EROD = 7-ethoxyresorufin-O-deethylase; F = female; (F) = food; (G) = gavage; (GO) = gavage in oil; (GW) = gavage in water; Gd = gestation day(s); GI = gastric intubation; gastro = gastrointestinal; Hemato = hematological; hr = hour(s); Ld = lactation day; LD50, lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); NDMA-d = N-nitrosodimethylamine demethylase; NOAEL = no-observed-adverse-effect level; Pc = post conception; PROD = 7-pentoxyresorufin-O-dealkylase; Resp = respiratory; wk = week(s); x = time(s); yr = year(s)

Figure 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - Oral

Acute (≤14 days)

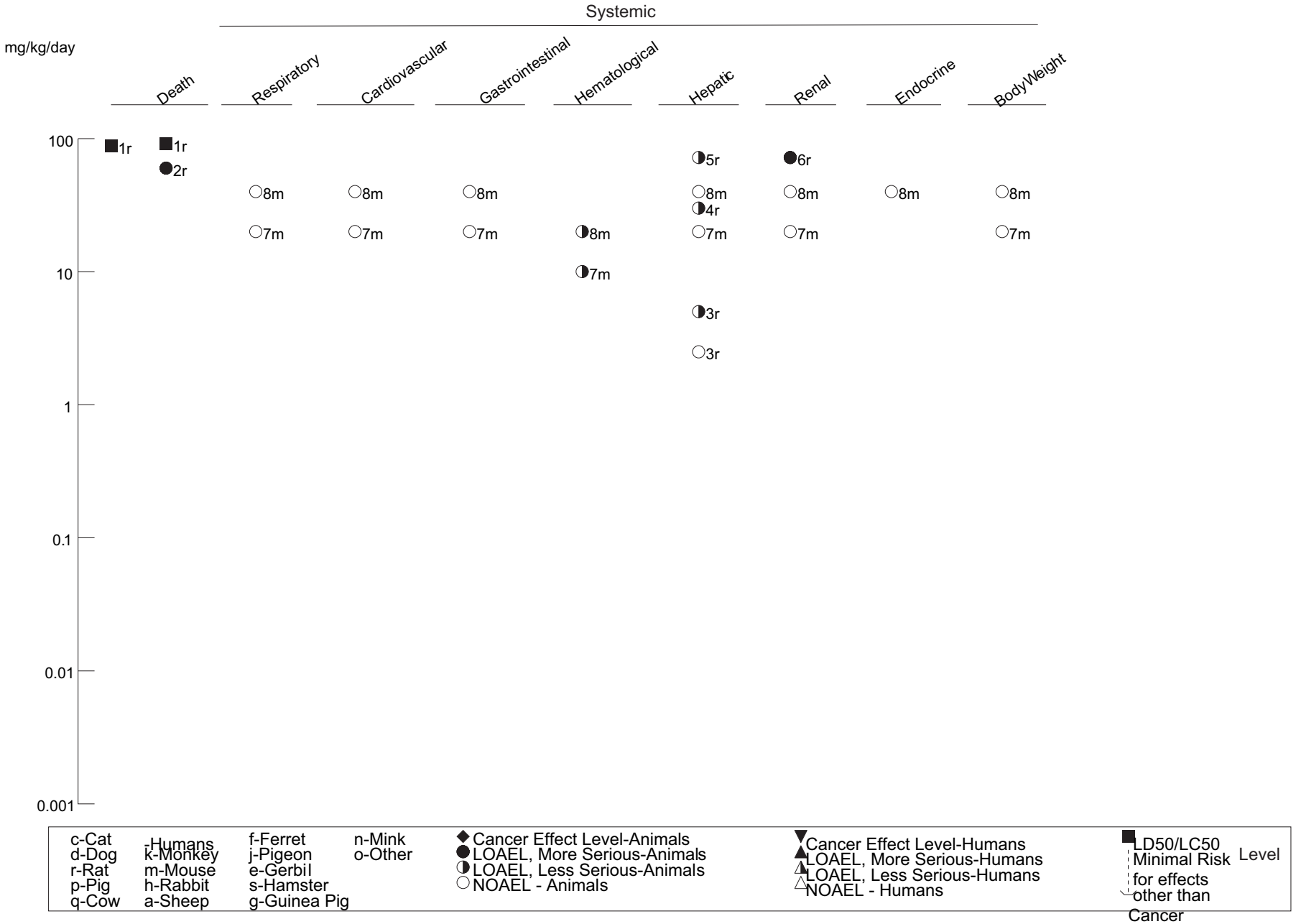


Figure 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - Oral (Continued)

Acute (≤14 days)

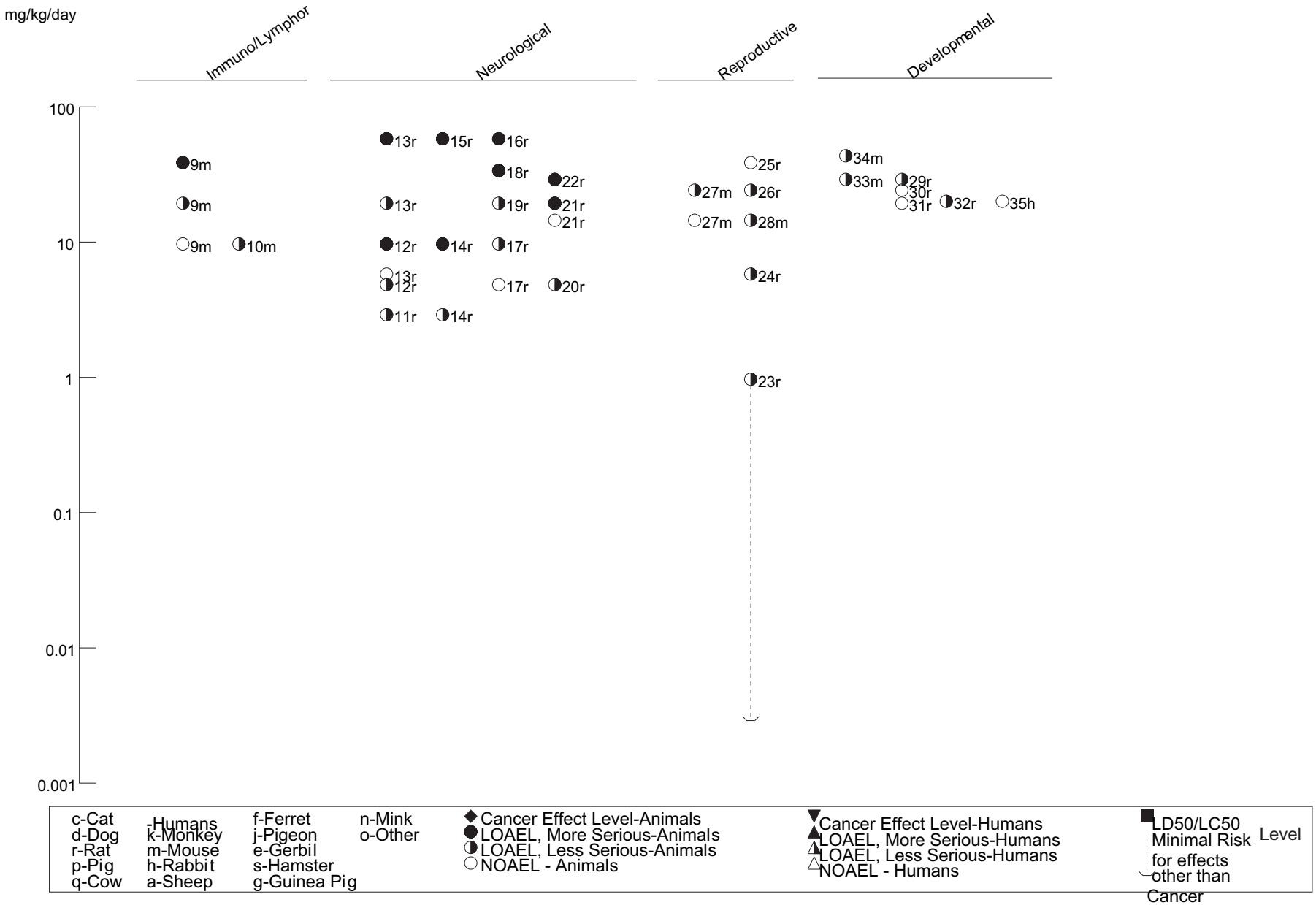


Figure 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - Oral (Continued)

Intermediate (15-364 days)

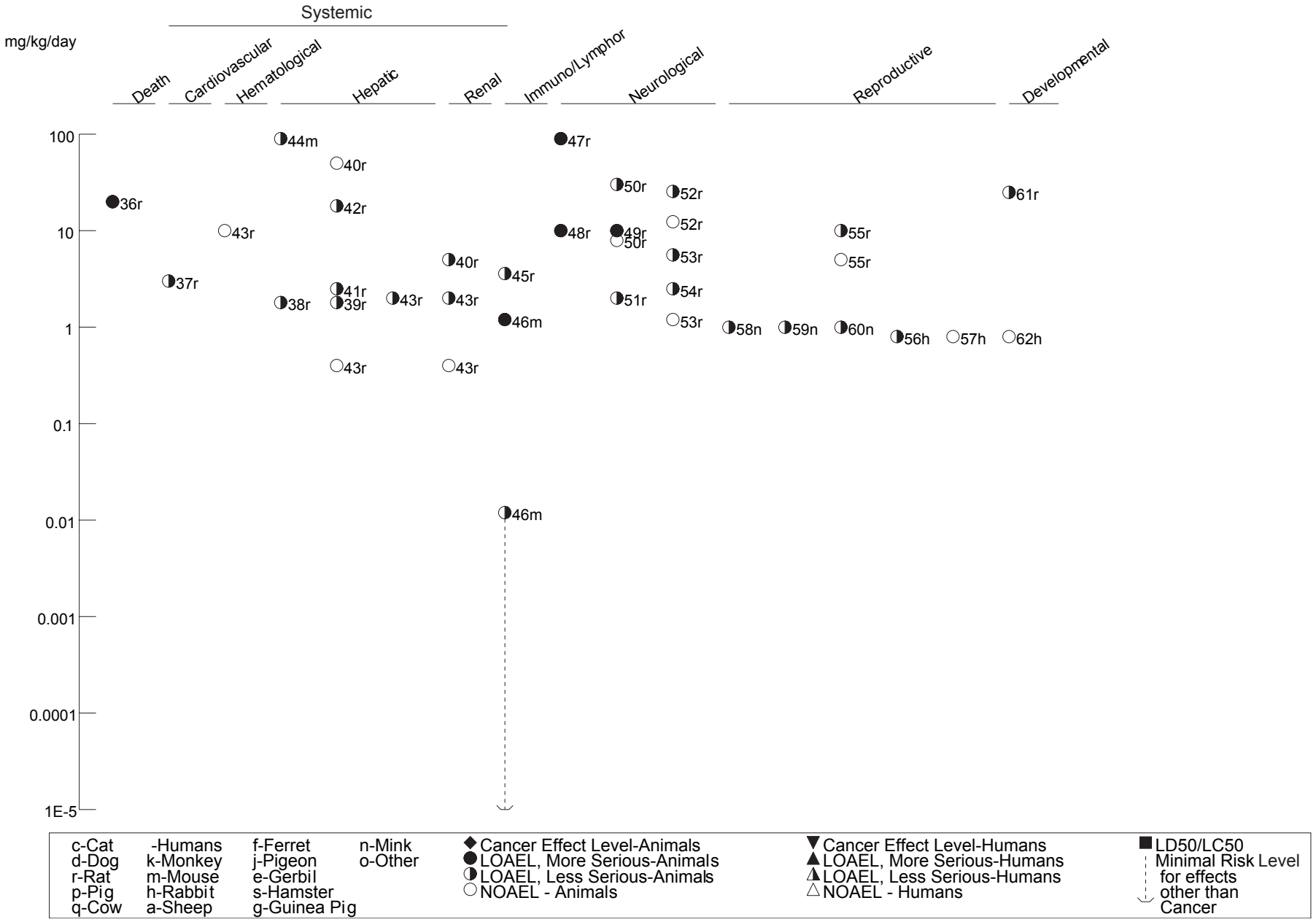


Figure 3-2 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - Oral (*Continued*)

Chronic (≥365 days)

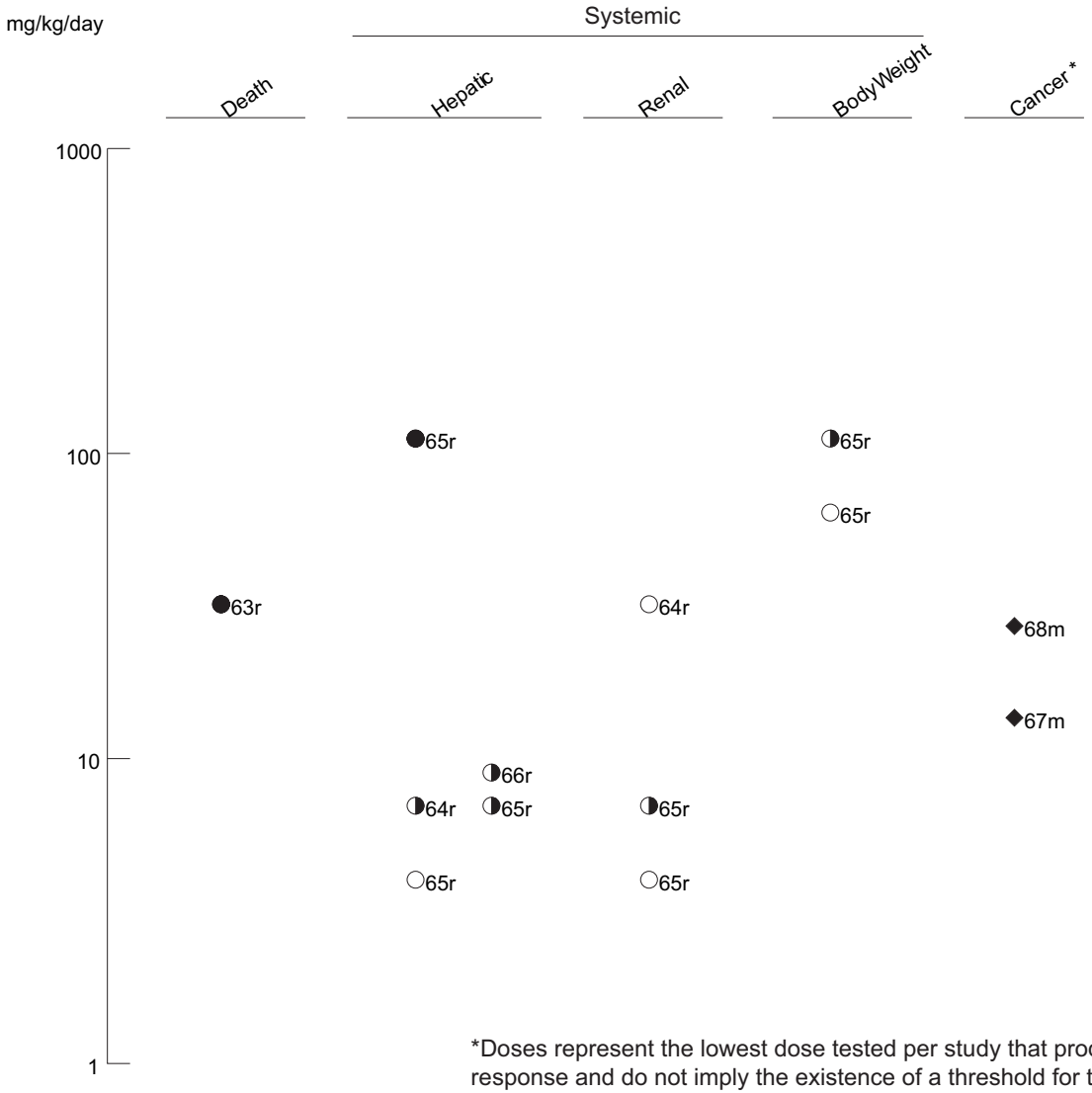


Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - Oral

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
ACUTE EXPOSURE							
Death							
1	Rat (CFT-Wistar)	once (GO)				2428 M (LD50)	Joseph et al. 1992a technical
Systemic							
2	Rat (NS)	once (GO)	Metab		100 F (increased phosphoinositide turnover in erythrocyte membranes)		Agrawal et al. 1995 technical
3	Rat (Sprague-Dawley)	2 wk ad libitum (F)	Hepatic		90 M (increased triglycerides, phospholipids and cholesterol, increased cytochrome C reductase and decreased glutathione peroxidase)		Ikegami et al. 1991a beta
4	Rat (Sprague-Dawley)	2 wk ad libitum (F)	Hepatic		90 M (increased relative liver weight and cytochrome P-450 levels and decreased hepatic vitamin A levels)		Ikegami et al. 1991b beta
5	Rat (Wistar)	14 d ad libitum (F)	Renal			72 M (tubular degeneration, distention of glomeruli, swelling of tubular epithelia, 22% increase in kidney weight, altered excretion patterns)	Srinivasan et al. 1984 beta

Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - 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
6	Mouse (Swiss albino)	Gd 9 once (GO)	Hepatic		5 F (significantly decreased GOT and lactate dehydrogenase (LD) activities)		Dikshith et al. 1990 technical
7	Mouse (NS)	1, 5, 15 d 1x/d (GO)	Hepatic			50 (congestion of portal vessels and central vein, fatty changes, granular degeneration)	Philip et al. 1989 technical
			Renal			50 (congestion of blood vessels and glomeruli, fatty changes, interstitial hemorrhaging)	
8	Mouse (Swiss albino)	2 wk ad libitum (F)	Hepatic		72 M (127% increase in relative liver weight, increased serum alanine and aspartate aminotransferases and ALP, increased hepatic phosphatases and acid cathepsin)		Ravinder et al. 1989 technical
9	Mouse (Swiss albino)	2 wk ad libitum (F)	Hepatic		72 M (cellular hypertrophy, centrilobular degeneration, focal necrosis)		Ravinder et al. 1990 technical

Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - 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
Neurological							
10	Rat (Wistar)	2 wk ad libitum (F)		5 M 4.5[b] F		22.5 M (ataxia, inactivity) 25 F (ataxia, inactivity)	Van Velsen et al. 1986 beta
11	Mouse (B6C3F1)	1 wk ad libitum (F)		19 F	57 F (ataxia)	190 F (lateral recumbancy)	Cornacoff et al. 1988 beta
Reproductive							
12	Mouse (Swiss albino)	Gd 9 once (GO)		5 F		25 F (increased fetal resorptions)	Dikshith et al. 1990 technical
INTERMEDIATE EXPOSURE							
Death							
13	Rat (NS)	360 d ad libitum (F)				0.4 M (4/20 deaths)	Dikshith et al. 1991a technical
14	Rat (NS)	90 d 1x/d (GO)				5 (6/12 M, 4/12 F died)	Dikshith et al. 1991b technical
Systemic							
15	Rat (NS)	3-6 mo 5 d/wk (GO)	Metab		5 F (significant reductions in phosphoinositide levels in erythocyte membranes and cerebrum)		Agrawal et al. 1995 technical

Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - 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
16	Rat (Wistar)	15 d ad libitum (F)	Hepatic		1.8 M (increased cytochrome P-450 level, superoxide dismutase, catalase, and lipid peroxidation activities)		Barros et al. 1991 alpha
17	Rat (Wistar)	30 d ad libitum (F)	Hepatic		1.8 M (increased cytochrome P-450 level, superoxide dismutase, catalase, NADPH-cytochrome P-450 reductase activities, and lipid peroxidation)		Barros et al. 1991 alpha
18	Rat (NS)	30 d 1x/d (GO)	Hemato	60 M			Dikshith et al. 1989a technical
			Hepatic		60 M (decreased GOT and LDH activities, increased ALP activity, 65% increase in liver weight)		
			Renal	60 M			

Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - 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
19	Rat (NS)	360 d ad libitum (F)	Hepatic	0.4 M	2 M (increased liver weight)	20 M (focal necrosis, enlargement of hepatocytes, nuclear pyknosis, vacuolation, margination)	Dikshith et al. 1991a technical
			Renal	2 M		20 M (tubular necrosis, glomerular degeneration)	
20	Rat (NS)	90 d 1x/d (GO)	Hepatic		5 M (decreased liver and serum GOT and alkaline phosphatase activities)		Dikshith et al. 1991b technical
21	Rat (Charles Foster)	180 d 1x/d (G)	Bd Wt		3 M (17% decrease in body weight gain)		Gautam et al. 1989 technical
22	Rat (CFT-Wistar)	7 wk ad libitum (F)	Hepatic		90 M (Decreased hepatic vitamin A content, increased GPT and beta-GLR activities, 56% increase in liver weight)		Joseph et al. 1992b technical
23	Rat (CFT-Wistar)	7 wk ad libitum (F)	Hemato		90 M (decreased white blood cell counts)		Joseph et al. 1992c technical

Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - 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
24	Rat (NS)	30 d 1x/d (GO)	Hepatic	50 M			Khanna et al. 1990 technical
			Renal	50 M			
25	Rat (Wistar)	90 d ad libitum (F)	Bd Wt		20 F (significantly decreased body weight gain)		Nagaraja and Desiraju 1994 technical
26	Rat (Wistar)	13 wk ad libitum (F)	Hemato	5 F	22.5 M (decreased red blood cells, leukocyte and hemoglobin concentrations)		Van Velsen et al. 1986 beta
			Hepatic		0.18[c] M (hyalinization of centrilobular cells)	4.5 M (hyalinization of centrilobular cells, focal cell necrosis, increased mitoses)	
			Renal	4.5 M	22.5 M (calcinosis in males)		
			Bd Wt	5 F	22.5 M (15% decrease in body weight)		
27	Mouse (dd)	32 wk ad libitum (F)	Hepatic	20 F	54 M (nuclear irregularities in foci of enlarged hepatocytes)		Hanada et al. 1973 beta

Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - 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
28	Mouse (dd)	24 wk ad libitum (F)	Hepatic		45 M (centrilobular hypertrophy)		Ito et al. 1973 beta
29	Mouse (dd)	24 wk ad libitum (F)	Hepatic		90 M (centrilobular hypertrophy)		Ito et al. 1973 delta
30	Mouse (dd)	24 wk ad libitum (F)	Hepatic		18 M (centrilobular hypertrophy)		Ito et al. 1973 alpha
31	Mouse (Swiss)	2-8 mo ad libitum (F)	Hepatic		90 (100% increase in liver weight, decreased G6P and FDP activity, glycogen accumulation, smooth endoplasmic reticulum proliferation)		Karnik et al. 1981 technical
32	Mouse (HPB)	50 wk ad libitum (F)	Hepatic		90 M (hyperplastic nodules)		Tryphonas and Iverson 1983 alpha
Immuno/ Lymphoret							
33	Rat (Wistar)	13 wk ad libitum (F)		5 F		22.5 M (cortical atrophy in thymus)	Van Velsen et al. 1986 beta

Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - 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
34	Mouse (B6C3F1)	30 d ad libitum (F)		20 F	60 F (decreased lymphoproliferative responses to T-cell mitogens, decreased natural killer cytolytic activity)		Cornacoff et al. 1988 beta
Neurological							
35	Rat (NS)	90 d 6 d/wk 1x/d (GO)			50 M (increased dopamine and decreased serotonin and norepinephrine. Behavioral changes, increased brain wave frequency)		Anand et al. 1991 technical
36	Rat (NS)	360 d 1x/d (F)		0.04 M		0.4 M (convulsions, tremors, hindlimb paralysis, salivation)	Dikshith et al. 1991a technical
37	Rat (NS)	120 d 1x/d (GO)			50 M (increased motor activity, decreased resting stereotypic time)		Gopal et al. 1992 technical
38	Rat (Wistar)	30 d ad libitum (F)		106.2 M			Muller et al. 1981 alpha

Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - 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
39	Rat (Wistar)	30 d ad libitum (F)			66.3 M (reduced tail nerve conduction velocity)		Muller et al. 1981 beta
40	Rat (Wistar)	90 d ad libitum (F)			20 F (increased GABA levels, increased GAD activity, decreased glutamate levels)		Nagaraja and Desiraju 1994 technical
41	Rat (Wistar)	13 wk ad libitum (F)		5 F		22.5 M (ataxia, coma)	Van Velsen et al. 1986 beta
Reproductive							
42	Rat (NS)	360 d 1x/d (F)		2 M		20 M (testicular degeneration)	Dikshith et al. 1991a technical
43	Rat (Charles Foster)	180 d 1x/d (GO)			3 M (6% decrease in vas deferens weight, degeneration of inner muscle and cell layers)		Gautam et al. 1989 technical

Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - 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
44	Rat (Charles Foster)	180 d 1x/d (G)			3 M (decreased seminiferous tubular diameter and Leydig cell nuclear population)	6 M (seminiferous tubular degeneration)	Roy Chowdhury and Gautam 1990 technical
45	Rat (Wistar)	13 wk ad libitum (F)		0.9 M 0.2 F[d]	4.5 M (decreased testes weight) 1 F[d] (increased absolute ovary and uterus weights)	22.5 M[d] (atrophy of testes) 25 F (atrophy of ovary; hyperplastic and vacuolized endometrium epithelium in uterus)	Van Velsen et al. 1986 beta
46	Mouse (B6C3F1)	30 d ad libitum (F)		60 F			Cornacoff et al. 1988 beta
47	Mouse (Swiss)	3 mo ad libitum (F)				90 M (increased testis weight, degeneration of seminiferous tubules, decreased spermatocytes)	Nigam et al. 1979 technical
Developmental							
48	Rat (Wistar)	60 d ad libitum			10 F (alterations in levels of dopamine, serotonin, and noradrenaline in pup brains)		Nagaraja and Desiraju 1994 technical

Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - 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
49	Rat (Wistar)	Gd 0-21 Ld 1-28 (F)			5 (increased liver weight in pups exposed during gestation and lactation)	20 (increased pup mortaility)	Srinivasan et al. 1991a beta
Cancer							
50	Rat (Wistar)	20 wk ad libitum (F)				2 F (CEL: increase in preneoplastic hepatic foci)	Schroter et al. 1987 alpha
51	Rat (Wistar)	20 wk ad libitum (F)				3 F (CEL: increase in preneoplastic hepatic foci)	Schroter et al. 1987 beta
52	Mouse (dd)	32 wk ad libitum (F)				18 M (CEL: hepatoma)	Hanada et al. 1973 alpha
53	Mouse (dd)	24 wk ad libitum (F)				45 M (CEL: hepatocellular carcinoma)	Ito et al. 1973 alpha
54	Mouse (DDY)	16-36 wk ad libitum (F)				90 M (CEL: hepatocellular carcinoma)	Ito et al. 1976 alpha

Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - 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
55	Mouse (Swiss)	2-4 mo ad libitum (F)				90 F (CEL: hepatocellular carcinoma)	Karnik et al. 1981 technical
56	Mouse (DDY, ICR, DBA/2, C57BL/6, C3H/He)	24 wk ad libitum (F)				90 M (CEL: hepatocellular carcinoma)	Nagasaki et al. 1975 alpha
57	Mouse (Swiss)	2-8 mo ad libitum (F)				90 (CEL: hepatocellular carcinoma)	Thakore et al. 1981 technical
58	Mouse (HPB)	50 wk ad libitum (F)				90 M (CEL: hyperplastic nodules and adenomas in liver)	Tryphonas and Iverson 1983 alpha
59	Mouse (DD)	16-36 wk ad libitum (F)				90 M (CEL: hepatoma)	Tsukada et al. 1979 alpha

Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - 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
CHRONIC EXPOSURE							
Systemic							
60	Rat (Wistar)	107 weeks ad libitum (F)	Hepatic		0.8 F (Very slight microscopic damage in the absence of gross liver damage, 33% increase in liver weight)	56 M[d] (Moderate microscopic damage [hepatic cell atrophy, fatty degeneration, and focal necrosis] in the presence of marked gross liver damage)	Fitzhugh et al. 1950 beta
						64 F	
			Renal	8 F	56 M (focal nephritis)		
			Bd Wt	56 M 0.8 F[d]	8 F (12% decrease in body weight gain)		
61	Rat (Wistar)	107 weeks ad libitum (F)	Hepatic	0.8 F	3.5 M (very slight to slight microscopic damage in the absence of gross liver damage)	56 M (Moderate microscopic damage [hepatic cell atrophy, fatty degeneration, and focal necrosis] in the presence of moderate gross liver damage. 36% increase in liver weight)	Fitzhugh et al. 1950 technical
			Renal	8 F	56 M (focal nephritis)		
			Bd Wt	8 F	56 M (decreased body weight gain)		

Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - 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
62	Rat (Wistar)	107 weeks ad libitum (F)	Hepatic	0.8[e] F	3.5 M (very slight to slight microscopic damage in the absence of gross liver damage; 32% increased liver weight)	56 M (Moderate microscopic damage [hepatic cell atrophy, fatty degeneration, and focal necrosis] in the presence of moderate gross liver damage)	Fitzhugh et al. 1950 alpha
			Renal	8 F	56 M (focal nephritis)		
			Bd Wt	8 F	56 M (18% decrease in body weight gain)		
Neurological							
63	Mouse (Swiss)	80 wk ad libitum (F)				17 (convulsions)	Kashyap et al. 1979 technical
64	Mouse (Swiss)	80 wk 1x/d (GO)				10 (convulsions)	Kashyap et al. 1979 technical
Cancer							
65	Rat (NS)	72 wk ad libitum (F)				75 M (CEL: hepatocellular carcinoma)	Ito et al. 1975 alpha
66	Mouse (Swiss)	80 wk 1x/d (GO)				10 (CEL: hepatocellular carcinoma)	Kashyap et al. 1979 technical

Table 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, And Tech-grade HCH - 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
67	Mouse (Swiss)	80 wk ad libitum (F)				17	(CEL: hepatocellular carcinoma)	Kashyap et al. 1979 technical
68	Mouse (Swiss)	20 mo ad libitum (F)				21.3 M	(CEL: hepatocellular carcinoma)	Munir et al. 1983 technical
69	Mouse (CF1)	104 wk ad libitum (F)				34	(CEL:hepatocellular carcinoma)	Thorpe and Walker 1973 beta

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

b Used to derive an acute-duration oral minimal risk level (MRL) of 0.05 mg/kg/day for beta-HCH; 19 mg/kg/day divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, 10 for human variability).

c Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.0006 mg/kg/day for beta-HCH; 0.18 mg/kg/day divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans, 10 for human variability).

d Differences in levels of health effects and cancer effects between male and females are not indicated in Figure 3-3. Where such differences exist, only the levels of effect for the most sensitive gender are presented.

e Used to derive a chronic-duration oral minimal risk level (MRL) of 0.008 mg/kg/day for alpha-HCH; 0.8 mg/kg/day divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, 10 for human variability).

