CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of 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. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

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

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 by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figures 2-1, 2-2, and 2-3 provide an overview of the database of studies in humans or experimental animals for α-, β-, and γ-HCH included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to HCH, but may not be inclusive of the entire body of literature. A systematic review of the scientific evidence of the health effects associated with exposure to HCH was also conducted; the results of this review are presented in Appendix C.

Tabulated human studies of specific health endpoints are presented in the corresponding subsections of this Chapter. Animal inhalation studies of γ-HCH are presented in Table 2-1 and Figure 2-5. There were no inhalation studies of other HCH isomers or mixtures of isomers. Animal oral studies are presented in Table 2-2 and Figure 2-6 (α-HCH), Table 2-3 and Figure 2-7 (β-HCH), Table 2-4 and Figure 2-8 (γ-HCH), and Table 2-5 and Figure 2-9 (δ-HCH and technical-grade HCH or unspecified isomers). Animal dermal studies are presented in Table 2-6 (γ-HCH) and Table 2-7 (technical-grade or unspecified isomers). There were no dermal studies of other HCH isomers.

Levels of significant exposure (LSEs) 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. Effects have been classified into “less serious LOAELs” or “serious LOAELs (SLOAELs).” "Serious" effects (SLOAELs) 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 endpoint 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 endpoints (ATSDR 2018). 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. Levels of oral exposure associated with cancer (Cancer Effect Levels, CELs) of HCH are indicated in Tables 2-2 through 2-5 and Figures 2-6 through 2-9.

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

The discussion of the available data for health effects in this chapter is organized into human and animal data, with isomer-specific subsections on the animal data provided in the following order: α-HCH, β-HCH, γ-HCH, δ-HCH, and technical-grade and mixtures of HCH isomers. Case reports of effects in humans are limited to γ-HCH and technical-grade HCH and are discussed under the isomer-specific subsections. If there are no case reports or animal data for a given isomer or for technical grade/mixtures, there is no corresponding subsection.

Effects of HCH isomers have been evaluated in epidemiological studies and in laboratory animals exposed under controlled conditions. Most of the human epidemiological studies used measures of HCH isomers in blood or tissues to assess exposure, so the route is unknown; for the purpose of enumerations, these studies are considered to reflect oral exposure (e.g., through contaminated food). In addition, there

are several case reports of health effects in humans exposed by inhalation, oral, or dermal exposure to γ-HCH. The human data were not considered adequate for identification of sensitive target organs for any of the HCH isomers or mixtures.

As shown in Figure 2-1 (α-HCH), there were a small number of human studies examining a handful of endpoints; the largest number of studies were devoted to developmental endpoints. There were no inhalation or dermal animal studies of α-HCH, and few oral studies. The available animal studies primarily examined liver effects and cancer. Animal studies suggest that hepatic effects are a sensitive target of α-HCH toxicity.

- **Hepatic endpoints:** Hepatic toxicity is a presumed health effect for humans based on a high evidence level in animals showing increased liver weight and histopathological lesions after oral exposure to α-HCH. No information was located on hepatic effects in humans exposed to α-HCH.

Figure 2-2 provides an overview of the health effects data for β-HCH. For this isomer, human studies examined a wide range of outcomes, with more studies of endocrine endpoints (thyroid hormone levels) developmental outcomes, other noncancer endpoints (diabetes and metabolic perturbations), and cancer than other outcomes. Animal studies are limited to oral exposures, and the endpoints examined were largely focused on liver, kidney, body weight, nervous system, and cancer. Animal studies suggest that neurological and hepatic effects are sensitive targets of β-HCH toxicity after acute-duration exposures and intermediate- or chronic-duration exposures, respectively.

- **Neurological endpoints:** Neurotoxicity is a presumed health effect in humans based on human and animal studies. There is a moderate level of evidence in humans suggesting associations between serum β-HCH and risk of Parkinson disease, Alzheimer's disease, and cognitive deficits. There is a high level of evidence in animal studies of oral exposure showing clinical signs of neurotoxicity in rats and mice after acute durations and reduced nerve conduction velocity in rats after an intermediate duration. Clinical signs showed a dose-related increase in severity.

  Hepatic endpoints: Hepatic toxicity is a presumed health effect for humans based on a high level of evidence in animals showing increased liver weight and histopathology changes in rats and mice exposed by dietary administration for intermediate and chronic durations. In humans, there is a very low level of evidence for a minimal liver toxicity based on two cross-sectional studies reporting no association between serum or adipose levels of β-HCH and hepatic clinical chemistry endpoints except for increased serum bilirubin.

An overview of health effects data for γ-HCH is presented in Figure 2-3. Most of the human studies evaluated developmental, reproductive, renal, endocrine, or cancer endpoints. Studies of occupational exposure via pesticide application are considered to reflect primarily inhalation exposure. Most of the

animal studies used oral administration, and the available studies examined comprehensive noncancer and cancer endpoints. The effects seen at the lowest doses in the animal studies were developmental and immune system effects. Animal studies suggest that developmental and immune system effects are sensitive targets of γ-HCH toxicity after acute-duration exposures (developmental) and intermediate-duration exposures (developmental and immune system). Available studies of chronic-duration oral exposure to γ-HCH were limited and identified effects on other systems (hepatic and renal) at much higher doses than those associated with developmental and immune system effects in acute- and intermediate-duration exposure studies.

- **Developmental endpoints:** Developmental toxicity is a presumed health effect in humans based on human and animal evidence. There is a low level of evidence in humans based on associations between γ-HCH in maternal or fetal blood (or tissue) and fetal growth retardation, preterm birth, and cryptorchidism or hypospadias. There is a high level of evidence in animals based on studies in a variety of species exposed orally to γ-HCH for acute or intermediate durations during gestation or postnatal development demonstrating adverse effects on a wide range of developmental endpoints, including birth outcomes and development of the male and female reproductive tracts, central nervous system, heart, thymus, and spleen.

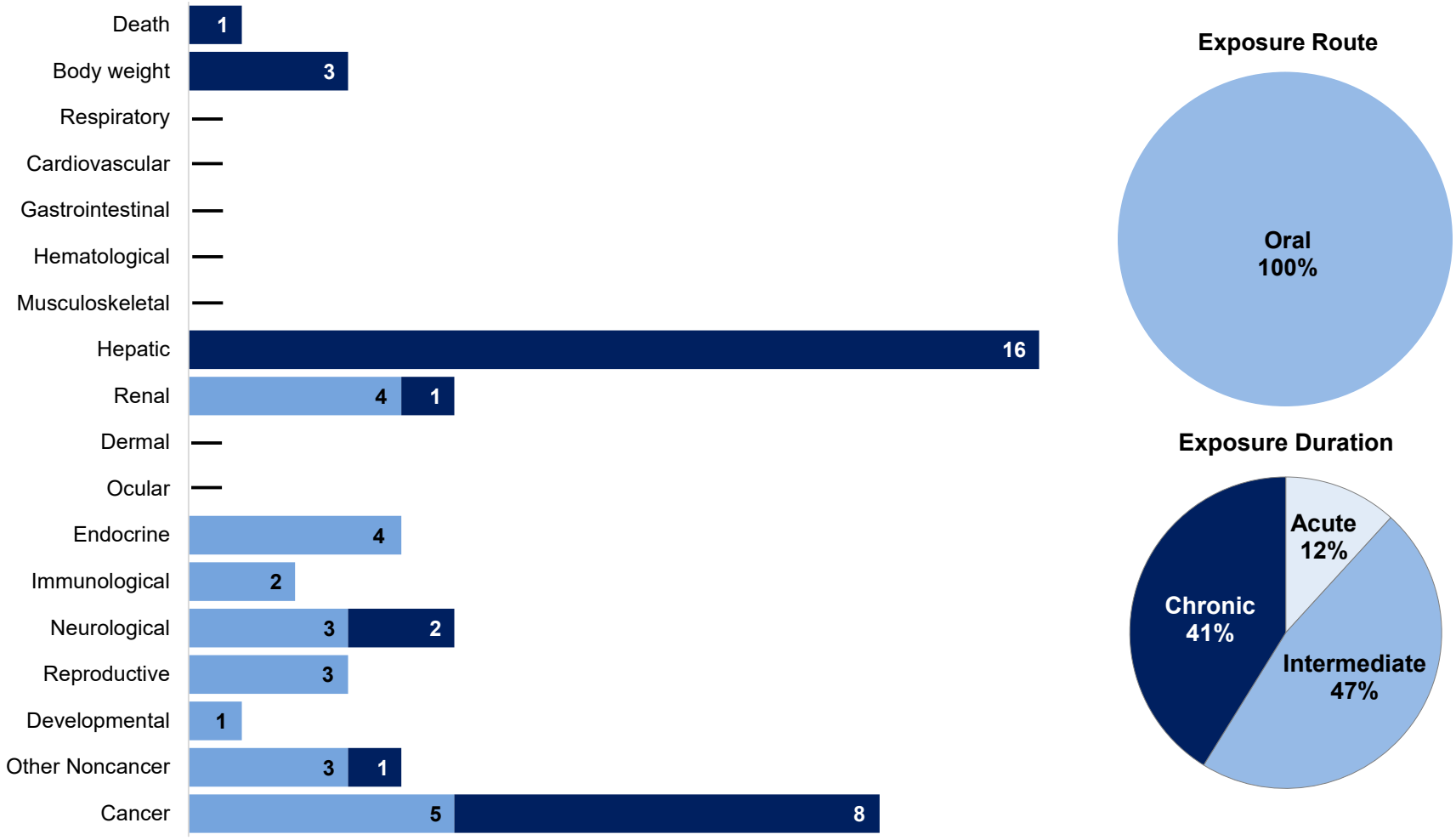
- **Immune system endpoints:** Immunotoxicity is a presumed health effect in humans based primarily on animal evidence. There is a low level of evidence in humans based on an observed association between asthma and plasma levels of γ-HCH in children and no evidence for increased prevalence of monoclonal gammopathy of undetermined significance in male pesticide applicators. There is a high level of evidence in animals based on acute- and intermediate-duration studies of γ-HCH administered orally to rats, mice, rabbits, and sheep showing suppression of the immune system and effects on thymus, spleen, and lymph node weights or histology.

Figure 2-4 shows the limited health effects data available for δ-HCH and unspecified HCHs. The human studies primarily evaluated other noncancer, developmental, reproductive, and neurological endpoints. The small number of animal studies used oral or dermal administration and were focused on hepatic and cancer endpoints. Data were not adequate to identify sensitive targets of δ-HCH.

Figure 2-1. Overview of the Number of Studies Examining α-Hexachlorocyclohexane (α-HCH) Health Effects*

Most studies examined the potential body weight, hepatic, and cancer effects of α-HCH

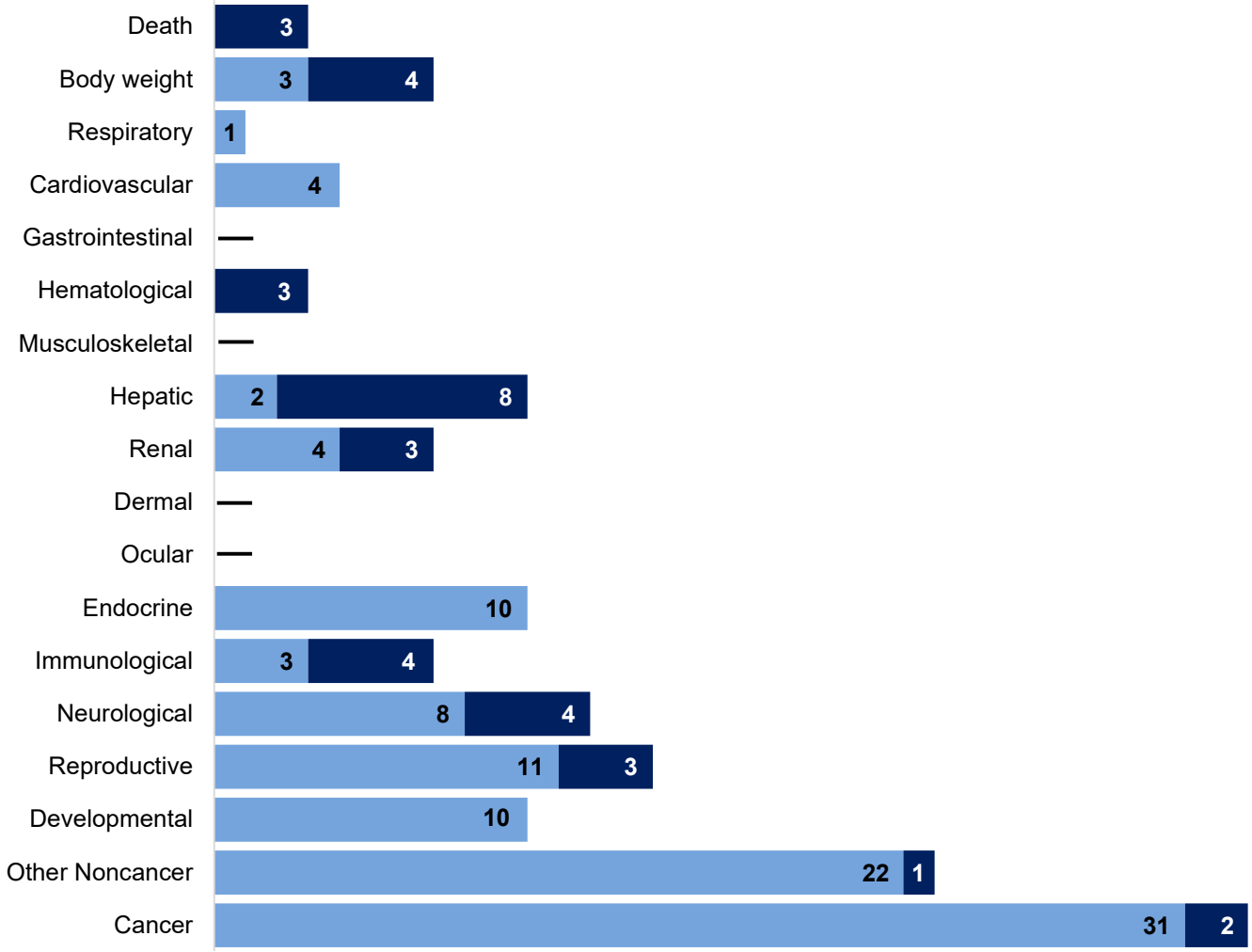
Fewer studies evaluated health effects in humans than animals (counts represent studies examining endpoint)

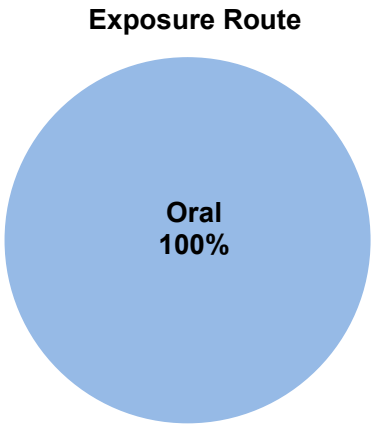


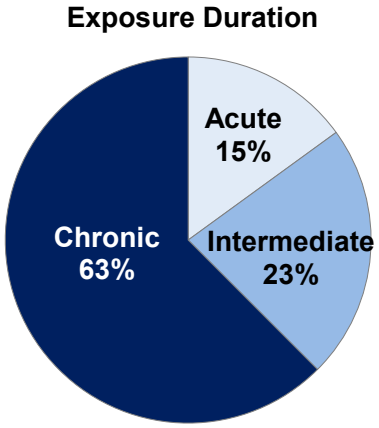
*Includes studies discussed in Chapter 2. A total of 40 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints. Human studies of unknown route and/or duration were classified as chronic oral studies for the purpose of this figure.

Figure 2-2. Overview of the Number of Studies Examining β-Hexachlorocyclohexane (β-HCH) Health Effects*

Most studies examined the potential developmental, other noncancer, and cancer effects of β-HCH
More studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)



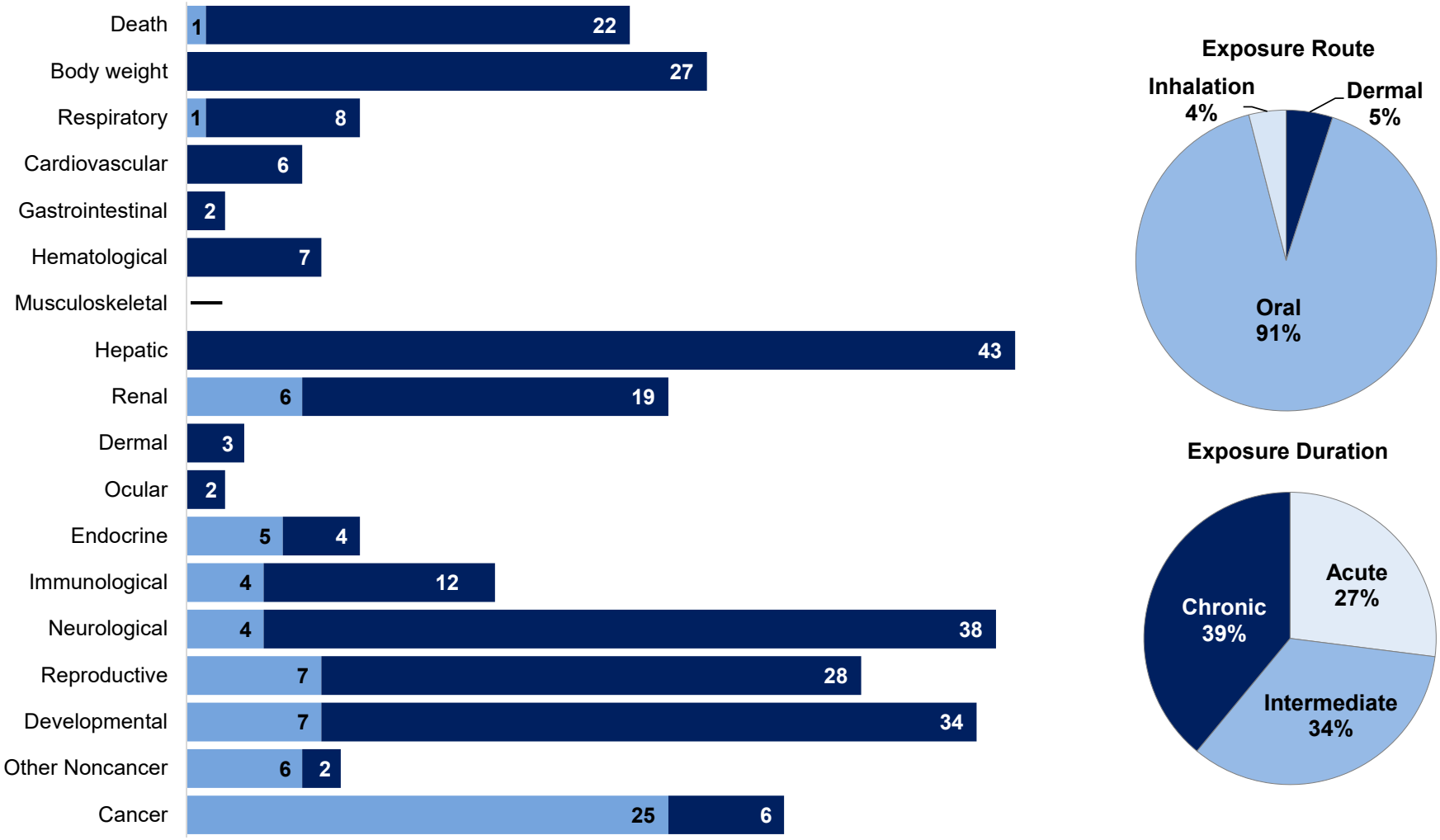




*Includes studies discussed in Chapter 2. A total of 41 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints. Human studies of unknown route and/or duration were classified as chronic oral studies for the purpose of this figure.