ALP = alkaline phosphatase; Bd Wt = body weight; CEL = cancer effect level; d = day(s); (F)= feed; F = female; FDP = fructose-1,6-diphosphatase; (G) = gavage; (GO) = gavage in oil; Gd = gestation day; GABA = gamma-aminobutyric acid; GAD = glutamate decarboxylase; GLR = glucorinidase; GOT = glutamate oxaloacetate transaminase; G6P = glucose-6-phosphatase; GPT = glutamate pyruvate transaminase; Hemato = hematological; Ld = lactation day; LD50, lethal dose, 50% kill; LDH = lactate dehydrogenase; LOAEL = lowest-observed-adverse-effect level; M = male; metab = metabolism; mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s); x = time(s)

Figure 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Tech-grade HCH - Oral

Acute (≤14 days)

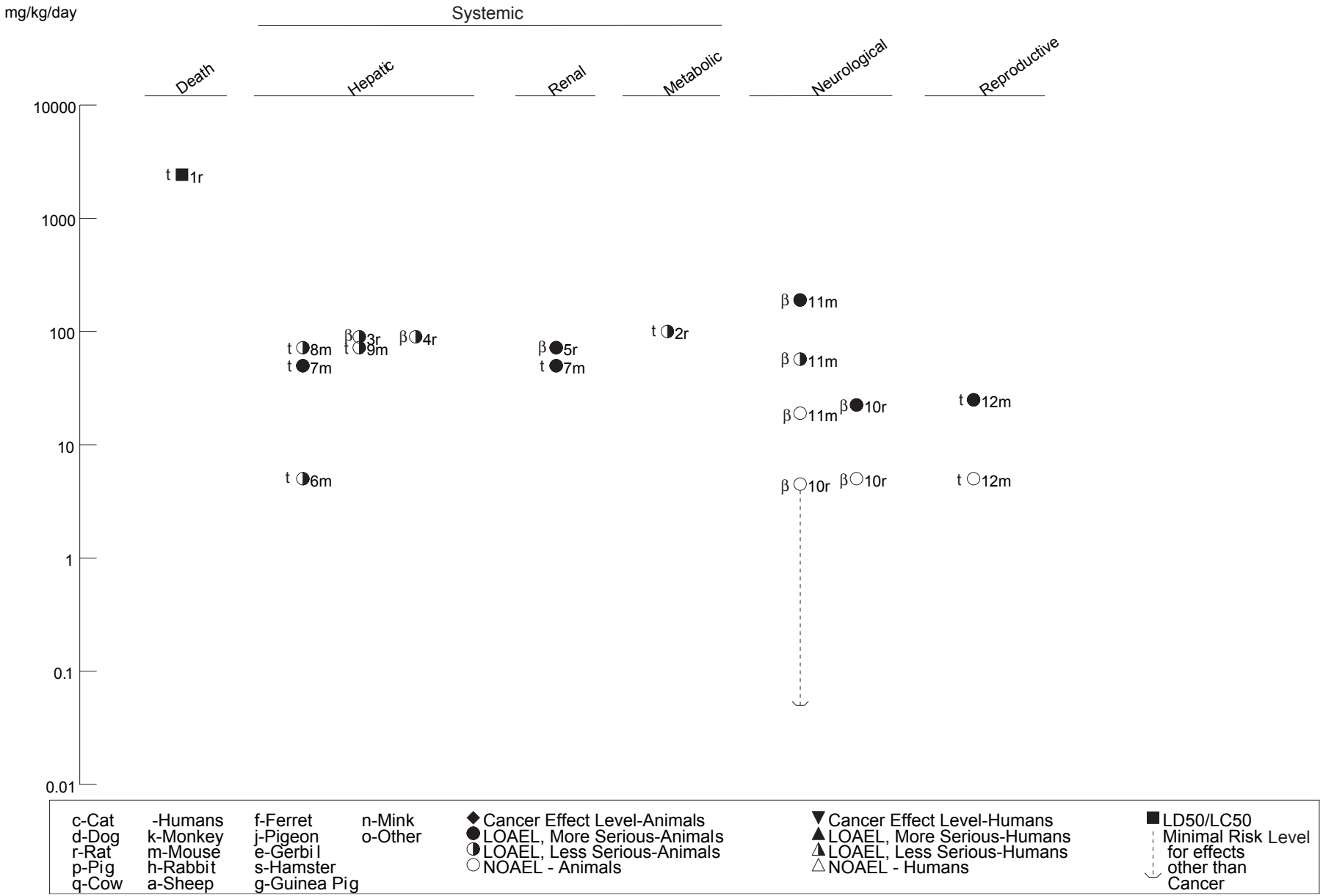


Figure 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Tech-grade HCH - Oral (*Continued*)

Intermediate (15-364 days)

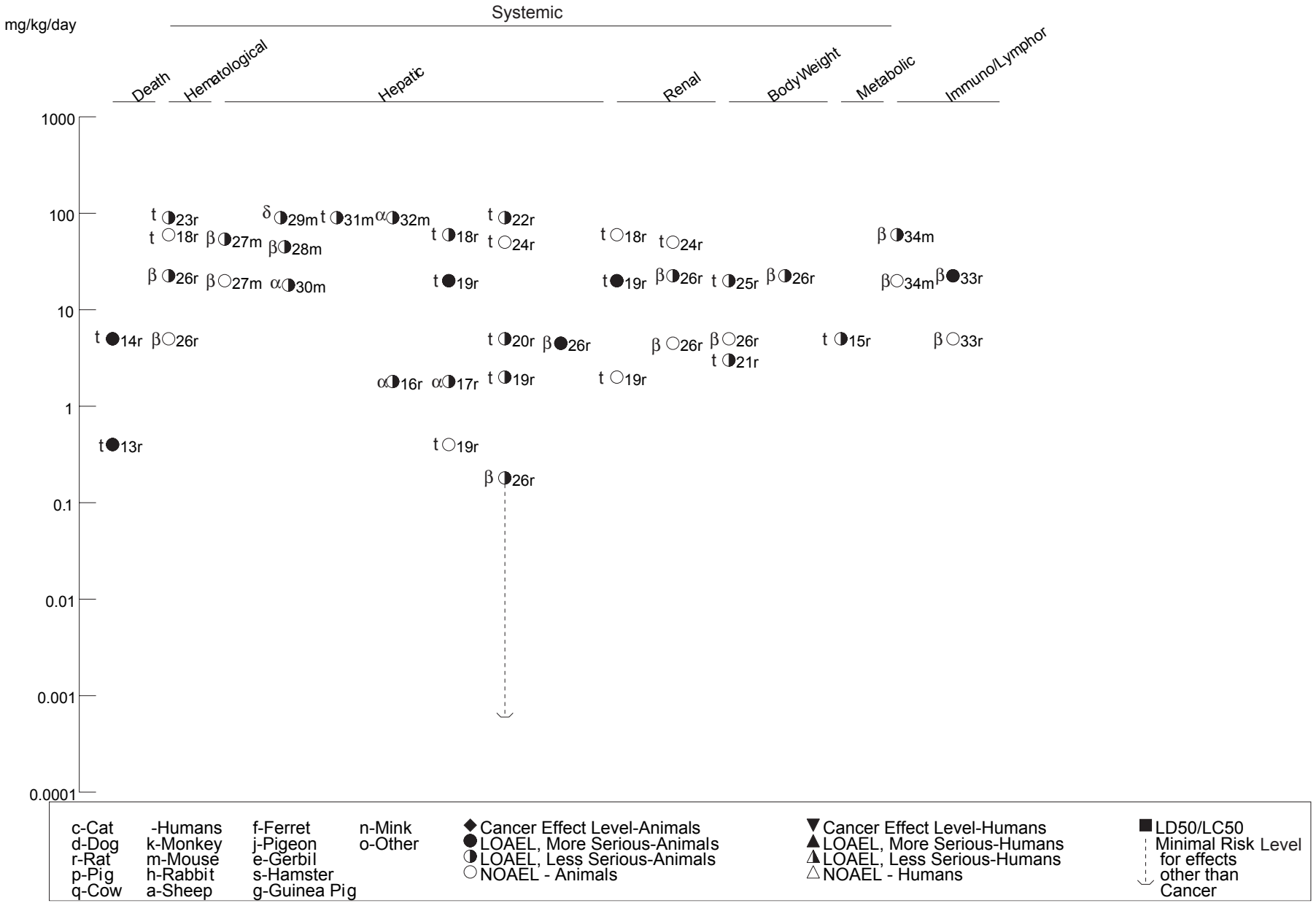


Figure 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Tech-grade HCH - Oral (*Continued*)

Intermediate (15-364 days)

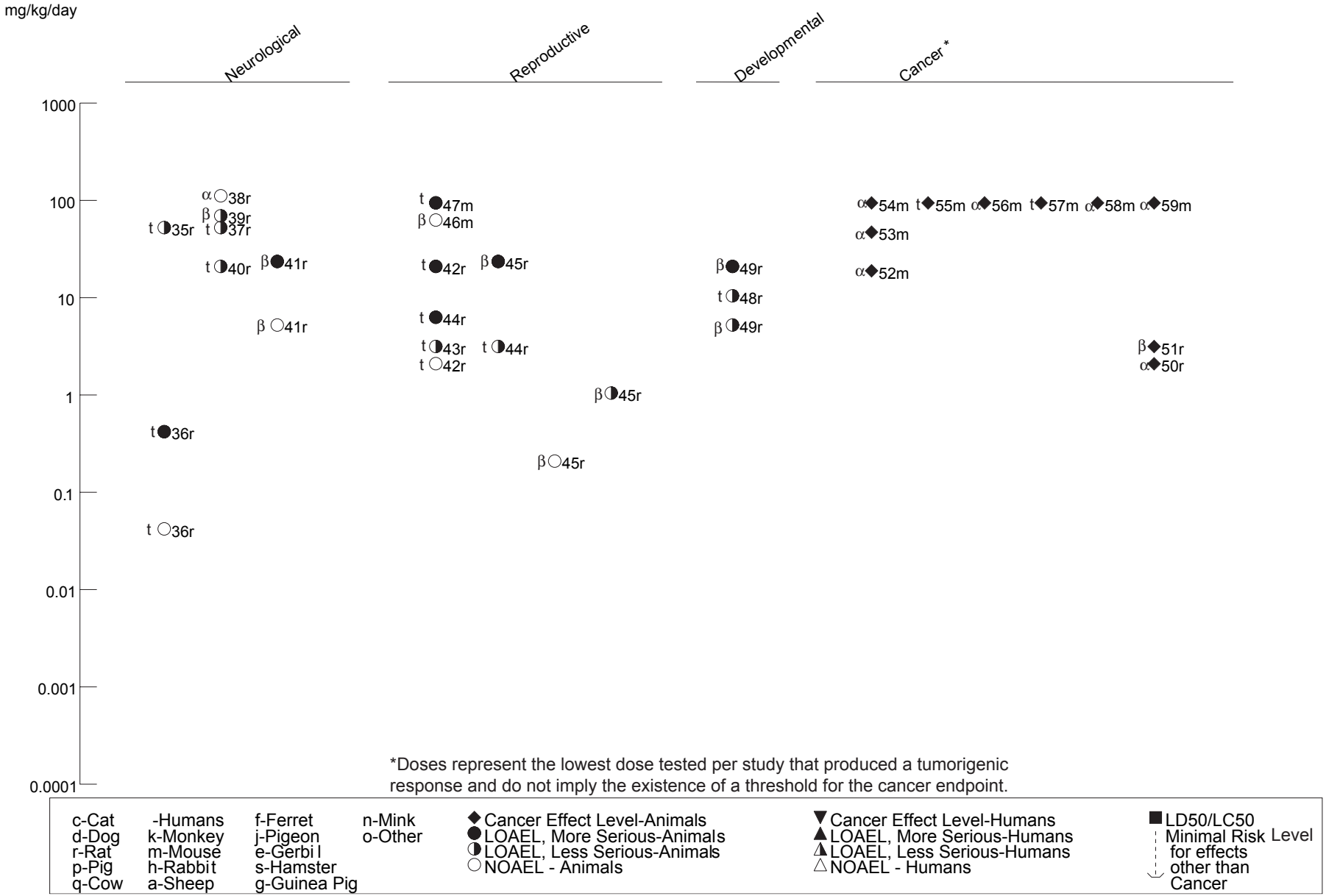
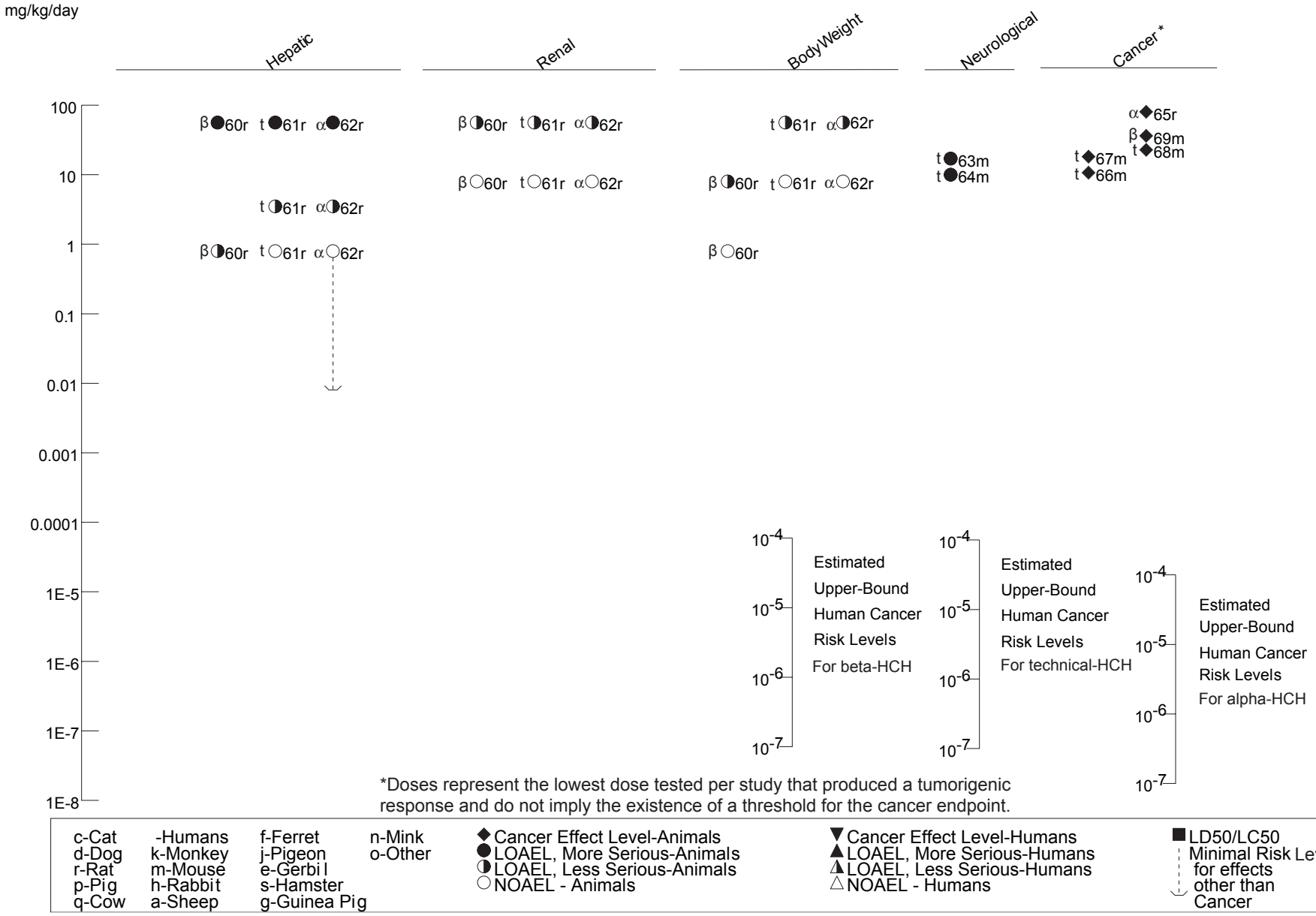


Figure 3-3 Levels of Significant Exposure to Alpha-, Beta-, Delta-, and Tech-grade HCH - Oral (*Continued*)

Chronic (≥365 days)



3.2.2.2 Systemic Effects

No studies were located regarding respiratory, dermal, or ocular effects in humans or animals following oral exposure to HCH. The animal studies in which systemic effects of HCH were examined, in most cases, used isomers of >99% purity.

The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Tables 3-2 and 3-3 and plotted in Figures 3-2 and 3-3.

Gastrointestinal Effects. Decreased appetite, vomiting, nausea, and diarrhea have been observed in humans following ingestion of food contaminated with unknown amounts of γ-HCH; exposure was inferred from levels of γ-HCH measured in urine (Nantel et al. 1977). Vomiting and nausea are usual manifestations of γ-HCH ingestion (Sunder Ram Rao et al. 1988).

γ-HCH has been shown to affect the absorption of substances such as glucose, glycine, and calcium in the gastrointestinal tract of rats (Labana et al. 1997), and the effect depends on the nutritional status of the animals. Additional reports of gastrointestinal effects after oral administration of γ-HCH were not located; however, γ-HCH administered subcutaneously at 20 mg/kg/day to rats for 15 days reduced (Na^+-K^+)-ATPase activity in the rat jejunum (Moreno et al. 1996).

Cardiovascular Effects. No studies were located regarding cardiovascular effects in humans following inhalation exposure to HCH.

Rats receiving gavage doses of 3 mg/kg/day γ-HCH for 6 weeks exhibited tachycardia, increased blood pressure and plasma calcium levels, an increase in myocardial calcium influx, and decreased Ca,K-ATPase activity. Electrocardiographic changes included increased ST segment and T-wave amplitude and reduced R-R interval and P-wave (Anand et al. 1995).

Hematological Effects. A woman who committed suicide by drinking γ-HCH was found to have disseminated (dispersed) intravascular coagulation during the period when serum γ-HCH levels were elevated (Sunder Ram Rao et al. 1988). No other reports were found on the possible effect of γ-HCH on blood-clotting factors in humans.

No hematological effects were noted in beagle dogs exposed to 12.5 mg γ-HCH/kg/day in the diet for 32 weeks or to 2.9 mg γ-HCH/kg/day in the diet for 104 weeks (Rivett et al. 1978). A 12-week study in rats using 10 mg γ-HCH/kg/day, support this finding (Suter 1983). However, exposure to 22.5 mg β-HCH/kg/day in the diet for 13 weeks in rats was found to be more toxic, resulting in a statistically significant decrease in numbers of red blood cells and white blood cells and reduced hemoglobin and packed cell volume values (Van Velsen et al. 1986). Significant decreases in total white blood cell counts and clotting time were reported in rats fed vitamin A-free diets containing technical-grade HCH at a dose level of 90 mg/kg/day for 7 weeks (Joseph et al. 1992c). In rats fed a vitamin A-supplemented diet containing the same dose level of technical-grade HCH, a significant reduction in total white blood cell count, but not red blood cell count, was observed (Joseph et al. 1992c). Significant suppression in bone marrow cellularity, erythrocyte precursors, and granulocyte-macrophage progenitor cells, and residual progenitor cell damage were reported in male $B6C3F_1$ mice given 20 or 40 mg γ-HCH/kg/day by gavage in corn oil for 3 days (Hong and Boorman 1993). Following 10 days of exposure to 10 or 20 mg γ-HCH/kg/day, dose-dependent decreases in bone marrow cellularity, granulocyte-macrophage progenitor cells, and pluripotent bone marrow stem cells were noted (Hong and Boorman 1993).

No hematological effects were seen in rats following oral exposure to 60 mg/kg/day technical-grade HCH for 30 days (Dikshith et al. 1989a).

Musculoskeletal Effects. In humans, ingestion of a single dose of approximately 15–30 mL γ-HCH powder (amount not reported by weight) was associated with seizures and limb muscle weakness and necrosis in an adult man (Munk and Nantel 1977); a muscle biopsy conducted 15 days after ingestion showed no evidence of denervation or neuropathy. Widespread striatal muscle necrosis was seen in a woman who died 11 days after intentionally ingesting 8 ounces of a 20% γ-HCH solution (Sunder Ram Rao et al. 1988).

Decreased cross-sectional bone area was found in young rats treated with 20 mg/kg/day of γ-HCH by gavage for 10 weeks (Andrews and Gray 1990). Myelotoxicity, manifested as significant, dose-dependent decrease in marrow progenitor numbers, was seen in mice exposed to 10 or 20 mg/kg/day γ-HCH for 10 days (Hong and Boorman 1993).

Hepatic Effects. No studies were located regarding hepatic effects in humans following oral exposure to HCH.

Significantly increased liver microsomal 7-ethoxycoumarin-O-dealkylase activity was found in Osborne-Mendel rats exposed to 11.2 mg γ-HCH/kg/day and in CF_1 and $B6C3F_1$ strain mice exposed to 23.6 and 50.5 mg/kg/day in the diet for 3 days (Oesch et al. 1982). Although no histopathological examinations were performed to confirm the presence or absence of toxicity, no significant increase in liver weight or other adverse effects were noted in rats exposed to 10 mg γ-HCH/kg/day for a minimum of 4 days (Joy et al. 1982). Rats exposed to 15 mg γ-HCH/kg/day for 5 days, and 2.5 mg γ-HCH/kg/day for 21 days, showed a significant increase in absolute liver weight (Parmar et al. 2003). A dose- and time-dependent increase of P-450 and P-450-dependent enzyme levels was observed in the liver of rats exposed to γ-HCH (Parmar et al. 2003). P-450 content was significantly increased in rats exposed to 10 mg γ-HCH/kg/day for 5 days, and in rats exposed to 2.5 mg γ-HCH/kg/day for 15 and 21 days. There was no significant increase in P-450 content in rats exposed to <10 mg γ-HCH/kg/day for 5 days. Several P-450- dependent enzymes, 7-ethoxyresorufin-O-deethylase (EROD), 7-pentoxyresorufin-O-dealkylase (PROD), and N-nitrosodimethylamine demethylase (NDMA-d), significantly increased in rats exposed to 5 mg γ-HCH/kg/day for 5 days, and in rats exposed to 2.5 mg γ-HCH/kg/day for 15 and 21 days (Parmar et al. 2003). Hepatocellular damage as indicated by elevation in serum aminotransferases and decrease in hepatic soluble enzymes was found in rats given 72 mg/kg/day γ-HCH for 2 weeks (Srinivasan and Radhakrishnamurty 1988). Significant increases in hepatic microsomal cytochrome P-450 levels and increases in hepatic microsomal superoxide anion production and cytoplasmic superoxide dismutase activity and lipid peroxidation were found in Wistar rats fed diets containing 1.8 mg/kg/day γ-HCH for 15 or 30 days (Barros et al. 1991). Male Wistar rats fed 13.5 mg γ-HCH/kg/day in their diet for 12 days exhibited decreased activities of liver lipogenic enzymes and increased levels of serum triglycerides (Boll et al. 1995).

Focal degeneration of hepatocytes was noted in rabbits given γ-HCH at a dose of 7 mg/kg/day by gavage for 4 weeks (Grabarczyk et al. 1990; Kopec-Szlezak et al. 1989). Rabbits treated with 4.21 mg γ-HCH/kg/day by gavage for 28 days exhibited a significant increase of plasma alkaline phosphatase and alanine aminotransferase (ALT) activities immediately following initiation of dosing; these activities returned to control levels by day 14 (Cerón et al. 1995). Activity of aspartate aminotransferase (AST) also increased immediately following dosing and remained elevated up to 7 days postexposure (day 35). γ-HCH residues were detected in the fat 28 days after dosing.

Treatment of female rats with ≥10.6 mg γ-HCH/kg/day or of male and female mice with ≥21.1 mg/kg/day in the diet for 3 months resulted in increases in liver microsomal mixed-function oxidase activity and in

significant increases in absolute and relative liver weights; histopathological examinations were not performed (Oesch et al. 1982). A dose-dependent increased incidence of liver centrilobular hypertrophy was reported in Wistar rats dosed with ≥0.4 mg γ-HCH/kg/day in the diet for 12 weeks (Suter 1983). Liver cell lipospheres were reported in rats fed 2.5 mg γ-HCH/kg/day in the diet for 32 weeks (Ortega et al. 1957). In mice, administration of 90 mg γ-HCH/kg/day in the diet for 24 weeks was reported to result in centrilobular hypertrophy (Ito et al. 1973). Other studies of intermediate-duration exposure (3–48 weeks) have reported slight liver effects or increased liver weight in mice exposed to 18 mg/kg/day of α-HCH, 45 mg/kg/day of β-HCH, and 90 mg/kg/day for δ-HCH and γ-HCH (Ito et al. 1973). These studies were limited by either a small sample size or lack of statistical analysis.

Chronic exposure of rats to 112–128 mg/kg/day γ-HCH in the diet for 107 weeks resulted in liver necrosis and fatty degeneration (Fitzhugh et al. 1950). A dose-related increase in periacinal hepatocytic hypertrophy was seen in Wistar rats given 7–8 mg γ-HCH/kg/day in the diet for 104 weeks (Amyes 1990). No liver effects were reported in dogs exposed to 2.9 mg/kg/day for 104 weeks (Rivett et al. 1978). In mice, chronic administration of 13.6–27.2 mg γ-HCH/kg/day in the diet was associated with an increased incidence of liver cancer (NCI 1977; Wolff et al. 1987) (see Section 3.2.2.8).

Similar liver effects were reported in animals following intermediate- or chronic-duration exposure to α-HCH in the diet. Administration of 1.8 mg/kg/day α-HCH in the diet to rats for 15 or 30 days resulted in increases in hepatic cytochrome P-450 content, hepatic lipid peroxidation, and hepatic microsomal superoxide production (Barros et al. 1991). Ito et al. (1975) reported liver cell hypertrophy and hyperplasia in rats exposed to 45 mg/kg/day α-HCH for 24–48 weeks. Hypertrophied liver cells were reported in mice fed 18 mg/kg/day α-HCH and 45 mg/kg/day β-HCH for 24 weeks (Ito et al. 1973), and hepatomegaly was reported in mice exposed to 90 mg/kg/day in the diet for 50 weeks (Tryphonas and Iverson 1983). Liver cancer has also been reported in mice given 18–90 mg α-HCH/kg/day for 16–36 weeks (Hanada et al. 1973; Ito et al. 1973, 1976; Nagasaki et al. 1975; Tsukada et al. 1979) (see Section 3.2.2.8). Long-term exposure to lower doses of α-HCH was reported to result in fatty degeneration and focal necrosis in rats exposed to 56–64 mg/kg/day for 107 weeks (Fitzhugh et al. 1950), and liver cancer was reported in rats administered 50 mg/kg/day in the diet for 72 weeks (Ito et al. 1975).

Significant increases in liver weight and in the levels of hepatic cytochrome P-450, triglycerides, phospholipids, and cholesterol were observed in rats administered 90 mg/kg/day β-HCH in the diet for 2 weeks (Ikegami et al. 1991a, 1991b); decreases in cytochrome c reductase activity were also reported. Intermediate and chronic exposure to β-HCH in the diet is also associated with liver effects in animals. A

dose-dependent increase in liver weight was noted in rats exposed for 13 weeks to 0.18–4.5 mg β-HCH/kg/day; the increase was significant at doses of >1 mg/kg/day (Van Velsen et al. 1986). Liver cell hypertrophy was reported in rats fed 25 or 50 mg/kg/day in the diet for 24 or 48 weeks (Ito et al. 1975). In mice, exposure to 45 mg/kg/day for 24 weeks resulted in liver cell hypertrophy (Ito et al. 1973), and exposure to 54–57 mg/kg/day for 32 weeks resulted in hepatic foci of degeneration (Hanada et al. 1973). β-HCH was not found to be carcinogenic in rats or mice exposed for 24–48 weeks (Hanada et al. 1973; Ito et al. 1975). Chronic exposure to lower doses of β-HCH resulted in fatty degeneration and necrosis in the liver of mice fed 56–64 mg/kg/day for 107 weeks (Fitzhugh et al. 1950), and Thorpe and Walker (1973) reported liver cancer in mice fed 34 mg/kg/day for 26 months.

Liver hypertrophy was observed in rats fed with 45 mg/kg/day of α-, β-, or δ-HCH in the diet for 24 or 48 weeks (Ito et al. 1975) and in mice fed 18 mg/kg/day α-HCH in the diet for 24 weeks (Ito et al. 1973). The toxicity of ingested δ-HCH has not been investigated following chronic exposure.

Technical-grade HCH was reported to cause increases in liver weight and enzymatic activity (e.g., alkaline phosphatase, aminotransferases) in male Swiss mice given 72 mg/kg in the diet for 2 weeks (Ravinder et al. 1989). The same dosing regime also caused significantly increased serum triglycerides, phospholipids, and cholesterol, as well as hypertrophy of hepatocytes with enlargement of nuclei, centrilobular degeneration, and focal necrosis (Ravinder et al. 1990). Statistically significant decreases in the liver activity of glutamic oxaloacetate transaminase (GOT), also known as aspartate aminotransferease (AST), and lactate dehydrogenase (LD) were observed in pregnant mice administered a single dose of technical-grade HCH (5 mg/kg) on gestation day 9 (Dikshith et al. 1990). Pregnant mice dosed with 25 mg/kg technical-grade HCH experienced a statistically significant decrease in glutamic pyruvic transaminase (GPT), also known as alanine aminotransferase (ALT), and alkaline phosphatase (AP) activity. Virgin mice administered a single dose of 5–200 mg/kg technical-grade HCH had statistically significant decreases in liver activity of ALT and AST. Statistically significant increases in liver AP activity were observed in the virgin mice administered 25–200 mg/kg technical-grade HCH. However, with the exception of AST activity in pregnant mice, the dose response relationships were questionable (Dikshith et al. 1990). There were also no corresponding pathological changes in the liver. Similar effects were seen in male, but not female, rats given 5 or 25 mg/kg/day by gavage for 90 days (Dikshith et al. 1991b). A 65% decrease in liver weight, decreased liver aspartate aminotransferase and lactate dehydrogenase activities, and increased alkaline phosphatase activity were noted in male rats given 60 mg/kg by gavage for 30 days, but animals had normal liver histology (Dikshith et al. 1989a). However, enlargement of hepatocytes, nuclear pyknosis, margination, and vacuolation were observed in

rats fed 20 mg/kg/day technical-grade HCH in the diet for 360 days (Dikshith et al. 1991a). No adverse hepatic effects were seen in rats treated with 50 mg/kg/day technical-grade HCH for 30 days (Khanna et al. 1990).

Technical-grade HCH was reported to deplete the hepatic vitamin A content in male rats fed a diet containing 90 mg/kg/day HCH for 7 weeks (Joseph et al. 1992b). Pronounced fatty degeneration and necrosis of the liver were found in rats exposed to 56–64 mg/kg/day of technical-grade HCH for 107 weeks (Fitzhugh et al. 1950). Mice treated daily with 50 mg/kg/day technical-grade HCH for 1, 5, or 15 days by oil gavage exhibited congestion of hepatic portal vessels and central vein, swollen hepatic cells with vacuolar or parenchymatous degeneration, and fatty changes in periportal and centrilobular cells (Philip et al. 1989). Mice fed diets containing 90 mg/kg/day of HCH for 8 months exhibited increased liver weight, glycogen accumulation, and decreased glucose-6-phosphatase and fructose-1,6-di-phosphatase activities (Karnik et al. 1981). Technical-grade HCH was also reported to cause liver cancer in mice following exposure to 90 mg/kg/day in the diet for 2–8 months (Karnik et al. 1981; Thakore et al. 1981) or exposure to 10–50 mg/kg/day for 80–88 weeks (Kashyap et al. 1979; Munir et al. 1983) (see Section 3.2.2.8).

Based on the occurrence of hepatic effects in rats and mice exposed to β-HCH, an intermediate MRL of 0.0006 mg/kg/day has been calculated from the LOAEL of 0.18 mg β-HCH/kg/day (Van Velsen et al. 1986), as described in the footnote in Table 3-3.

An MRL of 0.008 mg/kg/day has been derived for chronic-duration oral exposure to α-HCH, based on a NOAEL of 0.08 mg/kg/day for hepatic effects in female rats (Fitzhugh et al. 1950).

Renal Effects. Progressive renal failure was seen in a woman who died 11 days after intentionally ingesting 8 ounces of a 20% γ-HCH solution (Sunder Ram Rao et al. 1988). The myoglobin release resulting from muscle lysis in this case led to kidney shutdown, which was the ultimate cause of death.

Male Fischer-344 rats receiving gavage doses of 10 mg/kg/day of γ-HCH for 4 days showed α-2μ-globulin staining in the kidney cortex. Histopathological changes in the proximal tubule epithelial cells included accumulation of protein droplets, hypertrophy and necrosis, pyknotic nuclei, cellular exfoliation, and regenerative epithelium (Dietrich and Swenberg 1990, 1991). These effects did not occur or were seen to a very slight extent in Fischer-344 male controls, Fischer-344 female exposed rats, or exposed NBR rats (a strain that does not synthesize α-2μ-globulin). These results indicate that damage to

male rat kidneys by γ-HCH may be caused by α-2μ-globulin, a protein that is not present in humans. Thus, it is unlikely that humans are at risk for developing this type of pathology from γ-HCH (EPA 1991a). Other biochemical changes indicative of kidney injury, such as significantly increased excretion of glucose in urine, and histological changes, such as hypertrophy and degeneration of the renal tubular epithelia, were observed in Wistar rats exposed to 72 mg/kg/day of γ-HCH for up to 2 weeks (Srinivasan and Radhakrishnamurty 1988; Srinivasan et al. 1984).

However, no renal effects other than significantly increased kidney weight were observed in rats exposed to up to 5–50 mg γ-HCH/kg/day in the diet for up to 40 days (Desi 1974); histological examination of the kidney did not reveal any changes. Slight kidney damage (calcified tubular casts) was reported in rats exposed to 9–10 mg γ-HCH/kg/day for an average of 39.7 weeks (Fitzhugh et al. 1950); the results of this study are limited by poor survival in control and treated animals at all doses. Male rats exposed for 2 years to γ-HCH in their diet exhibited hyaline droplets in the renal proximal tubules at 0.07 mg/kg/day, and pale kidneys, increased kidney weights and urine volumes, and higher urinary protein excretions and tubular necrosis at 7 mg/kg/day (Amyes 1990). Hyaline droplet formation also occurred in a dose-dependent manner in rats treated with 0.02–10 mg γ-HCH/kg/day in their diets for 12 weeks (Suter 1983). Dose-dependent incidents of renal tubular distension and degeneration were seen in this study beginning at a dose of 2 mg γ-HCH/kg/day.

Fitzhugh et al. (1950) reported kidney damage (nephritis and basal vacuolation) in rats fed 72–80 mg α-HCH/kg/day for an average of 35.9 weeks; no such effects were observed in rats fed 5 mg/kg/day. Poor survival was noted in both control and treated animals.

Renal effects have also been noted in rats exposed to β-HCH in the diet. Srinivasan et al. (1984) reported significantly increased excretion of glucose in urine and increased excretion of creatinine and urea as well as hypertrophy and degeneration of the renal tubular epithelia in rats exposed to 72 mg β-HCH/kg/day for up to 2 weeks. Van Velsen et al. (1986) reported significantly increased kidney weights in female rats exposed to 0.18 mg β-HCH/kg/day for 13 weeks; males did not show a significant increase until they were exposed to a dose of 4.5 mg/kg/day. At 22.5 mg/kg/day, both males and females exhibited renal calcinosis in the outer medulla; however, the female controls also exhibited calcinosis. The study authors noted that renal calcinosis is common in female rats but that this finding was of significance in males (Van Velsen et al. 1986). Fitzhugh et al. (1950) also examined the renal effects of exposure to β-HCH in rats that died after an average of 4.4 weeks and found nephritis and basal vacuolation similar to that

described in rats exposed to α-HCH; poor survival due to unspecified causes was reported in both control and treated animals.

Nephritis, pigmentation, and basal vacuolation were also observed in rats fed 56–64 mg technical-grade HCH/kg/day (64% α-HCH, 10% β-HCH, 13% γ-HCH, 9% δ-HCH, and 1.3% ε-HCH) in the diet for an average of 32.9–64.6 weeks (Fitzhugh et al. 1950); poor survival (for which there was no explanation) was noted in both control and treated animals. Tubular necrosis and glomerular degeneration was seen in animals exposed for 360 days to 20 mg/kg/day of technical-grade HCH (Dikshith et al. 1991a), but no renal effects were seen in rats exposed to 60 mg/kg/day technical-grade HCH for 30 days by oil gavage (Dikshith et al. 1989a). Mice treated daily with 50 mg/kg/day technical-grade HCH for 1, 5, or 15 days by oil gavage exhibited congestion of blood vessels and glomerular tufts, swollen tubules with hyaline casts, cystic dilation, fatty changes, some interstitial hemorrhaging in the medulla, and epithelial cell vacuolation (Philip et al. 1989). No adverse effects were seen in rats treated with 50 mg/kg/day technical-grade HCH for 30 days (Khanna et al. 1990).

Endocrine Effects. No studies were located regarding endocrine effects in humans following oral exposure to HCH.

The endocrine effects of γ-HCH were reported in ewe lambs (Beard and Rawlings 1999) and young rams (Beard et al. 1999a) given 1 mg/kg/day in treated feed from conception to sexual maturity. In ewes, effects included increased pulse frequency of serum luteinizing hormone, slower increase and earlier decrease of progesterone levels, and lower T4 levels. In young rams, observed endocrine effects included lower serum luteinizing hormone and estrodiol concentrations. While serum testosterone levels were similar across treatment groups, the γ-HCH treated rams showed attenuated testosterone response to stimulation with gonadotropin releasing hormone.

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

Significantly decreased body weight gain has been seen in rats treated orally with 800 ppm α-HCH (Fitzhugh et al. 1950), 250 mg/kg β-HCH (Fitzhugh et al. 1950; Van Velsen et al. 1986), 40 mg/kg/day γ-HCH (Fitzhugh et al. 1950; Laws et al. 1994), and 3 or 20 mg/kg/day technical-grade HCH (Gautam et al. 1989; Nagaraja and Desiraju 1994).

Metabolic Effects. No studies were located regarding metabolic effects in humans following oral exposure to HCH.

Increased phosphoinositide turnover and generation of second messengers from phosphoinositides were seen in erythrocyte membranes from female rats treated by gavage with a single dose of 100 mg/kg technical-grade HCH, or with doses of 5 mg/kg/day technical-grade HCH for 3–6 months, 5 days/week (Agrawal et al. 1995). The latter exposure regime also resulted in a significant decrease in phosphatidylinositol, phosphatidylinositol 4-phosphate, and phosphatidylinositol 4,5-bisphosphate in erythrocyte membrane and cerebrum; the levels decreased with increased time of treatment (3–6 months).

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans following oral exposure to HCH.

Some evidence of possible immunotoxic effects of γ-HCH is available from acute- and intermediate-duration studies in animals. Dose-related decreases in thymus and spleen weights were observed in mice gavaged with 10–20 mg/kg/day γ-HCH for 10 days and decreased thymus weight was observed in mice gavaged with 20–40 mg/kg/day γ-HCH for 3 days (Hong and Boorman 1993). Immunosuppression, as measured by decreased agglutinin titers against typhoid vaccine and *Salmonella* vaccine, was reported in rats exposed by gavage to 6.25 and 25 mg γ-HCH/kg/day for 5 weeks (Dewan et al. 1980) and in rabbits exposed by capsules 5 times each week to 1.5, 6, and 12 mg/kg/day for 5–6 weeks (Desi et al. 1978). Humoral immune response, as indicated by serum antibody response to injected sheep red blood cells (SRBC), was suppressed in rats that were exposed to γ-HCH in estimated dietary doses of 3.6 or 7 mg/kg/day for 8 weeks (Koner et al. 1998). The primary antibody response to SRBC was also suppressed in albino mice after exposure to 9 mg/kg/day γ-HCH in the diet for 12 weeks (Banerjee et al. 1996). Suppression of secondary antibody response was also observed after 3 weeks of exposure to 9 mg/kg/day γ-HCH and after 12 weeks of 5.4 mg/kg/day γ-HCH exposure. Decreased lympho-proliferative responses to mitogens were seen in mice exposed to 60 mg/kg/day β-HCH in the diet for 30 days (Cornacoff et al. 1988). There were no associated changes in immunoglobulins, red blood cell counts, or histology of the thymus, spleen, or lymph nodes. Cortical atrophy of the thymus was observed in rats fed 22.5–25 mg/kg/day β-HCH (Van Velsen et al. 1986). A biphasic dose-dependent immunological effect of γ-HCH on components of cell- and humoral-mediated immunity, characterized by initial stimulation followed by immunosuppression, was reported in mice fed 0.012, 0.12, or 1.2 mg

γ-HCH/kg/day for 24 weeks (Meera et al. 1992). In addition, histological examinations revealed decreased lymphocyte populations in the thymus and lymph nodes and a reduction in overall cellularity in the spleen and necrosis of the thymus at 1.2 mg/kg/day. Cell-mediated immune response, as measured by delayed type hypersensitivity reaction to dinitroflurobenzene antigen, was suppressed in sheep that were exposed to 1.25 ppm γ-HCH in the diet for 6 months (Khurana et al. 1999). The LOAEL values for immunological effects are recorded in Tables 3-2 and 3-3 and plotted in Figures 3-2 and 3-3.

Based on immunological effects of γ-HCH on components of cell- and humoral-mediated immunity in mice, an intermediate MRL of $1x10^{-5}$ mg/kg/day has been calculated from the LOAEL of 0.012 mg γ-HCH/kg/day (Meera et al. 1992), as described in the footnote in Table 3-2.

3.2.2.4 Neurological Effects

In humans, the most commonly reported effects associated with oral exposure to γ-HCH are neurological. Most of the information is from case reports of acute γ-HCH poisoning. No studies were located regarding neurological effects in humans following long-term ingestion of α-, β-, γ-, or δ-HCH. Seizures and convulsions have been observed in individuals who have accidentally or intentionally ingested γ-HCH in insecticide pellets, liquid scabicide, or contaminated food (Davies et al. 1983; Harris et al. 1969; Munk and Nantel 1977; Nordt and Chew 2000; Powell 1980; Starr and Clifford 1972; Storen 1955). Several case studies of acute γ-HCH exposure to children ingesting liquid scabicide report similar neurological effects, including vomiting, tremors, and tonic/clonic seizures (Aks 1995; Lifshitz et al. 2002; Wheeler 1977). In these cases, the affected children returned to normal health in 6–48 hours following exposure. In most cases, exposure to γ-HCH was inferred from the presence of γ-HCH in the urine or blood. Also, the actual amount of γ-HCH ingested could not be determined because the γ-HCH was present in solution or in pellets in which other substances were present. Liquid scabicide has been reported to contain approximately 1% γ-HCH (Davies et al. 1983; Powell 1980).

Neurotoxic effects have been reported in several species of animals exposed to γ-HCH. The most serious effects were seizures following a single intragastric administration of approximately 15–60 mg/kg in rats (Martinez and Martinez-Conde 1995; Martinez et al. 1991; Tilson et al. 1987; Tusell et al. 1987; Vendrell et al. 1992a, 1992b; Woolley and Griffith 1989). Less-serious effects in rats included increased anxiety following a single gavage dose of 20 mg/kg (Llorens et al. 1990b) and increased spontaneous motor behavior observed at 10 mg/kg (Llorens et al. 1989).

Kindling, the induction of seizures with repeated application of subthreshold electrical or chemical stimuli to the brain, has been used as a method of investigating neurological response to HCH poisoning. A single oral dose of 5–20 mg γ-HCH/kg to rats previously kindled by electrical stimulus produced incidences of myoclonic jerks and clonic seizures, which increased in a dose-dependent manner (Gilbert and Mack 1995). Nonkindled animals displayed these symptoms at a dose of 10 mg γ-HCH/kg. Enhanced susceptibility to kindled seizures brought on by electrical stimulation was seen in rats exposed for 10 weeks to 10 mg γ-HCH/kg/day, 3 days/week (Gilbert 1995). Increased rates of acquisition of kindled seizures were observed following dosing of rats with 3–10 mg γ-HCH/kg/day for 4 days (Joy et al. 1982).

Epileptiform seizures have been reported in male rats fed milk, from dams that were gavaged with 20 mg γ-HCH/kg, on postnatal days 3–15 (Albertson et al. 1985). These data suggest that γ-HCH can be transferred in the dam's milk and can elicit neurological effects in offspring. It is not possible to determine the doses received by the pups. Avoidance response latency was statistically increased in rats administered a single dose of 15 mg/kg by gavage (Tilson et al. 1987). Acquisition of a passive avoidance task was improved in 15-day-old rat pups that were treated with non-convulsant levels of γ-HCH by gavage as either a single 20 mg/kg dose or 7-day repeated 10 mg/kg/day doses, although changes in motor activity and brain monoaminergic levels (e.g., ratios of 5-HIAA/serotonin and DOPAC/dopamine) depended on the treatment schedule (Rivera et al. 1998). No clinical signs of behavioral effects were seen in suckling Wistar rats treated once with 20 mg/kg γ-HCH by gavage at postnatal days 8, 15, 22, or 29, although regional changes in brain noradrenaline and serotonin were seen, with differential effects depending on age at the time of exposure (Rivera et al. 1991).

Changes in levels of brain norepinephrine (Rivera et al. 1991) and serotonin (Attia et al. 1991; Rivera et al. 1991) have also been reported in rats administered acute oral doses of γ-HCH. Decreased dopamine levels were seen in rats treated by gavage with 10 doses totaling 60 mg γ-HCH/kg (half the LC_{50}) over a period of 30 days (Martinez and Martinez-Conde 1995). Increase in the levels of brain catecholamines, particularly norepinephrine and dopamine, and associated signs of toxicity such as mild tremor, lacrimation, salivation, and dysnea were observed in female rats given oral doses of 100 mg/kg/day of technical-grade HCH for 7 days (Raizada et al. 1993). The activity of monoamine oxidase (MAO) in the cerebrum showed a marginal decrease, while the cerebellum and spinal cord indicated a significant increase and decrease in MAO, respectively. Rats treated with 20 mg technical-grade HCH/kg/day in food for 90 days exhibited increased γ-aminobutyric acid (GABA) levels, increased glutamate decarboxylase (GAD) activity, and decreased glutamate levels in the brain (Nagaraja and Desiraju 1994).

No significant changes were seen in lipid peroxidation in brain tissue from rats treated for 90 days with 90 mg γ-HCH/kg/day in food, indicating that the tonic convulsions observed throughout the exposure period were probably not brought on by oxidative stress in the brain (Arisi et al. 1994). Decreased myelin basic protein was observed in rats exposed to 5 mg/kg/day by gavage for 3 days (Serrano et al. 1990a).

The neurotoxicity of γ-HCH has also been assessed in acute, subchronic, and developmental exposure screening batteries in rats (Hughes 1999a, 1999b; Myers 1999). In the acute study, a single 0, 6, 20, or 60 mg/kg dose of γ-HCH was administered to Crl:CDBR rats (Hughes 1999a). End points included functional observational battery (FOB) and motor activity (MA) tests performed prior to treatment, within 3 hours of dosing (time of peak effect), and on post-exposure days 7 and 14, as well as histopathology of nervous system tissues at study termination. No clinical signs or other effects were observed at 6 mg/kg. Exposure to 20 mg/kg caused decreased motor activity 3 hours post-treatment in females at ≥20 mg/kg and in males at 60 mg/kg. Females also had increased forelimb grip strength and decreased grooming behavior at 20 mg/kg, and an absence of grooming behavior at 60 mg/kg. Other effects at 60 mg/kg, included clinical signs (e.g., piloerection, urine-stained fur, tremors, and/or convulsions) in both sexes and increased hindlimb foot splay in males. Gavage administration of 2.5, 5, 10, or 15 mg γ-HCH/kg/day for 5 days produced a dose-dependent increase in the activities of EROD, PROD, and NDMA-d in the brain of Wistar rats (Parmar et al. 2003). Compared with the liver, the magnitude of the induction of the P-450 enzymes in the brain was much smaller. In the same study, Parmar et al. (2003) examined the effect of metabolism on the convulsive effect of γ-HCH in rats. A single dose of 35 mg/kg of γ-HCH induced convulsions in 4 out of 10 animals. Pretreatment of the rats with 3-methylcholanthrene (MC), an inducer of P-4501A1/1A2, had no significant effect in the incidence of convulsions induced by γ-HCH. However, pretreatment with phenobarbitol (PB), an inducer of P-4502B1/2B2, or ethanol, an inducer of P-4502E1, or blocking P-450-mediated metabolism with cobalt chloride, significantly increased the incidence of convulsions caused by γ-HCH. Taken together, the results suggest that the convulsive activity is due to γ-HCH *per se* and/or to metabolites formed by PB- or ethanol-inducible P-450 isoenzymes.

In the subchronic neurotoxicity screening battery, Crl:CDBR rats were exposed to 0, 20, 100, or 500 ppm γ-HCH in the diet for 13 weeks (Hughes 1999b). Due to severe toxicity, the high concentration was reduced to 400 ppm on day 11. Reported average daily intake levels of γ-HCH for the entire study were 0, 1.4, 7.1, and 28.1 mg/kg/day for the males and 0, 1.6, 7.9, and 30.2 mg/kg/day for the females. End points included FOB and MA tests performed prior to administration and after 4, 8, and 13 weeks of treatment, and histopathology of nervous system tissues at study termination. No clinical signs or other changes were observed in females at 1.6 mg/kg/day or males at ≤7.1 mg/kg/day. Effects in females at

7.9 mg/kg/day included decreased body weight gain and food consumption (40 and 16% lower than controls, respectively, during the first week). Both systemic and neurotoxic effects occurred in both sexes at the high dose, including clinical signs (e.g., staining of urogenital region, piloerection, abnormal grooming behavior), increased rearing, walking on tiptoes, hypersensitivity to touch, hunched posture, weight loss, and several deaths.

In the developmental neurotoxicity study, Han Wistar rats were exposed to 0, 10, 50 or 120 ppm γ-HCH in the diet from gestation day 6 through lactation day 10 (Myers 1999). Reported daily maternal dose levels were 0, 0.8–0.9, 4.2–4.6, or 8.0–10.5 mg/kg/day during gestation, and 0, 1.2–1.7, 5.6–8.3, or 13.7–19.1 mg/kg/day during lactation. The F_1 offspring were evaluated for FOB, motor activity, auditory startle response, learning and memory, developmental landmarks (e.g., vaginal perforation and balanopreputial separation), and brain end points (weight, histology, and morphometrics) on postpartum days 11 and 65. Maternal toxicity occurred at 13.7 mg/kg/day as shown by effects that included decreased body weight gain (64–79% less than controls on gestation days 6–20), decreased food consumption, and increased reactivity to handling. The offspring showed effects at the two highest dose levels, including increased motor activity (both sexes), decreased habituation of motor activity (females), decreased body weights (12–20% less than controls), and decreased body weight gains (60–84% less than controls) during lactation days 1–11 (both sexes) at ≥5.6 mg/kg/day. Effects observed at 13.7 mg/kg/day included reduced auditory startle response habitation in both sexes, increased stillbirths (live birth index of 77% compared to 99% in controls), and increased neonatal mortality (postnatal day 4 viability index of 71% compared to 89% in controls). This study was classified as an unacceptable developmental neurotoxicity study by EPA (2000) because there was no laboratory validation of the neurobehavioral tests and the number of animals (six per dose level) was insufficient.

There is evidence that γ-HCH exposure causes functional impairment of the developing blood brain barrier (BBB) in young rats (Gupta et al. 1999). The integrity (permeability) of the BBB was studied by assessing uptake of sodium fluorescein (a micromolecular tracer dye) into the brain of neonatal rats following single or repeated acute gavage doses of γ-HCH. The brain uptake index of fluorescein was significantly increased in 10-day-old pups treated with a single 2 mg/kg dose (72 and 23% higher than controls after 2 hours and 3 days, respectively), as well as in those treated with 2 mg/kg/day for 8 days (50% higher than controls 7 days after the first exposure, with recovery 20 days after the first exposure). The effect appeared to be age-related because the brain uptake index was lower when rats were administered a single 2 mg/kg dose at 15 days of age (20% higher than controls after 2 hours) or a higher dose of 4 mg/kg/day for 3 days as adults (no effect on brain permeability).

Longer exposures to lower doses of γ-HCH were reported to result in significantly altered Skinner box behavior (operant conditioning) in a small number of rats exposed to 2.5 mg/kg/day for 40 days (Desi 1974), and significantly decreased nerve conduction velocity in rats exposed to 25.4 mg/kg/day for 30 days (Muller et al. 1981). The latter study did not examine any behavioral parameters.

Similar neurological effects have not been reported in animals treated with α-HCH. Muller et al. (1981) reported no delay in tail nerve conduction velocity in rats fed 5.1, 54.2, or 106.2 mg α-HCH/kg/day for 30 days. However, neurological effects have been reported in rats exposed to β-HCH. Mice treated with 57 or 190 mg/kg/day β-HCH for 30 days developed ataxia within 1 week of treatment (Cornacoff et al. 1988). Muller et al. (1981) reported a significant delay in tail nerve conduction velocity in rats fed 66.3 mg β-HCH/kg/day for 30 days. An acute-duration oral MRL of 0.05 mg/kg/day was derived based on a NOAEL of 4.5 mg/kg/day for ataxia, progressive inactivity, and coma in male rats exposed to 25 mg β-HCH/kg/day for 2 weeks (Van Velsen et al. 1986).

Behavioral and neurochemical changes were evaluated in rats that were administered technical-grade HCH in doses of 10 or 20 mg/kg/day in oil by gavage for 7–30 days (Sahoo et al. 1999). Assessment of open-field behavior (horizontal motor activity, vertical exploratory rearing, and grooming activities) and brain biochemistry (ATPases and acetylcholinesterase) showed effects that included reduced brain total ATPase and Na^+-, K^+-, and/or Mg^{2+}-ATPase activities after 7–30 days at ≥10 mg/kg/day, reduced brain acetylcholinesterase activity after 15 and 30 days at 20 mg/kg/day, increased motor activity after 7 days at 20 mg/kg/day, and reduced grooming behavior after 30 days at 20 mg/kg/day. Increase motor activity was also observed in rats exposed to technical-grade HCH at a level of 50 mg/kg/day for 120 days (Gopal et al. 1992). Alterations in neurotransmitter levels, increased brain wave frequency, and behavioral changes were reported in male rats administered 50 mg/kg/day technical-grade HCH by gavage for 1 or 3 months (Anand et al. 1991b). Exposure to 0.4 mg/kg/day technical-grade HCH for 360 days resulted in convulsions, tremors, and paralysis in male rats after 270 days, although the number of animals affected or the severity of the symptoms were not reported (Dikshith et al. 1991a). This study also found degeneration of the cerebellum and cerebellar cortex in animals sacrificed after a 1-year exposure to 20 mg/kg/day. Seizures were noted in mice exposed to technical-grade HCH through feed or gavage at levels of 10–17 mg/kg/day in the feed for 80 weeks (Kashyap et al. 1979).

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following oral exposure to HCH.

Increased length of estrous cycle and decreased sexual receptivity were found in female rats treated with a single dose of γ-HCH (25 mg/kg) given by gavage (Uphouse and Williams 1989). Inhibition of the formation of estradiol-receptor complex in the rat uterus cytosol was reported in female rats administered 30 mg γ-HCH/kg/day by oral intubation for 7 days (Tezak et al. 1992). Female mink treated with 1 mg/kg/day γ-HCH in their diet from 3–6 weeks before mating until weaning at 8–10 weeks postpartum showed effects on reproductive efficiency that included reduced receptivity to a second mating and reduced whelping rate, although litter size was not affected (Beard et al. 1997). This decreased fertility effect was primarily a result of embryo mortality after implantation. Mouse dams treated with γ-HCH (6.2 mg/kg) during gestation period days 6–12 had increased numbers of resorbed fetuses (Sircar and Lahiri 1989). A lack of implantation sites and pups death were observed following treatment with 10.8 mg/kg/day on gestation days 1–4 and 3.6 mg/kg/day on gestation days 14–19, respectively. Statistically significant increases in the glycogen content of the uterus, cervix, and vagina (but no increase in organ weight) were reported in female rats exposed to 20 mg γ-HCH/kg/day in the diet for 30 days (Raizada et al. 1980). Antiestrogenic properties were found in female rats given oral gavage doses of 10 mg/kg/day γ-HCH for 15 weeks (Chadwick et al. 1988). These responses were not seen at 5 mg/kg/day. Ovariectomized rats exposed for 5 days and sexually immature female rats exposed for 7 days to 40 mg γ-HCH/kg/day showed no effects on the number of estrogen and estrogen-dependent progesterone receptors (Laws et al. 1994). Thus, γ-HCH's antiestrogenic effects in reproductive tissue do not appear to be due to direct action on estrogen receptors or its induction of progesterone receptors.

Acute preovulatory exposure to γ-HCH caused embryonic effects in mice (Scascetelli and Pacchierotti 2003). Three consecutive daily doses of γ-HCH in olive oil were administered to female mice either before mating (during the preovulatory period) or immediately after mating. Oocyte maturation, ovulation, and fertilization were evaluated by assessing percentage of vaginal plug positive females, number of embryos/female, percentage of one-cell embryos (corresponding to unfertilized oocytes or zygotes that did not undergo cleavage), and gross morphologic alterations of two-cell embryos. Preimplantation embryonic development was evaluated by morphological examinations of morulae for determinations of one-cell embryos (unfertilized eggs or zygotes that did not undergo cleavage), embryos retarded in their cleavage, and abnormal embryos, as well as by cytological examinations of morulae for determinations of interphase nuclei, meta-anaphases, apoptotic nuclei, micronuclei, and mitotic index.

Preovulatory exposure caused a significant increase of degenerating two-cell embryos (lysis or fragmentation of blastomeres), but there were no exposure-related effects of post-fertilization treatment. Female rabbits exposed to 0.8 mg γ-HCH/kg/day, 3 days/week for 12 weeks, had a reduced ovulation rate (Lindenau et al. 1994). However, rabbits given the same treatment regime followed by artificial insemination exhibited no effects on the fertilization rate or on pre- or postimplantation losses (Seiler et al. 1994).

Gavage administration of 15 mg γ-HCH/kg/day (only dose tested) in corn oil to pregnant CD-1 mice on gestational days 9–16 did not affect the relative or absolute weights of the uterus, ovaries, or mammary gland, monitored on postnatal day 23 (Maranghi et al. 2003). Microscopic examination of sections from these organs showed no significant treatment-related alterations. Female offspring from treated mice exhibited a slight increase (4.3%) in relative uterus weight when sacrificed on postnatal day 60. In addition, earlier vaginal opening (2 days) and increased branching of villi and oedema in the endometrial stroma was observed in the offspring from treated mice when compared to controls. Neither the ovary nor mammary glands from female offspring showed significant alterations (Maranghi et al. 2003).

In male rats, oral administration of 6 mg/kg for 5 days or a single dose of 30 mg/kg of γ-HCH resulted in a reduction in the number of testicular spermatids and epididymal sperms of both treated groups 2 weeks after treatment (Dalsenter et al. 1996). γ-HCH was detected in the testes of both groups 24 hours and 2 weeks after the last treatment. Histological examination by electron microscopy revealed ballooning of the Sertoli cells with fragmentation or loss of organelles. Similarly, Shivanandappa and Krishnakumari (1983) reported testicular atrophy, degeneration of seminiferous tubules, and disruption of spermatogenesis in male rats fed 75 mg γ-HCH/kg/day for 90 days. Significant reductions in the relative weight of testicles and epididymis, spermatid and sperm counts, and testosterone levels were observed in pubescent or adult rats fed milk as neonates from dams gavaged with 6 mg/kg γ-HCH on lactation day 9 or 14 or 1 mg/kg γ-HCH on lactation days 9–14 (Dalsenter 1997b). Histopathological observations included a reduction in Leydig cell numbers and spermatogenesis, but fertility, as measured by impregnation of female rats, was unaffected. The results of another study with γ-HCH, reported only as an abstract, indicate that the male reproductive system may be a particularly sensitive target of toxicity in rats (Pages et al. 2000). Male Sprague-Dawley rats were exposed to γ-HCH in drinking water for 12 weeks from the beginning of gestation, lactation, or weaning at concentrations that provided estimated doses of 0.000075, 0.00015, or 0.0003 mg/kg/day. Body weight gain, plasma testosterone, sperm number, and sperm mobility values were approximately 18, 38, 40, and 52% reduced compared to controls, respectively, in groups exposed to 0.0003 mg/kg/day during gestation or lactation. The pup rate

was normal when treated males were mated with untreated females, although the rate decreased and newborn mortality was higher when treated males were exposed to treated females. Given the lack of a complete report, the results of this study cannot be regarded as conclusive.

Effects of prenatal exposure on spermatogenesis were evaluated in adult offspring of mice that were administered 15 or 25 mg/kg/day doses of γ-HCH in olive oil by gavage on gestation days 9–16 (Traina et al. 2003). F_1 offspring were assessed on postnatal day (pnd) 60 (both dose levels) and pnd 100 (25 mg/kg/day); end points included litter size, growth and sexual maturation indices, and male reproductive indices (e.g., sperm number and concentration, testicular biochemistry and histology, and testicular cytotoxicity and germ cell damage). Statistically significant effects included testicular histological alterations at ≥15 mg/kg/day on pnd 60 (increased number and size of Leydig cells), reduced sperm head count (sperm/testis) at ≥15 mg/kg/day on pnd 60, reduced sperm head concentration (sperm/g testis) at 25 mg/kg/day on pnds 60 and 100, reduced activities of testicular serum sorbitol dehydrogenase (SDH) at ≥ 15 mg/kg/day and lactate dehydrogenase (LDH) at 25 mg/kg/day (only evaluated on pnd 60), altered testicular germ cell distribution at 25 mg/kg/day on pnds 60 and 100, and increased number of epididymal sperm with chromatin abnormalities at ≥15 mg/kg/day on pnd 60.

Multigeneration reproduction studies were conducted in rats exposed to technical HCH or γ-HCH (King 1991; Srivastava and Raizada 2000). In the study with technical HCH, male and female Druckrey rats were exposed via diet and drinking water to estimated total daily doses of 0, 16, or 32 mg/kg/day throughout three generations (Srivastava and Raizada 2000). Toxicity occurred in the P_0 parental animals, as shown by effects that included reduced body weight gain in both sexes at ≥16 mg/kg/day, and hepatic histopathological changes and some deaths at 32 mg/kg/day. There were no signs of toxicity in the subsequent parental generations (F_{1b} or F_{2b}), no exposure-related effects on reproduction in any of the three parental generations, and no morphological or teratological changes in any of the offspring generations (F_{1b}, F_{2b}, or F_{3b}). In the study with γ-HCH, Charles River CD rats were exposed to estimated dietary doses of 0, 0.09, 1.7, or 13.1 mg/kg/day for two generations during the mating periods only (King 1991). No treatment-related clinical signs of toxicity, effects on body weight or food consumption were observed in the F_0 or F_1 males or females during premating. Body weight gain decreased in the high-dose F_0 parental females during gestation, however, indicating that systemic toxicity occurred at 13.1 mg/kg/day. Other indications of systemic toxicity included renal histopathological changes characteristic of alpha 2μ globulin accumulation in F_0 and F_1 males at ≥1.7 mg/kg/day; however, this syndrome is specific to male rats and not relevant to humans. No gross or histopathological changes were observed in females in either generation. There were no effects on mating, fertility, gestation survival,

liveborn indices, or mean litter sizes in either generation, although offspring toxicity occurred at 13.1 mg/kg/day, as shown by reduced body weight and decreased viability in pups of both generations and delayed maturation of F_2 pups. Body weights of the high-dose pups of both generations were significantly lower than controls on lactation days 1 and 25. Viability indices (survival on lactation day 4) for the high dose F_1 and F_2 pups were 81 and 85%, respectively, compared with ≥96% for the controls. The onset and completion of tooth eruption and completion of hair growth were 10.5, 11.6, and 24% delayed in the high dose F_2 pups, respectively, compared to controls.

A two-generation reproduction study of γ-HCH was also conducted in mink that were exposed to dietary doses of 0 or 1 mg/kg/day (Beard and Rawlings 1998). The parental (P_0) generation was exposed from 3 weeks before breeding until weaning of the offspring. Following weaning, the F_1 females were exposed throughout growth and mating (to untreated males), and subsequently throughout pregnancy and lactation until 3 months post-lactation. The F_2 females were exposed until they reached full adult body size at 30 weeks of age. The F_1 and F_2 males were exposed until the time their testis development was maximal (sexual maturity) at about 42 weeks of age. In addition to standard reproductive indices, serum hormone levels (estradiol, thyroxine, cortisol, testosterone) and histology, including male and female reproductive and endocrine tissues (e.g., thyroid, parathyroid, adrenal, pituitary, and pancreas), were evaluated in offspring of both generations. There were no overt signs of toxicity or effects on mating percentage. Fertility was reduced in both generations, as shown by reductions in whelping rate and litter size, such that exposed mink produced approximately 60% fewer kits than controls. Other effects included reduced testis size and serum thyroxine concentration in F_2 males.

Oral exposure to 60 mg β-HCH/kg for 30 days resulted in normal uteri and reproductive cycling in female mice (Cornacoff et al. 1988). Atrophy of the ovaries and testes, hyperplastic and vacuolized endometrial epithelium, degeneration of the seminiferous tubules, and disruption of spermatogenesis were seen in rats exposed to 22.5–25 mg β-HCH/kg/day in the diet for 13 weeks (Van Velsen et al. 1986). Technical-grade HCH caused transient changes in testes' weights and decreased sperm counts in a 7-week study (Pius et al. 1990), degeneration of seminiferous tubules and Leydig cells (Roy Chowdhury and Gautam 1990), and changes in the muscle layer of the vas deferens (Gautam et al. 1989). None of these studies provide adequate evidence for the effects of technical-grade HCH on sperm function in animals or humans.

Testicular oxidative stress was studied in immature (15-day-old) and mature (90-day-old) rats that were administered technical-grade HCH in doses of 10 or 20 mg/kg/day in oil by gavage for 7, 15, or 30 days (Samanta et al. 1999). End points that were evaluated included testicular protein and lipid peroxidation,

testicular levels of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase) and non-enzymatic antioxidants (reduced glutathione, ascorbic acid, hydrogen peroxide), weights of testis and accessory sex organs, and testicular histology including epididymal sperm counts and sperm anomalies. Exposure to ≥10 mg/kg/day for 7 days caused effects that included reduced epididymis weight in immature rats and reduced seminal vesicle and ventral prostate weights in adult rats. Effects observed following exposure to ≥10 mg/kg/day for 7–30 days included reduced total sperm count and increased frequencies of damaged sperm and sperm with anomalous heads in adult rats. Testes from immature and adult rats exposed to ≥10 mg/kg/day for 7–30 days also showed increased lipid peroxidation and changes in glutathione peroxidase, ascorbic acid, and hydrogen peroxide levels. In mice, exposure to 90 mg technical-grade HCH/kg/day (isomer composition unknown) for 3 months led to increased testicular weight and degeneration of seminiferous tubules (Nigam et al. 1979). Testicular degeneration was reported in male rats exposed to 20 mg/kg/day technical-grade HCH in the diet for 360 days (Dikshith et al. 1991a). A dose-related increase in fetal resorptions was seen in pregnant female mice treated once with 25–200 mg/kg technical-grade HCH by gavage on the ninth gestation day (Dikshith et al. 1990).

The reproductive effects of γ-HCH were reported in ewe lambs (Beard and Rawlings 1999) and young rams (Beard et al. 1999a) given 1 mg/kg/day in treated feed from conception to sexual maturity. In estrus synchronized ewes, treated animals had significantly shorter estrous cycle length and lower number and less total volume of corpus lutea. No other detrimental fertility effects were observed. The subjectively-scored sexual behavior in young rams was significantly reduced in treated animals presented with estrous ewes.

3.2.2.6 Developmental Effects

In a study of women from India, 30 pregnant women diagnosed with intra-uterine fetal growth retardation (IUGR) had higher mean serum levels of γ-HCH (OR=1.38, 95% CI 1.05–1.80), α-HCH (OR=1.22, 95% CI 1.02–1.46), δ-HCH (OR=1.61, 95% CI 1.01–2.54), and total HCH (OR=1.07, 95% CI 1.01–1.13) than 24 mothers of non-IUGR babies after adjusting for potential confounders (Siddiqui et al. 2003). Similarly, γ-HCH, δ-HCH, and total HCH cord blood levels of IUGR babies were higher than the cord blood levels in the normal-weight babies (OR=1.14, 95% CI 1.00–1.31; OR=1.31, 95% CI 1.00–1.75; and OR=1.07, 95% CI 1.00–1.14, respectively) (Siddiqui et al. 2003). Since other organochlorine pestides, including DDT and its metabolites, were also present in the blood, the role of HCH, if any, cannot be ascertained. In this study, exposure is likely due to consumption of the pesticides in food; however, other

environmental contamination pathways, including inhalation and dermal exposure, may be possible routes of exposure as well.