Figure 2-3. Overview of the Number of Studies Examining γ-Hexachlorocyclohexane (γ-HCH) Health Effects*

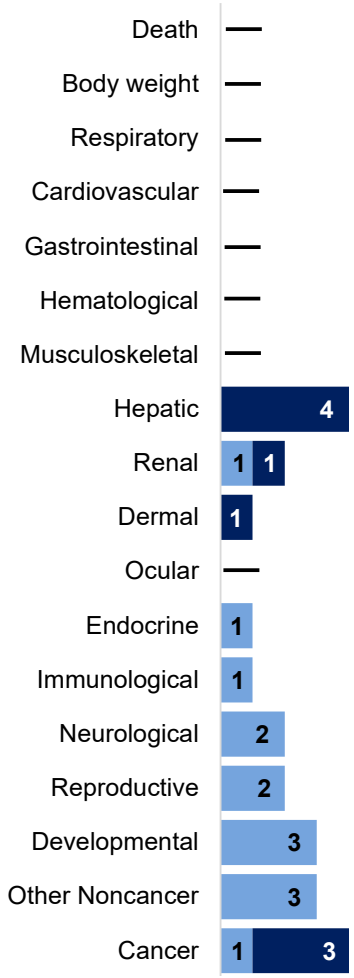
Most studies examined the potential body weight, hepatic, and neurological effects of γ-HCH
Fewer studies evaluated health effects in humans than animals (counts represent studies examining endpoint)

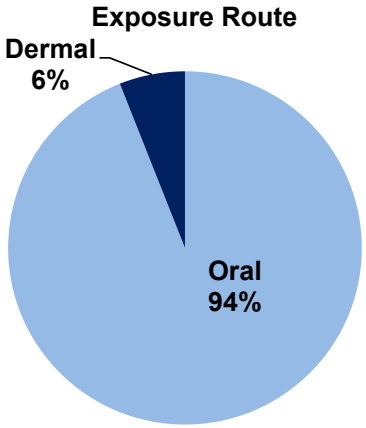


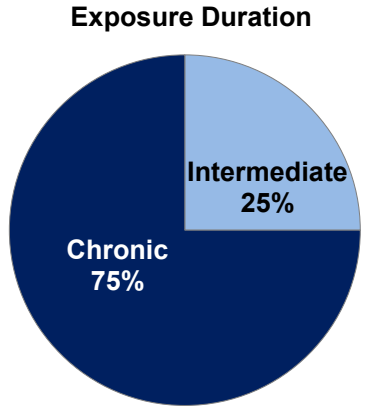
*Includes studies discussed in Chapter 2. A total of 158 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints. Human studies of unknown route and/or duration were classified as chronic oral studies for the purpose of this figure.

Figure 2-4. Overview of the Number of Studies Examining δ-Hexachlorocyclohexane (δ-HCH) and Unspecified Hexachlorocyclohexanes Health Effects*

Most studies examined the potential hepatic and cancer effects of δ-HCH and/or Unspecified HCHs
Fewer studies evaluated health effects in **animals** than **humans** (counts represent studies examining endpoint)







*Includes studies discussed in Chapter 2. A total of 16 studies (including those finding no effect) have examined toxicity; most studies examined multiple endpoints. Human studies of unknown route and/or duration were classified as chronic oral studies for the purpose of this figure.

Table 2-1. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effects
ACUTE EXPOSURE									
Oldiges et al. 1980									
1	Rat (Wistar) 5 M, 5 F	4 hours	0, 273, 603	LE, CS, GN, OW	Bd wt		603 F		Body weight loss in females during first week of observation
					Neuro		273	603	LOAEL: clinical signs of restlessness, hyperactivity Serious LOAEL: marked somnolence
Ullmann 1986b									
2	Rat (Wistar) 5 M, 5 F	4 hours	0, 101, 378, 642, 2,104	LE, CS, BW, GN	Death			378	20% of rats died (LC_{50}: 1,560 mg/m^3)
					Neuro		101		Clinical signs (sedation, curved body position)
Klonne and Kintigh 1988									
3	Mouse (CD-1) 45 M, 45 F	1 week 5 days/week 6 hours/day	0, 0.3, 1, 5, 10	LE, CS	Death			10	12/45 females and 2/45 males died during first week of 13-week study
INTERMEDIATE EXPOSURE									
Oldiges et al. 1983									
4	Rat (Wistar) 12 M, 12 F	90 days 7 days/week 6 hours/day	0, 0.02, 0.1, 0.5, 5	LE, CS, BW, FI, WI, HE, UR, OW, HP	Bd wt	5			
					Resp	5			
					Cardio	5			
					Gastro	0.5	5		Diarrhea
					Hemato	0.5	5		Bone marrow myelogram changes (increased reticulocytes, stem cells and myeloblasts; decreased lymphocytes)
					Hepatic	5			
					Renal	5 F	0.5 M		Dilated tubules with protein-containing contents; proliferated tubules
					Endocr	5			

Table 2-1. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effects
					Neuro	5			
					Repro	5			
Klonne and Kintigh 1988									
5	Mouse (CD-1) 45 M, 45 F	14 weeks 5 days/week 6 hours/day	0, 0.3, 1.0, 5	LE, CS, BW, FI, WI, HE, BC, UR, GN, OW, HP	Death			1	1/45 males and 1/45 females died at 1 mg/m^3; 5/45 males and 15/45 females died at 5 mg/m^3
					Bd wt	5			
					Resp	5			
					Cardio	5			
					Gastro	5			
					Hemato	5			
					Hepatic	5			
					Renal	5			
					Endocr	5			
					Repro	5			No histopathology changes in reproductive organs

[a]The number corresponds to entries in Figure 2-5; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-5. Where such differences exist, only the levels of effect for the most sensitive sex are presented.

BC = serum (blood) chemistry; Bd wt or BW = body weight; Cardio = cardiovascular; CS = clinical signs; Endocr = endocrine; F = female(s); FI = food intake; Gastro = gastrointestinal; GN = gross necropsy; HE = hematology; Hemato = hematological; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Neuro = neurological; NOAEL = no-observed-adverse-effect level; OW = organ weight; Repro = reproductive; Resp = respiratory; UR = urinalysis; WI = water intake

Figure 2-5. Levels of Significant Exposure to γ-Hexachlorocyclohexane (Lindane) – Inhalation
Acute (≤14 days)

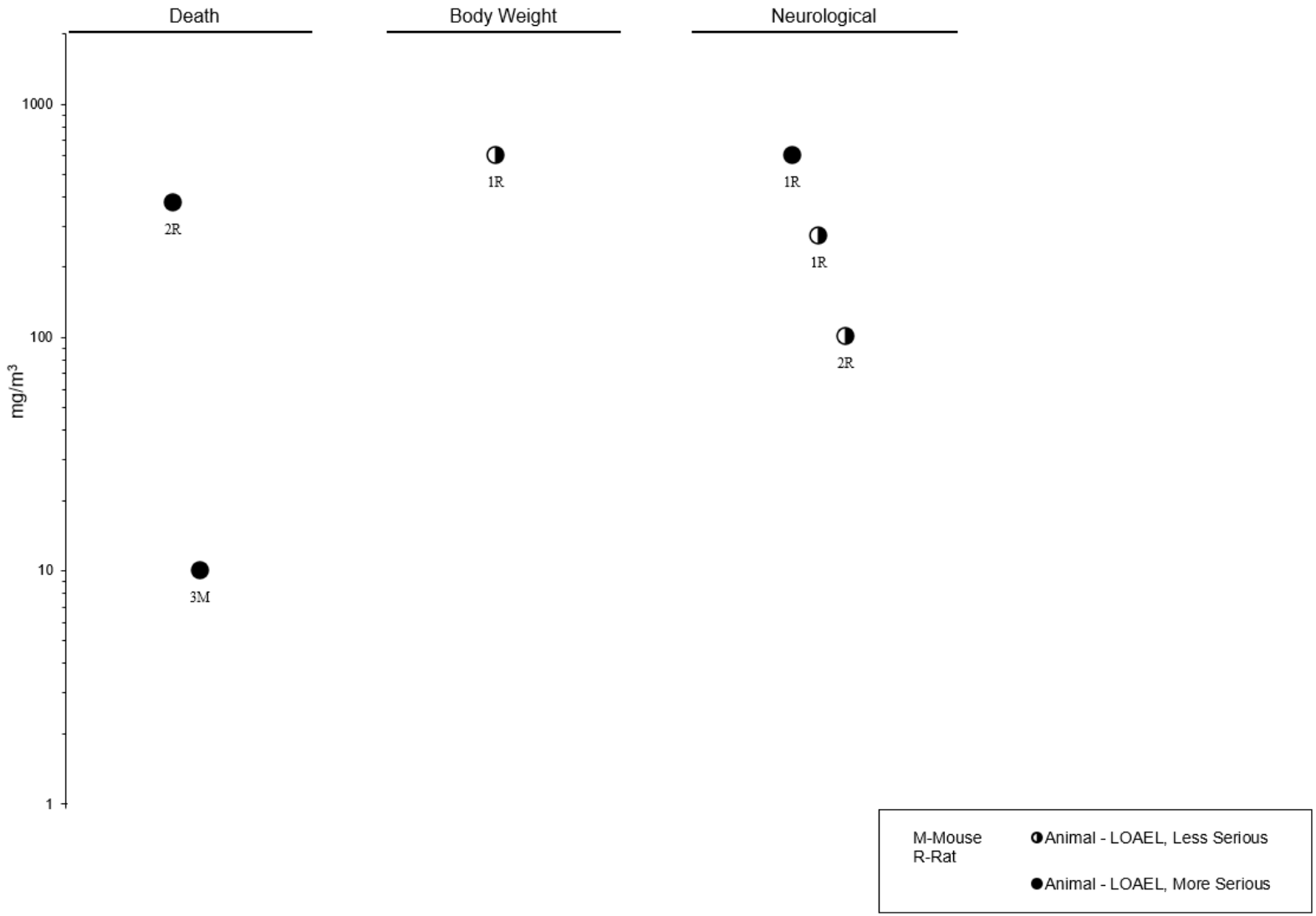


Figure 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane (Lindane) – Inhalation

Intermediate (15–364 days)

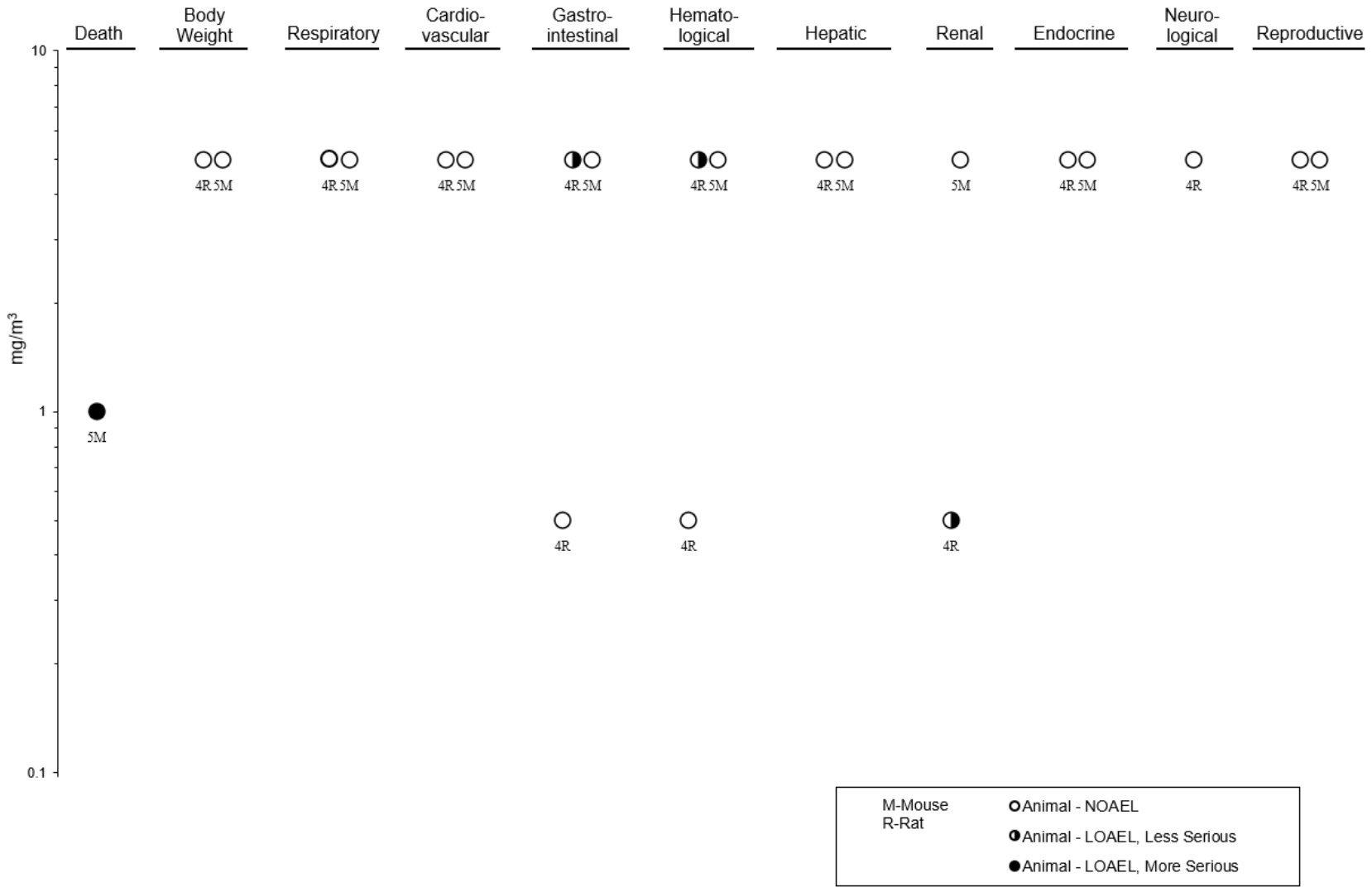


Table 2-2. Levels of Significant Exposure to α-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
ACUTE EXPOSURE									
Sumida et al. 2007									
1	Rat (Fischer-344) 4 M	1, 3, 7, or 14 d (GO)	0, 2, 20	BW, BC, OW	Bd wt	20			
					Hepatic	2	20		24% increase in relative liver weight
INTERMEDIATE EXPOSURE									
Fitzhugh et al. 1950									
2	Rat (Wistar) 10 F, 10 M	6–9 months (F)	Males: 0, 60 Females: 0, 70	LE, BW, FI, GN, OW, HP	Death			60 M 70 F	Mean survival was 35.9 weeks versus 58.3 weeks in controls
					Bd wt		60 M 70 F		11–15% decrease in body weight gain
					Resp	60 M 70 F			
					Cardio	60 M 70 F			
					Gastro	60 M 70 F			
					Hemato	60 M 70 F			
					Musc/skel	60 M 70 F			
					Hepatic		60 M 70 F		Moderate histopathology changes (focal necrosis, fatty degeneration); >2-fold increase in liver weight
					Renal		60 M 70 F		Slight to moderate histopathology changes including tubular dilatation, hyaline tubular casts, glomerular fibrosis or atrophy, pigment deposition

Table 2-2. Levels of Significant Exposure to α-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Endo	60 M 70 F			
					Repro	60 M 70 F			
Ito et al. 1975									
3	Rat (W strain) 18–24 M	48 weeks (F)	0, 35, 70	BW, OW, HP	Hepatic		35		Hepatocellular hypertrophy
					Cancer			70	CEL: liver tumors after 48 weeks
Muller et al. 1981									
4	Rat (Wistar) 15 M	30 days (F)	0, 5.1, 54.2, 106.2	NX	Neuro	106.2			No reduction in motor conduction velocity
Nagasaki et al. 1975									
5	Rat (Wistar) 8 M	24 weeks (F)	0, 45	BW, OW, HP	Bd wt		45		15% decrease in terminal body weight
					Hepatic		45		Mild hypertrophy; ~2-fold increase in absolute and relative liver weight
Sumida et al. 2007									
6	Rat (Fischer-344) 4 M	28 days (GO)	0, 2, 20	BW, BC, OW, HP	Bd wt	20			
					Hepatic	2[b]	20		Increased relative liver weight (25%); centrilobular hepatocellular hypertrophy
Hanada et al. 1973									
7	Mouse (dd) 10–11 M, 10–11 F	32 weeks (F)	M: 0, 18, 54, 108 F: 0, 20, 60, 120	BC, GN, HP	Cancer			18 M 60 F	CEL: hepatoma