A single oral dose of 25 mg/kg technical-grade HCH caused increased resorptions of the fetus in female mice, but fetal development was normal (Dikshith et al. 1990). Srivastava and Raizada (1993) further studied the prenatal effect of orally administered technical-grade HCH. While mice exposed to HCH during the preimplantation period (days 2–6 of gestation) did not show fetolethality, exposure during the postimplantation period (days 6–12 of gestation) to 25 and 50 mg/kg/day HCH produced significant increases in resorption of fetuses, inhibition of maternal serum progesterone levels, and higher levels of HCH in fetal tissues. Oral exposure to Benesan (a pesticidal formulation containing 50% γ-HCH) given at doses of 6.25, 12.5, or 25 mg/kg/day by gavage on days 6–15 of gestation failed to produce teratogenic effects in rats (Khera et al. 1979). When minks were treated with 1 mg/kg/day γ-HCH in their diet (Beard et al. 1997), the proportion of embryos lost after implantation was increased. A multigeneration study in mink exposed to 1 mg/kg/day γ-HCH in the diet observed that testis size was reduced in F3 males, although there were no effects on testicular development in the second generation (Beard and Rawlings 1998).

Another study of γ-HCH was conducted in which the compound was administered to pregnant mice by gastric intubation on day 12 of gestation (Hassoun and Stohs 1996a). At doses of 30 and 45 mg/kg body weight in C57BL/6J mice, significant decreases in fetal weight, fetal thymic weight, and placental weight were observed. When given to DBA/2J mice at a dose of 45 mg/kg body weight, γ-HCH caused significant reductions in fetal and placental weight. No malformations in the fetuses of both strains of mice were observed, even though the administered doses caused maternal deaths. Increases in the production of lipid metabolites in maternal sera and the amniotic fluids were found to parallel the observed fetotoxicities (Hassoun et al. 1996). Superoxide production, lipid peroxidation and DNA-single strand breaks were increased in fetal and placental tissues 48 hours after administration of single dose of 30 mg/kg γ-HCH to pregnant mice on day 12 of gestation (Hassoun and Stohs 1996b). Significant increases in lipid peroxidation also occurred in fetal livers collected on day 18 of gestation. Thus, it was suggested that fetotoxic effects of γ-HCH may be due to induced oxidative stress, enhanced lipid peroxidation, and DNA-single strand breaks in the fetal and placental tissues of mice.

Developmental/reproductive effects of γ-HCH were studied in male offspring of rats that were exposed during lactation (Dalsenter et al. 1997b). Females were treated with γ-HCH in peanut oil by gavage as a single 6 mg/kg dose on day 9 or day 14 of lactation, or as daily 1 mg/kg/day doses on days 9–14 of

lactation. Male offspring were evaluated on pnd 65 (puberty) or 140 (adulthood) and evaluated for testis and epididymis weights, spermatid and sperm numbers, serum testosterone level, sexual behavior at 130 days of age during mating with unexposed females, reproductive indices (mating, pregnancy and fertility), pregnancy end points (numbers of litters, implantations/litters, fetuses/litter, resorptions), and testicular histology (6 mg/kg offspring). The 1 mg/kg/day offspring had statistically significant reductions in relative testicular weight at pnd 140, relative epididymis weight at pnd 65, spermatid number at pnds 65 and 140, sperm number at pnd 140, and serum testosterone at pnd 65. Effects were generally similar in type and magnitude in the 6 mg/kg offspring exposed on lactation day 9 or 14. There were no significant effects on sexual behavior or fertility in the 1 mg/kg/day or 6 mg/kg offspring. The testicular histological examinations of the 6 mg/kg/day offspring showed large areas of normal tissue, although some areas had distinct changes ranging from small alterations to a pronounced effect, including necrotic changes and reductions in Leydig cell numbers and spermatogenesis. An acute oral MRL of 0.003 mg/kg/day has been derived for γ-HCH based on the 1 mg/kg/day minimal LOAEL for reproductive effects in rats (Dalsenter et al. 1997b).

An isomer comparison study in rats found that dietary exposure to 25 mg/kg/day of γ-HCH during gestation and lactation did not cause developmental effects in pups, whereas 20 mg/kg/day of β-HCH during gestation caused increased fetal deaths within 5 days of birth and 5 mg/kg/day of β-HCH during gestation and lactation resulted in increased liver weights of pups (Srinivasan et al. 1991a). A dose-related increase in the incidence of fetuses with an extra 14th rib was reported in CFY rats exposed to 5, 10, or 20 mg/kg γ-HCH by gavage during gestation days 6–15; statistical significance was attained only at 20 mg/kg (Palmer et al. 1978a). The incidence of fetuses with an extra 13^{th} rib was statistically increased in rabbits exposed to 20 mg/kg γ-HCH by gavage during gestation days 6–18 (Palmer et al. 1978a). In both rats and rabbits, the incidences of extra ribs were within or just greater than the ranges recorded for the control groups, and therefore, may not be sufficient evidence of teratogenicity of γ-HCH. Maternal toxicity (reduced body weight gain and food consumption) occurred at doses ≥10 mg/kg/day in the rats, but not in rabbits (highest tested dose 20 mg/kg/day). No effects on embryonic development were seen in rabbits treated by oral gavage with 0.8 mg γ-HCH/kg, 3 times/week for 12–15 weeks before artificial insemination and throughout gestation (Seiler et al. 1994). A two-generation study of γ-HCH was conducted in rats exposed to estimated dietary doses of 0, 0.09, 1.7, or 13.1 mg/kg/day (King 1991). As detailed in Section 3.2.2.5 (Reproductive Effects), developmental toxicity occurred at 13.1 mg/kg/day, as shown by reduced body weight and decreased viability in pups of both generations and delayed maturation of F_2 pups.

Regional changes in brain noradrenaline, serotonin and the dopamine metabolite 3,4-dihydroxyphenyl-acetic acid (DOPAC) levels were noted in suckling rats treated with 20 mg/kg/day γ-HCH, as a single dose (Rivera et al. 1991). Alterations in levels of brain dopamine, serotonin, gamma-aminobutyric acid ($GABA_B$), glutamate, glutamate decarboxylase, and noradrenaline were seen in various areas of the brains of female rat pups treated with 10 mg technical-grade HCH/kg/day for 60 days (Nagaraja and Desiraju 1994).

3.2.2.7 Cancer

In a study of women from India, blood levels of HCH, and its isomers (α, β, and γ), were found to be higher in women with breast cancer when compared to healthy women without the disease (Mathur et al. 2002). In this study, 135 breast cancer patients and 50 females without cancer filled out questionnaires and were evaluated for their body burden of pesticides through blood testing. α- and γ-HCH blood levels were significantly higher in breast cancer patients, 41–50 years of age, compared to women of the same age without the disease. β-HCH blood levels were significantly higher in breast cancer patients, 31–50 years of age, compared to those without the disease (Mathur et al. 2002). Other organochlorine pesticides, including DDT and its metabolites, were also present in the blood and could have contributed to the incidence of breast cancer. In contrast to the Mathur et al. (2002) findings, there were no positive associations between serum levels of β-HCH and incidence of breast cancer in studies of 95 Mexican women (Lopez-Carrillo et al. 2002), 150 Norwegian women (Ward et al. 2000), or 240 women from Denmark (Hoyer et al. 1998). In addition, Hoyer et al. (1998) did not find an association between serum levels of γ-HCH and the incidence of breast cancer. The risk for endometrial cancer was also not associated with β-HCH serum concentrations in a study of 90 women from the United States (Sturgeon et al. 1998). Levels of β- and γ-HCH in surgically removed breast tissue samples from 65 women in Germany were not indicative of malignant breast disease; there was no significant difference between the levels of β- and γ-HCH in the breast tissue surrounding malignant and benign breast disease (Guttes et al. 1998). Exposure to HCH, and other organochlorine pesticides, to the populations of these studies is mainly through food where the pesticides are primarily used for agricultural applications; however, other possible environmental contamination pathways include inhalation and dermal routes of exposure.

α-HCH, β-HCH, γ-HCH, and technical-grade HCH have been shown to be liver carcinogens in rats and mice; however, in some studies, the liver was the only organ examined. Ito et al. (1973) examined the carcinogenicity of HCH isomers in dd mice exposed to 45 mg/kg/day of each isomer (total dosage was 90 mg/kg/day) for 24 weeks. Exposure to β-, γ-, or δ-HCH alone did not result in hepatocellular

carcinoma. However, when these isomers were mixed with α-HCH, hepatocellular carcinoma was observed. These results suggest that α-HCH is itself a hepatocellular carcinogen or acts synergistically with the other isomers.

In Wistar rats, exposure to 25 mg γ-HCH/kg/day in the diet for 24 or 48 weeks did not result in any identifiable carcinogenic effect (Ito et al. 1975); however, high mortality in the control and treatment groups precludes determination that γ-HCH is not carcinogenic to rats under this experimental protocol. Mice (dd strain) exposed to as much as 90 mg γ-HCH/kg/day in the diet for 24 weeks did not exhibit any carcinogenic effects (Ito et al. 1973). Although an increased incidence of malignant hepatomas was reported in male dd mice exposed to 108–120 mg/kg/day in the diet for 32 weeks (Hanada et al. 1973), this dose level may have exceeded the maximum tolerated dose (MTD), based on effects of γ-HCH on survival. Liver nodules developed following doses of 39 mg/kg/day. The study was limited by the lack of statistical analysis (Hanada et al. 1973).

Information concerning the cancer effects of γ-HCH following chronic-feeding exposure is equivocal. No statistically significant increases in endocrine, thyroid, pituitary, adrenal gland, liver, or ovary tumors were observed in male and female Osborne-Mendel rats fed 10.8–33 mg/kg/day in the diet for 80 weeks (NCI 1977) and in Wistar rats fed 0.07–32 mg γ-HCH/kg/day in the diet for 104 weeks (Amyes 1990); however, poor survival rates limit the significance of these results. On the other hand, hepatocellular carcinomas have been reported in F_1 and B6C3F_1 mice exposed to 13.6–27.2 mg/kg/day in the diet for 80 or 104 weeks, respectively (NCI 1977; Wolff et al. 1987). Hepatocellular carcinomas were also increased in yellow (YS/UY)F-1 mice exposed to 27.2 mg/kg/day in the diet for 96 weeks (Wolff et al. 1987); this strain of mouse has a dominant mutation at the agouti locus (A^{vy}) that results in an increased susceptibility to formation of strain-specific neoplasms. Chronic dietary studies of γ-HCH additionally showed that incidences of benign lung adenomas were increased in female Agouti and Pseudoagouti mice exposed to 27.2 mg/kg/day for 24 months (Wolff et al. 1987) and in female CD-1 mice exposed to ≥26.8 mg/kg/day for 78 weeks (Chase 2000; Huntington Life Sciences Ltd. 2001). The EPA has classified γ-HCH (lindane) into the category “suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential” (EPA 2001a, 2002b). No cancer potency factors have been estimated for γ-HCH or γ-HCH (EPA 2001a, 2002b; IRIS 2005).

No evidence of liver carcinogenicity was reported in Wistar rats exposed to 45 or 90 mg α-HCH/kg/day in the diet for 24 or 48 weeks (Ito et al. 1975; Nagasaki et al. 1975); high mortality was observed in both the treated and control groups. However, α-HCH appears to be carcinogenic in mice following intermediate-

duration exposure. Hepatomas and hepatocellular carcinomas have been reported in a number of strains of mice exposed to 13–95 mg/kg/day for 16–36 weeks (Hanada et al. 1973; Ito et al. 1973, 1976; Nagasaki et al. 1975; Tsukada et al. 1979). Tryphonas and Iverson (1983), however, reported no evidence of a carcinogenic effect in male mice exposed to 90 mg α-HCH/kg/day in the diet for 50 weeks. Ito et al. (1975) reported an increased incidence of hepatocellular carcinoma in male rats exposed to 50 and 75 mg α-HCH/kg/day in the diet for 72 weeks, suggesting that α-HCH may be carcinogenic in rats after long-term exposure. A study of enzyme-altered liver foci in rats treated first with the tumor initiator *N*-nitrosomorpholine, and then 20 mg α-HCH/kg/day in food for 49 weeks, found that the tumor promoter activity of HCH is apparently due to increased cell proliferation caused by a lowering of the cell death (apoptosis) rate (Luebeck et al. 1995). In another study in rats, additional administration of 35 mg/kg/day of α-HCH in the diet for 65 weeks inhibited the induction of liver tumors by 0.07 mg/kg/day of aflatoxin B_1 (Angsubhakorn et al. 1981). IRIS (2005) lists α-HCH as a probable human carcinogen and estimated an oral cancer potency factor for α-HCH of 6.3 $(mg/kg/day)^{-1}$ based on the incidence of hepatic nodules and hepatocellular carcinomas observed in male mice administered α-HCH in the diet (Ito et al. 1973). The doses corresponding to cancer risk levels ranging from 10^{-4} to 10^{-7} are $1.6x10^{-5}$–$1.6x10^{-8}$ mg/kg/day, respectively, as indicated in Figure 3-3. The oral cancer potency factor is a plausible upper-bound estimate of the lifetime probability of an individual developing cancer as a result of oral exposure per unit intake of the chemical.

β-HCH has not been found to be carcinogenic in Wistar rats exposed to 25 or 50 mg/kg/day in the diet for 24 or 48 weeks (Ito et al. 1975) or in dd mice exposed to 18–120 mg/kg/day in the diet for 24 or 32 weeks (Hanada et al. 1973; Ito et al. 1973). However, Thorpe and Walker (1973) reported an increased incidence of hepatocellular carcinomas in CF1 mice exposed to 26 mg/kg/day in the diet for 104 weeks. The studies with negative results were, in general, of short duration, used a small number of animals, or failed to examine all of the animals. IRIS (2005) lists β-HCH as a possible human carcinogen and estimated an oral cancer potency factor for ingested β-HCH of 1.8 $(mg/kg/day)^{-1}$ based on the incidence of hepatic nodules and hepatocellular carcinomas observed in male mice administered β-HCH at a single dose level in the diet (Thorpe and Walker 1973). The doses corresponding to cancer risk levels ranging from 10^{-4} to 10^{-7} are $5.6x10^{-5}$–$5.6x10^{-8}$ mg/kg/day, respectively, as indicated in Figure 3-3. This is the only chronic study from which to estimate cancer risk from exposure to β-HCH. The study is limited by the use of only one nonzero dose group. Also, the use of incidence of liver tumors alone in mice to predict a compound's carcinogenicity in humans may be equivocal (Vesselinovitch and Negri 1988). Diversity of factors has been shown to influence the development of liver cell tumors in mice, such as the

strain of the mice (Nagasaki et al. 1975), the protein or calorific value of the diet (Tannenbaum and Silverstone 1949), and the microbial flora of the animals (Roe and Grant 1970).

δ-HCH has not been found to be carcinogenic in male Wistar rats exposed to 45 or 90 mg/kg/day in the diet for 24 or 48 weeks (Ito et al. 1975) or in male dd mice exposed to 18–90 mg/kg/day in the diet for 24 weeks (Ito et al. 1973). However, these studies were of relatively short-exposure duration. δ-HCH is structurally related to carcinogenic HCH isomers, but it is currently listed as not classifiable for human carcinogenicity (IRIS 2005).

Increased incidence of carcinoma was reported in Swiss mice following exposure to 90 mg technical-grade HCH/kg/day in the diet for 8–32 weeks (Thakore et al. 1981). Increased incidences of hepatocellular carcinoma were also reported in Swiss mice exposed to 21.3–85 mg/kg/day in the diet for 20 months (Munir et al. 1983) and in Swiss mice exposed to 10 or 17 mg/kg/day through gavage or the diet, respectively, for 80 weeks (Kashyap et al. 1979). The EPA has derived a cancer potency estimate for oral exposure to technical HCH (IRIS 2005). The oral slope factor is 1.8 per (mg/kg)/day and the doses corresponding to risk levels ranging from 10^{-4} to 10^{-7} are $5.6x10^{-5}$–$5.6x10^{-8}$ mg/kg/day, respectively, as indicated in Figure 3-3.

The DHHS has determined that γ-HCH and other HCH isomers may reasonably be anticipated to cause cancer in humans (NTP 2002). IARC has determined that HCH is possibly carcinogenic to humans (IARC 2003). As previously mentioned, the EPA has classified technical HCH and α-HCH as probable human carcinogens, β-HCH as a possible human carcinogen, and δ- and ε-HCH as not classifiable as to human carcinogenicity (IRIS 2005). The EPA has additionally classified γ-HCH into the category "suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential" (EPA 2001a, 2002b).

3.2.3 Dermal Exposure

Studies examining the dermal toxicity of HCH in humans are limited. Most of the available information is derived from cases in which γ-HCH was dermally applied as a scabicide. γ-HCH in topical creams and lotions is efficiently absorbed through the skin (Ginsburg et al. 1977). Although it has been reported that these lotions contain 1% γ-HCH, it is not possible to quantify the amount of γ-HCH to which these individuals were exposed, because of the different areas of skin treated.

3.2.3.1 Death

No studies were located regarding lethal effects in humans following dermal exposure to α-, β-, or δ-HCH. An acute whole-body dermal application of 1% γ-HCH lotion to a 2-month-old infant for the treatment of scabies was reported to result in death (Davies et al. 1983), and a concentration of 110 ppb γ-HCH was identified in the brain. The death of an elderly woman was reported following a 6-hour dermal application of γ-HCH-containing lotion (approximately 40 mg total γ-HCH) to the head for the treatment of scabies (Katsumata and Katsumata 2003). No data were reported for blood or tissue levels of γ-HCH. The causal association between HCH exposure and death is difficult to establish since the women had numerous pre-existing health problems. In general, most humans dermally poisoned with γ-HCH have recovered with no apparent adverse effects (Fagan 1981).

In animals, acute dermal exposures to high doses of γ-HCH were reported to result in death. The dermal LD_{50} values for γ-HCH are 900 mg/kg in female rats and 1,000 mg/kg in male rats (Gaines 1960). Rats exposed to moistened γ-HCH for 24 hours exhibited no mortality at 250 mg/kg, 20% mortality at 600 mg/kg, 40% mortality at 1,000 mg/kg, and 30% mortality at 2,000 mg/kg (Ullmann 1986a). Significant lethality (47%) was seen in female rats, but not male rats, exposed dermally to 400 mg γ-HCH/kg/day for 6 hours/day, 5 days/week, for 13 weeks (Brown 1988). Calves dermally exposed to 33.3 mg/kg γ-HCH died within 5 months (Venant and Sery 1991). Dikshith et al. (1978) reported that guinea pigs dermally exposed to 200 mg technical-grade HCH/kg died within 5–12 days. Four of 20 rats died from exposure to technical-grade HCH at 100 mg/kg/day for 15–30 days (Dikshith et al. 1991c). Weanling rabbits were more sensitive to γ-HCH treatment than young adults, as seen by increased mortality rates accompanied by excitement and convulsions after a single whole-body treatment with a 1% solution at a dose of 60 mg γ-HCH/kg (Hanig et al. 1976). This suggests that children might be at a greater risk than adults for toxic responses to dermal absorption of HCH. Rabbits treated with 25 mg/kg/day technical-grade HCH for 30 days by skin painting on shaved dorsal, ventral, or thigh areas exhibited no deaths in the group exposed by dorsal application, but two of eight rabbits died in the group exposed by ventral application, and four of eight died in the group exposed by thigh application (Dikshith et al. 1989b). These and other values are in Tables 3-4 and 3-5.

3.2.3.2 Systemic Effects

No studies were located regarding musculoskeletal, endocrine, ocular, or body weight effects in humans or animals after inhalation exposure to inorganic tin or organotin compounds. Reliable LOAELs for

Table 3-4 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious		LOAEL Serious		Reference Chemical Form
ACUTE EXPOSURE								
Death								
Rat (Sherman)	10 d (GO)					1000 M mg/kg/day	(LD50)	Gaines 1960 lindane
						900 F mg/kg/day	(LD50)	
Rat (Wistar)	24 hr once					1000 mg/kg/day	(LD50)	Ullmann 1986a lindane
Systemic								
Rat (Wistar)	24 hr once	Resp	600 mg/kg/day	1000 mg/kg/day	(dyspnea)			Ullmann 1986a lindane
Rabbit (New Zealand)	once	Ocular		40 mg/kg/day	(mild eye irratation)			Ullmann 1986c lindane
Rabbit (New Zealand)	4 hr once	Dermal	200 mg/cm²/kg					Ullmann 1986d lindane
Neurological								
Rat (Wistar)	24 hr once		600 mg/kg/day	1000 mg/kg/day	(slight sedation)	2000 F mg/kg/day	(severe spasms)	Ullmann 1986a lindane

Table 3-4 Levels of Significant Exposure to Gamma-hexachlorocyclohexane (lindane) - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious		LOAEL Serious		Reference Chemical Form
INTERMEDIATE EXPOSURE								
Death								
Rat (Crl:(WI)BR)	13 wk 5 d/wk 6 hr/d		60 F mg/kg/day			400 F mg/kg/day	(23 deaths out of 49)	Brown 1988 lindane
Systemic								
Rat (Crl:(WI)BR)	13 wk 5 d/wk 6 hr/d	Resp		10 mg/kg/day	(rapid respiration or wheezing)			Brown 1988 lindane
		Hepatic	10 mg/kg/day	60 mg/kg/day	(centrilobular hypertrophy)			
		Renal	10 F mg/kg/day	10 M mg/kg/day	(hyaline droplet formation)			
				60 F mg/kg/day	(basophilic tubules)			
Rat	once for 25 days			180 F mg/kg	(mild dermatitis)			Dikshith et al. 1973 lindane
Neurological								
Rat (Crl:(WI)BR)	13 wk 5 d/wk 6 hr/d			10 mg/kg/day	(hyperactivity)	60 F mg/kg/day	(ataxia, tremors, convulsions)	Brown 1988 lindane

d = day(s); F = female; hr = hour(s); LD50, lethal dose; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

Table 3-5 Levels of Significant Exposure to Technical - Grade Hexachlorocyclohexane - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL			Reference Chemical Form
				Less Serious		Serious	
ACUTE EXPOSURE							
Death							
Gn Pig (NS)	5-12 d 1x/d				200 M mg/kg/day	(24/24 deaths)	Dikshith et al. 1978 technical - grade
INTERMEDIATE EXPOSURE							
Death							
Rat (Wistar)	15 d 1x/d				100 F mg/kg/day	(2/10 deaths)	Dikshith et al. 1991c technical - grade
Rabbit (NS)	30 d 1x/d				25 M mg/kg/day	(6/24 deaths)	Dikshith et al. 1989b technical - grade

Table 3-5 Levels of Significant Exposure to Technical - Grade Hexachlorocyclohexane - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL				Reference Chemical Form
				Less Serious		Serious		
Systemic								
Rat (Wistar)	30 d 1x/d	Hepatic				100 F mg/kg/day	(hypertrophy, fatty degeneration, nuclear pyknosis of hepatocytes, diffuse and focal liver necrosis)	Dikshith et al. 1991c technical - grade
		Renal				100 F mg/kg/day	(tubular necrosis)	
		Dermal		100 F mg/kg/day	(hyperkeratosis, epidermal cell vacuolization, thickening of collagen fibers)			
Gn Pig (NS)	30 d 1x/d	Hepatic		100 M mg/kg/day	(38% increase in liver weight, hepatic hypertrophy, pycnotic nuclei in cytoplasm, foca fatty inclusions, increased GOT and ALF activity)			Dikshith et al. 1978 technical - grade
		Renal	100 M mg/kg/day					

Table 3-5 Levels of Significant Exposure to Technical - Grade Hexachlorocyclohexane - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form
Rabbit (NS)	30 d 1x/d	Hepatic		25 M mg/kg/day (hepatocyte degeneration, pycnotic nuclei, enlarged liver, altered GOT, GPT, LDH and ALP activities)		Dikshith et al. 1989b technical - grade
		Renal			25 M mg/kg/day (altered epithelial lining o proximal convoluted tubules, loss of brush borders of tubules, atrophy of glomerular capsules)	
		Dermal		25 M mg/kg/day (thickened epidermis, hyperkeratinization, and infiltration of mononuclear cells)		
CHRONIC EXPOSURE						
Cancer						
Mouse (Swiss)	80 wk 2 d/wk				2.4 mg/kg/day (CEL: liver tumors)	Kashyap et al. 1979 technical - grade

ALP = alkaline phosphatase; CEL = cancer effect level; d = day(s); F = female; Gn pig = guinea pig; GOT = glutamate oxaloacetate transaminase; GPT = glutamate pyruvate transaminase; LDH = lactate dehydrogenase; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; NS = not specified; wk = week(s); x = time(s)

respiratory, hepatic, and renal effects in animals after acute and intermediate exposure to γ-HCH are shown in Table 3-4. Reliable LOAELs for hepatic, renal, and dermal effects in animals after intermediate exposure to technical-grade HCH are shown in Table 3-5.

Respiratory Effects. An acute dermal poisoning of a 2-month-old infant exposed to a whole-body application of 1% γ-HCH lotion resulted in death. The autopsy revealed pulmonary petechiae (tiny reddish spots that contain blood) (Davies et al. 1983). Slight dyspnea was observed in rats exposed dermally for 24 hours to 1,000 or 2,000 mg γ-HCH/kg on a shaved patch of dorsal skin (Ullmann 1986a). The dyspnea was severe in one female administered the high dose. Rapid respiration or wheezing was noted in rats exposed dermally to 10 mg γ-HCH/kg/day for 13 weeks (Brown 1988).

Cardiovascular Effects. An acute dermal poisoning of a 2-month-old infant exposed to a whole-body application of 1% γ-HCH lotion resulted in death. The autopsy findings were minimal but revealed epicardial petechiae (Davies et al. 1983).

No studies were located regarding cardiovascular effects in animals following dermal exposure to HCH.

Gastrointestinal Effects. Vomiting and diarrhea occurred in a child (Ramchander et al. 1991) and a woman (Hall and Hall 1999) who had 1% γ-HCH applied to the skin to treat a rash and to treat scabies.

No studies were located regarding gastrointestinal effects in animals following dermal exposure to HCH.

Hematological Effects. Aplastic anemia was documented in a man who applied γ-HCH to his skin for 3 weeks for treatment of scabies (Rauch et al. 1990). Excessive dermal exposure to HCH was reported to result in aplastic anemia and bone marrow hyperplasia in a woman who bathed her dog once a week for 2 years in a preparation that reportedly contained 2% HCH (Woodliff et al. 1966). Reduced hemoglobin and hematocrit values and a nearly complete absence of red blood cell precursors in bone marrow were reported in a 2-year-old boy exposed to a family dog that was dipped regularly in mange treatment containing 12% γ-HCH (Vodopick 1975).

No studies were located regarding hematological effects in animals following dermal exposure to any of the HCH isomers.

Hepatic Effects. No studies were located regarding hepatic effects in humans following dermal exposure to HCH.

Liver pathology, including dilation of sinusoids, focal fatty inclusions, hypertrophy of hepatocytes, thickened blood vessels, swelling, and proliferation of epithelial cells of bile ducts, was observed in guinea pigs treated with 100 mg technical-grade HCH/kg/day for 30 days (Dikshith et al. 1978). The patch of the abdomen on which the HCH was applied was not covered to prevent licking, so oral exposure may also have occurred. In rabbits exposed to 25 mg technical-grade HCH/kg/day for 30 days, there were degenerative changes in hepatocytes along with increased liver and serum GPT and alkaline phosphatase (Dikshith et al. 1989b). Liver cell hypertrophy, fatty degeneration, nuclear pyknosis, and focal and diffuse necrosis were found in female rats treated with 100 mg/kg/day technical-grade HCH for 7–30 days, but the time that it took for these lesions to occur, the severity, and the number of animals affected were not reported (Dikshith et al. 1991c). Centrilobular hypertrophy was reported in male and female rats exposed dermally to 60 mg γ-HCH/kg/day for 6 hours/day, 5 days/week, for 13 weeks (Brown 1988).

Renal Effects. No studies were located regarding renal effects in humans following dermal exposure to HCH.

Female rats treated with 100 mg/kg/day of technical-grade HCH for 7, 15, or 30 days had necrosis and atrophy of the renal tubules and glomeruli, although the number of animals affected and the severity of the lesions were not reported (Dikshith et al. 1991c). Similar effects were noted in male rabbits treated with 25 mg/kg/day technical-grade HCH (Dikshith et al. 1989b). Male rats treated dermally with 10 mg/kg/day γ-HCH for 13 weeks exhibited hyaline droplet formation, and urinalysis showed increased cast formation and positive scores for protein, blood, and turbidity in treated males (Brown 1988). Females in the same study exhibited a slight increase in the incidence of tubular basophilia at 60 mg/kg/day.

Dermal Effects. Rashes were observed in a boy following treatment with shampoo containing γ-HCH (Fagan 1981). No exposure level was reported, but the shampoo was rinsed over the boy's entire body.

Mild dermatitis was observed in rats after 15 skin paintings with 180 mg/kg/day γ-HCH/kg for 25 days (Dikshith et al. 1973). Rabbits exposed to 200 mg/kg moistened γ-HCH for 4 hours showed no primary skin irritation or other toxic symptoms (Ullmann 1986d). Rabbits exposed to technical-grade HCH

(25 mg/kg/day for 30 days) had hyperkeratinization of the epidermal layer and swollen collagen fibers in the dermis, but no scoring level was provided (Dikshith et al. 1989b). Dermal treatment of rats with 100 mg/kg/day technical-grade HCH for 7–30 days resulted in hyperkeratosis, epidermal cell vacuolization, and thickening of collagen fibers (Dikshith et al. 1991c).

Ocular Effects. No studies were located regarding ocular effects in humans following dermal exposure to HCH.

Mild eye irritation was seen in rabbits exposed to 40 mg/kg γ-HCH in the conjunctival sac for up to 72 hours, giving a primary irritation score of 0.6 out of a maximum possible cumulative score of 16 (Ullmann 1986c).

3.2.3.3 Immunological and Lymphoreticular Effects

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

3.2.3.4 Neurological Effects

There have been several reports of human intoxication involving convulsions in adults and children after excessive topical application of γ-HCH (Boffa 1995; Fischer 1994; Hall and Hall 1999; Lee and Groth 1977; Matsuoka 1981; Ramchander et al. 1991; Telch and Jarvis 1982; Tenenbein 1991); exposure levels were not reported. Heiberg and Wright (1955) reported convulsions in a woman who had treated calves with an insecticide containing 11% γ-HCH and 16% other HCH isomers. Central nervous systems symptoms of severe γ-HCH poisoning, including incontrollable shaking and myoclonic jerking and tonic-clonic movements of the extremities, developed in a woman following three dermal applications of a considerable amount (not quantified) of an antiscabies product over a period of approximately 2 weeks (Hall and Hall 1999). Fever, tachycardia, grand mal seizure, and hallucinations were reported in a teenager treated with a 1% γ-HCH lotion for 3 consecutive nights (Boffa 1995). Weakness of the left and right limbs, dysarthria, and dysphagia were seen in an agricultural worker exposed by inhalation and dermal contact to unspecified levels of several organochlorine pesticides, including γ-HCH (Fonseca et al. 1993). A man with human immunodeficiency virus (HIV) exhibited generalized tonic-clonic seizure activity after a single topical application of a 1% γ-HCH lotion to treat scabies (Solomon et al. 1995).

Studies in animals have substantiated the neurological symptoms resulting from γ-HCH application. Manifestations such as excitability, seizures, and convulsions have been observed in rabbits following a single topical application of 60 mg γ-HCH/kg in a 1% solution (Hanig et al. 1976); young rabbits were more susceptible than older rabbits. Slight sedation was observed in rats exposed once for 24 hours to 1,000 mg/kg γ-HCH through shaved dorsal skin (Ullmann 1986a). Sedation was severe in one female receiving the highest dose (2,000 mg/kg). This female also showed severe spasms. Damage to Purkinje cells in the cerebellum and tremors were found in female Wistar rats treated with 100 mg/kg/day technical-grade HCH for 7–30 days (Dikshith et al. 1991c). Aggressiveness or hyperactivity was noted in female rats exposed dermally for 13 weeks to 10 mg γ-HCH/kg/day, while ataxia and tremors were seen at 60 mg/kg/day (Brown 1988).

3.2.3.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following dermal exposure to HCH. Dikshith et al. (1978) reported testicular hypertrophy and atrophy and complete inhibition of spermatogenesis in guinea pigs dermally treated with technical-grade HCH for 7, 15, or 30 days at doses as low as 100 mg/kg/day. The patch of the abdomen on which the HCH was applied was not covered to prevent licking, so oral exposure more than likely occurred. In a similar study, the backs of male rats were sprayed with 50 or 100 mg/kg/day technical-grade HCH for 120 days and the rats were housed in separate cages to prevent licking (Prasad et al. 1995). Depletion of germ cells and impaired function of Leydig and Sertoli cells was suggested by significant dose-related changes in activities of testicular enzymes such as sorbitol dehydrogenase, glucose-6-P-dehydrogenase, γ-glutamyl transpeptidase, and β-glucoronidase. Significant reductions in sperm count and motility and increased percentages of abnormal sperm were also observed in both groups. A significant reduction in testosterone level was observed in the high dose group.

3.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals following dermal exposure to HCH.