Table 2-2. Levels of Significant Exposure to α-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Ito et al. 1973									
8	Mouse (dd) 20–40 M	24 weeks (F)	0, 18, 45, 90	BW, OW, GN, HP	Bd wt	90			
					Hepatic		18		Increased relative liver weight (33%); hepatocellular hypertrophy
					Cancer			45	CEL: hepatocellular carcinoma
Ito et al. 1976									
9	Mouse (DDY) 13–20 M	16–36 weeks (F)	0, 90	BW, OW, HP	Cancer			90	CEL: hepatocellular carcinoma
Nagasaki et al. 1975									
10	Mouse (DDY, ICR, DBA/2, C57BL/6, C3H/He) 20 M, 20 F	24 weeks (F)	Males: 0, 90 Females: 0, 100	BW, OW, HP	Bd wt		90 M		17% decrease in terminal body weight of male C57BL/6 mice
					Hepatic		90 M 100 F		Parenchymal cell hypertrophy, bile duct proliferation, oval cells; nodular hyperplasia; 2-fold increase in liver weight
					Cancer			90 M 100 F	CEL: hepatocellular carcinomas
Tryphonas and Iverson 1983									
11	Mouse (HPB) 75 M	50 weeks (F)	0, 90	BW, GN, OW, HP	Hepatic		90		Hepatomegaly; megalocytosis
					Cancer			90	CEL: neoplastic nodules of the liver after 21 weeks
Tsukada et al. 1979									
12	Mouse (DD) 6 M	16–36 weeks (F)	0, 90	GN HP	Cancer			90	CEL: hepatomas after 28 weeks

Table 2-2. Levels of Significant Exposure to α-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Nagasaki et al. 1975									
13	Hamster (Golden Syrian) 6–10 M	24 weeks (F)	0, 45	BW, OW, HP	Bd wt		45		14% decrease in terminal body weight
					Hepatic		45		20–38% increase in liver weight; liver cell hypertrophy
CHRONIC EXPOSURE									
Fitzhugh et al. 1950									
14	Rat (Wistar) 10 F, 10 M	107 weeks (F)	M: 0, 0.7, 4, 7 F: 0, 0.9, 4, 9	LE, BW, FI, GN, OW, HP	Resp	9 F 7 M			
					Cardio	9 F 7 M			
					Gastro	9 F 7 M			
					Hemato	9 F 7 M			
					Musc/skel	9 F 7 M			
					Hepatic	0.7 M 0.9[c] F	4		32% increase in relative liver weight and very slight to slight microscopic damage
					Renal	9 F 7 M			
					Endo	9 F 7 M			
					Repro	9 F 7 M			

Table 2-2. Levels of Significant Exposure to α-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Ito et al. 1975									
15	Rat (W strain) 18–24 M	72 weeks (F)	0, 70, 105	BW, OW, HP	Cancer			70	CEL: hepatocellular carcinoma

[a]The number corresponds to entries in Figure 2-6; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-6. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an intermediate-duration oral minimal risk level (MRL). The NOAEL of 2 mg/kg/day was divided by an uncertainty factor of 100 (10 for human variability and 10 for animal to human extrapolation) and a modifying factor of 10 (for lack of data on developmental toxicity, immunotoxicity, and neurotoxicity), resulting in an MRL of 0.002 mg/kg/day ($2x10^{-3}$ mg/kg/day).
[c]Used to derive a chronic-duration oral MRL. The NOAEL of 0.9 mg/kg/day was divided by an uncertainty factor of 100 (10 for human variability and 10 for animal to human extrapolation) and a modifying factor of 10 (for lack of data on immunotoxicity and neurotoxicity), resulting in an MRL of 0.0009 mg/kg/day ($9x10^{-4}$ mg/kg/day).

BC = serum (blood) chemistry; Bd wt or BW = body weight; CEL = cancer effect level; (F) = feed; F = female(s); FI = food intake; GN = gross necropsy; (GO) = gavage in oil; HP = histopathology; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = muscular/skeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NX = neurotoxicity; OW = organ weight; (W) = drinking water

Figure 2-6. Levels of Significant Exposure to α-Hexachlorocyclohexane – Oral
Acute (≤14 days)

Figure 2-6. Levels of Significant Exposure to α-Hexachlorocyclohexane – Oral

Intermediate (15–364 days)

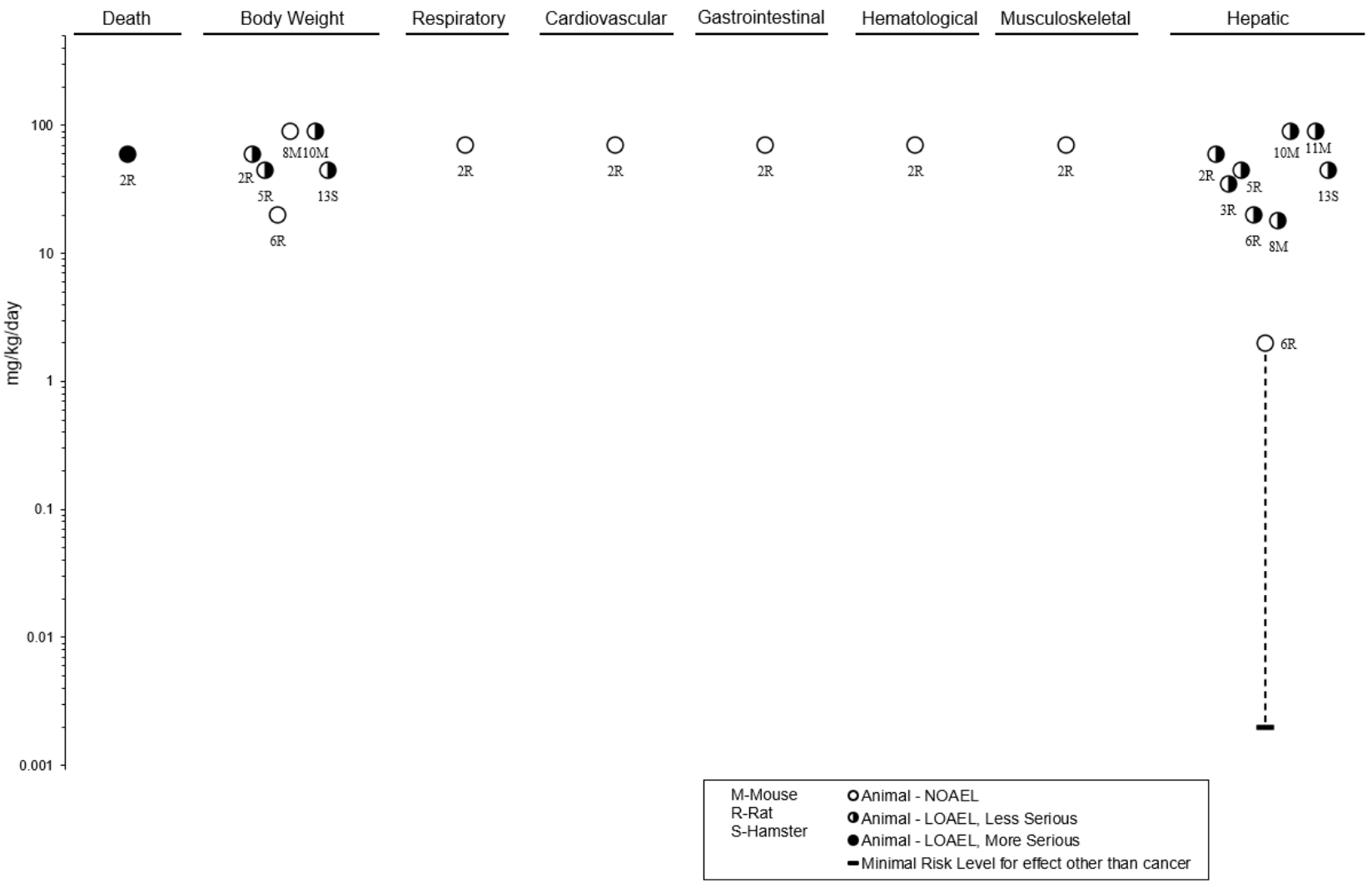


Figure 2-6. Levels of Significant Exposure to α-Hexachlorocyclohexane – Oral

Intermediate (15–364 days)

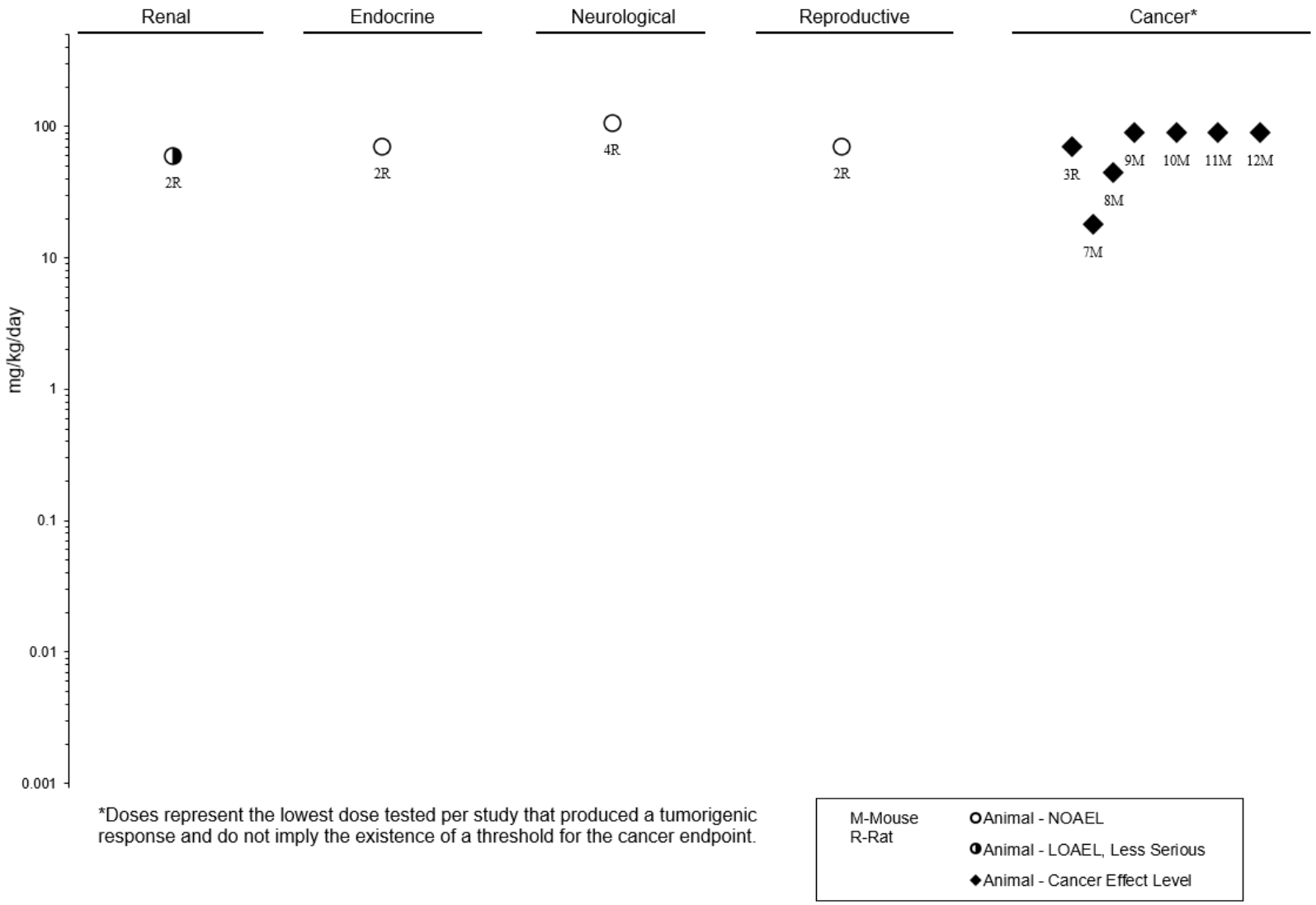


Figure 2-6. Levels of Significant Exposure to α-Hexachlorocyclohexane – Oral

Chronic (≥365 days)

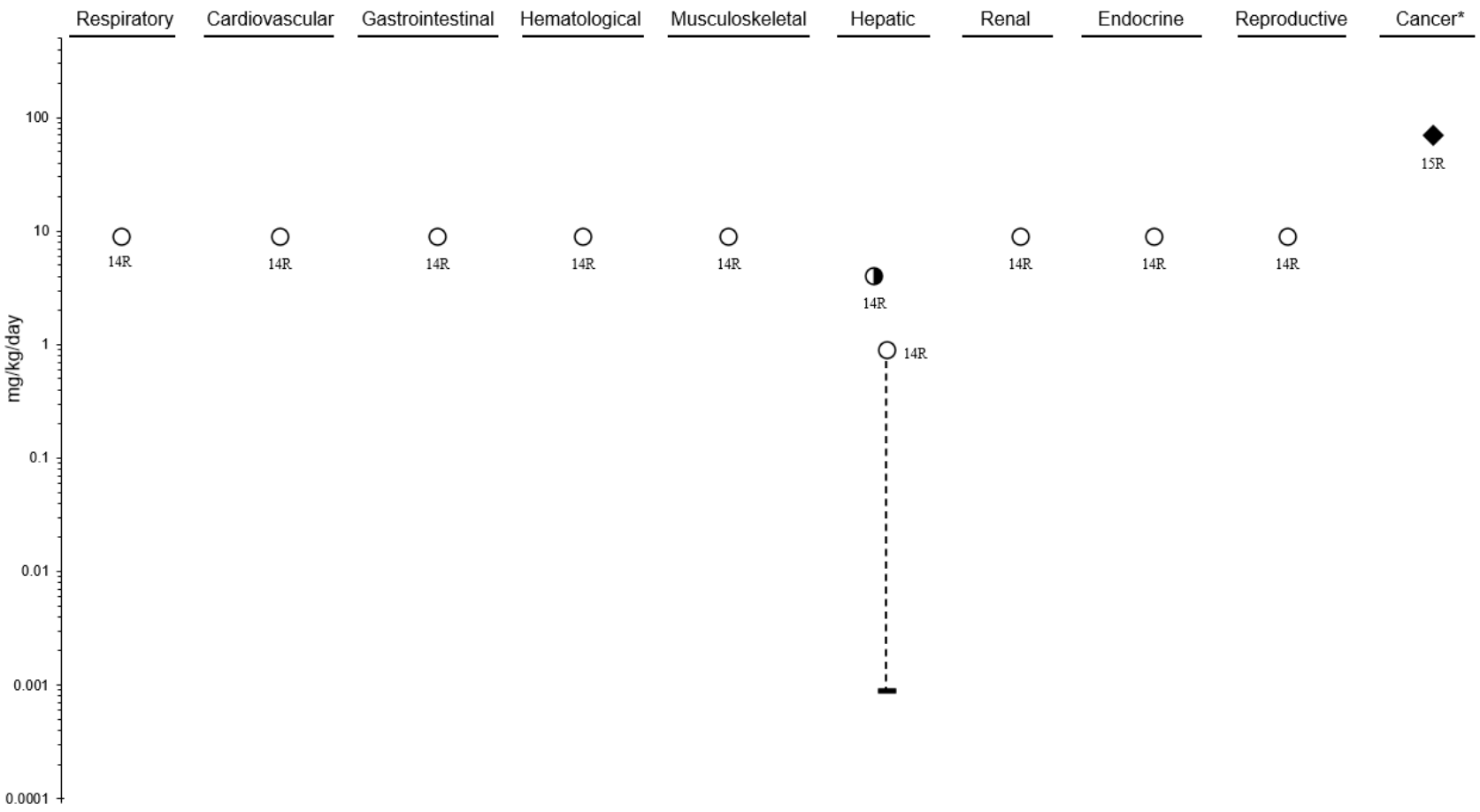


Table 2-3. Levels of Significant Exposure to β-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
ACUTE EXPOSURE									
Srinivasan et al. 1984									
1	Rat (Wistar) 6 M	2 weeks (F)	0, 72	BW, BC, UR, HP	Renal		72		Tubular degeneration, distention of glomeruli, swelling of tubular epithelia, 22% increase in kidney weight, glucosuria, increased urinary excretion of urea and creatinine, decreased excretion of protein
Van Velsen et al. 1986									
2	Rat (Wistar) 10 F, 10 M	2 weeks (F)	0, 8, 38	CS	Neuro	8[b]		38	Ataxia, hypoactivity
Cornacoff et al. 1988									
3	Mouse (B6C3F1) 6 F	1 weeks (F)	0, 20, 60, 200	CS	Death			200	Lateral recumbency leading to humane sacrifice in 80% of mice
					Neuro	20	60		Ataxia resolving within a few days
INTERMEDIATE EXPOSURE									
Fitzhugh et al. 1950									
4	Rat (Wistar) 10 F, 10 M	10 weeks (F)	Males: 0, 60 Females: 0, 70	LE, BW, FI, GN, OW, HP	Death			70 F 60 M	All animals died by 10 weeks of exposure; mean age at death was 4.4 weeks
					Resp	70 F 60 M			
					Cardio	70 F 60 M			
					Gastro	70 F 60 M			