3.2.3.7 Cancer

A case-control study surveying childhood brain cancer cases among Missouri residents found that the odds ratios for the use of Kwell, a shampoo containing γ-HCH for lice control, were slightly elevated during the first 7 months of age to diagnosis (Davis et al. 1992). Thus, Kwell use was significantly associated with childhood brain cancer compared to controls. However, this study was limited by small sample sizes, potential recall bias in questionnaires, multiple comparisons, and the lack of detailed exposure information.

In mice, dermal exposure to a 0.5% solution of γ-HCH in acetone applied twice a day for 60 days was reported to result in no treatment-related tumors (Orr 1948). Increases that were not statistically significant were reported in the incidences of hyperplastic and preneoplastic areas in the liver and hepatic tumors in Swiss mice exposed to 2.4 mg technical-grade HCH/kg/day for 80 weeks (Kashyap et al. 1979). Limitations of these studies, including less-than-lifetime exposure and study duration, the testing of only one dose, and the potential for ingestion of some of the compound from the skin, preclude determination that dermally applied HCH is noncarcinogenic in mice.

3.3 GENOTOXICITY

The available genotoxicity data indicate that γ-HCH and other individual HCH isomers have some genotoxic potential, but the evidence for this is not conclusive.

No appreciable increase in the frequency of chromosome aberrations was observed in humans exposed primarily to γ-HCH by inhalation in a pesticide production factory (Kiraly et al. 1979). These individuals had been exposed for 8 hours/day for at least 6 months. Other studies are available regarding genotoxic effects in humans exposed to a wide variety of pesticides, including γ-HCH, when they were used on farms (Rupa et al. 1988, 1989a, 1989b, 1989c). The specific effects of HCH, apart from the effects due to the other exposures, are not known.

In animals, ingestion of technical-grade HCH was reported to induce dominant-lethal mutations in mice (Lakkad et al. 1982). It did not induce chromosome aberrations in bone marrow cells of Syrian hamsters (Dzwonkowska and Hubner 1986), but positive results were reported in bone marrow cells of rats exposed to β-HCH (Shimazu et al. 1972). Oral exposure to α-HCH was reported to result in mitotic disturbances including an increased mitotic rate and an increased frequency of polyploid hepatic cells in

rats (Hitachi et al. 1975). γ-HCH has also been tested *in vivo* in animals. Incidence of chromosome clastogeny in bone marrow cells was increased in mice exposed to 1.6 mg γ-HCH/kg body weight/day by gavage for 7 days (Kumar et al. 1995). γ-HCH was negative in a micronucleus assay in mice (Jenssen and Ramel 1980). α-HCH produces DNA fragmentation in primary cultures of rat and human hepatocytes, but not in mouse hepatocytes (Mattioli et al. 1996). DNA repair induction was absent in hepatocytes from all three species. Both α- and γ-HCH have been observed to bind to mouse liver DNA at a low rate (Iverson et al. 1984).

γ-HCH has been tested in several *in vitro* genotoxicity studies. In bacteria, it was not observed to induce gene mutations in assays with or without a metabolic activation system (Moriya et al. 1983; Nagy et al. 1975), and it did not produce DNA damage, although a mammalian metabolic activation system was not present (Shirasu et al. 1976). γ-HCH was also not mutagenic in yeast (Shahin and von Borstel 1977) or algae (Kar and Singh 1979a). Mitotic disturbances (c-mitosis which is characterized by spindle breakdown as that produced by colchicine) and chromosome aberrations were observed in onion root tip cells exposed to commercial γ-HCH (Nybom and Knutsson 1947). In mammalian cells, γ-HCH (purity not reported) induced a marginal increase in the frequency of chromosome aberrations (including chromosomal gaps) in Chinese hamster cells, which was interpreted by the authors of the study as providing suggestive, but not conclusive, evidence of an effect (Ishidate and Odashima 1977). DNA damage was observed in cultures of rat nasal and gastric mucosa cells, and human nasal mucosa cells (Pool-Zobel et al. 1994). γ-HCH (NTP 1984) and technical-grade γ-HCH (Murli 1990) were both reported to be negative for cytogenetic effects in Chinese hamster ovary cells. α-HCH and γ-HCH were reported to bind to calf thymus DNA in the presence of metabolic activation (Iverson et al. 1984). γ-HCH was found inactive for inducing unscheduled DNA synthesis in human SV-40 fibroblasts, with and without activation (Ahmed et al. 1977), while it was found to induce unscheduled DNA synthesis in human peripheral lymphocytes (Rocchi et al. 1980). More recent studies by Kalantzi et al. (2004) with human mammary carcinoma MCF-7 and human prostate carcinoma PC-3 cell lines showed that incubation of the cells with low concentrations of γ-HCH (10-12–10-10 M) induced increases in micronuclei in both cell lines in the absence of DNA damage or cytotoxicity, suggesting a clastogenic effect for this chemical. The α- and β- stereoisomers were less active than γ-HCH. Cultured human lymphocytes taken from three healthy males showed a dose-dependent increase in chromosomal aberrations (gaps, breaks, and fragments) with significant increases at 0.1 μL/mL technical-grade HCH (6.5% γ-HCH) for 48-hour treatment and at 0.05 and 0.1 μL/mL for 72-hour treatment (Rupa et al. 1989d). In addition, sister chromatid exchanges increased in a dose-dependent manner with the high dose (0.1 μL/mL) producing the only significant result. These results suggest mild mutagenic activity at high

doses in humans (Rupa et al. 1989d). Technical-grade γ-HCH was also found inactive for inducing unscheduled DNA synthesis in rat primary hepatocytes *in vitro* (Cifone 1990).

Tables 3-6 and 3-7 present the results of *in vivo* and *in vitro* genotoxicity studies on HCH isomers, respectively.

3.4 TOXICOKINETICS

Absorption of the various HCH isomers following inhalation, oral, or dermal exposure has been inferred from humans who have become ill or who had increased serum levels of the various isomers following exposure by these routes. No animal data are available from the inhalation route to quantify the extent or rate of absorption. Technical-grade HCH has been shown to be well absorbed from the gastrointestinal tract of animals (Albro and Thomas 1974). The distribution of HCH isomers in humans and animals is primarily to the adipose tissue but also to the brain, kidney, muscle, blood, and other tissues (Baumann et al. 1980; Siddiqui et al. 1981a). β-HCH accumulates to a much greater extent than γ-HCH. The excretion of HCH isomer metabolites is primarily through the urine. The isomers have also been detected in breast milk (Ejobi et al. 1996; Schoula et al. 1996) and semen (Szymczynski and Waliszewski 1981). The primary urinary metabolites are chlorophenols and 1,2,4-trichlorocyclohexane-4,5-epoxide. The conversion occurs mainly by the action of hepatic enzymes.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Evidence exists that humans absorb γ-HCH vapor or dusts via inhalation. This can be inferred from occupational studies in which adverse health effects, including hematological abnormalities and neurological effects, have been reported in workers exposed to γ-HCH in workplace air (Brassow et al. 1981; Czegledi-Janko and Avar 1970; Kashyap 1986; Samuels and Milby 1971). In addition, α-, β-, γ-, and δ-HCH have been detected in the blood serum, adipose tissue, and semen of occupationally and environmentally exposed individuals, indicating that absorption does take place (Baumann et al. 1980; Czegledi-Janko and Avar 1970; Kashyap 1986; Nigam et al. 1986; Saxena et al. 1980, 1981a, 1981b). There are no specific studies that have quantified the rate or extent of absorption of the HCH isomers following inhalation exposure. No information is available on the absorption of α-, β-, γ-, and δ-HCH following inhalation exposure in experimental animals.

Table 3-6. Genotoxicity of Hexachlorocyclohexane Isomers *In Vivo*

Species (test system)	End point	Results	Isomer	Reference
Mammalian cells:				
Human (peripheral lymphocytes)	Chromosomal aberrations	–	Gamma	Kiraly et al. 1979
Human hepatocytes	DNA fragmentation	+	Alpha	Mattioli et al. 1996
Syrian hamster (bone marrow)	Chromosomal aberrations	–	Gamma	Dzwonkowska and Hubner 1986
Rat (bone marrow)	Chromosomal aberrations	+	Beta	Shimazu et al. 1972
Rat (primary cultures)	DNA fragmentation	+	Alpha	Mattioli et al. 1996
Mouse (germ cells)	Dominant lethal	+	Technical	Lakkad et al. 1982
Mouse	Micronuclei	–	Gamma	Jenssen and Ramel 1980
Mouse (bone marrow)	Chromosomal aberrations	+	Gamma	Kumar et al. 1995
Mouse (liver)	DNA binding	(+)	Alpha/ gamma	Iverson et al. 1984
Mouse (hepatocytes)	DNA fragmentation	–	Alpha	Mattioli et al. 1996
Rat (liver)	Mitotic disturbances	+	Alpha	Hitachi et al. 1975

– = negative result; + = positive result; (+) = weakly positive result; DNA = deoxyribonucleic acid

Table 3-7. Genotoxicity of Hexachlorocyclohexane Isomers *In Vitro*

Species (test system)	End point	Results		Isomer	Reference
		With activation	Without activation		
Prokaryotic organisms:					
Salmonella typhimurium (TA100, TA98, TA1535, TA1537, TA1538)	Gene mutation	–	–	Gamma	Moriya et al. 1983
Escherichia coli (WP2/spot test)	Gene mutation	NT	–	Gamma	Nagy et al. 1975
E. coli (WP2 *hcr*)	Gene mutation	–	–	Gamma	Moriya et al. 1983
Bacillus subtilis (rec assay)	DNA damage	NT	–	Gamma	Shirasu et al. 1976
Eukaryotic organisms:					
Fungi and plant cells:					
Saccharomyces cerevisiae	Gene mutation	–	–	Gamma	Shahin and von Borstel 1977
Nostoc muscorum	Gene mutation	NT	–	Gamma	Kar and Singh 1979a
Allium cepa	Mitotic disturbances	NT	+	Gamma	Nybom and Knutsson 1947
Mammalian cells:					
Human (SV-40 fibroblasts)	Unscheduled DNA synthesis	–	–	Gamma	Ahmed et al. 1977
Human (peripheral lymphocytes)	Unscheduled DNA synthesis	NT	+	Gamma	Rocchi et al. 1980
Human (mammary carcinoma MCF-7)	Micronuclei	NT	+	Gamma	Kalantzi et al. 2004
Human (prostate carcinoma PC-3)	Micronuclei	NT	+	Gamma	Kalantzi et al. 2004
Human (mammary carcinoma MCF-7)	DNA damage	NT	–	Gamma	Kalantzi et al. 2004
Human (prostate carcinoma PC-3)	DNA damage	NT	–	Gamma	Kalantzi et al. 2004
Human (peripheral lymphocytes)	Sister chromatid exchange	NT	+	Technical	Rupa et al. 1989d
Human (peripheral lymphocytes)	Chromosomal aberrations	NT	+	Technical	Rupa et al. 1989d
Chinese hamster (CHL cells)	Chromosomal aberrations	NT	(+)	Gamma	Ishidate and Odashima 1977
Chinese hamster (CHL cells)	Chromosomal aberrations	NT	–	Gamma	NTP 1984
Chinese hamster (CHL cells)	Sister chromatid exchange	NT	–	Gamma	NTP 1984
Chinese hamster (CHL cells)	Chromosomal aberrations	–	–	Technical	Murli 1990

Table 3-7. Genotoxicity of Hexachlorocyclohexane Isomers *In Vitro*

Species (test system)	End point	Results		Isomer	Reference
		With activation	Without activation		
Rat (primary hepatocytes)	Unscheduled DNA synthesis	NT	–	Technical	Cifone 1990
Calf (thymus DNA)	DNA binding	(+)	NT	Alpha/ gamma	Iverson et al. 1984

– = negative result; + = positive result; (+) = weakly positive result; DNA = deoxyribonucleic acid; NT = not tested

3.4.1.2 Oral Exposure

In humans, HCH is absorbed following oral exposure. Many accidental poisonings have occurred in humans as a result of γ-HCH ingestion, and high blood concentrations have been demonstrated in a number of acute poisoning cases (Berry et al. 1987; Harris et al. 1969; Khare et al. 1977; Munk and Nantel 1977; Nantel et al. 1977; Powell 1980; Starr and Clifford 1972).

HCH is similarly absorbed following oral exposure in animals. Information concerning the rate of absorption from the gastrointestinal tract can be inferred from studies conducted in mice and rats. These studies indicated that γ-HCH is readily absorbed from the gastrointestinal tract (Ahdaya et al. 1981; Turner and Shanks 1980). Ahdaya et al. (1981) demonstrated that half of the administered dose was absorbed from the gastrointestinal tract of fasting mice approximately 14 minutes after administration of radiolabelled γ-HCH by stomach tube. Although this study demonstrates the rapid absorption of γ-HCH from the gastrointestinal tract, the use of fasted animals prevents an assessment of the effect of stomach contents on the rate of absorption. Turner and Shanks (1980) studied the rate of absorption of γ-HCH from the gastrointestinal tract and intestinal lymphatic system using rat intestinal loop preparations. Prepared loops were injected with γ-HCH, and the blood and lymph were sampled for 30 minutes. γ-HCH was readily absorbed from the intestine into the blood; however, only a small amount of γ-HCH entered the lymphatic system from the intestine.

Absorption of technical-grade HCH following oral exposure has been quantified in rats. The extent of absorption of technical-grade HCH has been estimated to be 95.8% in rats within 4 days following the oral administration of single doses of the substance (Albro and Thomas 1974). Variation of the dosages from 30 to 125 mg/kg had no effect on the percentage of absorption. The overall degree of absorption of technical-grade HCH administered in the feed for 14 days was similar (94.9%), but the average absorption values of α-, β-, γ-, and δ-HCH were 97.4, 90.7, 99.4, and 91.9%, respectively (Albro and Thomas 1974).

3.4.1.3 Dermal Exposure

The ready absorption of γ-HCH across human skin, due to its lipid solubility, has been demonstrated in several studies that examined the absorption of γ-HCH from an antiscabies lotion (Feldmann and Maibach

1974; Franz and Lehman 1996; Lange et al. 1981). Maximum serum levels in healthy volunteers and scabies patients were reported within 4–6 hours following whole-body application (Lange et al. 1981). However, the maximum serum levels of γ-HCH in scabies patients were greater than those reported for normal volunteers. Studies involving a single topical application of γ-HCH to the forearm, which was left for 24 hours before washing, indicate that at least 9% of the applied dose was absorbed; maximum absorption occurred during the first 12 hours after application of γ-HCH to the skin, but absorption continued for at least 5 days (Feldmann and Maibach 1974).

The absorption of γ-HCH through the skin was studied following application of two different preparations to the forearm of volunteers (Dick et al. 1997a). One with 120 mg γ-HCH/mL in acetone as the vehicle and the other, a commercial product, consisted of 3 mg γ-HCH/mL formulation, which primarily contained white spirit as the solvent base. The proportion of the applied dose absorbed into the systemic circulation in 6 hours was 5% for the dose applied in acetone and 60% of the applied dose in white spirit-based formulation. Thus, the white spirit enhanced the absorption of γ-HCH relative to acetone as the vehicle. The absorption of γ-HCH through human skin was also assessed in an *in vitro* study (Dick et al. 1997b). γ-HCH absorption was reported to be 15–25% in 24 hours for the two formulations that contained white spirit as the predominant solvent, 3% in 24 hours from an aqueous spray dilution, and <1% in 24 hours for the acetone preparation.

γ-HCH is similarly absorbed through the skin of animals. Toxicity was observed in guinea pigs and rabbits following dermal exposure to γ-HCH and following dermal exposure to technical-grade HCH (Dikshith et al. 1978; Hanig et al. 1976). Male rats treated dermally with radiolabelled γ-HCH (20% emulsifiable concentrate) on a 4.9 cm^2 shaved dorsal area exhibited absorption of radiolabel, which increased with time of exposure (Bosch 1987a). After 4 hours, 10.1, 5.3, and 2.0% were absorbed from doses of 0.06, 0.6, and 6 mg/cm^2/kg, respectively. After 24 hours, 27.7, 20.9, and 5.1% were absorbed from doses of 0.06, 0.6, and 6 mg/cm^2/kg, respectively. Male rabbits treated dermally with radiolabelled γ-HCH (20% emulsifiable concentrate) in a 28.3-cm^2 shaved dorsal area absorbed, after 4 hours, 29.6, 18.3, and 7.3% radiolabel from doses of 0.005, 0.05, and 0.5 mg/cm^2/kg, respectively, and, after 24 hours, 55.7, 40.0, and 16.6% from the same respective doses (Bosch 1987b).

The absorption of γ-HCH in infants and children who had received dermal treatment with 1% γ-HCH lotion was investigated in one study (Ginsburg et al. 1977). Maximum blood concentrations of γ-HCH were observed in 6 hours, and averaged at 0.028 μg/mL for the group infected with scabies and 0.024 μg/mL for the noninfected group.

3.4.2 Distribution

Placental transfer of HCH in humans has been well documented (Saxena et al. 1981a). The levels of HCH and other organochlorine insecticides were found to be higher in the maternal blood, placenta, and umbilical-cord blood of stillborn cases than those of live-born cases (Saxena et al. 1983). Similarly, γ-, α-, δ-, and total HCH maternal blood and umbilical-cord blood levels were higher in mothers who gave birth to IUGR babies than in women who gave birth to normal weight babies (Siddiqui et al. 2003). HCH has been shown to accumulate in amniotic fluid, placenta, and fetal tissues after oral treatment of pregnant mice (Srivastava and Raizada 1993) and can be related to fetolethality. HCH isomers have been detected in human breast-milk, particularly in developing countries that still use HCH as a pesticide. Detected concentrations in these studies are discussed in Section 6.6. In a study on rats, γ-HCH has been reported to be transferred in the breastmilk and to elicit neurological effects in neonates. Epileptiform seizures have been reported in male rats fed maternal milk for 12 days beginning on the third day after birth, from dams exposed daily to 20 mg γ-HCH/kg by gavage (Albertson et al. 1985). In another study, in which lactating females were treated orally with a single dose of 6 mg/kg of γ-HCH on days 9 or 14 of lactation, the testosterone level of the male offspring was reduced 50% when puberty was reached (day 65) when compared to the control group (Dalsenter et al. 1997b). When the offspring reached adulthood (postnatal day 140), the relative testicular weight was significantly lower (Dalsenter et al. 1997b). The number of sperm and spermatids was also significantly reduced. α-, β-, and γ-HCH have been found to be bioconcentrated and excreted in breast milk of women who have been exposed to technical-grade HCH in pesticide residues (Nair et al. 1996).

3.4.2.1 Inhalation Exposure

Information on the distribution of the HCH isomers, following inhalation by humans, comes from studies of humans exposed to HCH in the workplace. Air concentrations of α-HCH (0.002–1.99 mg/m^3), β-HCH (0.001–0.38 mg/m^3), and γ-HCH (0.004–0.15 mg/m^3) were associated with concurrent mean blood serum levels in workers of 69.6, 190.3, and 36.9 μg/L, respectively (Baumann et al. 1980). Serum levels of total HCH of 0.14–0.60 ppm were found in workers with unknown levels of exposure to technical-grade HCH (Nigam et al. 1986). HCH isomers have also been detected in the adipose tissues of workers occupationally exposed and individuals exposed via the ambient environment (Baumann et al. 1980; Siddiqui et al. 1981a). Accumulation of β-HCH has been shown to increase approximately linearly with

time of exposure (Baumann et al. 1980). Siddiqui et al. (1981a) found adipose levels of 0.1–1.5, 0.06–0.9, 0.7–3.0, and 0.97–5.8 ppm of α-, β-, γ-, and total HCH, respectively, in the tissues collected during an autopsy case study conducted in India.

In a study with Wistar rats exposed to air concentrations of 0.02–5 mg/m^3 γ-HCH for 90 days, male rats exhibited higher serum γ-HCH levels than females, but females had higher liver, brain, and fat levels (Oldiges et al. 1983). The organ levels of γ-HCH were dose-dependent, but had returned to baseline levels after a 4-week recovery period.

3.4.2.2 Oral Exposure

Information on the distribution of the HCH isomers following ingestion by humans comes from case reports. A fatal poisoning case confirmed that γ-HCH is, in part, distributed to the central nervous system. γ-HCH was detected in the cerebrospinal fluid of a young boy following ingestion of an unknown quantity of γ-HCH (Davies et al. 1983).

More detailed information on the distribution of HCH or its isomers is available from studies in which laboratory animals were exposed by ingestion (Chand and Ramachandran 1980; Eichler et al. 1983; Srinivasan and Radhakrishnamurty 1983b). These studies examined the overall distribution pattern of HCH isomers. γ- and β-HCH are primarily stored in the fat of rats acutely exposed for 5, 10, or 15 days (Srinivasan and Radhakrishnamurty 1983b). The overall distribution of γ-HCH was greatest in fat, followed by brain, kidney, muscle, lungs, heart, spleen, liver, and blood. More recently, γ-HCH has also been found in the adrenal glands of rats (Lahiri et al. 1990; Sulik et al. 1988). In an experiment lasting 12 days, the accumulation of γ-HCH in the brain of rats gavaged with 5 or 12 mg/kg/day began to decline after 8 days. This reduction was not observed in rats gavaged with 20 mg/kg/day (Tusell et al. 1988). In rats gavaged with γ-HCH on lactation days 9 or 14, γ-HCH levels were higher in their milk than plasma (Dalsenter et al. 1997b). Levels of γ-HCH in the offspring of those rats were approximately twice as high in kidneys and liver than in brain and testes. In the brain of rats, α-HCH has been found to accumulate preferentially in the white matter, an area containing lipid-rich myelin, as opposed to gray matter (Portig et al. 1989). However, the same brain distribution pattern was not noted for γ-HCH in mice, despite the fact that it is equally lipophilic. Differences in distribution of γ-HCH and α-HCH are most likely due to stereospecific forces.

The distribution pattern for β-HCH was found to be in the following order: fat > kidney > lungs > liver > muscle > heart > spleen > brain > blood. For γ-HCH, the distribution pattern was as follows: fat > brain > kidney > muscle > lungs > heart > spleen > liver > blood. β-HCH accumulates in tissues to a greater degree than γ-HCH except in the brain, where the γ-HCH accumulates at a higher concentration (Srinivasan and Radhakrishnamurty 1983b). This accumulation increases with increasing dose and treatment period for β-HCH more so than for γ-HCH. The greater accumulation of β-HCH in tissues is expected since this isomer is known to be metabolized more slowly. In addition, γ-HCH is known to induce the liver mixed-function oxygenase system, and thus, self-induced metabolism is an important factor that minimizes the accumulation of γ-HCH residues in animal tissues.

The preferential accumulation of HCH in fatty tissues is also observed following intermediate-duration exposure of rats to HCH (isomer unspecified) in the diet (overall distribution: fat > liver > serum) (Chand and Ramachandran 1980) or exposure to α- or γ-HCH by gavage (overall distribution: fat > kidney > liver > brain > blood) (Eichler et al. 1983).

3.4.2.3 Dermal Exposure

Information on the distribution of the HCH isomers in exposed humans comes from case reports. A fatal poisoning case indicated that γ-HCH is, in part, distributed to the brain following topical application. The isomer was detected in brain tissue (110 ppb) and heart blood (33.3 ppb) collected during the autopsy of an infant who was treated with a whole-body application of a 1% γ-HCH lotion after a hot bath (Davies et al. 1983). In another study, blood levels of γ-HCH peaked 6 hours following topical application of a 1% solution to 20 children (12 infected with scabies, 8 noninfected) (Ginsburg et al. 1977). Mean concentrations did not differ statistically between the two groups at 6 hours and were 0.024 μg/mL in healthy children and 0.028 μg/mL in infected children. The half-lives in blood were 17.9 and 21.4 hours in infected and healthy children respectively. Differences in dosage between the two groups of children were considered marginally significant (p=0.11). However, the infected children were younger. The mean ages for the infected and noninfected groups were 32.5 and 64.3 months, respectively.

The distribution of γ-HCH through the skin was studied following application of two different preparations to the forearm of volunteers (Dick et al. 1997a). The mean peak plasma concentrations of γ-HCH following exposure to the acetone and white-spirit based applications were 0.91 and 0.47 ng/mL, respectively; although the preparation in acetone contained a 40-fold higher concentration of γ-HCH. About 30% of the applied dose for the white-spirit based formulation was observed in the stratum

corneum at 6 hours of exposure and decreased by 90% at 24 hours. Fifteen percent of the applied dose for the acetone-based application was located in the stratum corneum.

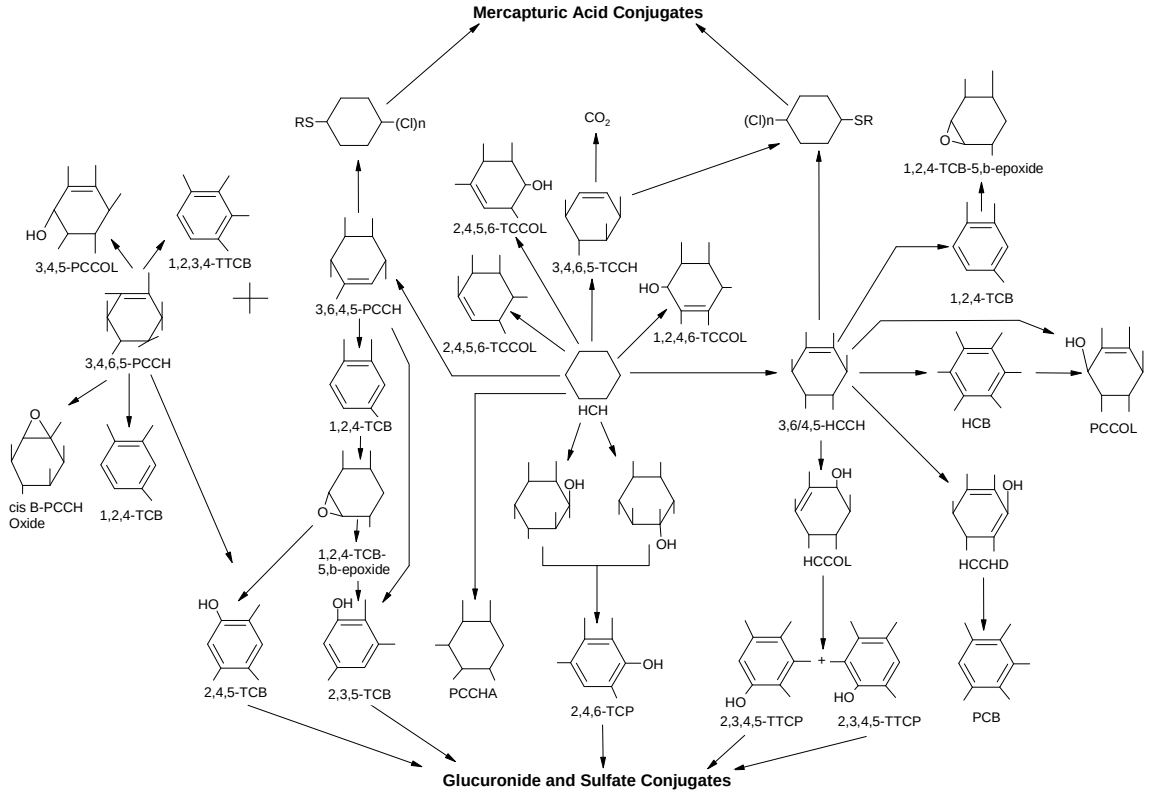
Some information on the distribution of γ-HCH is available from studies in which laboratory animals were exposed by dermal application (Bosch 1987a, 1987b; Hanig et al. 1976; Solomon et al. 1977a, 1977b). A study on the distribution of γ-HCH in guinea pigs following acute dermal exposure indicates that accumulation of γ-HCH in the brain is greater than in the blood after single and multiple topical applications (Solomon et al. 1977a, 1977b); the levels in both tissues increased with the number of applications. Experiments with radiolabelled γ-HCH in dermally treated rats (Bosch 1987a) and rabbits (Bosch 1987b) found that absorption of radiolabel increased with time of exposure, with greater absorption and subsequent excretion in the urine occurring at the lower treatment doses. In weanling rabbits, which appear to be more sensitive to γ-HCH toxicity from dermal exposure than young adults, levels of γ-HCH in the blood after a single application of a 1% solution (60 mg γ-HCH/kg) were 1.67 and 2.48 μg/mL in two rabbits that had been shaved and depilated, then stripped to remove the keratin layer (Hanig et al. 1976). In contrast, a blood level of only 0.67 μg/mL was seen in a rabbit that had only been shaved and depilated, indicating that absorption increases with loss of skin integrity.

Following dermal treatment of rats with 50 or 100 mg/kg/day technical-grade HCH for 120 days, α-, β-, γ-, and δ-HCH were accumulated in testicular tissue and sperm in a dose-related manner (Prasad et al. 1995). β-HCH was present at the highest concentration in testicular tissue and sperm.

3.4.3 Metabolism

The metabolism of γ-HCH is illustrated in Figure 3-4. Angerer et al. (1983) determined that chlorophenols were the primary urinary metabolites of γ-HCH excreted by workers involved in γ-HCH production. In the study, glucuronides and sulfates of chlorophenols were cleaved by acidic hydrolysis of urine samples. The metabolites 2,3,5-, 2,4,6-, and 2,4,5-trichlorophenol accounted for almost 57.7% of the γ-HCH metabolites identified in the urine collected during the last 2 hours of the workers' shifts. Other urinary metabolites identified included other trichlorophenols, dichlorophenols, tetrachlorophenols, and dihydroxychlorobenzenes. Pentachlorophenol has also been identified as a urinary metabolite in humans following occupational exposure (Engst et al. 1979). *In vitro* investigations indicate that human liver microsomes convert γ-HCH by dechlorination, dehydrogenation, dehydrochlorination, and hydroxylation to five primary metabolites: 3,6/4,5-hexachlorocyclohexene, pentachlorocyclohexene, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorobenzene (Fitzloff et al. 1982). Similar *in*

Figure 3-4. The Proposed Metabolism of Hexachlorocyclohexane*



Abbreviations:

HCB:	Hexachlorobenzene
HCCHD:	Hexachlorocyclohexadiene
HCCOL:	Hexachlorocyclohexenol
HCH:	Hexachlorocyclohexane
PCCHA:	Pentachlorocyclohexane
PCCOL:	Pentachlorocyclohexenol
PCCH:	Pentachlorocyclohexene
PCB:	Pentachlorobenzene
TCCOL:	Tetrachlorocyclohexenol
TCCH:	Tetrachlorobenzene
TTCP:	Tetrachlorophenol
TCB:	Trichlorobenzene
TCP:	Trichlorophenol
3,6/4,5-HCCH:	3,6/4,5-Hexachlorocyclohexene

*Adapted from Chadwick et al. 1979, 1985; Fitzloff and Pan 1984; Fitzloff et al. 1982

vitro studies have demonstrated that an epoxide forms during the metabolism of pentachlorocyclohexene. This stable halogenated hydrocarbon epoxide metabolite may be responsible for the mutagenic and carcinogenic effects of γ-HCH (Fitzloff and Pan 1984).

In animals, γ-HCH appears to be transformed by hepatic enzymes to form chlorophenols, chlorobenzene, chlorocyclohexanes, chlorocyclohexanols, and conjugates of mercapturic acid, glucuronide, and sulfate (Chadwick and Freal 1972a; Chadwick et al. 1978a; Engst et al. 1979; Kujawa et al. 1977). These metabolites have been identified in various tissues and in the urine of laboratory animals. Metabolites found in the liver of rats following intermediate exposure to γ-HCH via gavage or diet include di-, tri-, tetra-, and pentachlorobenzenes; pentachlorocyclohexenes; and pentachloro-2-cyclohexen-1-ol (Chadwick and Freal 1972a; Kujawa et al. 1977). Metabolites identified in the blood of these rats include di-, tri-, tetra-, and pentachlorophenols and pentachloro-2-cyclohexen-1-ol (Kujawa et al. 1977). Di-, tri-, and tetrachlorophenols; pentachlorocyclohexenes; and pentachloro-2-cyclohexen-1-ol have been identified in samples of kidney, spleen, heart, and brain tissue from rats fed γ-HCH (Kujawa et al. 1977). Metabolites found in the urine include tri-, tetra-, and pentachlorophenol; pentachloro-2-cyclohexen-1-ol; and isomers of tetrachloro-2-cyclohexen-1-ol (Chadwick and Freal 1972a; Chadwick et al. 1978c; Kujawa et al. 1977). The metabolism of γ-HCH in the intestine was reported to be very minor, or the metabolites were completely absorbed. No metabolites were detected in the feces or in the adrenal gland (Kujawa et al. 1977). *In vitro* preparations using rat liver slices have also found that γ-HCH is converted to hexachlorobenzene (Gopalaswamy and Aiyar 1984). However, these findings have not yet been confirmed in *in vivo* experiments.

The major urinary metabolites formed in rats, following intermediate oral exposure to α- or β-HCH, were identified as tri- and tetrachlorophenols; pentachlorocyclohexene was also identified as a metabolite of γ-HCH in kidney tissue (Macholz et al. 1982a, 1982b).