Table 2-3. Levels of Significant Exposure to β-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Hemato	70 F 60 M			
					Musc/skel	70 F 60 M			
					Hepatic			70 F 60 M	Moderate to marked liver damage including fatty degeneration and focal necrosis
					Renal		70 F 60 M		Very slight nephritis; basal vacuolation
					Endo	70 F 60 M			
					Repro	70 F 60 M			
Fitzhugh et al. 1950									
5	Rat (Wistar) 10 M, 10 F	6 months (F)	M: 0, 7 F: 0, 9	BW	Bd wt	7 M	9 F		11% decrease in body weight gain among females
Ito et al. 1975									
6	Rat (W strain) 18–24 M	48 weeks (F)	0, 35, 70	BW, OW, HP	Hepatic		35		Hepatocellular hypertrophy
Muller et al. 1981									
7	Rat (Wistar) 15 M	30 days (F)	0, 66.3, 270.6	NX	Neuro		66.3		Reduced tail nerve conduction velocity
Srinivasan et al. 1991									
8	Rat (Wistar) 6 F	GDs 0–21 (F)	0, 5, 20, 40, 80	DX	Death			80	None of the dams survived 3 weeks of treatment
					Develop	5		20	48% pup mortality before PND 5

Table 2-3. Levels of Significant Exposure to β-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Srinivasan et al. 1991									
9	Rat (Wistar) 6 F	GDs 0–21 and LDs 1–28 or LDs 1–28 only (F)	0, 5, 25	RX, DX	Develop		5	25	LOAEL: increased liver weight in pups at 28 days of age Serious LOAEL: 100% mortality before PND 5 in pups exposed *in utero*
Van Velsen et al. 1986									
10	Rat (Wistar) 10 F, 10 M	13 weeks (F)	Males: 0, 0.18, 0.9, 4.5, 22.5 Females: 0, 0.2, 1.0, 5, 25	CS, BW FI, HE, BC, BI, OW, HP	Death			22.5 M 25 F	50% of animals were moribund and sacrificed humanely
					Bd wt	5 F 4.5 M	25 F 22.5 M		≥10% decrease in body weight
					Hemato	5 F 4.5 M	25 F 22.5 M		Decreased red blood cells, leukocytes, and hemoglobin concentrations
					Hepatic	ND M 1 F	0.18[c] M 5 F		Hyalinization of centrilobular cells in males; increased mitoses in females
					Renal	4.5 M	22.5 M		Renal medullary calcinosis
					Endo	5 F 4.5 M	25 F 22.5 M		Adrenal cortical hypertrophy
					Immuno	5 F 4.5 M	25 F 22.5 M		Depletion of splenic lymphoid tissue; thymic cortical atrophy
					Repro	5 F 4.5 M		25 F 22.5 M	Atrophy of testes and ovaries

Table 2-3. Levels of Significant Exposure to β-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Cornacoff et al. 1988									
11	Mouse (B6C3F1) 6 F	30 days (F)	0, 20, 60	CS, BW, HE, OW, HP, NX	Bd wt	60			
					Immuno	20	60		Decreased lymphoproliferative responses to T-cell mitogens, decreased natural killer cell activity
					Repro	60			No changes in ovarian or uterine histology
Hanada et al. 1973									
12	Mouse (dd) 10–11 M, 10–11 F	32 weeks (F)	Males: 0, 20, 50, 100 Females: 0, 20, 60, 100	GN, HP	Hepatic	20	60 F 50 M		Nuclear irregularities in foci of enlarged hepatocytes
Ito et al. 1973									
13	Mouse (dd) 20–40 M	24 weeks (F)	0, 18, 45, 90	BW, OW, HP	Bd wt	90			
					Hepatic		18		18% increase in relative liver weight with histopathology changes (liver cell hypertrophy) at higher doses
Thorpe and Walker 1973									
14	Mouse CF1 30 M, 30 F	3 months (F)	0, 34	LE	Death			34	12% of males and 25% of females died during the first 3 months of a chronic study

Table 2-3. Levels of Significant Exposure to β-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
CHRONIC EXPOSURE									
Fitzhugh et al. 1950									
15	Rat (Wistar) 10 F, 10 M	107 weeks (F)	M: 0, 0.7, 7 F: 0, 0.9, 9	LE, BW, FI, GN, OW, HP	Resp	7 M 9 F			
					Cardio	7 M 9 F			
					Gastro	7 M 9 F			
					Hemato	7 M 9 F			
					Musc/skel	7 M 9 F			
					Hepatic		0.7 M 0.9 F		34% increase in relative liver weight; very slight histopathology changes
					Renal	7 M 9 F			
					Endo	7 M 9 F			
					Repro	0.7 M 9 F		7 M	Slight testicular atrophy

Table 2-3. Levels of Significant Exposure to β-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Thorpe and Walker 1973									
16	Mouse (CF1) 30 M, 30 F	104 weeks (F)	0, 34	CS, GN, HP	Cancer			34	CEL: liver tumors in males; unspecified tumors in females.

[a]The number corresponds to entries in Figure 2-7; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-7. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an acute-duration oral minimal risk level (MRL). The NOAEL of 8 mg/kg/day was divided by an uncertainty factor of 100 (10 for human variability and 10 for animal to human extrapolation) resulting in an MRL of 0.08 mg/kg/day ($8x10^{-2}$ mg/kg/day).
[c]Used to derive an intermediate-duration oral MRL. The LOAEL of 0.18 mg/kg/day was divided by an uncertainty factor of 300 (10 for human variability, 10 for animal to human extrapolation, and 3 for use of a minimal LOAEL) resulting in an MRL of 0.0006 mg/kg/day ($6x10^{-4}$ mg/kg/day).

Bd wt or BW = body weight; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; GD = gestation day; GN = gross necropsy; Hemato = hematological; HP = histopathology; Immuno = immunological; LD = lactation day; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = muscular/skeletal; ND = not determined; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NX = neurotoxicity; OW = organ weight; PND = postnatal day; Repro = reproductive; RX = reproductive toxicity; UR = urinalysis

Figure 2-7. Levels of Significant Exposure to β-Hexachlorocyclohexane – Oral
Acute (≤14 days)

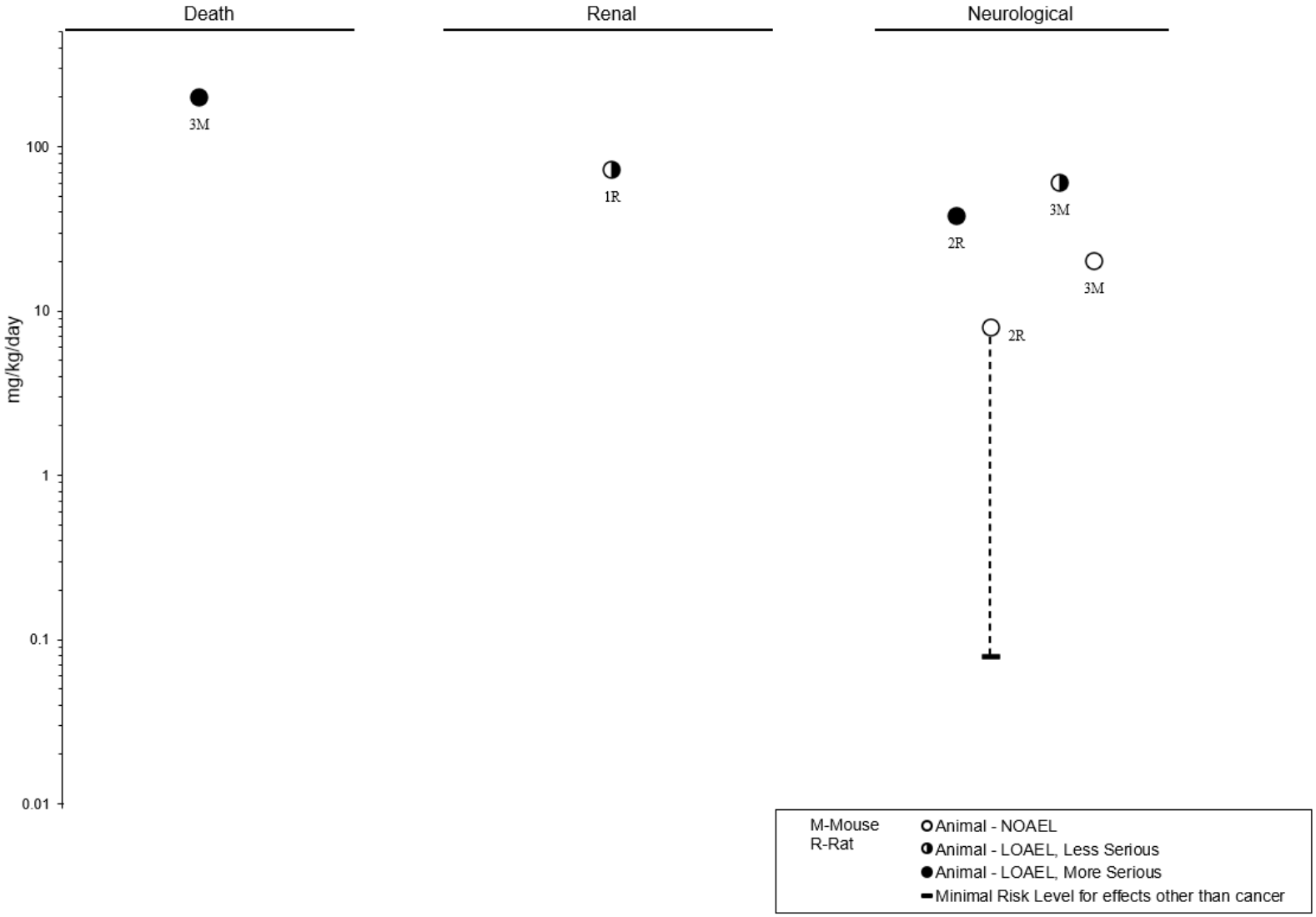


Figure 2-7. Levels of Significant Exposure to β-Hexachlorocyclohexane – Oral

Intermediate (15–364 days)

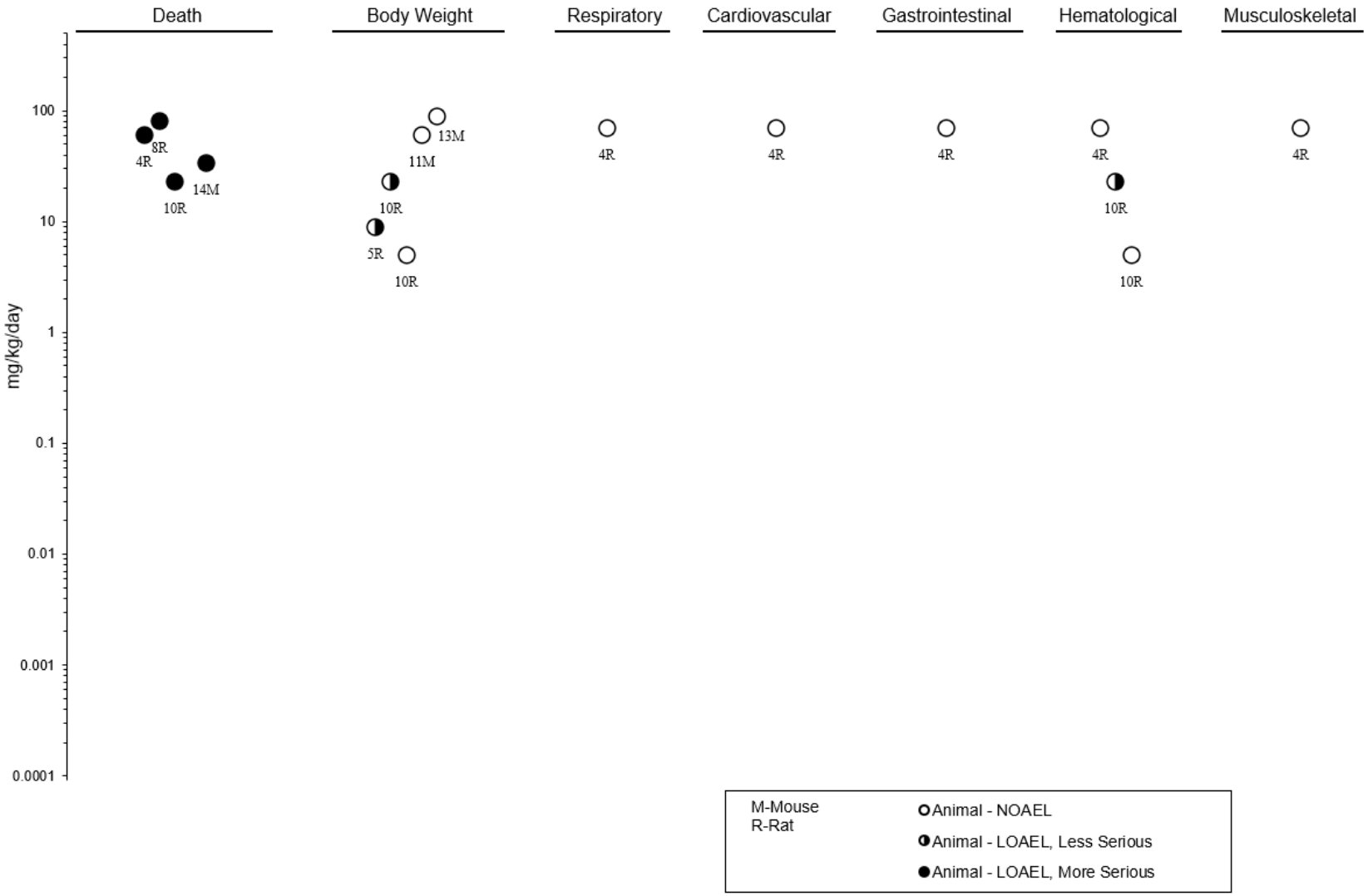


Figure 2-7. Levels of Significant Exposure to β-Hexachlorocyclohexane – Oral

Intermediate (15–364 days)

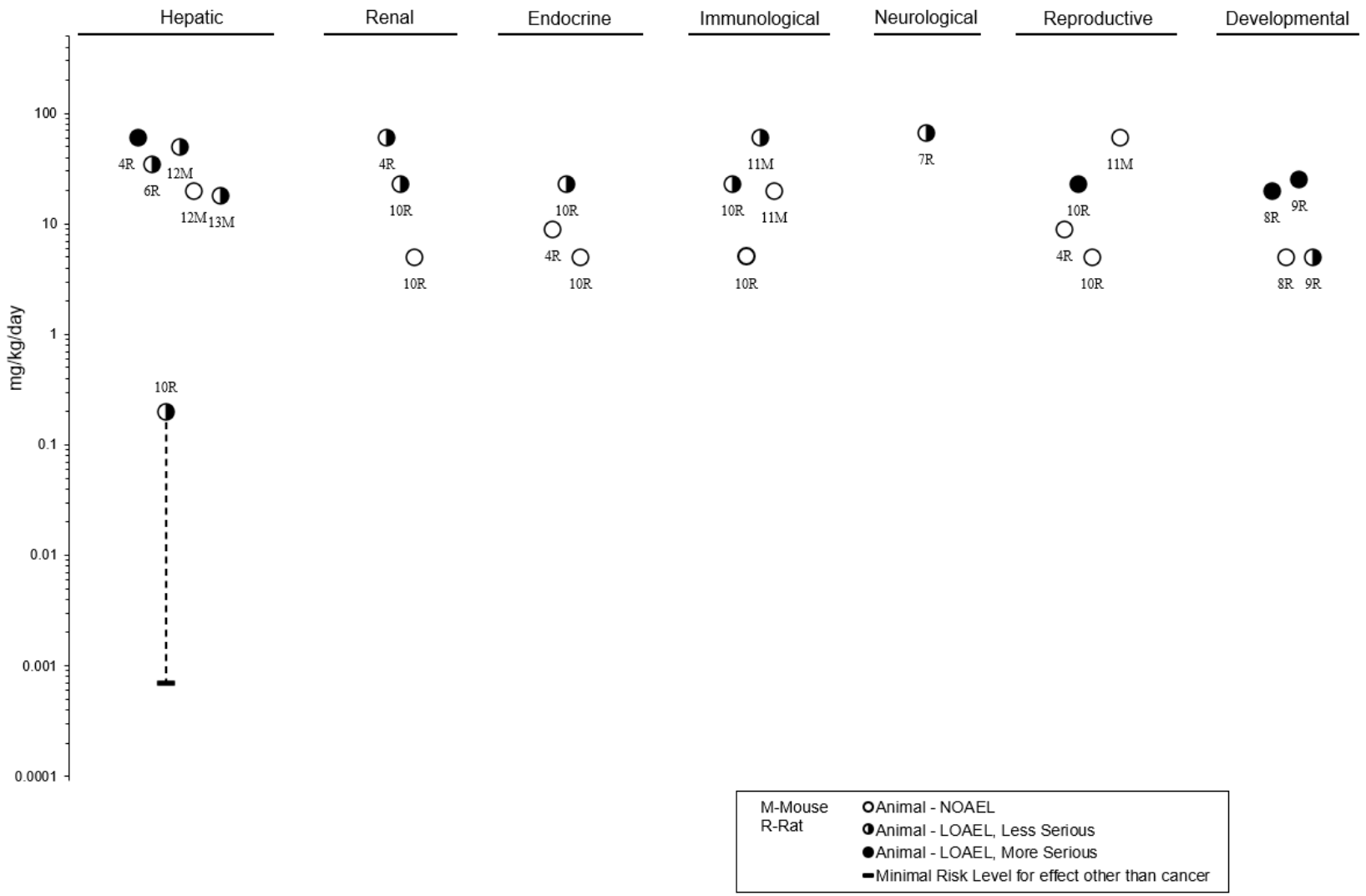


Figure 2-7. Levels of Significant Exposure to β-Hexachlorocyclohexane – Oral

Chronic (≥365 days)