The toxicity of γ-HCH appears to be dependent on the P-450 oxidative system. Intermediate exposure to γ-HCH resulted in greater toxicity in DBA/2 (D2) mice than in C57BL/6 (B6) mice; the former are unresponsive to microsomal enzyme induction by γ-HCH (Liu and Morgan 1986). Increased toxicity was associated with higher blood and brain concentrations in D2 mice than in B6 mice at the time of sacrifice. In addition, D2 mice were found to have more 2,4,6-trichlorophenol in the liver, kidney, and spleen than the less-susceptible B6 mice. The inability of D2 mice to undergo enzyme induction to increase the rate of detoxification led to γ-HCH's enhanced toxicity in this strain. Other investigators have demonstrated the importance of the hepatic microsomal enzymes in the toxicity of γ-HCH (Baker et al. 1985; Chadwick

and Freal 1972a; Chadwick et al. 1981; Chand and Ramachandran 1980; Tanaka et al. 1979). Chadwick et al. (1981) demonstrated that pretreatment of rats with inducers of hepatic enzymes significantly influenced the metabolism and excretion of γ-HCH and its metabolites by altering specific metabolic pathways; excretion of γ-HCH metabolites in the urine increased nearly 4-fold following pretreatment with Aroclor 1254 or phenobarbitol. Following pretreatment with Aroclor 1254, a 7-fold increase in expired metabolites was observed. Naphthoflavone had no effect on the excretion rate.

Metabolic transformations also affect the neurotoxicity of γ-HCH. Experiments in rats pretreated with 3-methylcholanthrene (MC), an inducer of P-4501A1/1A2, phenobarbitol (PB), an inducer of P-4502B1/2B2, or ethanol, an inducer of P-4502E1, suggested that the convulsive activity of γ-HCH is due to γ-HCH *per se* and/or to metabolites formed by PB- or ethanol-inducible P-450 isoenzymes (Parmar et al. 2003).

Metabolism of HCH has not been studied in children. However, although it is unknown whether the ability to metabolize HCH specifically differs between children and adults, some enzymes that belong to the enzyme superfamilies involved in phase II HCH metabolism are developmentally regulated in humans. The development of UDP-glucuronosyltransferase (responsible for glucuronide conjugation) depends on the enzyme isoform but, in general, adult activity is attained by 6–18 months of age (Leeder and Kearns 1997). Development of sulfotransferase (responsible for sulfate conjugates) activity is also substrate specific and is usually earlier than UDP-glucuronosyltransferase. In fact, levels of some sulfotransferases may be greater during infancy and early childhood than during adulthood (Leeder and Kearns 1997). A series of enzymes are involved in the production of mercapturic acid conjugates: γ-glutamyltranspeptidase, glutathione S-transferase, cysteinyl glycinase, and N-acetyl transferase (Sipes and Gandolfi 1991). There are two superfamilies of N-acetyltransferases, and one (i.e., the N-acetyl-transferase 2 superfamily) has members that are developmentally regulated in humans. There is some N-acetyltransferase 2 activity in fetuses by 16 weeks of gestation. Infants up to 2 months of age have the slow metabolizer phenotype (there is a genetic polymorphism in this enzyme in adults). The adult distribution of slow and fast metabolizer phenotypes is reached by 4–6 months of age and full adult activity is achieved at 1–3 years of age (Leeder and Kearns 1997).

3.4.4 Elimination and Excretion

Excretion of hexachlorocyclohexane has not been studied in children.

3.4.4.1 Inhalation Exposure

Humans excrete γ-HCH and its metabolites in urine, milk, and semen (Angerer et al. 1981). Chromatographic analysis of urine from humans occupationally exposed to HCH showed the presence of chlorinated phenols and all isomers of di-, tri-, and tetrachlorophenol (Angerer et al. 1981). In another study, the elimination of β-HCH was investigated in a group of 40 former workers of a γ-HCH-producing plant by analyzing at least two blood specimens from different time points between 1952 and 1980. The median half-life of β-HCH was 7.2 years, calculated by concentrations in whole blood, and 7.6 years, calculated by concentrations in extractable lipids (Jung et al. 1997), assuming first order kinetics for excretion. HCH is commonly detected in low concentrations (0.015 mg/kg fat) in the breastmilk of women exposed to HCH in the environment (Fytianos et al. 1985). All five of the HCH isomers discussed in this profile have been detected in human semen following environmental exposure, suggesting another route of elimination (Szymczynski and Waliszewski 1981). No animal studies using the inhalation route of exposure were located.

3.4.4.2 Oral Exposure

Excretion of γ-HCH and its metabolites in laboratory animals has been well documented. Data indicate that its major route of elimination is via the urine following intermediate and chronic oral feeding in mice (Chadwick et al. 1985). Very little is eliminated in exhaled air (Ahdaya et al. 1981; Chadwick et al. 1985) or in feces (Chadwick et al. 1985) following acute, intermediate, and chronic oral administration in rodents. Because of its high lipid solubility, γ-HCH is excreted through the dam’s milk (Dalsenter et al. 1997b).

Very little γ-HCH is excreted unaltered. Various phenylmercapturic acid derivatives have been detected in the urine of rats, formed by the conjugation of γ-HCH metabolites with glutathione subsequent to dechlorinations and dehydrochlorinations (Allsup and Walsh 1982; Kurihara et al. 1979). *In vitro* investigations using rat liver cells indicate that β-HCH seems to resist, to some extent, conversion to the glutathione derivative; γ-HCH and α-HCH are readily conjugated (Fitzloff and Pan 1984; Fitzloff et al. 1982). γ-HCH derivatives are not only excreted in the form of phenylmercapturic acids; there is ample evidence that they are also excreted in the form of glucuronides and sulfate conjugates (Chadwick et al. 1978a).

No studies were located regarding genotoxic effects in animals following oral exposure, in humans following inhalation exposure, or in humans or animals following dermal exposure to HCH.

3.4.4.3 Dermal Exposure

Nonmetabolized γ-HCH was excreted in the urine and feces of healthy volunteers and scabies patients acutely exposed to a 0.3% γ-HCH emulsion by whole-body application. The cumulative excretion of nonmetabolized γ-HCH was almost the same in the healthy volunteers and the scabies patients (Zesch et al. 1982).

The elimination of γ-HCH was studied following application of two different preparations to the forearm of volunteers (Dick et al. 1997a). The elimination half-life was between 50 and 111 hours for the acetone-based application, and 25–58 hours for the white-spirit based formulation. Absorbed γ-HCH was excreted in the urine as conjugates of 2,4,6-; 2,3,5-; and 2,4,5-trichlorophenol. Only 0.01–0.15% of the dose was excreted in the urine in 72 hours following dermal exposure for 6 hours.

In a study in which children infected with scabies and their noninfected siblings were treated dermally with 1% γ-HCH lotion, the blood level was found to diminish rapidly after application, with a half-life of 17.9 hours in infected children and 21.4 hours in noninfected children.

In male rats treated dermally with radiolabelled γ-HCH, 0.28, 0.08, and 0.02% radiolabel were excreted in urine 4 hours after doses of 0.06, 0.6, and 6 mg/cm^2/kg, respectively (Bosch 1987a). After 24 hours, 4.4, 3.2, and 0.6% radiolabel were excreted in urine from the same respective doses. In a similar study with male rabbits, 3.8, 2.6, and 1.3% radiolabel were excreted in urine 4 hours after doses of 0.005, 0.05, and 0.5 mg/cm^2/kg, respectively (Bosch 1987b). After 24 hours, 25.5, 11.6, and 6.8% radiolabel were excreted in urine from the same respective doses.

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

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry

models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

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

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

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) 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 3-5 shows a conceptualized representation of a PBPK model.

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.

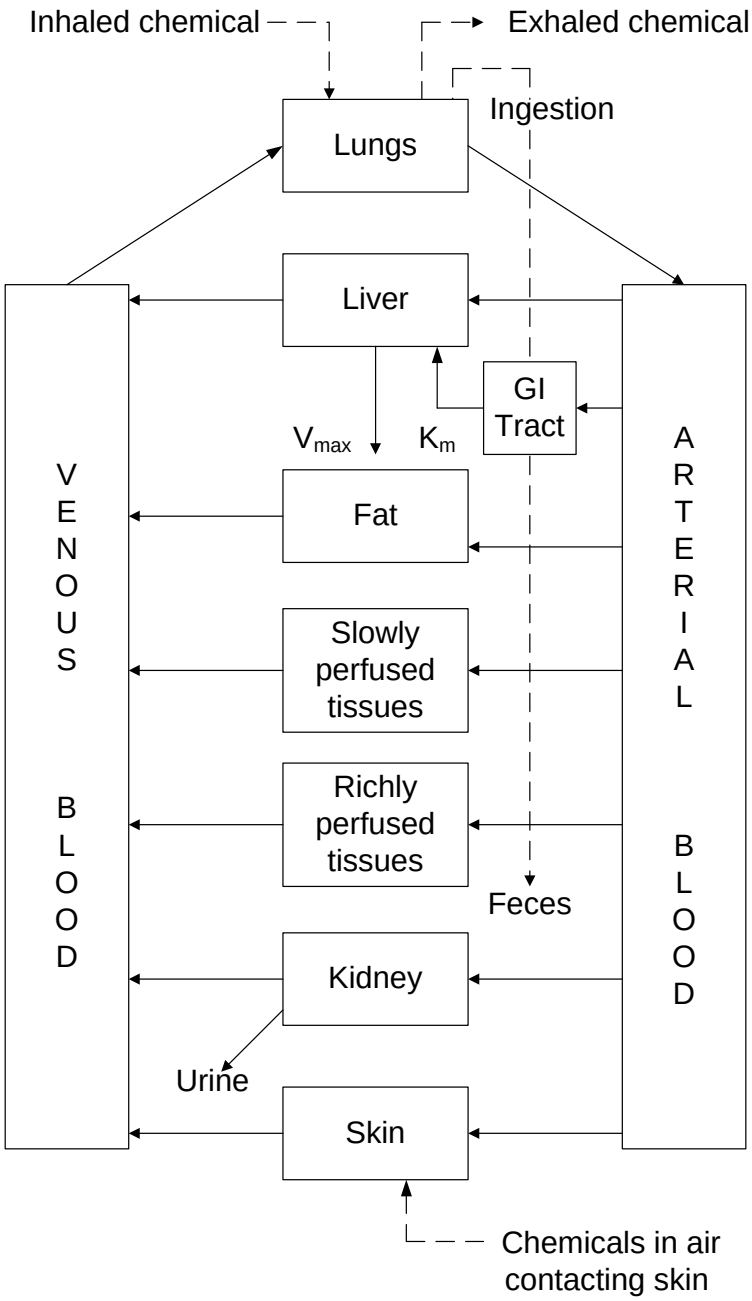
DeJongh and Blaauboer (1997) simulated the toxicokinetics of γ-HCH in rats with a PBPK model. A five-compartment model for the rat as presented in Figure 3-5 was constructed, including (1) the liver, serving as the metabolizing organ; (2) blood; (3) fat; (4) brain; and (5) a lumped compartment representing all other tissues, consisting mainly of muscle tissue. Values for the physiological parameters, tissue-blood partition coefficients, were obtained from the literature and are presented in Figure 3-6 and Table 3-8. The model was calibrated on a dataset from the literature on the disposition of γ-HCH from blood *in vivo* after single oral dosage and first-order biotransformation and gastrointestinal absorption constants for γ-HCH were obtained.

The model was validated by simulating the disposition of γ-HCH *in vivo* after single intraperitoneal and chronic oral dosing and comparing simulated with experimental results. Simulated γ-HCH concentrations in blood, brain, muscle, and fat after single intraperitoneal and chronic oral dosage compared adequately well with experimental results. However, the model is not validated via biological evaluation of kinetic parameters.

There are no PBPK models for HCH in children.

Currently, the Agency of Toxic Substances and Disease Registry is assessing the feasibility of using tools such as PBPK modeling and pharmacodynamic modeling to extrapolate data across routes or durations of exposure. Such extrapolation may be done on a substance-by-substance basis after adequate toxicokinetic information has been collected.

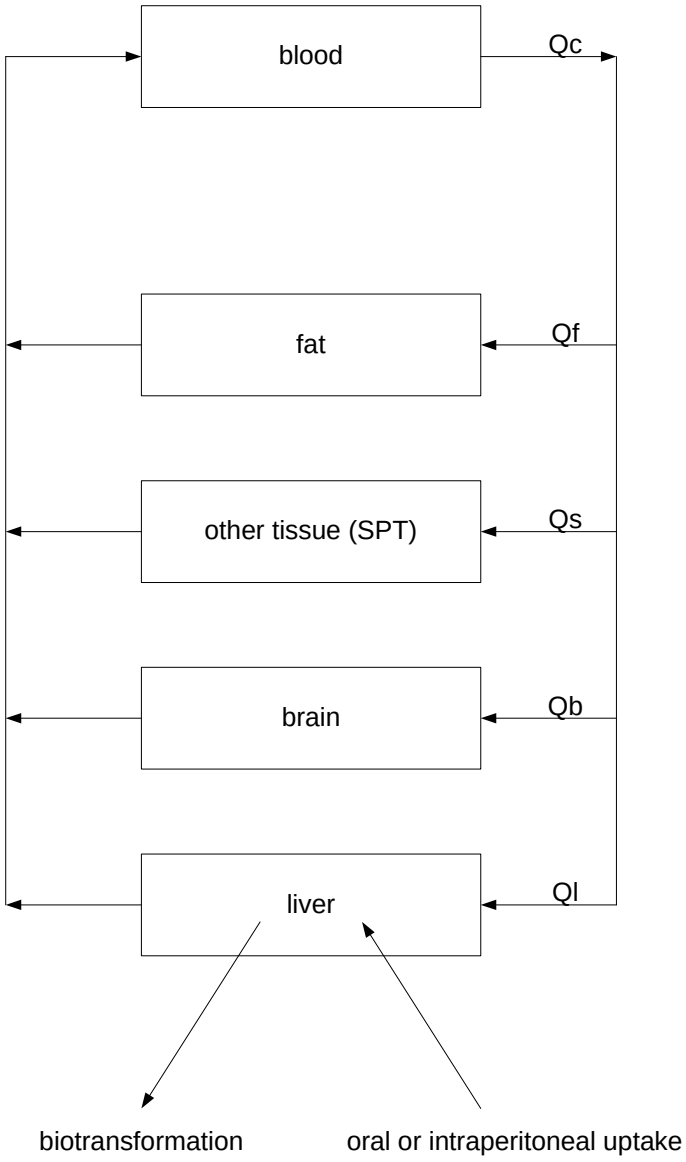
Figure 3-5. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

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

Figure 3-6. Structure of the PBPK Model for γ-HCH*



Source: DeJongh and Blaauboer (1997)

*Model parameters are described in Table 3-8.

Table 3-8. Parameters for a PBPK Model for γ-Hexachlorocyclohexane in Rats

Parameter	Value	Scaling factor
Body weight (kg)	0.135–0.313	
Cardiac output (L/hour kg)[a] $BW^{0.74}$	14	
Blood flow fractions[a]		
Liver	0.25	–
Fat	0.09	–
Other tissues (SPT)	0.63	–
Brain	0.03	–
Tissue group volume fractions		
Blood[a]	0.06	–
Liver[a]	0.04	–
Brain[a]	0.0006	–
Fat[b]	0.2xBW+0.0166	–
Remaining tissues (SPT)	0.894-VFC	–
Partition coefficients for lindane		
Liver-blood[c]	4.2	–
Fat-blood[c]	95.3	–
SPT-blood[c]	1.6	–
Brain-blood[d]	4.1	–
Metabolic and uptake constants		
Biotransformation rate[e] ($hour^{-1}kg^{-1}$)	4.5	$BW^{-0.3}$
Oral/intraperitoneal uptake rate[e] ($hour^{-1}$)	0.035	–
Oral/intraperitoneal uptake efficiency[d]	0.8	–

Source: DeJongh and Blaauboer 1997

BW = body weight; SPT = slowly perfused tissue; VFC = relative adipose tissue mass where VFC=0.2*BW+0.0166

[a]Reference values (Arms and Travis 1988)
[b]Calculated as a function of body weight (Bailey et al. 1980)
[c]Measured *in vitro* (Jepson et al. 1994)
[d]Measured *in vivo* (Oshiba 1972)
[e]Value obtained by calibration

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Information is available to assess the extent and rate of HCH absorption following oral and dermal exposure (Ahdaya et al. 1981; Albro and Thomas 1974; Turner and Shanks 1980). However, inhalation absorption of HCH can only be inferred from toxicity studies and studies assessing the distribution and excretion of γ-HCH. No quantitative information is available to assess the rate and extent of inhalation absorption.

Following oral exposure to γ-HCH in rats, little, if any, metabolism was observed in the gut as indicated by the absence of metabolites in the feces (Kujawa et al. 1977). Rather, it is readily absorbed, where the majority is transported via the blood rather than the lymphatics (Turner and Shanks 1980). HCH, a lipophilic compound, is well distributed in adipose tissue, though whether these compounds are absorbed into tissues via active or passive mechanisms is unknown. HCH metabolism is mediated primarily in the liver by the cytochrome P-450 oxygenase system. The many metabolites of HCH, mostly polychlorophenols, are eliminated primarily in the urine.

Additional data concerning the mechanisms of inhalation and dermal absorption of HCH in animals may provide information to assist in characterizing absorption of HCH in humans.

3.5.2 Mechanisms of Toxicity

In the nervous system, γ-HCH is thought to interfere with GABA neurotransmitter function by interacting with the $GABA_A$ receptor-chloride channel complex at the picrotoxin binding site (Abalis et al. 1985; Anand et al. 1998; Casida and Lawrence 1985; Lawrence and Casida 1984; Pomès et al. 1994). Thus, the seizures caused by γ-HCH can be antagonized by $GABA_A$ mimetics. The δ-HCH isomer has also been shown to act at the picrotoxin binding site, but to a lesser extent (Fishman and Gianutsos 1988). Intraparataneal doses of γ-HCH or picrotoxin administered to rats resulted in epileptic events, but different levels of extracellular excitatory amino acids were observed in the hippocampus, suggesting a difference in mechanism of action (Nyitria et al. 2002). In rat cortical neurons, expression of the protooncogene *c-fos*, which is associated with seizure activity and is induced by elevated intracellular calcium levels, was increased by γ-HCH treatment but decreased by δ-HCH treatment (Barrón et al. 1995). Treatment-related changes in *c-fos* expression suggested that γ-HCH induces seizures through the

activation of calcium channels, while inhibition of calcium channels by δ-HCH results in anticonvulsant effects. The α-HCH isomer, another nonconvulsant, has been shown, like δ-HCH, to suppress c-*fos* induction (Vendrell et al. 1992a). In a study on the cytotoxic action of δ-HCH and γ-HCH in cultured rat cerebellar granule neurons (Rosa et al. 1997), both isomers were found to induce an increase in the free intracellular Ca^{2+} concentration. However, the γ-isomer mainly caused this increase by a release from intracellular Ca^{2+} stores. On the other hand, δ-HCH may exert its action by stimulating a large influx of Ca^{2+}. δ-HCH was found to be more potent and active as a cytotoxic agent than γ-HCH, and the differences in cytotoxicity and neurotoxic action may be related to their action on the different Ca^{2+} pools. Other suggestive data concerning mechanisms by which HCH causes neurological effects in animals include enhanced synaptic activity (Joy 1982; Joy and Albertson 1985) altered GABA functional activity (Bhatt and Panchal 1994; Cattabeni et al. 1983; Fishman and Gianutsos 1987, 1988; Hulth et al. 1978; Joy and Albertson 1985), and inhibition of (McNamara and Krop 1948a; Nakajima 1983; Uchida et al. 1974) or oxidative damage to Na^{+}-K^{+}-ATPase activity (Sahoo and Chainy 1998). In general, the mechanism of toxicity of HCH on the nervous system appears to be similar to those of other neurotoxic organochlorine insecticides.

γ-HCH interacts with cellular membranes and may produce several generalized cytotoxic effects associated with impaired membrane function. In rat renal cortical tubules, glucose uptake and cyclic AMP accumulation were altered by γ-HCH treatment (López-Aparicio et al. 1994). Transport of D-galactose and L-leucine across enterocytes was decreased in chickens injected daily with γ-HCH for 7 days (Moreno et al. 1994). Rats exposed orally to 5 mg/kg/day technical-grade HCH 5 days/week, for 3–6 months, exhibited significantly decreased levels of phosphatidylinositol, phosphatidylinositol 4-phosphate, and phosphatidylinositol 4,5-bisphosphate in the erythrocyte membrane and cerebrum (Agrawal et al. 1995). An *in vitro* study showed that γ-HCH altered the action potential and transmembrane currents in frog heart (atrial) myocytes (Sauviat et al. 2002). γ-HCH also has been shown to block gap junctional intercellular communication in Sertoli cells by inducing the aberrant endocytosis of Connexin 43 and zonula occludens-1 within Rab5 positive endosomes via the activation of the extracellular signal-regulated kinases (Defamie et al. 2001; Mograbi et al. 2003). Inhibition of intercellular communication could potentially lead to uncontrolled cell growth and tumor promotion. γ-HCH inhibited gap junction and intercellular communication in myometrial cell cultures isolated from rats on gestation day 10 by creating an oxidative stress environment (Kreiger and Loch-Caruso 2001; Loch-Caruso et al. 2003). γ-HCH also inhibited spontaneous phasic contractions in late gestation rat uterus (Loch-Caruso et al. 2003).

Oxidative stress in the liver has been suggested as a mechanism of γ-HCH-induced hepatotoxicity (Azzalis et al. 1995; Barros et al. 1988, 1991; Junqueira et al. 1997; Puri and Kohli 1995; Srinivasan and Radhakrishnamurty 1983a; Videla et al. 1991). This condition is characterized in the rat liver by a reduction in hepatic glutathione content, lipid peroxidation, the microsomal generation of superoxide radical coupled to cytochrome P-450 induction, and a decrement in superoxide dismutase and catalase activity (Junqueira et al. 1993). Dose-dependent inhibition of intercellular communication in cultured rat hepatocytes, with subsequent reversal by addition of vitamin E or superoxide dismutase, indicates oxidative stress as a hepatotoxic mechanism (Leibold and Schwarz 1993). Species differences exist in the activities of hepatic metabolizing enzymes, and it has been demonstrated that γ-HCH at a dose of 10 mg/kg/day for 6 days increased the hepatic cytochrome P-450 as well as glutathione-*S*-transferase in the rat, but not in the rabbit or monkey (Puri and Kohli 1995). Thus, oxidative stress and hepatotoxicity are produced with γ-HCH treatment in rats, but not in the rabbit and monkey (Puri and Kohli 1995). Inhibition of Mg^{2+}-ATPase activity has also been observed in rat liver tissue, suggesting an ATPase enzyme sensitivity to the action of γ-HCH (Gopalaswamy and Aiyar 1984). The researchers suggested that some toxic effects appearing in mammals as a result of γ-HCH exposure may arise from its influence on this ATPase activity (Gopalaswamy and Aiyar 1984). An *in vitro* study in mammalian CHO-K1 cells indicated that both γ-HCH and an unspecified HCH isomer mixture induced glutathione peroxidase and glutathione reductase activities as a defense mechanism against oxidative stress (Garcia-Fernandez et al. 2002).

The toxic mechanisms acting on prenatal development are poorly understood. Some studies of γ-HCH in rodents suggest that oxidative stress and depletion of GSH may be developmentally significant. Mouse fetal and placental tissues exhibited increased superoxide production, lipid peroxidation, and DNA-single strand breaks at 48 hours after administration of single dose of 30 mg/kg γ-HCH to pregnant dams on day 12 of gestation (Hassoun and Stohs 1996b). *In vitro* exposures of rat conceptuses to γ-HCH solutions of ≥50 μM on gestational day 10 resulted in significantly lower intracellular levels of GSH compared to controls (McNutt and Harris 1994). Data were not available, however, to determine whether a relationship exists in the prenatal rat between oxidative stress and GSH depletion.

3.5.3 Animal-to-Human Extrapolations

Extrapolating animal toxicity data to predict human risk from HCH exposure appears to be reasonable since similar effects are seen in both species.

Metabolism of HCH isomers is believed to be carried out primarily by the P-450 monooxygenase system in humans and rodents. The presence of chlorophenols and chlorobenzenes in urine of workers occupationally exposed to γ-HCH (Angerer et al. 1983; Engst et al. 1979) was similar to observations of rats experimentally exposed to γ-HCH (Chadwick and Freal 1972a; Chadwick et al. 1978a; Engst et al. 1976; Kujawa et al. 1977). *In vitro* investigations indicate that human liver microsomes convert γ-HCH to chlorocyclohexenes, chlorophenols, and chlorobenzenes (Fitzloff et al. 1982). Both human and rat microsomes have been shown to form an identical epoxide *in vitro* following γ-HCH exposure (Fitzloff and Pan 1984). An important difference in interspecies metabolism of γ-HCH is the production of α-2μ-globulin in the male rat (Dietrich and Swenberg 1990, 1991), a protein not present in humans, which is well known for its role in renal toxicity.

Similar clinical toxic effects resulting from HCH exposure have been observed in laboratory animals dosed experimentally and humans experiencing occupational, therapeutic, and accidental domestic exposures to HCH. These include neurological, hepatic, hematological, and dermatological effects. Though reproductive, immunological, and carcinogenic effects have been reported in occupationally exposed humans and in animals, the human cases (Blair et al. 1998; Kashyap 1986; Tomczak et al. 1981) lack both quantitative exposure data and strong causal associations and also involve concurrent exposures to other chemicals. While rodents appear to be adequate models for a variety of human effects of HCH exposure, care must be taken in interpreting data from reproductive toxicity feeding studies in sheep (Beard and Rawlings 1999; Beard et al. 1999a), since significant differences exist in the gastrointestinal physiology of ruminants and humans.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Clement (1992), was also used in 1996 when Congress mandated the 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 1997). 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).

Studies indicating that γ-HCH may act as an endocrine disruptor are summarized below. The amount of evidence is limited and further investigation is necessary to ascertain the relevance and impact to public health.

Estrogen influences the growth, differentiation, and functioning of various target tissues, including male and female reproductive systems such as mammary gland, uterus, vagina, ovary, testes, epididymis, and prostate. Findings indicative of antiestrogenic activity of oral exposure to γ-HCH include reduced embryo implantation in mice (Sircar and Lahiri 1989), reduced ovulation rate in rabbits (Lindenau et al. 1994), and delayed vaginal opening, disrupted estrous cycling, and reduced uterine weight in rats (Chadwick et al. 1988). Conversely, Raizada et al. (1980) indicated induction of estrogenic activity by γ-HCH based on increased glycogen content of the uterus, cervix, and vagina. Inconsistencies in the classification of estrogenic activity for γ-HCH may have been due to variations in experimental protocols, examination of different end points, and controversy in the interpretation of hormonal effects (Chadwick et al. 1988). Ovariectomized rats exposed for 5 days and sexually immature female rats exposed for 7 days to 40 mg γ-HCH/kg/day showed no effects on the number of estrogen and estrogen-dependent progesterone receptors (Laws et al. 1994), indicating that the antiestrogenic effects of γ-HCH in rat reproductive tissues do not appear to be due to direct action on estrogen receptors or the induction of

progesterone receptors. This is consistent with *in vitro* tests showing that γ-HCH had no significant agonistic action on the estrogen receptor (ER) in the MCF-7 human cell line (Soto et al. 1995), or activity in ER-mediated assays with luciferase reporter systems transfected to MCF-7 and HeLa human cells (Balaguer et al. 1999).

Nativelle-Serpentini et al. (2003) showed that γ-HCH can modulate the activity of human aromatase, the enzyme that catalyzes the aromatization of androgens to estrogens, thus potentially affecting sexual maturation in developing organisms. The study showed that short-term (10 minutes to 18 hours) incubation of human placental JEG-3 cells with γ-HCH increased aromatase activity, whereas longer-term (18 hours) incubation produced dose-related inhibition (Nativelle-Serpentini et al. 2003). This occurred at dose levels that were not cytotoxic. Because aromatase is an enzyme that catalyzes the aromatization of androgens to estrogens, alterations in aromatase activity can have widespread consequences, particularly in developing organisms.

Studies with β-HCH in ovariectomized mice showed that mobilization of this isomer from fat during fasting produced estrogenic effects including stimulation of uterine growth in mice (Bigsby et al. 1997), and that blood and fat levels of the isomer were correlated with the estrogenic end points uterine epithelial height and vaginal epithelial thickness (Ulrich et al. 2000). The blood concentrations of β-HCH that induced these effects in mice were within the same order of magnitude of blood levels of this isomer in some subjects in the general human population. β-HCH has estrogenic action in transfected MCF-7 cells, although there is evidence that this activity is mediated through ligand-independent activation of the ER (Hatakeyama et al. 2002).

The male gonad is a highly sensitive target organ for γ-HCH in animals as discussed in Section 3.2.2.5 (Reproductive Effects). For example, spermatogenesis was reduced in rats as shown by reductions in serum testosterone levels, testicular weight, and/or spermatid and sperm counts, following exposure as adults (6 mg/kg/day for 5 days or a single 30 mg/kg dose) or during gestation (1 mg/kg/day for 5 days or a single 6 or 30 mg/kg dose) (Dalsenter et al. 1996, 1997a, 1997b). Similarly, oral exposure of rats to 15 mg/kg/day γ-HCH for 5 days during gestation caused effects in adult male offspring that included testicular histological alterations, reduced sperm head counts, and increased chromatin abnormalities in epididymal sperm (Traina et al. 2003). Oral exposure to β- or technical-grade-HCH also caused degenerative changes in male reproductive tissues and sperm abnormalities in rats and mice (Dikshith et al. 1991a; Gautam et al. 1989; Nigam et al. 1979; Pius et al. 1990; Roy Chowdhury and Gautam 1990; Van Velsen et al. 1986), and similar effects on male reproductive tissues and spermatogenesis occurred in

rats and guinea pigs following dermal treatment with technical-grade HCH (Dikshith et al. 1978; Prasad et al. 1995).

In vitro exposure to γ-HCH caused depolarization, influx of extracellular Ca^{2+}, and other cell membrane changes in rat testis peritubular myoid cells (PMCs, the smooth muscle cell layer surrounding the seminiferous tubules), suggesting that interference with hormone-regulated PMC function might be involved in testicular toxicity of γ-HCH (Silvestroni et al. 1999). Other *in vitro* effects of γ-HCH included altered sperm responsiveness to progesterone (Silvestroni and Palleschi 1999) and inhibition of testicular steroidogenesis in rat Leydig cells (Ronco et al. 2001). Testing of HCH isomers for activity in an *in vitro* androgen receptor assay using a human PC-3 LUCAR- prostate carcinoma cell line showed that α- and β-HCH interacted with the human androgen receptor as agonists, whereas γ- and β-HCH had no agonist or antagonist activity (Schrader and Cooke 2000). Another isomer comparison study found that *in vitro* exposure to γ-, α-, and δ-HCH (only isomers tested) inhibited (Bu2) cAMP-stimulated progesterone production by mouse MA-10 Leydig tumor cells (Walsh and Stocco 2000).

3.7 CHILDREN'S SUSCEPTIBILITY

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

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

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics

and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

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

Limited information is available on the specific health effects resulting from HCH exposure in children. Generally, health effects observed in adults should also be of potential concern in children. Occasional deaths of children have been reported following ingestion of γ-HCH (Storen 1955). Although a causal relationship between exposure to γ-HCH and hematological effects in humans has not been established, there is one case report of hypochromic anemia and another of aplastic anemia in children exposed to γ-HCH by inhalation (Morgan et al. 1980; Rugman and Cosstick 1990). There are also sporadic reports of adverse effects of γ-HCH including convulsions in children after excessive topical application of γ-HCH (Lee and Groth 1977; Matsuoka 1981; Nordt and Chew 2000; Ramchander et al. 1991; Telch and

Jarvis 1982; Tenebien 1991). Based on animal data as discussed below, it can be inferred that children may be more susceptible than adults to some effects of HCH isomers.

Neurological effects have been observed in immature animals exposed to γ-HCH via gestation and/or lactation. A developmental neurotoxicity study found several changes, including increased motor activity and reduced auditory startle response habituation, in 11-day-old offspring of maternal rats that were exposed to ≥5.6 mg/kg/day doses of γ-HCH (γ-HCH) in the diet from gestation day 6 through lactation day 10 (Myers 1999). Epileptiform seizures occurred in rat pups that were exposed to maternal milk from dams that were exposed to 20 mg γ-HCH/kg by gavage for 12 days on postnatal days 3–15 (Albertson et al. 1985). Weanling rabbits were more sensitive to γ-HCH than young adults, as seen by increased mortality rates accompanied by excitement and convulsions after a single whole-body treatment with a 1% solution (60 mg γ-HCH/kg) that was absorbed dermally (Hanig et al. 1976). Although the data from these studies suggest that γ-HCH can be transferred via the placenta and maternal milk and elicit functional neurological effects in offspring, the actual doses received by the young animals is not known.

There is evidence that γ-HCH caused functional impairment of the developing blood brain barrier (BBB) in young rats (Gupta et al. 1999). The integrity (permeability) of the BBB was studied by assessing uptake of sodium fluorescein (a micromolecular tracer dye) into the brain of neonatal rats following single or repeated acute gavage doses of γ-HCH. The brain uptake index of fluorescein was significantly increased in 10-day-old pups treated with a single 2 mg/kg dose (72 and 23% higher than controls after 2 hours and 3 days, respectively), as well as in those treated with 2 mg/kg/day for 8 days (50% higher than controls 7 days after the first exposure, with recovery 20 days after the first exposure). The effect appeared to be age-related because the brain uptake index was lower when rats were administered a single 2 mg/kg dose at 15 days of age (20% higher than controls after 2 hours) or a higher dose of 4 mg/kg/day for 3 days as adults (no effect on brain permeability).

Alterations in cerebral levels of noradrenaline, serotonin, and dopamine were observed in suckling rats treated intragastrically with a single dose of 20 mg/kg γ-HCH during the postnatal period (Rivera et al. 1991). Levels of noradrenalin were reduced in the mesencephalon. Concentrations of a serotonin metabolite were increased in the frontal cortex primarily on postnatal days 8 and 15, but the results were not statistically significant. Levels of a dopamine metabolite were decreased in the mesencephalon, but statistical significance was only obtained on postnatal day 15 (+44%, $p<0.05$). According to the authors, earlier experiments demonstrated that higher doses of γ-HCH were required to increase serotonin in adult rats. Alterations in levels of brain dopamine, serotonin, GABA, glutamate, glutamate decarboxylase, and

noradrenaline were seen in various areas of the brains of female rat pups treated orally with 10 mg technical-grade HCH/kg/day for 60 days (Nagaraja and Desiraju 1994). Acquisition of a passive avoidance task was improved in 15-day-old rat pups that were orally treated with γ-HCH as either a single 20 mg/kg dose or 7-day repeated 10 mg/kg/day doses, although changes in motor activity and brain monoaminergic levels (e.g., ratios of 5-HIAA/serotonin and DOPAC/dopamine) depended on the treatment schedule (Rivera et al. 1998).

No direct information is available regarding the effects of HCH on the developmental process in humans. However, developmental studies in animals indicated few effects from exposure to γ-HCH (Khera et al. 1979; Hassoun and Stohs 1996a; Srinivasan et al. 1991a); significant teratogenic effects were not observed (Khera et al. 1978). The proportion of embryos lost after implantation was increased after minks were treated with 1 mg/kg/day γ-HCH in the diet (Beard et al. 1997). An increase in the incidence of fetuses with extra ribs was reported in rats exposed to 20 mg/kg/day γ-HCH during gestation days 6–16 and in rabbits exposed during days 6–18 (Palmer et al. 1978a). However, the incidence of extra ribs were within or just greater than the ranges recorded for the control groups, and therefore, may not be significant evidence of teratogenicity caused by exposure to γ-HCH (Hassoun and Stohs 1996a). β-HCH given to rat dams at 20 mg/kg/day during gestation caused increased fetal deaths within 5 days of birth (Srinivasan et al. 1991a). In another study, cadmium interacted with γ-HCH to cause significant embryotoxic and teratogenic effects in the developing rat fetus when administered together at a dosage that for either toxin alone is insufficient to cause any deleterious effects in development (Saxena et al. 1986).

β-HCH is lipophilic and accumulates in maternal adipose tissue and may be mobilized during pregnancy and lactation. HCH residues have been measured in human skin lipids (Dua et al. 1998) and in breastmilk (Czaja et al. 1997; Dua et al. 1997; Nair et al. 1996); HCH also crosses the placenta (Saxena et al. 1981b). Its levels in placenta, maternal blood, and umbilical-cord blood were higher in cases of stillbirths than in live-born cases; however, many other organochlorine pesticides were present that could have contributed to stillbirths (Saxena et al. 1983). γ-, α-, δ-, and total HCH maternal blood and umbilical-cord blood levels were also higher in mothers who gave birth to IUGR babies (Siddiqui et al. 2003). Similar to the Saxena et al. (1983) study, other organochlorine pestides were also present in the blood that could have contributed to the IUGR. In a study in rats, γ-HCH has been reported to be transferred in the maternal milk and to elicit neurological effects in neonates. Following intraperitoneal dosing of dams with γ-HCH on days 12–17 of gestation, $GABA_A$ receptors in rat fetuses were studied with radiolabelled t-butylbicyclophosphorothionate (TBPS), a ligand that binds to the $GABA_A$ receptor (Brannen et al.

1998). Treatment with γ-HCH significantly reduced the TBPS binding affinity in fetal brainstems and it was concluded that the effect could potentially lead to abnormal brain activity, increased susceptibility to seizures, and behavioral effects. Also noted in the study, was reduced TBPS binding in brains of fetuses when compared to adults. In another study, lactating female rats were treated orally with a single dose of 6 mg/kg of γ-HCH on days 9 or 14, or with 1 mg/kg on days 9–14 of lactation; the testosterone level of the male offspring was reduced 50% at puberty (day 65) when compared to the control group (Dalsenter et al. 1997b). When the offspring reached adulthood (day 140 postnatal), the relative testicular weight was significantly lower (Dalsenter et al. 1997b). The number of sperm and spermatids was also significantly reduced.

Differences in oxidative effects have been observed in the testes of young versus mature rats, 15 and 90 days old respectively, following intrapertitoneal injection with 10 or 20 mg/kg technical-grade HCH (Samanta and Chainey 1997b). Lipid peroxidation occurred to a greater extent in mature rats. However, the percent decrease in cytosolic superoxide dismutase activity was greater in young rats, which have increased baseline activity of the enzyme. Based on the findings of this study, it does not appear that young rats are at increased risk of oxidative testicular damage.

Although it is unknown whether the ability to metabolize HCH specifically differs between children and adults, some enzymes, which belong to the enzyme superfamilies involved in phase II HCH metabolism, are developmentally regulated in humans. The development of UDP-glucuronosyltransferase (responsible for glucuronide conjugation) depends on the enzyme isoform, but, in general, adult activity is attained by 6–18 months of age (Leeder and Kearns 1997). Development of sulfotransferase (responsible for sulfate conjugates) activity is also substrate specific and is usually earlier than UDP-glucuronosyl-transferase. In fact, levels of some sulfotransferases may be greater during infancy and early childhood than during adulthood (Leeder and Kearns 1997). A series of enzymes are involved in the production of mercapturic acid conjugates: γ-glutamyltranspeptidase, glutathione S-transferase, cysteinyl glycinase, and N-acetyl transferase (Sipes and Gandolfi 1991). There are two superfamilies of N-acetyltransferase, and the N-acetyltransferase 2 superfamily has members that are developmentally regulated in humans. There is some N-acetyltransferase 2 activity in fetuses by 16 weeks of gestation. Infants up to 2 months of age have the slow metabolizer phenotype (there is a genetic polymorphism in this enzyme in adults). The adult distribution of slow and fast metabolizer phenotypes is reached by 4–6 months of age and full adult activity is achieved at 1–3 years of age (Leeder and Kearns 1997).

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

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

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to HCH 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 HCH 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 Hexachlorocyclohexane

There are few quantitative data to correlate levels of any of the HCH isomers in human tissue or fluids with environmental levels. A study in which children infected with scabies and their noninfected siblings were treated dermally with 1% γ-HCH lotion found no correlation between the dose applied and the subsequent level of γ-HCH in blood (Ginsburg et al. 1977). The blood level was also seen to diminish rapidly after application, with a half-life of 17.9 hours in infected children and 21.4 hours in noninfected children.

In contrast, β-HCH persists in the blood for a longer period of time than the other isomers. A study of workers in a γ-HCH -producing factory found that levels of β-HCH in blood serum were higher than those of other isomers, and there was a significant correlation between serum levels of β-HCH and length of employment (Baumann et al. 1980). Studies of populations with general HCH exposure have consistently found the level of the β-isomer to be higher than those of the other isomers (Kashyap 1986; Nigam et al. 1986; Ramachandran et al. 1984). This is probably due to the greater tendency of β-HCH to persist and accumulate in the body, while the other isomers are more rapidly metabolized or excreted. A survey of epidemiological studies involving workers occupationally exposed to "crude benzene hexachloride" as much as 10–15 years prior to sampling reported serum levels of 20–348 μg/L β-HCH (Morgan and Lin 1978). Unfortunately, none of the above studies specified exposure levels, so it is still questionable whether blood HCH levels can be used as biomarkers to quantify exposure.

There is also a direct correlation between HCH levels in the blood and human adipose tissue and semen (Baumann et al. 1980; Radomski et al. 1971a, 1971b; Szymczynski and Waliszewski 1981); concentrations of β-HCH in subcutaneous adipose tissues were found to be 300 times higher than blood levels (Baumann et al. 1980). Levels of β-HCH detected in skin lipids correlated with those found in human adipose tissue (Sasaki et al. 1991b). Although exposure levels were not known, the results of this study indicate that measuring β-HCH in skin lipids can be an easy means of determining relative levels or times of individual exposure. The method of collecting the skin lipid samples was noninvasive, involving washing the face with soap and wiping 3–4 hours later with fat-free cotton soaked in 70% ethanol. β- and γ-HCH have also been found in samples of human maternal adipose tissue, maternal blood, cord blood, and breast milk in women who were exposed to unknown levels of various organochlorine pesticides in

Kenya (Kanja et al. 1992). The metabolites of γ-HCH have been detected in human urine (Angerer et al. 1981). However, such findings are not specific to γ-HCH exposure, and these findings could follow from exposure to both γ-HCH and a number of structurally related compounds.

3.8.2 Biomarkers Used to Characterize Effects Caused by Hexachlorocyclohexane

The individual isomers of HCH can be detected in the blood serum, urine, adipose tissue, and semen of exposed individuals. However, the concentrations measured in these biological tissues have not been exclusively correlated with the degree of adverse health effects observed. Additionally, there are no general biomarkers of effect for HCHs analogous to red blood cell or plasma cholinesterase for organophosphorous insecticides.

Adverse effects such as neurophysiological and neuropsychological disorders and gastrointestinal disturbances have been reported in workers exposed to HCH during pesticide or fertilizer formulation. Nigam et al. (1986) and Kashyap (1986) reported that nonhandlers indirectly exposed and handlers directly exposed to HCH during pesticide manufacture and formulation were found to have mean serum levels of 0.27 ppm (nonhandlers) and 0.6 ppm (handlers) total HCH. As much as 60–100% of the total HCH measured in serum was β-HCH. The ranges of serum HCH levels measured in all exposed workers were 0.07–0.72 ppm β-HCH, 0.004–0.18 ppm α-HCH, 0–0.17 ppm γ-HCH, and 0–0.16 ppm δ-HCH. Both handlers and nonhandlers complained of paresthesia of the face and extremities, headache, and giddiness; other symptoms included malaise, vomiting, tremors, apprehension, confusion, loss of sleep, impaired memory, and loss of libido. Similar but less-severe effects were noted in 19 maintenance workers who visited the plant frequently. Serum HCH levels measured in these workers were 0.004–0.1 ppm α-HCH, 0.02-0.2 ppm β-HCH, 0–0.32 ppm γ-HCH, and 0–0.04 ppm δ-HCH. Kashyap (1986) also reported higher serum enzyme levels of alkaline phosphatase, lactate dehydrogenase, ornithine carbamyl transferase, γ-glutamyl transpeptidase, and leucine aminopeptidase and increased IgM in the handlers as compared with the nonhandlers and a control population of 14 workers with no occupational contact with HCH. Czegledi-Janko and Avar (1970) reported that γ-HCH blood levels of 0.024–0.16 ppm were associated with clinical symptoms including muscle jerking and variations in EEG in 37 workers exposed to γ-HCH in a fertilizer plant.

HCH and other organochlorine pesticides have been found in the blood serum of some individuals in a population of men attending an infertility clinic in Israel. Serum levels of organochlorine pesticides, including γ-HCH, have been found in men with low sperm counts to be two times higher than that of

fertile men (Pines et al. 1987). Maternal mean serum γ-HCH levels were reported to be higher in cases of premature delivery and spontaneous abortions than in controls (Saxena et al. 1980; Wassermann et al. 1982). Saxena et al. (1980) reported HCH levels of 69.3–550.4 ppb and γ-HCH levels of 30.8–113.6 ppb in the blood of women in India who had experienced spontaneous abortions or premature labor compared with blood HCH levels of 22.2–85.5 ppb and γ-HCH levels of 7.1–32.5 ppb in women who had undergone full-term pregnancy. β-HCH levels were significantly higher in German women with a history of miscarriages (Gerhard et al. 1999). Serum levels of a number of other pesticides including aldrin, PCP, PCB, HCB, DDE, DDT, and DDD were also found to be higher in cases of premature labor and spontaneous abortions. It was, therefore, not possible to establish a quantitative, causal relationship between the serum HCH levels and these adverse effects.

Blood serum levels of 1–17 ppb β-HCH were not found to be associated with the incidence of colorectal adenocarcinoma in 10 families (Caldwell et al. 1981). Serum levels of 0–49.5 ppb γ-HCH were not found to be associated with the occurrence of hematological syndromes such as pancytopenia, thrombocytopenia, plasma cell myoma, acute leukemia, chronic lymphocytic leukemia, and anemia in 103 patients (Traczyk et al. 1977).

For more information on biomarkers for renal and hepatic effects of chemicals, see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (1990) and for information on biomarkers for neurological effects, see OTA (1990).

3.9 INTERACTIONS WITH OTHER CHEMICALS

Guinea pigs maintained on diets deficient in vitamin C and protein showed altered γ-HCH metabolism and excretion. Vitamin C deficiency decreased the amount of γ-HCH and its metabolites excreted in the urine and increased the amount stored in the kidney (Chadwick et al. 1972c). Vitamin A supplements decreased HCH-induced toxicity in the rat testes, while deficiencies in vitamin A potentiated the toxicity (Pius et al. 1990).

Cadmium, which is known to inhibit hepatic drug-metabolizing enzymes in mammals, also inhibited the metabolism of γ-HCH in adult male Wistar rats exposed to the compound after short- and long-term pretreatment with cadmium (Chadwick et al. 1978b). Liver microsomal enzymes affected by exposure were γ-HCH dehydrogenase, γ-HCH dechlorinase, and hepatic cytochrome P-450 content. This action altered the profile of metabolites excreted in the urine. Cadmium may inhibit γ-HCH metabolism

indirectly by increasing levels of zinc and reducing levels of copper in the liver (Chadwick et al. 1978b). The addition of cadmium to the diet also increased the concentration of γ-HCH measured in the plasma and liver (Khanna et al. 1988). Cadmium also interacts with γ-HCH to cause significant embryotoxic and teratogenic effects in the developing rat fetus when administered together at a dosage that, for either toxin alone, is insufficient to cause any deleterious effects on development (Saxena et al. 1986).

A low-protein diet potentiated the effects of γ-HCH on reducing the weights of various organs in male rats (Khanna et al. 1990). Serum and liver lipid contents and cholesterol levels were increased in animals fed low-protein diets. The low-protein diet increased the levels of γ-HCH found in the various organ tissues.

The combined application of HCH (mixed isomers) and malathion to the skin of guinea pigs for 30 days showed no significant influence of either chemical on neurological signs of toxicity before dying (e.g., tremors, dyspnea, salivation, convulsions, and paralysis of the hind limbs) or mortality induced by the other (Dikshith et al. 1987). The study suggests that HCH isomers and malathion did not elicit any potentiation effects at the doses tested (50 and 100 mg/kg HCH, 200 and 400 mg/kg malathion).

γ-HCH is a central nervous system stimulant, whereas the α-, β-, and δ-isomers of HCH are mainly depressants (McNamara and Krop 1948a; Smith 1991). Isomeric interactions can occur, such that α-, β-, and δ-HCH counteract the effects of the γ-isomer (lindane); neurotoxicity is reduced when a dose of δ-HCH is accompanied by an equal or higher dose of the other isomers. These interactions likely account for differences in the neurotoxicity of γ-HCH and technical HCH, the majority of which is comprised of isomers other than γ-HCH (60–70% α-HCH, 5–12% β-HCH, 10–15% γ-HCH, 6–10% δ-HCH, and 3–4% ε-HCH [Baumann et al. 1980; Kutz et al. 1991]).

The metabolism of γ-HCH can be altered by exposure to other chlorinated hydrocarbon insecticides such as DDT. Exposure to various chlorinated hydrocarbon insecticides, including γ-HCH, is thought to produce generalized nonspecific induction of microsomal enzymes, including cytochrome P-450. Induction of these enzymes could affect the toxicokinetics of a variety of xenobiotics that are metabolized through microsomal oxidation. Induction of mixed-function oxidase activity by other chlorinated hydrocarbon insecticides stimulates the selective effect on the oxidative degradation of γ-HCH to the tetrachlorophenols and enhances its elimination in the urine (Chadwick and Freal 1972b). In addition, since HCH is hepatotoxic, therapeutic agents, which can produce liver toxicity, such as acetaminophen, might also enhance the symptoms of HCH exposure.

Single daily doses of 20 mg/kg γ-HCH in mice significantly reduced the convulsive threshold, as measured by the dose of pentylenetetrazol required to induce seizures 1–4 hours after treatment, but increased the convulsive threshold 48 hours following treatment (Hulth et al. 1978). A dose of 50 mg/kg γ-HCH significantly increased the convulsive threshold 2, 4, and 10 days following dosing. A single dose of α-HCH significantly increased the convulsive threshold 3 and 24 hours after dosing and resulted in a significant 17% increase in brain levels of γ-aminobutyric acid (GABA) 24 hours after dosing.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to HCH than will most persons exposed to the same level of HCH 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 HCH, or compromised function of organs affected by HCH. Populations who are at greater risk due to their unusually high exposure to HCH are discussed in Section 6.7, Populations with Potentially High Exposures. Based on information from animal studies as discussed in Section 3.7, Children's Susceptibility, it can be inferred that children may be more susceptible than adults to some effects of HCH isomers. Individuals possessing genetic polymorphisms affecting HCH metabolism and elimination may be more susceptible, though supporting data do not exist.

People with excoriated (peeling) skin exhibited higher levels in blood of γ-HCH following dermal exposure to γ-HCH lotion than those with normal skin (Ginsburg et al. 1977). It was not known if there were any increased toxic effects to individuals with excoriated skin. It is also not known with certainty if children are unusually susceptible to the toxic effects of HCH, but case reports of acute neurotoxicity in children treated for scabies with γ-HCH suggest that it should not be used on infants and young children (Telch and Jarvis 1982). The potential hazards of using γ-HCH dermal preparations on infants and young children are underscored by the fact that the very young have a large surface area-to-volume ratio, possibly less efficient hepatic detoxification abilities, and are more likely to lick treated skin (Kramer et al. 1980). Therefore, the use of γ-HCH as a scabicide on infants and very young children, especially those who have very little body fat, has been discouraged (Telch and Jarvis 1982).

Evidence suggests that pregnant women should exercise extreme caution in their exposure to γ-HCH (Ginsburg et al. 1977; Kramer et al. 1980; Solomon et al. 1977a). Refer to Section 3.7 for more detailed explanation. In pregnant animals and humans, γ-HCH crosses the placenta. HCH and γ-HCH body tissue

levels have also been associated with premature labor, spontaneous abortions, and IUGR in babies (Rasmussen 1980; Saxena et al. 1980, 1981a, 1981b; Siddiqui et al. 2003; Wassermann et al. 1982). However, no causal relationship has been established between blood and tissue levels of γ-HCH and premature termination of pregnancy.

Nair et al. (1996) demonstrated that there is a significant bioconcentration of the α-, β-, and γ- isomers of HCH in the breastmilk of mothers exposed to technical-grade HCH.

People with lowered convulsion thresholds due to epilepsy (treated or untreated), cerebrovascular accidents, or head injuries may be at greater risk of the central nervous system effects of γ-HCH toxicity and may suffer increased risk of or severity of seizures (Kramer et al. 1980; Matsuoka 1981). Those individuals suffering from malnutrition (e.g., low protein, low fiber, and low vitamin intake) may be more susceptible than the general public to the toxic effects of γ-HCH (Rasmussen 1987). Individuals with liver and/or kidney disease may be at risk because of compromised detoxification mechanisms in the liver and impaired excretory mechanisms in the kidney. Additionally, individuals with existing or suspected immunodeficiencies may be at risk because HCH isomers may enhance immunosuppression.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to HCH. 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 HCH. 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 HCH:

Reigart JR, Roberts JR. 1999. Recognition and management of pesticide poisonings. 5th edition. Washington, DC: U.S. Environmental Protection Agency, 55-61.

Goldfrank L, Flomenbaum N, Lewin N, et al. 2002. Goldfrank's toxicologic emergencies. 7th edition. New York, NY: McGraw-Hill, 1366-1378.

Ellenhorn MJ, Barceloux DG. 1988. Medical toxicology: Diagnosis and treatment of human poisoning. New York, NY: Elsevier, 1078-1080.

3.11.1 Reducing Peak Absorption Following Exposure

When a large amount of HCH has been swallowed, emetics have been used to induce vomiting. One of the problems with inducing vomiting is that the insecticidal form of HCH is often dissolved in an organic solvent, which presents an aspiration hazard. Activated charcoal can also be used to decrease gastrointestinal absorption. To avoid skin absorption after exposure, clothing should be removed, and the skin should be washed with water and mild soap (Ellenhorn and Barceloux 1988). There are no known methods for reducing absorption following inhalation exposure.

3.11.2 Reducing Body Burden

The traditional methods of increasing elimination or decreasing distribution (e.g., dialysis, diuresis, and hemoperfusion) are not useful because of the high volume of distribution of HCH into adipose tissue (Ellenhorn and Barceloux 1988). HCH accumulates in adipose tissue following all routes of exposure. However, peritoneal dialysis may be required if rhabdomyolysis (muscle necrosis) leads to myoglobinuria and kidney shutdown (Sunder Ram Rao et al. 1988).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Possible mechanisms of action of HCH on some of the target organs have been described. In the nervous system, γ-HCH is thought to interfere with the GABA system by interacting with the $GABA_A$ receptor-ionophore complex at the picrotoxin binding site (Portig and Schnorr 1988; Rivera et al. 1991; Sunol et al. 1988). Thus, the seizures caused by γ-HCH can be antagonized by $GABA_A$ mimetics; diazepam is the anticonvulsant of choice (Ellenhorn and Barceloux 1988). Phenobarbital and/or phenytoin or fosphenytoin may be used if seizures are uncontrollable (HSDB 1998). Use of anticonvulsants (especially in children and other susceptible individuals) should include careful monitoring of hypotension, respiratory depression, and the need for endotracheal interbation. In the liver, γ-HCH is thought to produce oxidative stress by inducing oxidative enzymes such as cytochrome P-450 and depleting hepatic glutathione content (Barros et al. 1988, 1991; Srinivasan and Radhakrishnamurty 1988; Videla et al. 1991). Another possible mechanism for hepatic toxicity is increased lipid metabolism (Ravinder et al. 1990; Srinivasan and Radhakrishnamurty 1988). It is possible that interfering with these mechanisms can decrease the toxicity of HCH.

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 HCH is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the iniiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of HCH.

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.

Most of the literature reviewed concerning the health effects of inhaled α-, β-, γ-, or δ-HCH in humans consists of case reports of individuals occupationally exposed or exposed in the home by a γ-HCH vaporizer. The predominant route of exposure in occupational studies is presumed to be inhalation, although dermal exposure is also likely. The health effects in humans associated with ingested HCH are reported primarily in case studies in which individuals ingested pesticide pellets or therapeutic lotions containing γ-HCH to control scabies, and in several epidemiological studies where exposure is likely through ingestion of pesticide residues in the diet. Information concerning the health effects of HCH in humans following dermal exposure is limited to case studies of individuals who have misused therapeutic lotions containing γ-HCH to control scabies and head and body lice. The duration and level of exposure to HCH generally cannot be quantified from the information presented in these reports. In addition, the case study reports in humans are limited because concomitant exposure to other toxic substances or other substances present in the atmosphere may have occurred.

Limited information was found regarding the health effects of γ-HCH following inhalation exposure in animals. The health effects of α-, β-, γ-, and δ-HCH following oral exposure have been well documented in a variety of species. Limited information is available concerning the health effects of technical-grade HCH and γ-HCH following dermal exposure.

γ-HCH is the isomer most thoroughly tested in intermediate- and chronic-duration studies. The carcinogenic effects of technical-grade HCH and α-, β-, and γ-HCH have been examined, but the carcinogenic potential of δ-HCH has not been as well studied. Studies on the long-term effects of dermal exposure to γ-HCH are inadequate for the determination of carcinogenicity status.

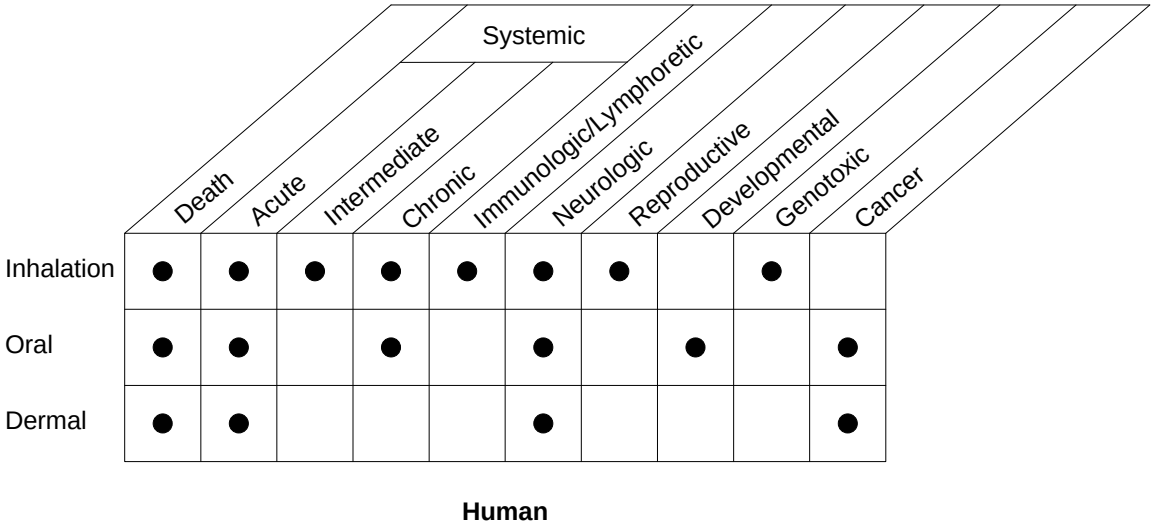
3.12.1 Existing Information on Health Effects of Hexachlorocyclohexane

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to HCH are summarized in Figure 3-7. The purpose of this figure is to illustrate the existing information concerning the health effects of HCH. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

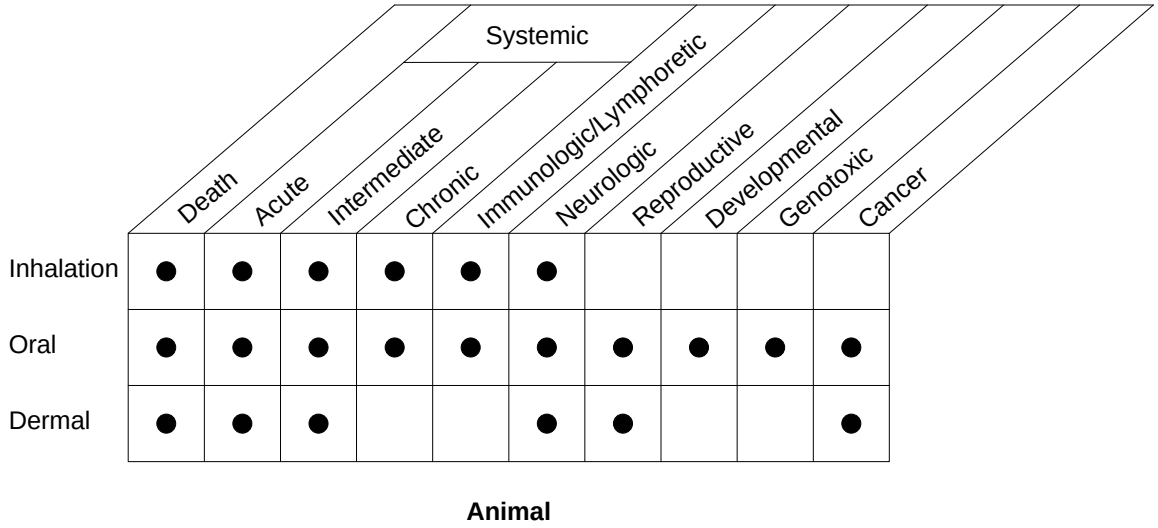
3.12.2 Identification of Data Needs

Acute-Duration Exposure. Occasional case reports are available for humans who have had adverse health effects, including irritation of the nose and throat and death, from excessive inhalation exposure from γ-HCH vaporizers (Conley 1952; Loge 1965). Oral exposure to large amounts has resulted in a few human deaths (Storen 1955; Sunder Ram Rao et al. 1988) and adverse neurological, musculoskeletal, and renal effects (Munk and Nantel 1977; Sunder Ram Rao et al. 1988). When applied dermally, γ-HCH has also been shown to have adverse effects such as pulmonary and epicardial petechiae, aplastic anemia, and rashes in a few humans (Davis et al. 1992; Fagan et al. 1981; Rauch et al. 1990). The level of exposure in the human studies generally cannot be quantified because the information is derived from anecdotal case reports. Therefore, there is little reliable information in humans associating dose with effect. Such information might allow investigators to establish thresholds for systemic toxicity due to acute exposure, although it is not necessarily a priority data need.

Figure 3-7. Existing Information on Health Effects of α-, β-, γ-, and δ-HCH



Human



Animal

● Existing Studies

Information on health effects (death and neurological) following acute inhalation of γ-HCH in animals (Klonne and Kintigh 1988; Oldiges et al. 1980; Ullmann 1986b) is limited. Neurological effects following acute inhalation exposure to γ-HCH include excitation, sedation, ataxia, and spasms (Ullmann 1986b). Acute inhalation studies for the other HCH isomers and technical-grade HCH are not available. No acute inhalation MRL was developed because of insufficient data. Additional acute inhalation data are needed for all isomers (e.g., threshold, dose-response, and target organ). This information is necessary for determining levels of significant human exposure to hexachlorocyclohexane and the associated effects following exposure.

Acute oral studies in animals exposed to γ-HCH have reported death in rats (Gaines 1960) and mice (Liu and Morgan 1986); neurological effects in rats including enhanced susceptibility to kindling (Gilbert and Mack 1995; Joy et al. 1982), reduced brain serotonin level (Attia et al. 1991), reduced brain barrier permeability in 10-day-old pups (Gupta et al. 1999), and neurobehavioral changes (Hughes 1999a); increased hepatic microsomal mixed-function oxidase activity in mice (Oesch et al. 1982); increased hepatic cytochrome P-450 and P-450-dependent enzyme levels and increased absolute liver weight (Parmar et al. 2003), and degeneration of renal tubular epithelia in rats (Srinivasan et al. 1984). Oral exposure to β-HCH has resulted in an increase in hepatic cytochrome P-450 levels, and renal tubular degeneration in rats (Ikegami et al. 1991b; Srinivasan et al. 1984). Exposure to technical-grade HCH has resulted in hepatic focal necrosis, fatty changes, and enzyme activation and renal hemorrhage (Dickshith et al. 1990; Phillip et al. 1989; Ravinder et al. 1989). An acute oral MRL of 0.5 mg/kg/day for β-HCH has been developed based on ataxia in mice (Van Velsen et al. 1986). Additional studies that examine systemic effects (e.g., cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, and renal) following acute oral exposure to all HCH isomers would be helpful. Acute dermal studies in rats are available on γ-HCH and technical-grade HCH (Dikshith et al. 1991c; Gaines 1960). Acute dermal exposure of rats to γ-HCH (Gaines 1960) or of guinea pigs to technical-grade HCH (Dikshith et al. 1978) was associated with lethality. Additional acute dermal data in animals are needed, for example, threshold, dose-response, and target organs. This information is necessary for determining levels of significant human exposure to HCH and the associated health effects following dermal exposure, and is particularly important for γ-HCH given the use of γ-HCH in shampoos and lotions for the pharmaceutical treatment of scabies and head lice.

Intermediate-Duration Exposure. Information on human health effects of repeated exposure to HCH is available from studies of occupationally exposed individuals (Kashyap 1986); no information is

available on the effects of repeated oral or dermal exposure in humans. EEG abnormalities and increased liver enzymes have been observed in factory workers involved in the production of technical-grade HCH (Kashyap 1986). The exact duration and level of exposure in the human studies are often not provided in the studies. Such information would allow investigators to determine health effects associated with known levels of exposure.

Intermediate-duration inhalation studies of γ-HCH have been performed in rats with mortality reported (Klonne and Kintigh 1988). Inhalation of 603 mg/m^3 γ-HCH for 4 hours or 5 mg/m^3 for 90 days has not resulted in adverse respiratory, hematological, hepatic, or renal effects in rats (Oldiges et al. 1983). However, the data are insufficient for developing an intermediate-inhalation MRL. Additional intermediate-inhalation data in animals are needed (e.g., threshold, dose-response, and target organs). This information is necessary for determining levels of significant human exposure to HCH and the associated health effects following inhalation.