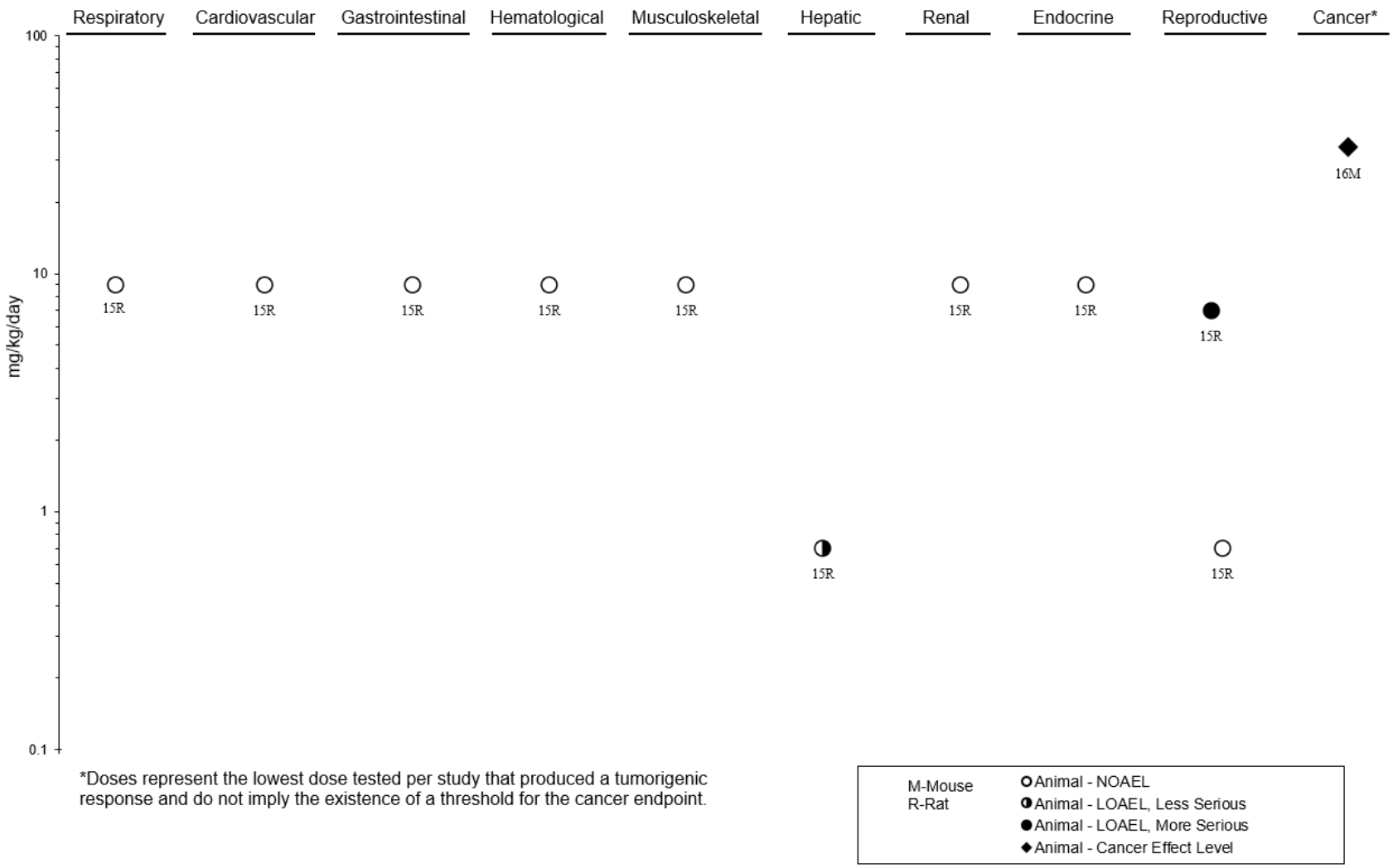


Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
ACUTE EXPOSURE									
Ali and Shakoori 1998									
1	Rat (Sprague-Dawley) 3–5, NS	2 days (F)	0, 30	HP	Hepatic		30		Reduced number of cells per field; increased cell, nucleus, and nucleolus size; slight cellular disorganization
Attia et al. 1991									
2	Rat (Sprague-Dawley) 9 M	6 days (GO)	0, 3	BI	Neuro		3		Increased pineal N-acetyl-transferase, decreased serotonin levels
Dalsenter et al. 1996									
3	Rat (Wistar) 15 M	1–5 days	0, 6, 30	RX	Repro		6		Decreased number of spermatids per epididymis
Dalsenter et al. 1997a									
4	Rat (Wistar) 15 M	GDs 15 once (GO)	0, 30	DX	Develop		30		Reduced serum testosterone in adult offspring
Dalsenter et al. 1997b									
5	Rat (BOR) 9 F	LD 9 or 14 once (GO)	0, 6	CS, BI, OW, HP, NX, RX	Develop		6		In male pups, reduced relative testicular and epididymis weight (~10%), spermatid and sperm counts (~8–10%), testosterone levels (~30–50%), Leydig cell numbers, and spermatogenesis at maturity, with no effect on fertility

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Dalsenter et al. 1997b									
6	Rat (BOR) 9 F	LDs 9–14 (GO)	0, 1	CS, BI, OW, HP, NX, RX	Develop		1[b]		In male pups, reduced relative testicular and epididymis weight (~10%), spermatid and sperm counts (~10%), and testosterone levels (30–50%) at maturity, with no effect on fertility
EPA 1999a									
7	Rat (CD) 10 M, 10 F	Once (G)	0, 6, 20, 60		Neuro	6 F 20 M	20 F	60 M	LOAEL: decreased motor activity and grooming behavior, increased forelimb grip strength in females Serious LOAEL: tremors and convulsions in one male
Gaines 1960									
8	Rat (Sherman) 89 M, 69 F	Once (GO)	NS	LE, CS	Death			91 F 88 M	LD_{50} LD_{50}
Gilbert 1995									
9	Rat (Long-Evans) 15–16 M	10 days 3 days/week (GO)	0, 10	CS	Neuro			10	Myoclonic jerks and clonic seizures
Gilbert and Mack 1995									
10	Rat (Long-Evans) 14 M	once (GO)	0, 5, 10, 20	CS	Neuro			5	Myoclonic jerks and single clonic seizure in naive animals

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Hfaiedh et al. 2012									
11	Rat (Wistar) 6 M	3 days (GO)	0, 5	BC, BI, HP	Hepatic		5		Fatty degeneration, vacuolation, and necrosis of the liver
Johri et al. 2008									
12	Rat (Wistar) 10 M	Once (GO)	0, 30	CS	Neuro			30	Convulsions in 5/10 animals
Joy et al. 1982									
13	Rat (Sprague-Dawley) 7–14 M	4 days (GO)	0, 1, 3, 10	CS, BW, BC, OW, HP, NX	Neuro	1	3	10	LOAEL: increased kindling acquisition Serious LOAEL: seizures
Khera et al. 1979									
14	Rat (Wistar) 20 F	GDs 6–15 (GO)	0, 6.25, 12.5, 25	DX	Develop	25			No teratogenic effects
Llorens et al. 1989									
15	Rat (Wistar) 9 M	Once (GO)	0, 10, 15, 30	CS, NX	Neuro		10		Increased spontaneous motor behavior
Llorens et al. 1990									
16	Rat (Wistar) 9 M	Once (GO)	0, 20	CS, NX	Neuro		20		Increased anxiety
Martinez and Martinez-Conde 1995									
17	Rat (Wistar) 8 M, 8 F	Once (GO)	0, 60	CS, NX	Neuro			60	Convulsions
Martinez et al. 1991									
18	Rat (Wistar) 7 M	Once (GO)	0, 60	LE, CS, NX	Death			60	1/7 died
					Neuro			60	Tonic-clonic seizures

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Mediratta et al. 2008									
19	Rat (Wistar) 8 M	14 days (NS)	0, 10	IX	Immuno		10		Reduced delayed-type hypersensitivity (43% decrease in foot pad thickness)
Palmer et al. 1978									
20	Rat (CFY) 20 F	GDs 6–16 (G)	0, 5, 10, 20	DX	Develop	20			
Parmar et al. 2003									
21	Rat (Wistar) 10 M	Once (GO)	0, 35	CS	Neuro			35	Convulsions in 4/10 rats
Parmar et al. 2003									
22	Rat (Wistar) 10 M	5 days (GO)	0, 2.5, 5, 10, 15	CS, BW, BI, OW	Bd wt	15			
Rivera et al. 1991									
23	Rat (Wistar) 4 M, 4 F	Once (GO)	0, 20	CS, BI, NX	Develop		20		Regional changes in brain noradrenaline, serotonin, and dopamine metabolite levels in suckling rats
Rivera et al. 1998									
24	Rat (Wistar) NS M, F	PND 15 once (G)	0, 20	DX	Develop		20		Altered acquisition of a passive avoidance task, decreased motor activity, altered neurotransmitter levels in brain
Rivera et al. 1998									
25	Rat (Wistar) NS M, F	PNDs 8–14 (G)	0, 10	DX	Develop		10		Altered acquisition of a passive avoidance task, increased motor activity, altered neurotransmitter levels in brain

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Serrano et al. 1990									
26	Rat (Wistar) 5 M, 5 F	PNDs 8–10 (GO)	0, 5, 10, 15, 20	BW, BI	Develop			5	Decreased myelin in developing brain
Sharma and Singh 2010									
27	Rat (Wistar) 6 M	14 days (GO)	0, 30	RX	Repro			30	Markedly decreased epididymis (27%) and testes (68%) weights; substantial and persistent reductions (≥85% less than controls) in sperm head count, motility, and percent live sperm; marked and persistent increases (4-fold) in percent abnormal sperm
Singh and Sharma 2011									
28	Rat (Wistar) NS M	1 day (G)	0, 60	BI, HP	Hepatic			60	Marked centrilobular necrosis
Sinha and Shukla 2003									
29	Rat (Druckrey) 8 M	3 days (GO)	0, 8.8	BW, OW	Bd wt	8.8			
Srinivasan et al. 1984									
30	Rat (Wistar) 6 M	2 weeks (F)	0, 72	BW, BC, UR, OW, HP	Renal		72		10% increase in kidney weight, distention of glomeruli, swelling of tubular epithelia, glucosuria, increased excretion of urea and creatinine, decreased excretion of protein

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Sumida et al. 2007									
31	Rat (Fischer-344) 4 M	1, 3, 7, or 14 days (GO)	0, 1, 10	BW, BC, OW	Bd wt	10			
					Hepatic	10			
Tusell et al. 1988									
32	Rat (Wistar) 4 M	3–12 days	0, 5, 12, 20	LE, CS	Death			20	2/18 died on 3rd day
					Neuro	5		12	Convulsions after 3 days
Uphouse and Williams 1989									
33	Rat (CDF-F344) 8–11 F	Once (G)	0, 12.5, 25, 33, 50	RX	Repro		25		Increased length of estrous cycle
Woolley and Griffith 1989									
34	Rat (Sprague-Dawley) 7 M	Once (GO)	0, 30, 40, 50	CS, NX	Neuro			30	Seizures
Di Consiglio et al. 2009									
35	Mouse (CD-1) 2–10 F	GDs 9–16 (GO)	0, 25	LE, CS, BW, BI, OW, HP, DX	Bd wt	25			
					Develop		25		Decreased sperm concentration (20%) and count (27%) in F1 males at PNDs 65–69

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Hassoun and Stohs 1996a									
36	Mouse (C57BL/6J and DBA/2J) NS F	GD 12 once (GO)	0, 30, 45	LE, DX	Death			30	14% of dams died at 30 mg/kg and 25% died at 45 mg/kg
					Develop		30	45	LOAEL: decreased fetal, placental, and thymic weights in C57BL/6J mice Serious LOAEL: increased early resorptions in C57BL/6J mice
Hong and Boorman 1993									
37	Mouse (B6C3F1) 7 M	3 days (GO)	0, 10, 20, 40	CS, BW, HE, OW HP	Bd wt	40			
					Hemato		20		Transient reductions in marrow progenitor cell numbers
					Immuno	10	20	40	LOAEL: decreased thymus weights Serious LOAEL: atrophy of thymic cortex
Hong and Boorman 1993									
38	Mouse (B6C3F1) 7 M	10 days (GO)	0, 10, 20	CS, BW, HE, OW HP	Bd wt	20			
					Hemato		10		Transient decrease in marrow progenitor cell numbers
					Immuno		10		Decreased relative thymus and spleen weights
La Sala et al. 2009									
39	Mouse (CD-1) NS F	3 days (GO)	0, 15, 30	DX	Develop		15		Decreased numbers of primordial germ cells in male and female offspring
Liu and Morgan 1986									
40	Mouse (DBA/2) 6 F	10 days	20	LE, CS, BC	Death			20	6/6 died

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Maranghi et al. 2007									
41	Mouse (CD) 12 F	GDs 9–16 (GO)	0, 15	BW, FI, BI, OW, HP, DX	Bd wt	15			
					Repro	15			
					Develop		15		Early vaginal patency; increased absolute (17%) and relative (13%) uterine weight at PND 22; decreased oocyte diameter (21%) in primary follicles at PND 60 in F1 females
Scascitelli and Pacchierotti 2003									
42	Mouse (CD-1) 16–29 F	3 days (GO)	0, 15, 25	RX	Repro	15	25		Increase in degenerating two-cell embryos following preovulatory exposure
Sinha and Shukla 2003									
43	Mouse Swiss 8 M	3 days (GO)	0, 5.9	BW, OW	Bd wt	5.9			
Traina et al. 2003									
44	Mouse (CD-1) 10–24 F	GDs 9–16 (GO)	0, 15, 25	BW, DX	Bd wt	25			
					Develop		15		14% decrease in sperm count in F1 offspring with more severe effects observed at higher dose
Palmer et al. 1978									
45	Rabbit (New Zealand) 13 F	GDs 6–18 (G)	0, 5, 10, 20	DX	Develop	20			

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
INTERMEDIATE EXPOSURE									
Ahmed et al. 2008									
46	Rat (Wistar) 10 M	4 weeks, 7 days/week (GO)	0, 30	CS, BW, FI, BC	Bd wt	30			
Ali and Shakoori 1998									
47	Rat (Sprague-Dawley) 3–5 NS	15 days (F)	0, 18	HP	Hepatic		18		Reduced number of cells per field; increased cell, nucleus, and nucleolus size; vacuoles in the cytoplasm and granulation; apparent fatty degeneration
Amyes 1990									
48	Rat (Wistar) 15 M, 15 F	Up to 52 weeks (F)	Males: 0, 0.07, 0.7, 7, 28 Females: 0, 0.08, 0.8, 8, 32	LE, CS, BW, FI, WI, HE, BC, BI, UR, GN, OW, HP, NX	Death			32 F	Statistically significant decreased survival
					Bd wt	32F 28 M			
					Hemato	32F 28 M			
					Hepatic	0.8 F 0.7 M	8 F 7 M		Periacinar hepatocytic hypertrophy
					Renal	32 F	0.07 M		Hyaline droplets, interstitial chronic nephritis, and regeneration in proximal tubules
					Neuro			32 F	Convulsions in 11/55 females

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Anand et al. 1995									
49	Rat (albino) NS M	6 weeks (G)	0, 3	BW, BC, OF	Cardio		3		Tachycardia (~30% increase in heart rate); increased blood pressure, plasma calcium levels, and myocardial calcium influx; decreased Ca, K-ATPase activity; ECG changes
Andrews and Gray 1990									
50	Rat (Long-Evans) NS M	10 weeks 7 days/week (G)	0, 10, 20	BW, OW, BC	Bd wt	20			
					Renal		10		Increased kidney weight; hyaline droplet accumulation and tubular regeneration
					Musc/skel	10	20		Decreased femur medullary area
Arisi et al. 1994									
51	Rat (Wistar) 4–12 M	90 days (F)	0, 90	CS	Neuro			90	Tonic convulsions
Chadwick et al. 1988									
52	Rat (Fischer-344) 6–12 F	15 weeks (GO)	0, 5, 10, 20, 40	LE, BW, FI, BI, OW	Death			20	2/12 died at 20 mg/kg/day; 7/12 died at 40 mg/kg/day
					Repro	5	10		Delayed vaginal opening, disrupted ovarian cycling
Desi 1974									
53	Rat (Wistar) 8–10 NS	40 days (F)	0, 2.5, 5, 10, 50	NX, OW, OF, HP	Hepatic	50			
					Neuro		2.5		Significantly altered Skinner box behavior

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Dewan et al. 1980									
54	Rat (Charles Foster) 5 M, 5 F	35 days	0, 6.25, 25	IX	Immuno		6.25		Immunosuppression (decreased antibody titers)
EPA 1991a									
55	Rat (CD) 30 M, 30 F	2 generations; 70 days prior to mating until sacrifice	0, 0.09, 1.7, 13.1	CS, BW, FI, HP, RX, DX	Bd Wt	1.7 F 13.1 M	13.1 F		Decreased body weight gain during gestation in F0 females
					Hepatic	0.09 M 13.1 F	1.7 M		In F1 males: hepatocellular hypertrophy
					Renal	0.09 M 13.1 F	1.7 M		In F0 and F1 males: Increased kidney weights; nephritis, tubular cell regeneration, hyaline droplets, tubular necrosis
					Repro	13.1			
					Develop		13.1		Reduced F1 and F2 pup weight and viability; delayed tooth eruption and hair growth in F2
EPA 1999b									
56	Rat (CD) 10 M, 10 F	13 weeks (F)	Males: 0, 1.4, 7.1, 28.1 Females: 0, 1.6, 7.9, 30.2	NX	Death			30.2 F	3/10 females died during the study
					Neuro	7.1 M 7.9 F	28.1 M 30.2 F		Increased rearing, walking on tiptoes, hypersensitivity to touch, and hunched posture

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
EPA 1999c									
57	Rat (Wistar) 10 M, 10 F	GD 6 to LD 10 (F)	Gestation: 0.8–0.9, 4.2–4.6, 8.0–10.5, Lactation:1.2–1.7, 5.6–8.3, 13.7–19.1	DX	Develop	1.2		5.6	Up to 18% reduction in pup body weight and 24% lower body weight gain during lactation
Fatih Fidan et al. 2008									
58	Rat (Sprague-Dawley) 10 M	30 days (GO)	0, 10, 20, 40	BC, BI, HP	Hepatic	10		20	Megalocytosis; vacuolar degeneration; severe venous and sinusoidal congestion; and lymphocytic infiltration
					Renal	10		20	Severe kidney congestion, medullary and cortical hemorrhage, and degeneration and vacuolation of proximal convoluted tubules
Fitzhugh et al. 1950									
59	Rat (Wistar) 10 F, 10 M	10 months (F)	Males: 0, 30, 60, 120 Females: 0, 30, 70, 140	LE, BW	Death			60 M 70 F	Mean age at death was 39.7 weeks versus 58.3 weeks in controls
					Bd wt	60 M 70 F	120 M 140 F		13–17% decrease in body weight gain at 6 months
					Resp	120 M 140 F			
					Cardio	120 M 140 F			
					Gastro	120 M 140 F			

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Hemato	120 M 140 F			
					Musc/skel	120 M 140 F			
					Hepatic		60 M 70 F		Slight microscopic damage
					Renal		60 M 70 F		Very slight microscopic damage
					Neuro			60 M 70 F	Convulsions
					Endo	120 M 140 F			
					Repro	120 M 140 F			
Gilbert 1995									
60	Rat (Long-Evans) 15–16 M	30 days (GO)	0, 10	CS	Neuro			10	Myoclonic jerks and clonic seizures
Hfaiedh et al. 2011									
61	Rat (Wistar) 6 M	30 days (W)	0, 50	BC, BI, OW, HP, RX	Endocr		50		85% increase in T4, 74% decrease in TSH
					Repro			50	Decreased testes (52%), epididymides (42%), prostate gland (50%), and seminal vesicles (5%) weights; 56% reduced sperm count; 37% reduced sperm motility; 74% decrease in FSH
Ito et al. 1975									
62	Rat (W strain) 18–24 M	48 weeks (F)	0, 35	BW, OW, HP	Hepatic		35		Hepatocellular hypertrophy

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Johri et al. 2007									
63	Rat (Wistar) 13–14 F	GDs 5–21 (GO)	0, 0.0625, 0.125, 0.25	DX, BI	Develop	0.125	0.25		Persistent hyperactivity
Johri et al. 2008									
64	Rat (Wistar) 13 F	GDs 5–21 and PND 45 (GO)	0, 0.25 (prenatal) and 30 (postnatal)	CS, BI	Develop			30	Convulsions in 8/10 animals
Koner et al. 1998									
65	Rat (Wistar) 8–10 M	8 weeks (F)	0, 3.6, 7.0	IX	Immuno		3.6		Reduced serum antibody response to SRBC
Martinez and Martinez-Conde 1995									
66	Rat (Wistar) 8 M, 8 F	30 days, every 3 days (GO)	0, 6	CS, NX	Neuro		6		Decreased brain dopamine levels
Matsuura et al. 2005									
67	Rat (Crj:CD [SD] IGS) 24 M, 24 F	~10 weeks (2-generation ; premating to PND 21) (F)	Males, F0: 0, 0.56, 3.4, 17.2 Males, F1: 0.74, 4.5, 23.3 Females, F0: 0, 0.88, 5.2, 26.1 Females, F1: 0.95, 5.6, 28.0	LE, CS, BW, FI, BC, BI, GN, OW, HP, RX, DX, NX	Death			26.1 F	2 F0 females died
					Bd wt	26.1 F 17.2 M			
					Hepatic	0.88 F 0.56 M	5.2 F 3.4 M		Hepatocellular hypertrophy in F0 and F1 male and female parents
					Renal	26.1 F	0.56 M		Basophilic tubules and hyaline droplets in the proximal tubules
					Endocr	5.2 F 3.4 M	26.1 F 17.2 M		Decreased absolute and relative pituitary weights in F0 and F1 females; altered serum thyroid hormone levels; thyroid follicular cell hypertrophy in F0 females and F1 males

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Neuro			28 F	Convulsions in two F1 females
					Repro	26.1 F 17.2 M			
					Develop	0.95	5.6	26.1	LOAEL: 10% decrease in F2 female offspring body weight at PND 4 Serious LOAEL: 49% decrease in F2 offspring viability (PNDs 0–4); 8–29% decreases in male and female F1 and F2 offspring weights on PNDs 0, 4, and 21; delayed sexual maturation (preputial separation in males and vaginal opening in females) in F1 generation
Mediratta et al. 2008									
68	Rat (Wistar) 8 M	21 days (NS)	0, 10	BC, IX	Immuno		10		Decreased anti-SRBC antibody titer (32%)
Mudawal et al. 2018									
69	Rat (Wistar) 6 M	3 weeks 7 days/week (NS)	0, 2.5	BI, NX, DX	Neuro		2.5		Decreased conditioned avoidance, alternations, and locomotor activity; ultrastructural changes in the hippocampus and substantia nigra in adult animals
Muller et al. 1981									
70	Rat (Wistar) 15 M	30 days (F)	0, 1.3, 12.3, 25.4	NX	Neuro	12.3	25.4		Reduced tail nerve conduction velocity

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Parmar et al. 2003									
71	Rat (Wistar) 10 M	15 or 21 days (GO)	0, 2.5	CS, BW, BI, OW	Bd wt	2.5			
Prasad et al. 2016									
72	Rat (Wistar) 6 M	15, 30, or 45 days (GO)	0, 20	BC, BI, GN, HP	Renal			20	Severe corticomedullary and glomerular congestion, intertubular hemorrhage, severe tubular degeneration, desquamation of tubular epithelium, cystic dilatation, mononuclear cell infiltrate, necrosis, and atrophic glomeruli
Sahaya et al. 2007									
73	Rat (Wistar) 10 M	6 weeks, 7 days/week (NS)	0, 15	BI, NX	Neuro		15		Impaired neurocognition (decreased step-down latency in passive avoidance test and prolonged transfer latency in elevated plus maze)
Saradha and Mathur 2006									
74	Rat (Wistar) 4 M	45 days (GO)	0, 1, 5, 50	BW, OW, RX	Repro	1	5	50	LOAEL: decreased sperm count (~27%) and motility (~25%) Serious LOAEL: decreased sperm count (~40%), motility (~40%), and viability (~15%); decreased epididymal weight (~10%)

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Sauviat et al. 2005									
75	Rat (Sprague-Dawley) NS F	~13 weeks (W)	0, 0.000076, 0.00015, 0.00030	GN, OW, HP	Develop	0.000076[c]	0.00015	0.0003	LOAEL: altered ventricular electrophysiology Serious LOAEL: 21% decrease in pup body weight; altered heart morphometry and electrophysiology; cardiac histopathology (hypertrophy in left ventricular area, unorganized collagen bundles and layers, fibroblast destruction)
Sharma and Singh 2010									
76	Rat (Wistar) 6 M	28 days (GO)	0, 30	RX	Repro			30	Decreased cauda epididymis (32%) and testes (70%) weights; ≥89% decreases in sperm head count, motility, and percent live sperm; 4-fold increase in percent abnormal sperm
Srinivasan et al. 1991									
77	Rat (Wistar) 6 F	GDs 0–21 and LDs 1–28 or LDs 1–28 (F)	0, 25	DX	Develop		25		Increased pup relative liver weights
Srivastava et al. 2019									
78	Rat (Wistar) 25 F	GDs 5–21 (GO)	0, 0.25	DX	Develop			0.25	Ultrastructural changes in the brain (moderately distorted mitochondria and demyelinated neurons)

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Srivastava et al. 2019									
79	Rat (Wistar) 6 M	21 days (GO)	0, 2.5	NX	Neuro			2.5	Reduced locomotor activity and spatial memory; ultrastructural changes in the hippocampus and substantia nigra (swollen mitochondria with disintegrated cristae, shortened fuzzy synapse, disintegrated myelin layer, and autophagosomes)
Sumida et al. 2007									
80	Rat (Fischer-344) 4 M	28 days (GO)	0, 1, 10	BW, BC, OW, HP	Bd wt Hepatic	10 10			
Suter 1983									
81	Rat (Wistar) 15 M, 15 F	12 weeks (F)	0, 0.02, 0.08, 0.4, 2.0, 10	LE, CS, BW, FI, HE, UR, OW, HP	Hemato Hepatic Renal	10 0.4 0.4 F	 2 0.4 M 2 F		 Centrilobular hypertrophy Basophilic proximal tubules; proximal tubular distention, hyaline droplet formation; and minimal to moderate interstitial nephritis in males; slight epithelial cell necrosis in proximal convoluted tubules in both sexes

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Vijaya Padma et al. 2011									
82	Rat (Wistar) 6 F	30 days (GO)	0, 100	BC, BI, HP	Hepatic			100	"Extensive" liver injuries consisting of vacuolar degeneration of hepatocytes and "massive" degradation of the central vein
					Renal		100		Glomerular degeneration and shrinkage; degeneration of proximal and distal tubules
Vijaya Padma et al. 2013									
83	Rat (Wistar) 6 M	30 days (GO)	0, 100	BC, BI, OW, HP	Cardio		100		Histopathology changes (inflammatory cells and separated muscle fibers)
Yang et al. 2014; Zhang et al. 2016									
84	Rat (Sprague-Dawley) 7–9 F	4 weeks, 7 days/week (GO)	0, 4, 8	BW, BC, OW, HP	Bd wt	8			
					Repro	4	8		Low columnar endometrial glandular epithelial cells in the uterus
Yuksel et al. 2009									
85	Rat (Sprague-Dawley) 10 M	30 days (GO)	0, 10, 20, 40	CS, BC, GN, OW, HP, RX	Death			20	1/10 rats died at 20 mg/kg/day and 1/10 died at 40 mg/kg/day
Banerjee et al. 1996									
86	Mouse (Hissar) NS M	3–12 weeks	0, 1.8, 5.4, 9	IX	Immuno	1.8	5.4		Immunosuppression (decreased splenic plaque-forming colonies) after 6 weeks; decreased antibody titers at 9 mg/kg/day

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Hanada et al. 1973									
87	Mouse (dd) 10–11 M, 10–11 F	32 weeks	Males: 0, 18, 54, 108 Females: 0, 20, 60, 120	BC, GN, HP	Cancer			120	CEL: hepatoma
Ito et al. 1973									
88	Mouse (dd) 20–40 M	24 weeks (F)	0, 18, 45, 90	BW, OW, HP	Bd wt	90			
					Hepatic	45	90		Relative liver weight increase of 33% and centrilobular hypertrophy
Meera et al. 1992									
89	Mouse (Swiss albino) 6 F	24 weeks (F)	0, 0.012, 0.12, 1.2	CS, HP, IX	Immuno		0.012	1.2	LOAEL: changes in cell- and humoral-mediated immune system Serious LOAEL: histopathology changes in thymus consisting of marked decrease in cortical lymphocytes, many necrosed cells in medulla, congestion of blood vessels, and severe loss of cortex and medulla distinction
Nagda and Bhatt 2011									
90	Mouse (Swiss) 8–10 M	60 days (GO)	0, 40	BI, OW, HP	Repro			40	10% decrease testes weight; histopathology changes in testes (shrunken and distorted seminiferous tubules, sparse Leydig cells, and oligospermia)

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Thorpe and Walker 1973									
91	Mouse (CF1) 30 M, 30 F	3 months (F)	0, 68	LE	Death			68	10% of males and 20% of females died in first 3 months of chronic study
Rivett et al. 1978									
92	Dog (Beagle) 1 M, 1 F	7 weeks	0.9, 2, 4, 7	CS, BW, HE, BC, UR, GN, OW, HP	Bd wt	4	7		Decreased body weight gain
Desi et al. 1978									
93	Rabbit (NS) 6 M	5–6 weeks 5 days/week (C)	0, 1.5, 3, 6, 12	LE, OF, IX	Death			12	“Numerous” deaths
Lindenau et al. 1994									
94	Rabbit (hybrid) 5 F	12 weeks 3 days/week (GO)	0, 0.8	CS, BC, BI	Repro		0.8		Reduced ovulation rate
Seiler et al. 1994									
95	Rabbit (New Zealand) 5 F	12–15 weeks 3 days/week (GO)	0, 0.8	DX, RX	Repro Develop	0.8 0.8			
Beard and Rawlings 1998									
96	Mink (NS) 8–10 F	3 generations (F)	0, 1	CS, BW, FI, GN, OW, HP, RX, DX	Repro			1	Reduced litter size in F2 females (~40% lower than controls); reduced testis size (11–13% shorter length and 34–36% lower mass) in F3 males

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Beard et al. 1997									
97	Mink (NS) 10 F	12 weeks 3 weeks premating to 8 weeks postpartum (F)	0, 1	CS, BW, FI, BC, GN, OW, HP, RX, DX	Repro		1		Reduced mating receptivity and whelping rate
Beard et al. 1997									
98	Mink (NS) 15 F	17 weeks 6 weeks premating to 10 weeks postpartum (F)	0, 1	CS, BW, FI, BC, GN, OW, HP, RX, DX	Repro		1		Reduced whelping rate and increased post-implantation embryo loss
CHRONIC EXPOSURE									
Ali and Shakoori 1998									
99	Rat (Sprague-Dawley) 3–5, NS	18 months (F)	0, 9	HP	Hepatic		9		Increased cell, nucleus, and nucleolus size; extensive cytoplasmolysis; slight cytoplasmic degeneration; increasing nuclear distortion
Fitzhugh et al. 1950									
100	Rat (Wistar) 10 F, 10 M	107 weeks (F)	Males: 0, 0.4, 0.7, 4, 7, 30 Females: 0, 0.4, 0.9, 4, 9, 30	LE, BW, FI, GN, OW, HP	Resp	30			
					Cardio	30			
					Gastro	30			
					Hemato	30			
					Musc/skel	30			
					Hepatic	4	9 F 7 M		Increased relative liver weight (35%); very slight microscopic liver damage
					Renal	4	9 F 7 M		Very slight microscopic kidney damage

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Endo	30			
					Repro	30			
EPA 2000a									
101	Mouse (CD-1) 50 M, 50 F	78 weeks (F)	Males: 0, 1.3, 5.2, 20.5 Females: 0, 1.8, 7.1, 26.8	LE, BW, FI, GN, OW, HP	Bd wt	20.5 M 26.8 F			
					Resp	20.5 M 26.8 F			
					Hemato	20.5 M			
					Hepatic	5.2 M 26.8 F	20.5 M		Centrilobular hepatocyte hypertrophy
					Renal	20.5 M 26.8 F			
					Endocr	20.5 M 26.8 F			
					Neuro	20.5 M 26.8 F			
					Cancer			26.8 F	CEL: bronchiolar-alveolar adenomas and carcinomas
Herbst et al. 1975; Weisse and Herbst 1977									
102	Mouse (NMRI) 50 M, 50 F	80 weeks (F)	Males: 0, 2.1, 4.1, 8.2 Females: 0, 2.0, 3.9, 7.8	BW, GN, HP	Bd wt	8.2 M 7.8 F			
					Hepatic	8.2 M 7.8 F			
NCI 1977									
103	Mouse (B6C3F1) 50 M, 50 F	80 weeks (F)	0, 13.6, 27.2	CS, BW, BI, HP	Cancer			13.6 M	CEL: hepatocellular carcinoma
Thorpe and Walker 1973									
104	Mouse (CF1) 30 M, 30 F	104 weeks (F)	0, 68	CS, GN, HP	Cancer			68	CEL: liver and other unspecified tumors in both sexes

Table 2-4. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Wolff et al. 1987									
105	Mouse (F-1 hybrid) 24–96 F	24 months (F)	0, 27.2	CS, BW, BI, HP	Cancer			27.2	CEL: hepatocellular carcinoma, lung tumors
Rivett et al. 1978									
106	Dog (Beagle) 4 M, 4 F	104 weeks (F)	0, 0.83, 1.60, 2.92	CS, BW, HE, BC, UR, GN, OW, HP, NX	Bd wt	2.92			
					Hepatic	2.92			Livers were dark but without histopathology changes
					Hemato	2.92			
					Ocular	2.92			

[a]The number corresponds to entries in Figure 2-8; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-8. Where such differences exist, only the levels of effect for the most sensitive sex are presented.
[b]Used to derive an acute-duration oral minimal risk level (MRL). The LOAEL of 1 mg/kg/day was divided by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for human variability, and 10 for animal to human extrapolation), resulting in an MRL of 0.003 mg/kg/day ($3x10^{-3}$ mg/kg/day).
[c]Used to derive an intermediate-duration oral MRL. The NOAEL of 0.000076 mg/kg/day was divided by an uncertainty factor of 100 (10 for human variability, and 10 for animal to human extrapolation), resulting in an MRL of 0.0000008 mg/kg/day ($8x10^{-7}$ mg/kg/day).