Intermediate-duration oral studies have been performed in animals. Oral γ-HCH did not affect the hematological parameter in rats (Suter 1983) and dogs (Rivett et al. 1978). A decrease in blood cell numbers was observed in rats fed β-HCH (Van Velsen et al. 1986) and technical-grade HCH (Joseph et al. 1992c). The endocrine effects of γ-HCH exposure were reported in ewe lambs (Beard and Rawlings 1999) and young rams (Beard et al. 1999a) given 1 mg/kg/day in treated feed from conception to sexual maturity. In ewes, effects included increased pulse frequency of serum luteinizing hormone, slower increase and earlier decrease of progesterone levels, and lower T4 levels. In young rams, observed endocrine effects included lower serum luteinizing hormone and estrodiol concentrations. While serum testosterone levels were similar across treatment groups, the γ-HCH treated rams showed attenuated testosterone response to stimulation with gonadotropin releasing hormone. Hepatic effects in animals following γ-HCH exposure included an increase in P-450-dependent enzymes, hypertrophy, necrosis, and cancer (Hanada et al. 1973; Ito et al. 1973; Parmar et al. 2003; Suter 1983). Hepatic effects in animals, following exposure to β-HCH, included cellular hypertrophy and necrosis (Hanada et al. 1973; Ito et al. 1973; Van Velsen et al. 1986); α-HCH induced hepatic effects included enzyme activation, hypertrophy, necrosis, and cancer (Barros et al. 1991; Hanada et al. 1973; Ito et al. 1973). Hepatic effects from technical-grade HCH exposure in animals included changes in enzyme activities and enlargement of hepatocytes, nuclear pyknosis, and vacuolation (Dikshith et al. 1989a, 1991a; Fitzhugh et al. 1950; Karnik et al. 1981; Joseph et al. 1992b). Renal effects from γ-HCH exposure included nephritis, accumulation of protein droplets, hypertrophy, and necrosis (Suter 1983); nephritis was observed following α-HCH exposure (Fitzhugh et al. 1950). Exposure to β-HCH has resulted in calcinosis and nephritis (Fitzhugh et

al. 1950; Van Velsen et al. 1986); technical-grade HCH exposure has resulted in nephritis and tubular necrosis (Dikshith et al. 1991a; Fitzhugh et al. 1950). Two MRLs have been derived for intermediate-duration oral exposure in animals. An intermediate oral MRL of 0.0006 mg/kg/day for β-HCH has been developed based on hepatic effects in rats (Van Velsen et al. 1986). An intermediate oral MRL for γ-HCH of 0.00001 mg/kg/day has also been developed based on immunological effects in mice (Meera et al. 1992). Insufficient data are available to derive an intermediate-duration oral MRL for α-HCH; additional studies using known or possible sensitive end points, including reproductive and immunological indices, could address this data need.

Intermediate-duration dermal studies have been performed in rabbits, guinea pigs, and rats; some deaths were observed following exposure to γ-HCH (Brown 1988). There are limited data pertaining to systemic effects (e.g., increased respiratory rate and wheezing, hepatic hypertrophy, and basophilic renal tubules) and neurological effects (e.g., hyperactivity, ataxia, and convulsions) in rats following intermediate-duration dermal exposure to γ-HCH (Brown 1988). Death and systemic effects (e.g., hepatic hypertrophy and fatty degeneration and renal tubular necrosis) have been observed in rats (Dikshith et al. 1991c); hepatic hypertrophy and enzyme activation were observed in guinea pigs (Dikshith et al. 1978) following intermediate-duration dermal exposure to technical-grade HCH. Additional intermediate-dermal data in animals are needed (e.g., threshold, dose-response, and target organs). This information is necessary for determining levels of significant human exposure to HCH and the associated health effects following dermal exposure.

Chronic-Duration Exposure and Cancer. Controlled epidemiological studies have been conducted in humans exposed to HCH, but are few in number and limited in scope. Hematological effects have been observed in persons exposed to γ-HCH in the workplace via the inhalation and/or dermal route (Brassow et al. 1981; Jedlicka et al. 1958). A number of case reports are available from individuals who had exposure to γ-HCH in the home, during the handling of the pesticide, or from a nearby formulating plant (Danopoulos et al. 1953; Friberg and Martensson 1953; Gewin 1939; Loge 1965; Mendeloff and Smith 1955). Effects that have been described in these case reports include hematological effects including granulocytopenia, aplastic anemia, paramyeloblastic leukemia, and pancytopenia. Blood levels of HCH, and its isomers (α, β, and γ), were found to be higher in women with breast cancer when compared to healthy women without the disease (Mathur et al. 2002). α- and γ-HCH blood levels were significantly higher in breast cancer patients, 41–50 years of age, compared to women of the same age without the disease. β-HCH blood levels were found significantly higher in breast cancer patients, 31–50 years of age, compared to those without the disease (Mathur et al. 2002). Other

organochlorine pesticides, including DDT and its metabolites, were also present in the blood and could have contributed to the incidence of breast cancer. Exposure to HCH, and other organochlorine pesticides, to the population is likely through food where the pesticides are primarily used for agricultural applications; however, other possible environmental contamination pathways include inhalation and dermal routes of exposure.

No chronic-duration inhalation studies in animals are available for any isomer. Altered renal excretions and hepatic hypertrophy and have been observed in chronic oral studies on rats with γ-HCH (Amyes 1990). A chronic oral MRL of 0.008 mg/kg/day for α-HCH has been developed based on hepatic effects in rats (Fitzhugh et al. 1950). Chronic dermal studies in animals are not available. Since there are insufficient data to develop inhalation and dermal chronic-duration MRLs, further data from the inhalation and dermal routes are needed (e.g., threshold, dose-response, and target organs). This information is needed for determining levels of significant human exposure to HCH and the associated health effects. However, the need for dermal studies is not a priority as data on skin absorption can be used to calculate equivalent oral doses.

Use of γ-HCH pesticides by farmers was associated with a 50% increased risk of non-Hodgkin's lymphoma (Blair et al. 1998). However, a causal relationship could not be determined due to confounding effects such as use of other pesticides. Limited chronic dermal data in humans are available (Davis et al. 1993), but chronic oral data in humans are not available. There are no inhalation studies in animals. Several chronic toxicity/carcinogenicity bioassays have been conducted in animals following oral exposure to technical-grade HCH and α-, β-, γ-, and δ-HCH (Hanada et al. 1973; Ito et al. 1975; Karnik et al. 1981; Kashyap et al. 1979; Munir et al. 1983; NCI 1977; Thorpe and Walker 1973; Wolff et al. 1987). Chronic dermal exposure to technical-grade HCH caused liver cancer in mice (Kashyap et al. 1979). However, the results were not useful in determining carcinogenic potential because of limitations of these studies, such as testing only one dose and the potential for oral ingestion. 2,4,6-Trichlorophenol, a metabolite of γ-HCH, may be responsible for some or all of the carcinogenic activity observed in mice. This metabolite has been classified by EPA as a group B2 carcinogen. Pentachlorocyclohexene epoxide, a metabolite of γ-HCH that has been identified in the liver of rats, may also be responsible for the carcinogenic effects of γ-HCH. Cancer classifications of several HCH isomers have been made by the U.S. Department of Health and Human Services (DHHS) and the EPA. EPA has classified technical-grade HCH, α-HCH, β-HCH, and δ-HCH as B2, B2, C, and D, carcinogens, respectively (EPA 1998a). γ-HCH has not been assigned a cancer classification by EPA. Additional carcinogenicity information would not be needed at this time. DHHS has classified γ-HCH and other HCH isomers as "reasonably

anticipated to be human carcinogen" in the 8th Report on Carcinogens (DHHS 1998). The International Agency for Research on Cancer (IARC) has classified HCH isomers as Group 2B, possibly carcinogenic to humans.

Genotoxicity. HCH did not produce chromosomal aberrations in humans exposed primarily by inhalation (Kiraly et al. 1979). Dominant lethal mutations occurred in mice orally exposed to technical-grade HCH (Lakkad et al. 1982). Increased frequency of polyploid cells occurred in rats exposed orally to α-HCH (Hitachi et al. 1975). Information on the genotoxic effects of γ-HCH is also obtained from *in vitro* studies. Gene mutations were observed in bacteria treated with γ-HCH (with and without metabolic activation) (Moriya et al. 1983; Nagy et al. 1975). γ-HCH was not mutagenic in yeast (Shahin and von Borstel 1977) or algae (Kar and Singh 1979a). Results of chromosomal aberration tests in γ-HCH-treated hamster cells were questionable (Ishidate and Odashima 1977). Technical-grade HCH produced chromosomal aberrations in cultured human lymphocytes (Rupa et al. 1989d) but did not produce cytogenetic effects in Chinese hamster cells (Murli 1990) or unscheduled DNA synthesis in rat hepatocytes (Cifone 1990). Human mammary carcinoma MCF-7 and human prostate carcinoma PC-3 cell lines showed that low concentrations of γ-HCH induced increases in micronuclei in both cell lines in the absence of DNA damage or cytotoxicity, suggesting a clastogenic effect for this chemical (Kalantzi et al. 2004). In general, the available information suggests that α-, β-, and γ-HCH may have some genotoxic potential; however, the evidence is not conclusive. Further testing in clastogenicity and genotoxicity tests *in vivo* would be valuable.

Reproductive Toxicity. The only available human data are from one study on hormone levels in pesticide workers in which increases in the levels of serum luteinizing hormone were noted following exposure to γ-HCH for 8 years (Tomczak et al. 1981). There are no inhalation data in animals for any HCH isomer. Anti-estrogenic properties were found in female rats given γ-HCH by the oral route (Chadwick et al. 1988), and female rabbits treated orally with γ-HCH had a reduced ovulation rate (Lindenau et al. 1994). γ-HCH exposure in female mice and their offspring on gestational days 9–16 resulted in slightly increased relative uterus weight in F1 females, and earlier vaginal opening and increased branching of villi and oedema in the endometrial stroma in treated mice (Maranghi et al. 2003). Reductions in testicular and epididymis weights, spermatid and sperm numbers, and serum testosterone level were found in male rats exposed to relatively low doses of γ-HCH during lactation and evaluated at puberty and adulthood (Dalsenter et al. 1997b). Effects on testicular histology and sperm numbers similarly occurred in adult male offspring of mice that were exposed to γ-HCH during gestation (Traina et al. 2003). Reproductive effects of γ-HCH exposure were reported in ewe lambs (Beard and Rawlings

1999) and young rams (Beard et al. 1999a) given 1 mg/kg/day in treated feed from conception to sexual maturity. In estrus synchronized ewes, treated animals had significantly shorter estrous cycle length and lower number and less total volume of corpus lutea. No other detrimental fertility effects were observed. The subjectively-scored sexual behavior in young rams was significantly reduced in treated animals presented with estrous ewes. Developmental/reproductive effects in male rats were used as the basis for an acute-duration MRL for oral exposure to γ-HCH. Results of single and multigeneration reproduction studies in rats and mink indicate that exposure to γ-HCH or technical HCH caused effects, such as decreased numbers of offspring at birth, reduced neonatal viability, and delayed maturation of pups, that were primarily results of prenatal and/or postnatal developmental toxicity (Beard and Rawlings 1998; Beard et al. 1997; King 1991; Srivastava and Raizada 2000). Oral exposure of rats and mice to β- or technical-grade-HCH has resulted in degeneration of male reproductive organs and sperm abnormalities (Dikshith et al. 1991a; Gautam et al. 1989; Nigam et al. 1979; Pius et al. 1990; Roy Chowdhury and Gautam 1990; Van Velsen et al. 1986), and ovarian atrophy was observed in rats exposed to β-HCH for 13 weeks (Van Velsen et al. 1986). Similar effects were also observed in reproductive organs of rats following dermal treatment with technical-grade HCH for 120 days (Prasad et al. 1995). The reproductive effects on guinea pigs after dermal exposure to technical-grade HCH (100–500 mg/kg/day) have also been investigated (Dikshith et al. 1978). Testicular hypertrophy and atrophy and complete inhibition of spermatogenesis were observed in the guinea pigs. Studies via the inhalation and dermal routes would provide information regarding the reproductive effects of HCH in animals for these exposure routes and could be useful in the assessment of potential reproductive effects in humans. Pharmacokinetic data suggest that HCH isomers might have the potential to affect reproduction across routes of exposure, although data are insufficient to predict effect levels.

Developmental Toxicity. Developmental effects in humans, specifically IUGR, may result from oral exposure to HCH (Siddiqui et al. 2003). The blood of mothers with IURG babies had higher levels of γ-, α-, δ-, and total HCH. Similarly, γ-, δ-, and total HCH cord blood levels of IUGR babies were higher than the cord blood levels in the normal-weight babies (Siddiqui et al. 2003). Other organochlorine pestides, including DDT and its metabolites, were also present in the blood, and could have contributed to IUGR. There are no inhalation data in animals for any isomer. No adverse prenatal developmental effects of γ-HCH from oral exposure have been found in rats or rabbits (Khera et al. 1979; Palmer et al. 1978a; Seiler et al. 1994) or from exposure to technical-grade HCH in mice (Dikshith et al. 1990). Alterations in neurotransmitter levels were noted in suckling rats treated once with γ-HCH by gavage (Rivera et al. 1991). γ-HCH exposure in F1 females of female mice treated on gestational days 9–16, resulted in slightly increased relative uterus weight (Maranghi et al. 2003). An acute oral MRL of

0.003 mg/kg/day has been developed from data on developmental/reproductive effects in mature male offspring of rats that were exposed to γ-HCH during lactation; these effects included reduced testicular and epididymis weights, reduced spermatid and sperm numbers, and alterations in mating behavior (Dalsenter et al. 1997b). Decreases in fetal weight, fetal thymic weight, and placental weight have been reported in mice exposed to a single oral dose of γ-HCH on day 12 of gestation (Hassoun and Stohs 1996a). No effects on embryonic development were seen in rabbits treated orally with γ-HCH (Seiler et al. 1994).

Alterations in neurotransmitter levels were observed in female rat pups treated orally with technical-grade HCH (Nagaraja and Desiraju 1994). No data on the developmental effects of α-, β-, or δ-HCH were located for the oral or dermal route and there is no information for dermal exposure to technical-grade HCH. Due to the lack of developmental toxicity studies in humans, as well as the lack of inhalation and dermal data in animals, insufficient information is available to indicate whether HCH affects development via all three routes of exposure. Pharmacokinetic data suggest that HCH isomers might have the potential to affect development across routes of exposure. Additional developmental studies in animals exposed to α-, β-, or δ-HCH would provide useful information concerning possible fetotoxic and teratogenic effects in animals, which might be relevant to humans.

Immunotoxicity. A statistically significant increase (approximately 18%) in IgM has been reported in individuals occupationally exposed to technical-grade HCH (Kayshap 1986). The HCH isomer concentrations in serum showed a 10-fold increase when compared to the control. There are no oral or dermal data in humans. Also, there are no inhalation or dermal data in animals. Depressed antibody response to *Salmonella* antigens was reported in rats (Dewan et al. 1980) and rabbits (Desi et al. 1978) exposed to γ-HCH via the oral route. γ-HCH exposure has been shown to result in thymus cortex atrophy, suppressed bone marrow cellularity, erythrocyte precursors, and granulocyte-macrophage progenitor cells in mice (Hong and Boorman 1993). Based on immunological effects of γ-HCH on components of cell- and humoral-mediated immunity in mice, an intermediate oral MRL has been developed (Meera et al. 1992). Decreased lymphoproliferative responses to T-cell mitogens were observed in mice treated by the oral route with β-HCH (Cornacoff et al. 1988). No immunological effects were observed in rats treated with β-HCH by the oral route for 13 weeks (Van Valsen et al. 1986). There are no immunotoxicity data for technical-grade HCH. The biological significance of increased immunoglobulin levels remains to be established. In addition, exposure to technical-grade or γ-HCH may also affect the immune system in humans (Kashyap 1986) and animals (Desi et al. 1978; Dewan et al.

1980). Further studies on all isomers using all three routes of exposure would be useful in the assessment of potential immunotoxic effects in humans.

Neurotoxicity. Exposure to γ-HCH and other isomers has been shown to be associated with neurological effects in both humans and animals, and there is no basis to suspect that these effects may be route-, species-, or age-dependent. Paresthesia has been reported in workers exposed via the inhalation or dermal routes (Fonseca et al. 1993; Kashyap 1986). Abnormal EEG patterns have also been noted in workers (Czegledi-Janko and Avar 1970). Seizures and coma have been observed in individuals who have ingested large amounts of γ-HCH (Davies et al. 1983; Harris et al. 1969; Munk and Nantel 1977; Nantel et al. 1977; Powell 1980; Starr and Clifford 1972; Storen 1955). Convulsions have been reported in children following dermal application of γ-HCH (Ramchander et al. 1991; Tenebein 1991). Neurological effects including sedation, restlessness, excitation, and ataxia were seen in rats exposed by inhalation to γ-HCH for 4 hours (Ullmann 1986b). Mice exposed via the inhalation route to γ-HCH in a chronic study did not display any neurotoxic signs (Klonne and Kintigh 1988). Convulsions have been observed in rats and mice following oral exposure to γ-HCH (Arisi et al. 1994; Attia et al. 1991; Gilbert 1995; Gilbert and Mack 1995; Joy et al. 1982; Martinez and Martinez-Conde 1995; Martinez et al. 1991; Parmar et al. 2003; Vendrell et al. 1992a; Wooley and Griffith 1989). Less serious neurological effects of oral exposure to γ-HCH in rats included reduced brain serotonin level, reduced brain barrier permeability in pups, decreased myelin and enzyme activity in brain, reduced tail nerve conduction velocity, enhanced susceptibility to kindling, motor activity changes, and other neurobehavioral alterations (Attia et al. 1991; Hughes 1999a; Joy et al. 1982; Muller et al. 1981; Serrano et al. 1990a). Oral exposure of mice and rats to β-HCH has resulted in lateral recumbency, coma, and reduced tail nerve conduction velocity (Cornacoff et al. 1988; Muller et al. 1981; Van Velsen et al. 1986). Rats and mice exposed orally to technical-grade HCH experienced convulsions, increased motor activity, and variations in neurotransmitter levels (Anand et al. 1991; Dikshith et al. 1991a; Gopal et al. 1992; Kashyap et al. 1979). Neurological effects were not observed in rats following oral exposure to α-HCH (Muller et al. 1981). Information is available on the neurotoxic effects of α-, β-, and γ-HCH in experimental animals following acute-duration oral exposure (Tilson et al. 1987; Tusell et al. 1987; Woolley and Griffith 1989) and intermediate-duration oral exposure (Desi 1974; Muller et al. 1981; Van Velsen 1986). An acute oral MRL of 0.2 mg/kg/day for β-HCH was developed based on ataxia in mice (Cornacoff et al. 1988). Studies in animals have substantiated the neurological symptoms resulting from dermal application of γ-HCH. Effects in rats included sedation, spasms (Ullmann 1986a), tremors, and convulsions (Brown 1988). Neurochemical and neurophysiological studies in animals exposed via the oral route would provide useful information regarding the mechanisms of HCH-related neurotoxic effects. Because an

MRL could not be developed for inhalation exposures and dermal data are limited, additional studies for all isomers for these two exposure routes are needed.

Epidemiological and Human Dosimetry Studies. Information on the adverse health effects of HCH in groups of humans comes from reports of occupationally exposed individuals (Brassow et al. 1981; Jedlicka et al. 1958; Kayshap 1986). Adverse health effects include EEG abnormalities, increased liver enzymes, and changes in hematological parameters. Limitations inherent in these studies include unquantified exposure concentrations and durations and concomitant exposure to HCH mixtures and other chemicals and pesticides. The few industrial surveys and studies of exposed individuals generally reported blood levels of HCH following exposure and the health effects associated with these levels (Czegledi-Janko and Avar 1970). However, the reported blood levels of HCH have not been quantitatively correlated with ambient HCH levels or health effects. Studies that provide information correlating exposure levels with body levels of HCH would allow investigators to monitor humans for exposure, including populations living near hazardous waste sites. Well-conducted studies are needed to determine and quantifying the effects of inhalation, oral, or dermal HCH exposure on human health including neurological, hematologic, and hepatic effects. However, considering the magnitude of the needed studies, possible difficulty in identifying a suitable potentially exposed subpopulation in the general populace or workplace, and lowered likelihood of exposure in present day society, the value of such studies is questionable.

Biomarkers of Exposure and Effect.

Exposure. Methods exist for the analysis of HCH in blood and urine (Angerer et al. 1981). Thus, biological monitoring for exposure to HCH is possible by measuring the levels of HCH in the blood or urine. In an occupational study, abnormal EEG changes were found to correlate with blood levels of γ-HCH (Czegledi-Janko and Avar 1970). Measurements of γ-HCH represent short-term exposure because it is metabolized and excreted rapidly. Due to its high lipid solubility and persistence, β-HCH level represents long-term exposures. β-HCH has been measured in numerous human tissues and is the isomer that is consistently detected at the highest concentration (Baumann et al. 1980; Kashyap 1986; Morgan and Lin 1978; Nigam et al. 1986; Ramachandran et al. 1984). However, the reported blood levels of HCH have not been quantitatively correlated with ambient HCH levels. Methods that measure the levels of HCH metabolites in urine are not specific enough to detect exposure to HCH alone. More information could be provided by studies designed to correlate biomarkers of exposure with exposure levels.

Effect. No biomarkers of effect, specific for HCH, have been identified in the literature. Nonspecific biomarkers of effect include EEG abnormalities, increases in liver enzymes, hematological effects, seizures and convulsions, neuropsychological, and gastrointestinal effects (Kashyup 1986; Nigam et al. 1986). Muscle spasms and EEG abnormalities have also been observed in workers exposed to γ-HCH (Czegledi-Janko and Avar 1970). High levels of HCH and other organochlorine insecticides have been detected in men with low sperm counts and in women who miscarry or deliver prematurely (Pines et al. 1987; Saxena et al. 1980; Wassermann et al. 1982). No quantitative correlation can be made between body levels of HCH and adverse health effects based on the existing data. Studies quantitatively correlating HCH exposure with body levels of HCH and the occurrence of specific adverse health effects are needed to monitor populations possibly exposed near hazardous waste sites. Studies designed to identify specific biomarkers of effect for HCH would be useful.

Absorption, Distribution, Metabolism, and Excretion. Information is available to assess the extent and rate of HCH absorption following oral exposure in animals and humans (Ahdaya et al. 1981; Albro and Thomas 1974; Turner and Shanks 1980). High blood concentrations of γ-HCH have been demonstrated in a number of acute poisoning cases in which humans were exposed to γ-HCH as the result of ingestion (Berry et al. 1987). Animal studies indicate that γ-HCH is readily absorbed from the gastrointestinal tract (Ahdaya et al. 1981). Both *in vivo* and *in vitro* studies that evaluate dermal absorption of γ-HCH in humans are available (Dick et al. 1997a, 1997b). However, absorption of HCH via inhalation can only be inferred from toxicity studies and studies assessing the distribution and excretion of γ-HCH. No quantitative information is available to assess the rate and extent of inhalation absorption in humans or animals. Additional data concerning the absorption of HCH in animals may provide information to assist in characterizing absorption of HCH in humans.

Information on the distribution of HCH isomers in humans is inferred from case studies, clinical studies, and industrial surveys (Baumann et al. 1980; Nigam et al. 1986; Siddiqui et al. 1981a). Air concentrations of α-, β-, and γ-HCH have been found to be associated with blood serum levels in workers (Baumann et al. 1980). HCH isomers have been detected in the adipose tissue of workers (Baumann et al. 1980). γ-HCH was detected in the cerebral spinal fluid of a young boy following ingestion of γ-HCH (Davies et al. 1983). γ-HCH was detected in brain tissue collected during the autopsy of an infant who was treated with a whole-body application of γ-HCH lotion (Davies et al. 1983). The distribution of HCH in animals following oral exposure has been well documented (Chand and Ramachandran 1980; Eichler et al. 1983; Srinivasan and Radhakrishnamurty 1983b). γ- and β-HCH were found to be primarily stored in

the fat of rats after acute oral exposure. Except in the brain, β-HCH accumulates in tissues to a greater degree than γ-HCH. α-HCH has been shown to accumulate preferentially in the white matter of the brain (Portig et al. 1989). Data exist on the rate and overall distribution of HCH in animals following dermal application. In guinea pigs, the accumulation of γ-HCH in the brain was greater than in the blood following acute dermal exposure (Solomon et al. 1977a, 1977b).

The metabolism of γ-HCH has been studied in mice and rats (Chadwick and Freal 1972a; Chadwick et al. 1978a; Engst et al. 1979; Kujawa et al. 1977). Researchers have identified the primary metabolites (di-, tri-, and tetrachlorophenols) in humans, rats, and mice. In humans, this information is obtained from urinary excretion studies in which individuals were occupationally exposed to γ-HCH (Angerer et al. 1983; Engst et al. 1979). *In vitro* studies using rat liver microsomes have helped to delineate the major metabolic processes and have demonstrated the formation of a reactive epoxide that may be indicative of similar processes in other mammals and humans (Fitzloff and Pan 1984). Investigations have not been conducted to examine the epoxide formation *in vivo* or its role in inducing mutagenic and carcinogenic effects. Extensive metabolic studies have been conducted in animals, and adequate studies exist identifying major metabolites in the tissues and urine (Chadwick and Freal 1972a; Kujawa et al. 1977; Macholz et al. 1982a, 1982b). Multiple detoxification pathways have been delineated (Chadwick et al. 1978a, 1981; Kujawa et al. 1977). Further information on the possible role of epoxide formation in carcinogenesis *in vivo*, as well as its rate of formation under various conditions, would be useful.

Information from occupational studies and studies in which γ-HCH was used as a therapeutic lotion is available to conclude that humans excrete HCH, principally as metabolites, in urine, breast milk, and semen (Angerer et al. 1981). Urinary excretion of γ-HCH metabolites by humans has been documented (Angerer et al. 1983). The primary urinary metabolites of γ-HCH are chlorophenols. Quantitative information also exists to conclude that the primary route of HCH excretion in animals, following oral exposure, is urine (Chadwick et al. 1985). There are no inhalation studies that have examined the excretion of HCH. In male rats treated dermally with radiolabelled γ-HCH, radiolabel was detected in the urine (Bosch 1987a).

Comparative Toxicokinetics. Evidence is available to suggest that rats and humans absorb HCH and store the isomers primarily in the fat and other body tissues (Chand and Ramachandran 1980; Eichler et al. 1983; Srinivasan and Radhakrishnamurty 1983b). Similar metabolites have been identified in the urine of exposed individuals and treated rodents, and in both, the primary route of excretion is the urine (Angerer et al. 1981; Chadwick et al. 1985).

Exposure to γ-HCH has been shown to be associated with neurological effects in both humans and animals (Czegledi-Janko and Avar 1970; Kashyap 1986; Van Velsen et al. 1986). The available human and animal data also suggest that HCH isomers may affect the blood system. In addition, HCH isomers may also affect the immune system in humans (Kashyap 1986) and animals (Desi et al. 1978; Dewan et al. 1980). Further studies are not needed at this time.

Methods for Reducing Toxic Effects. Seizures caused by γ-HCH can be antagonized by $GABA_A$ mimetics; diazepam is the anticonvulsant of choice (Ellenhorn and Barceloux 1988). Information is available to assess the extent and rate of absorption of HCH following oral and dermal exposure (Ahdaya et al. 1981; Albro and Thomas 1974; Turner and Shanks 1980), although the mechanism(s) of absorption is inadequately characterized. The available data indicate some ways in which peak absorption of HCH might be reduced following oral or dermal exposure (Ellenhorn and Barceloux 1988). Intestinal absorption can be reduced with activated charcoal, while washing with soap and water can decrease skin absorption. There are no known methods for reducing absorption following inhalation exposure.

Because of the high volume of distribution of HCH into adipose tissue, traditional methods of increasing elimination or decreasing distribution are not useful. Development of methods to enhance the excretion of HCH from adipose tissue, while minimizing toxicity, is needed for reducing the body burden.

There is some information on the mechanism (see Section 3.4) for the toxic effects of HCH on the brain (e.g., interference with the GABA system) (Abalis et al. 1985; Casida and Lawrence 1985; Lawrence and Casida 1984) and liver (e.g., disruption of oxidative defense mechanisms) (Barros et al. 1988, 1991; Srinivasan and Radhakrishnamurty 1988; Videla et al. 1991). Further studies in these areas might be helpful for developing methods for reducing toxic effects.

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.

Limited data are available on the health effects of HCH on exposed children.

It has been demonstrated that weanling rabbits were more sensitive to γ-HCH than young adults, as seen by increased mortality rate and associated excitement and convulsions after treatment (Hanig et al. 1976).

There is, however, no actual evidence that children are more sensitive to the neurotoxicity of γ-HCH. It would be useful to follow up on the weanling rabbits study and conduct additional studies on immature postnatal animals as an experimental model. Data needs relating to developmental effects are discussed above in developmental toxicity section. Replicating the Dalsenter et al. (1997b) study on lactational exposure and adult testosterone levels should be a priority. There is inadequate experimental evidence to determine if pharmacokinetics of HCH in children are different from adults. There is no experimental evidence to indicate whether metabolism of HCH or its mechanism of action is different in children compared with adults. Generally, it would be difficult to have data on the metabolism and mechanism of action of HCH in children (except in accidentally exposed children) to determine whether children are more vulnerable than adults to adverse health effects from exposure to HCH. There are no biomarkers of exposure or effect that have been validated in children or adults exposed as children. There are no data to determine whether there are any interactions with other chemicals that are unique to children or whether interactions observed in adults also occur in children. Although HCH is shown to have some genotoxic potential, it is not known whether parental exposure to HCH may affect children via parental germ cells, or whether HCH may indirectly affect the fetus during maternal exposure. Additional data are needed to determine the potential for genotoxicity in germ cells and adverse developmental effects.

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

Federally sponsored research regarding health effects of HCH that was reported in the CRIS/USDA (2003), CRISP (2003), and FEDRIP (2004) databases is summarized in Table 3-9.

Table 3-9. Ongoing Studies on Hexachlorocyclohexane Health Effects

Investigator	Affiliation	Research area	Reference
Adler, SR	Washington University	Examination of the regulatory potential of insecticides, plasticizers, and dioxins in estrogenic and non-estrogenic pathways	CRISP 2004
Alavanja, M	Not available	Epidemiologic investigations to identify and clarify cancer risks from pesticide exposure	FEDRIP 2004
Bloomquist, JR	Virginia Polytechnic Institute	Assessment of the ability of insecticide exposure to cause biomarkers indicating Parkinsonism	CRIS/USDA 2003; FEDRIP 2004
Casida, JE	University of California at Berkeley	Modes of toxic action, biochemical targets, mechanisms of selective toxicity, and health implications of exposure of selected insecticides	CRIS/USDA 2003
Clark, JM	University of Massachusetts Amherst	Detection of pyrethroid and γ-HCH resistance in head lice	FEDRIP 2004
Dietert, RR	Cornell University, Center for the Environment	Expansion of the database of Critical Evaluations on the current evidence of carcinogenicity for selected agricultural chemicals	FEDRIP 2004
MacDonald, JF	Cornell University, Center for the Environment	Establishment of a database of critical evaluations on evidence of breast carcinogenicity of selected pesticides	FEDRIP 2003
Misra, HP	Virginia Polytechnic Institute, College of Veterinary Medicine	Assessment of the role of pesticide mixtures in potentiating the genotoxicity in immune cells *in vitro*	FEDRIP 2004
Naeher, LP	University of Georgia, Environmental Health Sciences	Environmental and dietary monitoring for organophosphate and pyrethroid pesticides in children	CRIS/USDA 2003; FEDRIP 2004
Narahashi, T	Northwestern University	Determination of the mechanism by which neuroactive insecticides exert their toxic actions on mammals	CRIS 2003; CRISP 2004
Oman, GM	Department of Veteran Affairs, Medical Center	Assessment of the impacts of metal and organochlorine contaminants indigenous to Saginaw Bay, Lake Huron, on human and fish immune systems	FEDRIP 2003
Ostrea, EM	Wayne State University	Meconium analysis of fetal exposure to environmental toxins and infant outcome	CRISP 2003; FEDRIP 2004
Schwartz, SM	Fred Hutchinson Cancer Research Center	Determination of risk of testicular germ cell carcinoma in relation to serum levels of persistent organochlorines	CRISP 2004
Wong, PS	University of California at Davis	Establishment of the existence of "ligand independent" activation mechanism for various chlorinated pesticides in human cell systems (BG-1 ovarian cancer cells, and Ishikawa endometrial cells)	FEDRIP 2004

Table 3-9. Ongoing Studies on Hexachlorocyclohexane Health Effects

Investigator	Affiliation	Research area	Reference
Woolley, DE	University of California at Davis, Neurology, Physiology, and Behavior	Investigation of the neurotoxic effects and mechanisms of action produced by acute and chronic exposure to heptachlor and γ-HCH	CRIS/USDA 2003; FEDRIP 2004
Matsumura, F	University of California at Davis, Environmental Toxicology	Identification of the action of mechanism of selected pesticides and study of the basis of their differential toxicities against mammalian, insect, and acerine large proteins	FEDRIP 2004
Matsumura, F	University of California at Davis, Environmental Toxicology	Determination of whether c-Neu plays a pivotal role in mediating the estrogenic action of OC in MCF-7 cell transformation	FEDRIP 2004
Molony, D	University of Texas, Health and Science Center, Houston	Explanation of some of the cellular events and molecular mechanisms that participate in the induction of apoptosis of renal tubular epithelial cell in response to the inhibition of specific ion channels by toxicants	FEDRIP 2004

CRIS = Current Research Information System; CRISP = Computer Retrieval of Information on Science Projects; FEDRIP = Federal Research in Progress; OC = Organochlorine; USDA = U.S. Department of Agriculture