BC = serum (blood) chemistry; Bd wt or BW = body weight; BI = biochemical changes; (C) = capsule; Cardio = cardiovascular; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; ECG = electrocardiogram; Endocr = endocrine; (F) = feed; F = female(s); F0 = parental generation; F1 = first generation; F2 = second generation; F3 = third generation; FI = food intake; FSH = follicle stimulating hormone; (G) = gavage; GD = gestation day; GN = gross necropsy; (GO) = gavage in oil;; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; IX = immunotoxicity; LD = lactation day; LD_{50} = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = musculoskeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurotoxicity; OF = organ function; OW = organ weight; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; SRBC = sheep red blood cell; T4 = thyroxine; TSH = thyroid stimulating hormone; UR = urinalysis; (W) = drinking water; WI = water intake

Figure 2-8. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral
Acute (≤14 days)

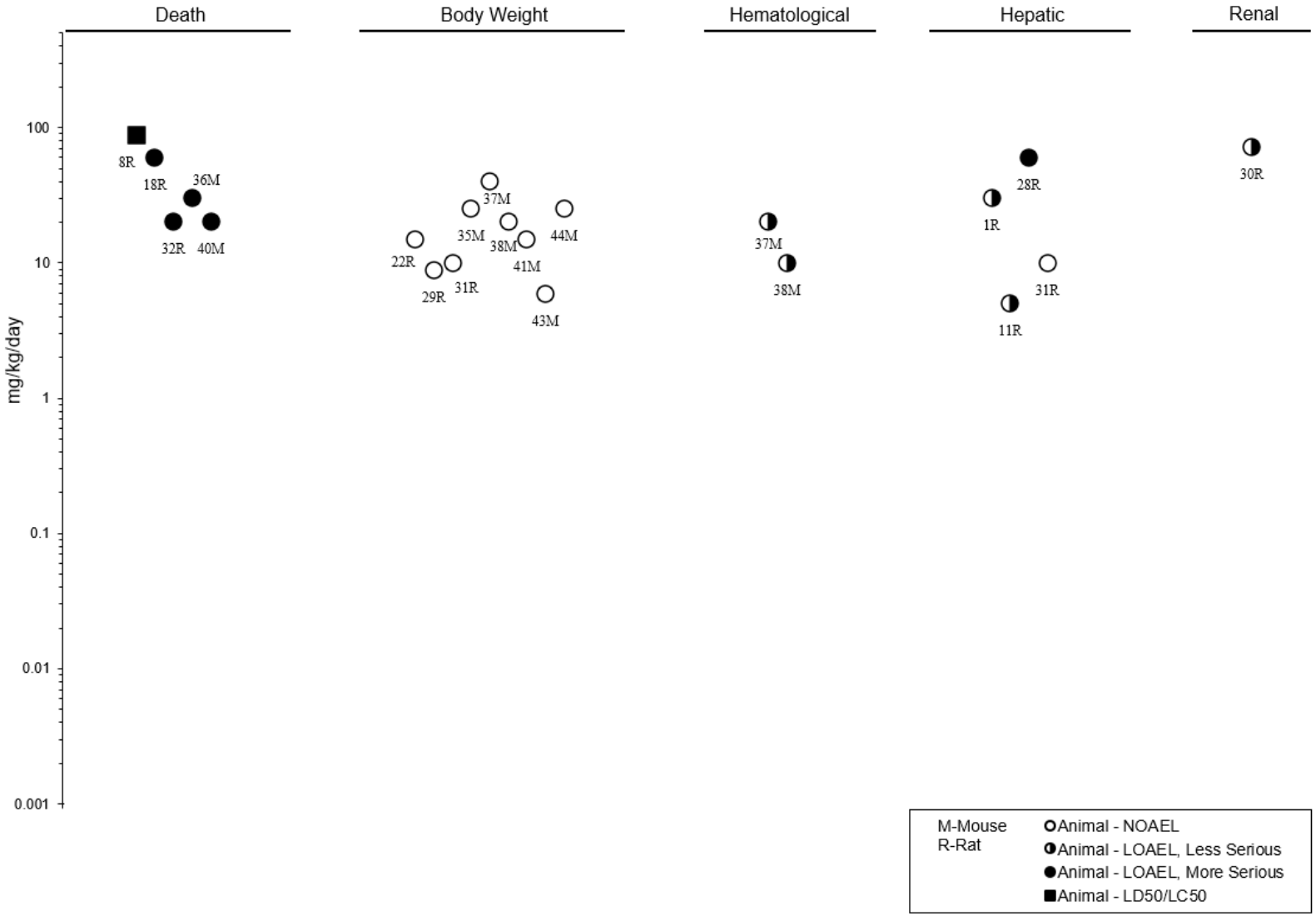


Figure 2-8. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Acute (≤14 days)

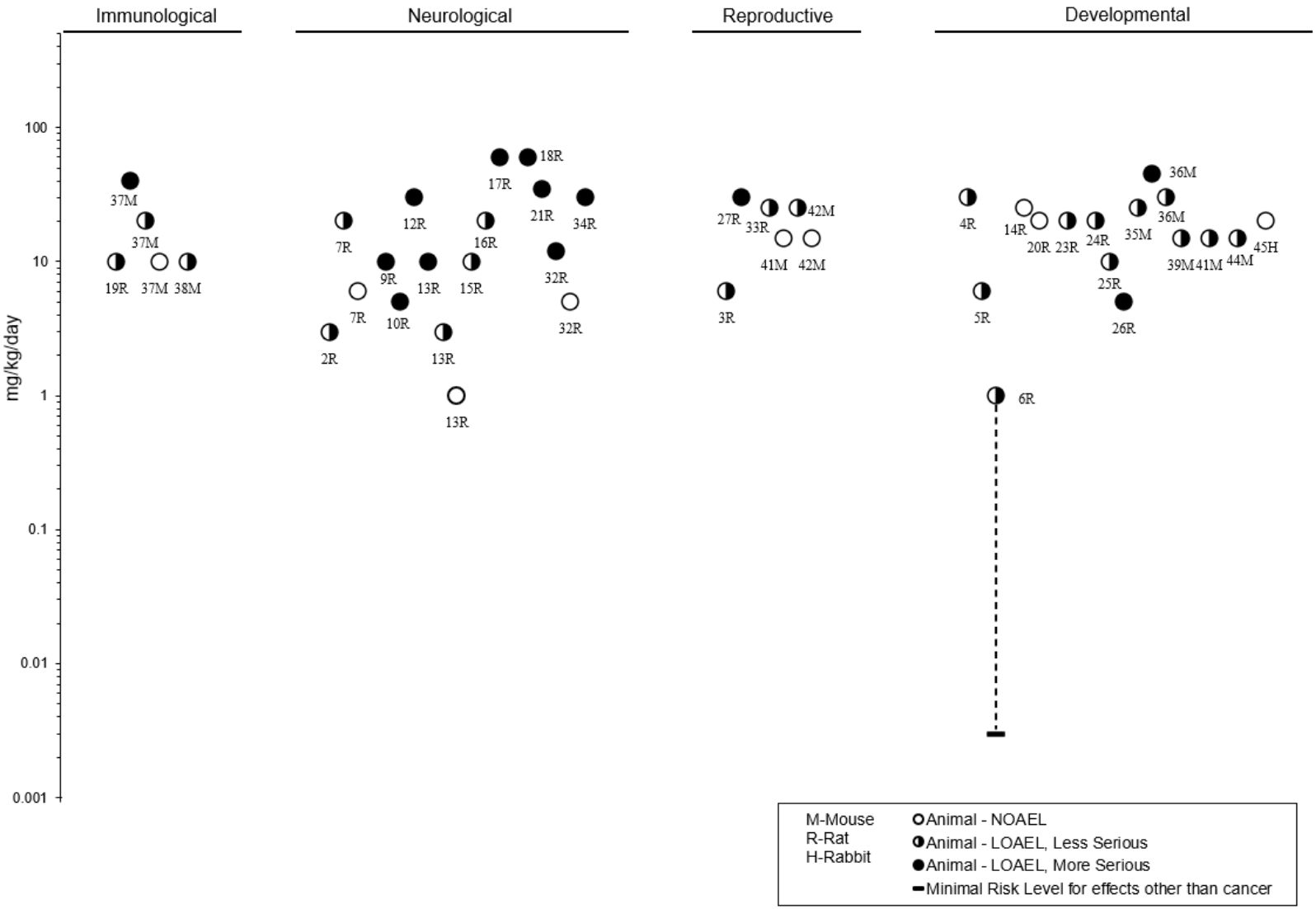


Figure 2-8. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Intermediate (15–364 days)

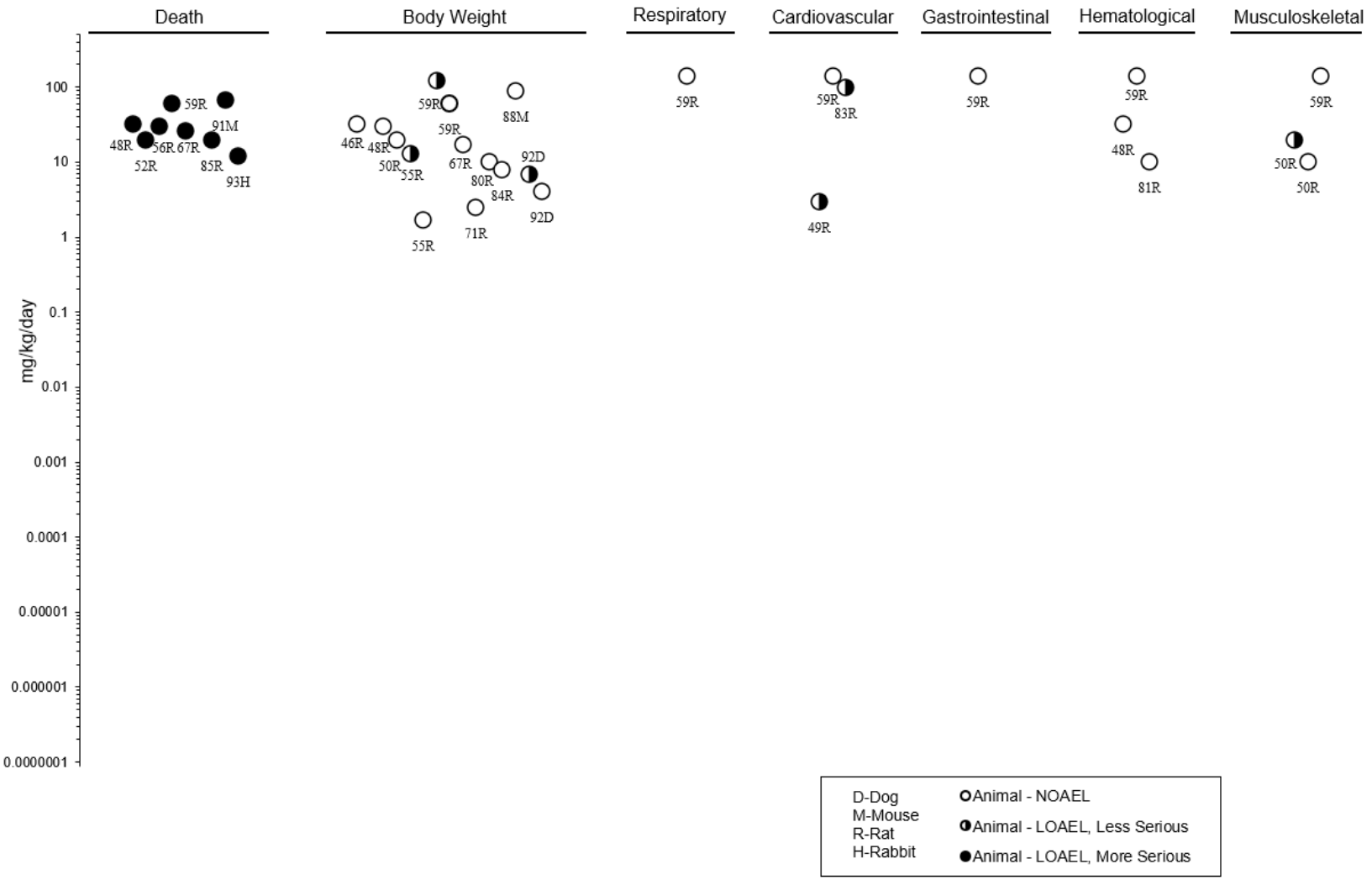


Figure 2-8. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Intermediate (15–364 days)

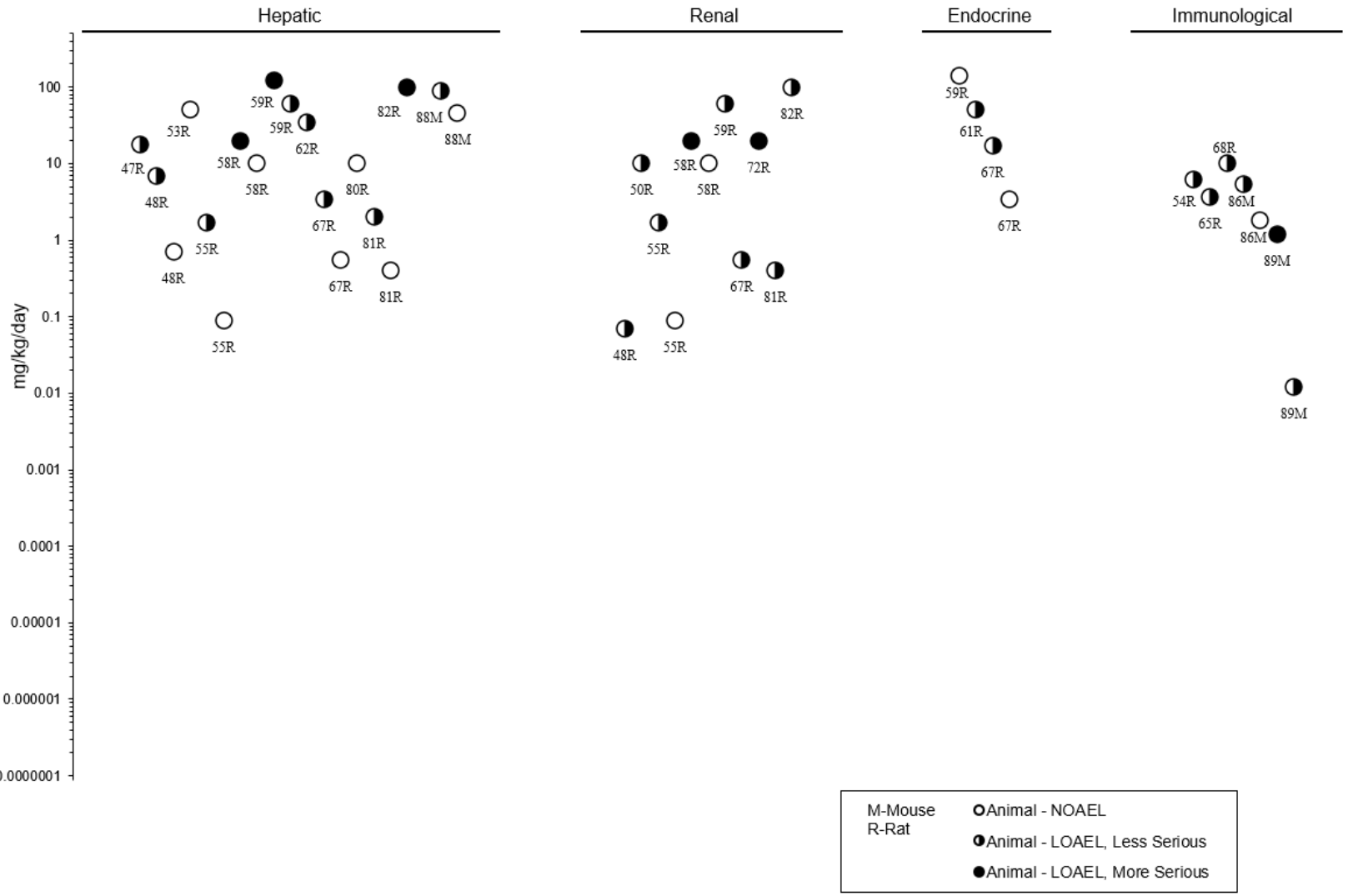


Figure 2-8. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Intermediate (15–364 days)

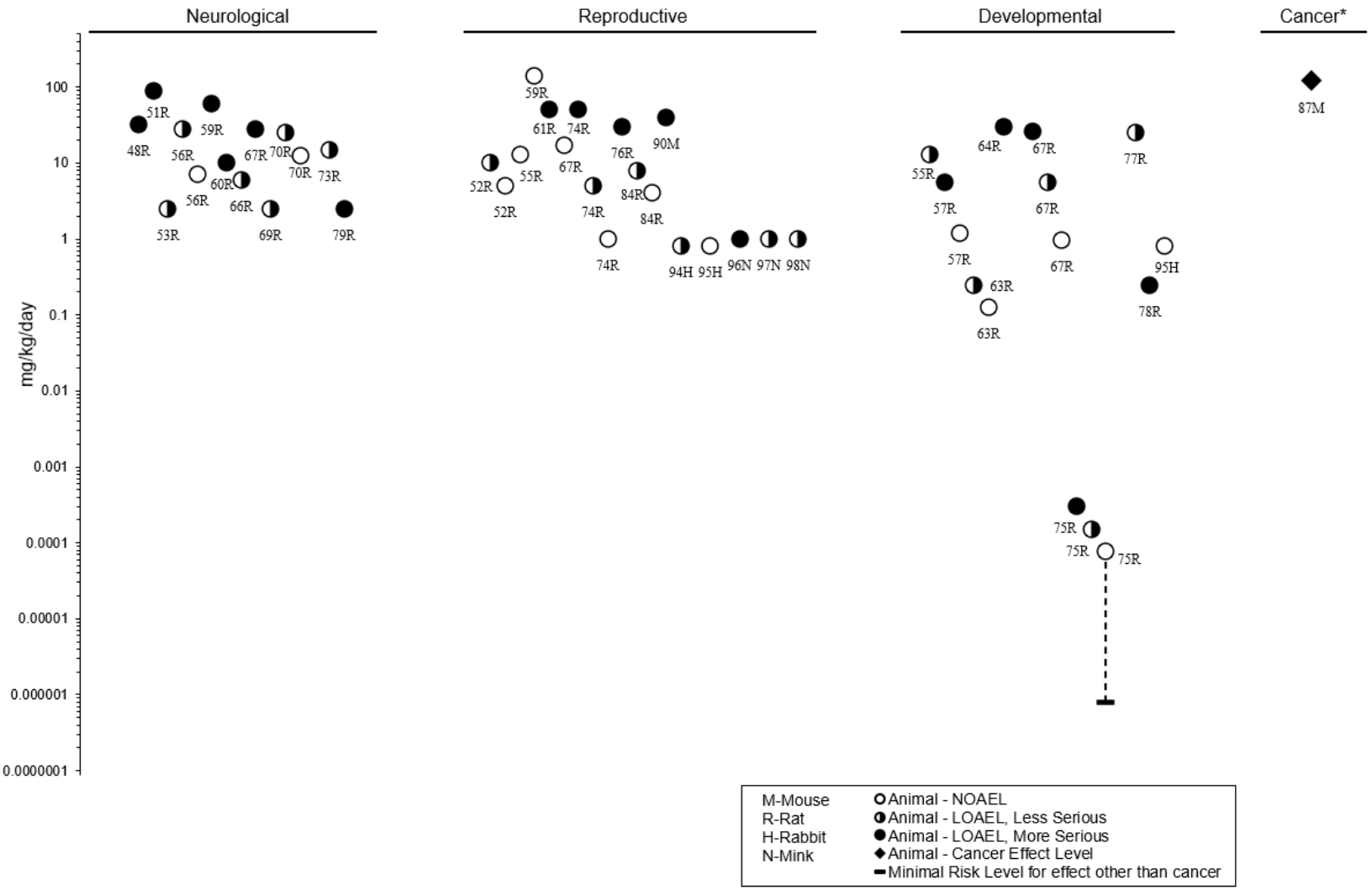


Figure 2-8. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Chronic (≥365 days)

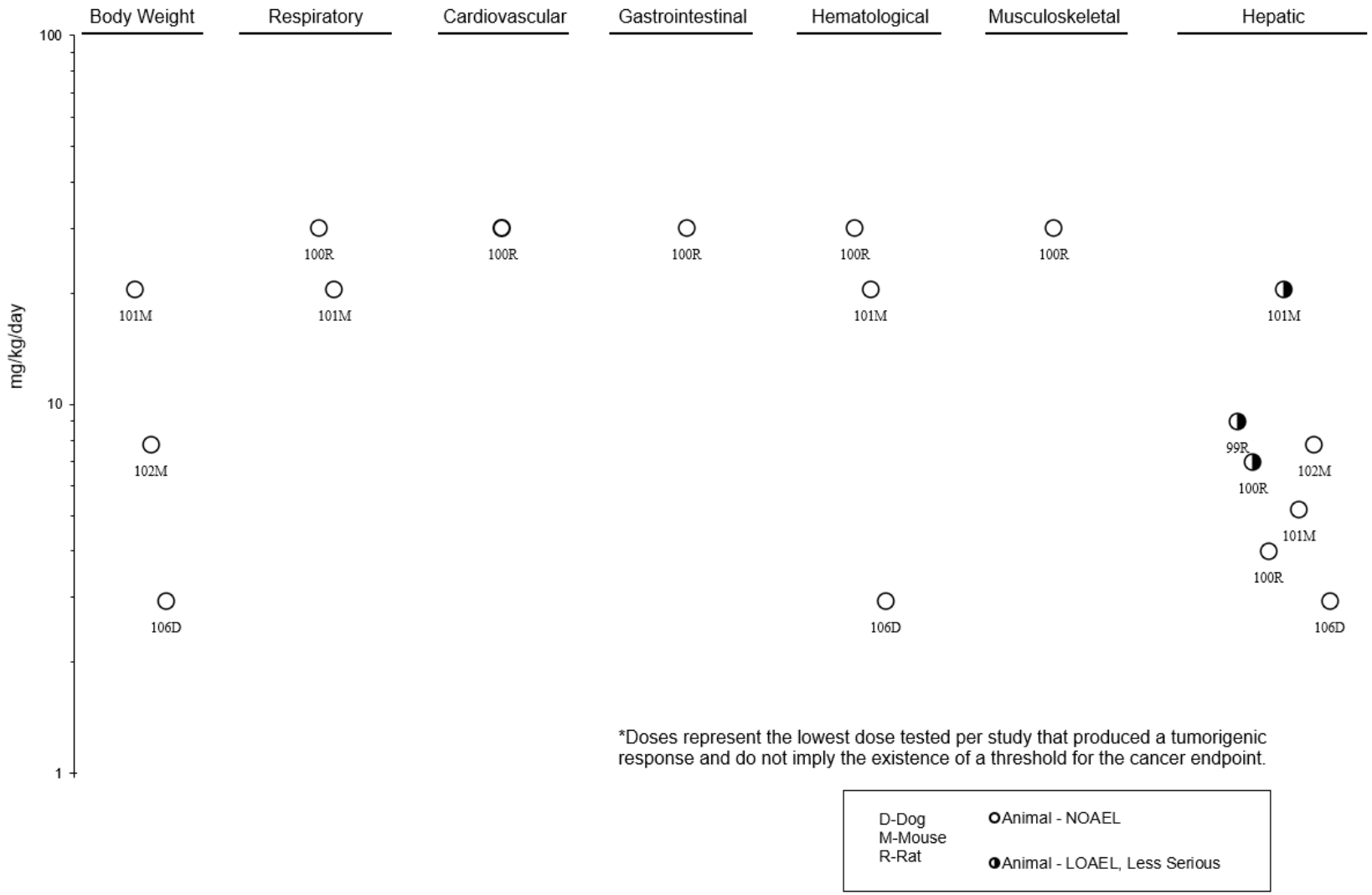


Figure 2-8. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Oral

Chronic (≥365 days)

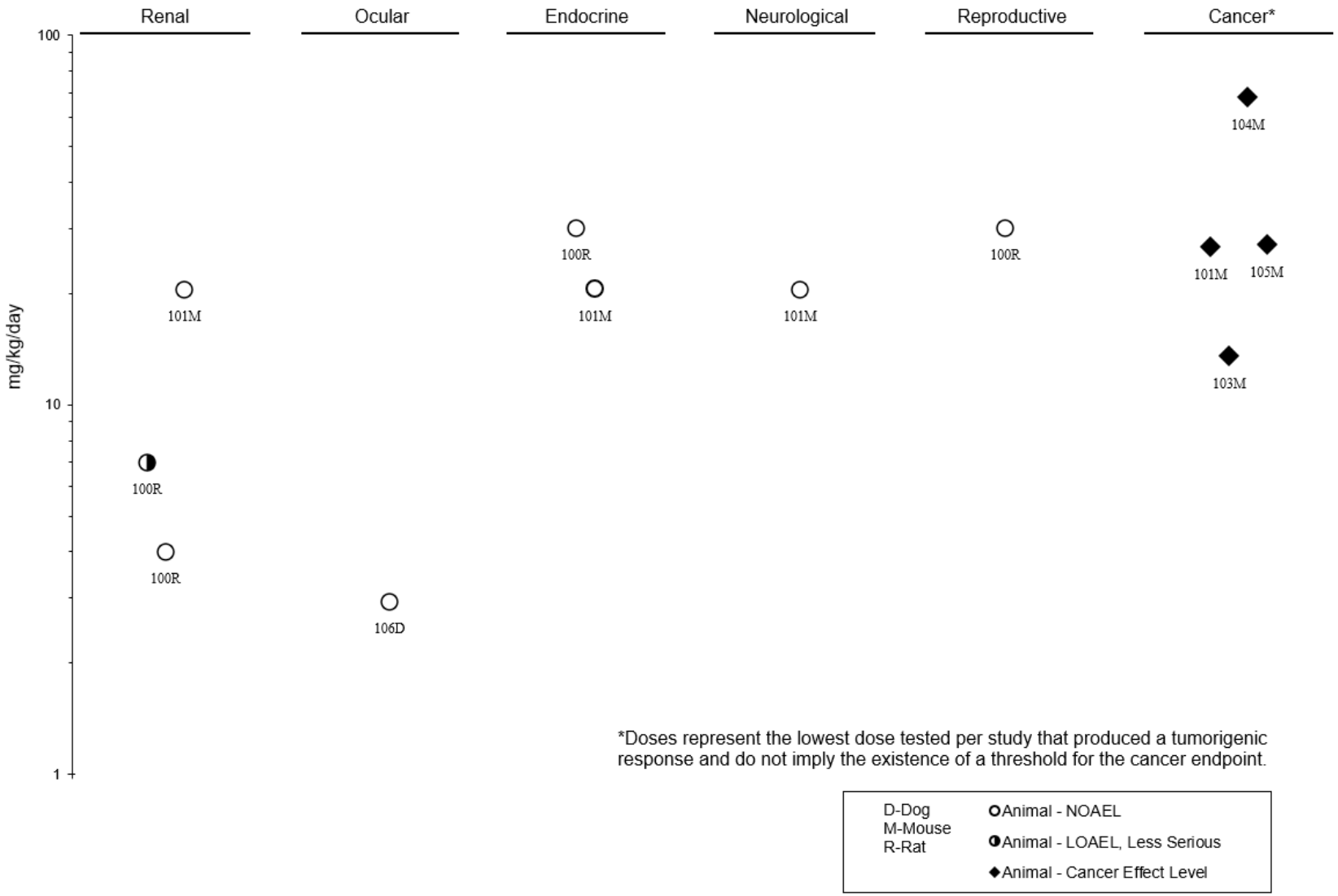


Table 2-5. Levels of Significant Exposure to δ- and Technical Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
ACUTE EXPOSURE									
Joseph et al. 1992a									**Technical HCH**
1	Rat (CFT-Wistar) 6 M	Once (GO)	0–4,000	LE	Death			2,428	LD_{50}
Sahoo et al. 1999									**Technical HCH**
2	Rat (Wistar) 10 M	7 days	0, 10, 20	BI, NX	Neuro		10		Reduced brain ATPase activities; 12% reduction in brain acetylcholinesterase activity; increased motor activity at 20 mg/kg/day
Samanta et al. 1999									**Technical HCH**
3	Rat (Wistar) 10 M	7 days (GO)	0, 10, 20	BW, BC, BI, OW, HP	Repro			10	54% reduction in total sperm count in adult rats; increased frequency of damaged sperm and sperm with anomalous heads
Dikshith et al. 1990									**Technical HCH**
4	Mouse (Swiss albino) 6 F	GD 9 once (GO)	0, 5, 25, 50, 100, 200	BW, DX	Repro			25	Increased fetal resorptions
Philip et al. 1989									**Technical HCH**
5	Mouse (NS) NS	1 or 5 days (GO)	0, 50	HP	Hepatic			50	Marked damage including severe congestion of portal vessels and central vein, severe fatty changes in periportal cells
					Renal		50		A few cases of interstitial hemorrhaging in medulla, cystic dilation of tubules, hyaline casts

Table 2-5. Levels of Significant Exposure to δ- and Technical Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Ravinder et al. 1989, 1990									**Technical HCH**
6	Mouse (Swiss albino) 10 M	2 weeks (F)	0, 72, 144	BI, OW, OF	Death			144	14% mortality
					Hepatic		72		>2-fold increase in relative liver weight, hepatocellular hypertrophy, mild centrilobular degeneration, focal necrosis in a few specimens
INTERMEDIATE EXPOSURE									
Anand et al. 1991									**Technical HCH**
7	Rat (NS) 45 M	90 days 6 days/week (GO)	0, 50	BI, NX	Neuro		50		Increased dopamine and decreased norepinephrine in brain; behavioral changes; increased brain wave frequency
Dikshith et al. 1989a									**Technical HCH**
8	Rat (NS) 10 M	30 days (GO)	0, 60	CS, HE, BC, BI, OW, HP	Hemato	60			
					Hepatic		60		65% increase in liver weight
					Renal	60			
					Repro	60			
Dikshith et al. 1991a									**Technical HCH**
9	Rat (NS) 20 M	360 days (F)	0, 0.04, 0.4, 2, 20, 40	BW, FI, BC, BI, OW, OF, HP	Death			0.4	4/20 deaths
					Hepatic	2	20		Focal necrosis, enlargement of hepatocytes, nuclear pyknosis, vacuolation, margination
					Renal	2	20		Debris cells in lumen, glomerular degeneration
					Neuro	0.04		0.4	Tremors, convulsions, hind limb paralysis

Table 2-5. Levels of Significant Exposure to δ- and Technical Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
					Repro	2		20	Testicular necrosis and degeneration
Dikshith et al. 1991b									**Technical HCH**
10	Rat (NS) 12 M, 12 F	90 days (GO)	0, 5, 25	LE, BW, BI, BC, OW, HP, OF	Death			5	33% mortality in females and 50% mortality in males
Fitzhugh et al. 1950									**Technical HCH**
11	Rat (Wistar) 10 F, 10 M	6 months (F)	Males: 0, 7, 60 Females: 0, 9, 70	LE, BW	Death			60 M 70 F	Decreased mean age at death (32.9 versus 58.3 weeks in controls)
					Bd wt	7 M 9 F	70 F	60 M	LOAEL: 16% decrease in body weight of females Serious LOAEL: 26% decrease in body weight of males
					Resp	60 M 70 F			
					Cardio	60 M 70 F			
					Gastro	60 M 70 F			
					Hemato	60 M 70 F			
					Hepatic		60 M 70 F		Moderate liver damage
					Renal		60 M 70 F		Slight kidney damage
					Musc/skel	60 M 70 F			
					Endo	60 M 70 F			
					Repro	70 F		60 M	Moderate testicular atrophy

Table 2-5. Levels of Significant Exposure to δ- and Technical Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Gautam et al. 1989									**Technical HCH**
12	Rat (Charles Foster) 10 M	180 days (GO)	0, 3, 6	BW	Bd wt		3		17% decrease in body weight gain
					Repro		3		6% decrease in vas deferens weight, degeneration of inner muscle and cell layers of vas deferens
Gopal et al. 1992									**Technical HCH**
13	Rat (NS) 50 M	120 days (GO)	0, 50	CS, NX	Neuro		50		Increased motor activity, decreased resting stereotypic time
Joseph et al. 1992c									**Technical HCH**
14	Rat (CFT-Wistar) 4 M	7 weeks (F)	0, 90	HE, BC	Hemato		90		Decreased white blood cell counts
Ito et al. 1975									**Delta HCH**
15	Rat (W strain) 18–24 M	48 weeks	0, 35, 70	BW, OW, HP	Hepatic	35	70		Hepatocellular hypertrophy
Mudawal et al. 2018									**Technical HCH**
16	Rat (Wistar) 6 M	3 weeks 7 days/week (NS)	0, 2.5	BI, NX, DX	Neuro		2.5		Statistically significant decreases in conditioned avoidance, alternations, and locomotor activity; ultrastructural changes in the hippocampus and substantia nigra in adult animals
Nagaraja and Desiraju 1994									**Technical HCH**
17	Rat (Wistar) 6–8 F	PNDs 2–60 (GO)	0, 10, 20	CS, BW, BI	Develop		10		Alterations in levels of dopamine, serotonin, and noradrenaline in pup brains

Table 2-5. Levels of Significant Exposure to δ- and Technical Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Nagaraja and Desiraju 1994									**Technical HCH**
18	Rat (Wistar) 6–7 F	90 days (F)	0, 20	CS, BW, BI	Bd wt			20	Significantly decreased (23%) terminal body weight
					Neuro		20		Increased GABA levels, increased GAD activity, decreased glutamate levels
Roy Chowdhury and Gautam 1990									**Technical HCH**
19	Rat (Charles Foster) 5–10 M	180 days (G)	0, 3, 6	BW, OW, HP	Repro		3	6	LOAEL: detachment of germinal cells from peritubular membrane of seminiferous tubules, atrophy of Leydig cells, and intertubular edema Serious LOAEL: “complete degeneration” of testicular tissue
Sahoo et al. 1999									**Technical HCH**
20	Rat (Wistar) 10 M	15 or 30 days 7 days/week	0, 10, 20	BI, NX	Neuro		10		Reduced brain ATPase activity; 39% decrease in acetylcholinesterase activity after 15 days, with reduced grooming behavior at 20 mg/kg/day after 30 days
Samanta et al. 1999									**Technical HCH**
21	Rat (Wistar) 10 M	15 or 30 days (GO)	0, 10, 20	BW, BC, BI, OW, HP	Repro			10	58–65% reduction in total sperm count in adult rats; increased frequency of damaged sperm and sperm with anomalous heads
Bhatt and Bano 2009									**Technical HCH**
22	Mouse (Swiss) 6–18 M	2, 4, or 6 months (F)	0, 90	BI, HP	Cancer			90	CEL: Liver tumors

Table 2-5. Levels of Significant Exposure to δ- and Technical Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Bhatt and Nagda 2012									**HCH Not further specified**
23	Mouse (Swiss) 18–20 M	2, 4, or 6 months (F)	0, 90	BI, HP	Hepatic		90		Hepatocyte degeneration, vacuolation, fatty changes; hypertrophy, and hyperplasia after 4 months
					Cancer			90	CEL: liver tumors
Ito et al. 1973									**Delta HCH**
24	Mouse (dd) 20–40 M	24 weeks (F)	0, 18, 45, 90	BW, OW, HP	Bd wt	90			
					Hepatic	45	90		23% increase in relative liver weight and centrilobular hypertrophy
Karnik et al. 1981									**Technical HCH**
25	Mouse (Swiss) 6 NS	2–8 months (F)	0, 90	OW, OF	Hepatic		90		100% increase in liver weight; glycogen accumulation, smooth endoplasmic reticulum proliferation
					Cancer			90	CEL: hepatocellular carcinoma
Nigam et al. 1979									**Technical HCH**
26	Mouse (Swiss) 6 M	3 months (F)	0, 90	BW, OW, HP	Repro			90	Increased (27%) relative testis weight, degeneration of seminiferous tubules, shrunken and edematous tubules (some completely hyalinized); decreased (sparse) and damaged spermatocytes

Table 2-5. Levels of Significant Exposure to δ- and Technical Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Philip et al. 1989									
27	Mouse (NS) NS	15 d (GO)	0, 50	HP	Hepatic			50	Marked damage consisting of congestion of portal vessels and central vein, granular degeneration
					Renal			50	Marked damage consisting of congestion of blood vessels and glomeruli, vacuolation of epithelial cells in glomeruli, fatty changes, cystic dilation of the tubules, and interstitial hemorrhaging
Thakore et al. 1981									**Technical HCH**
28	Mouse (Swiss) 6 NS	2–8 months (F)	0, 90	BW, BI, OW	Cancer			90	CEL: hepatocellular carcinoma
Trivedi et al. 2007, 2009									**Technical HCH**
29	Mouse (Swiss) 6 M	1–8 months (F)	0, 90	BC, BI, GN, HP	Hepatic			90	"Severe" liver damage after 6 months
					Cancer			90	CEL: liver tumors
Wang et al. 2006									**Technical HCH**
30	Pig (Duroc X Landrace X Large white) 12 M, 12 F	90 days (F)	0, 0.4, 0.8	BW, FI, BC, BI, OW, IX	Bd wt	0.8			
					Hepatic	0.8			No effect on liver weight; <30% increases in serum ALT and ALP
					Renal	0.4	0.8		24% increase in relative kidney weight

Table 2-5. Levels of Significant Exposure to δ- and Technical Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
CHRONIC EXPOSURE									
Fitzhugh et al. 1950									**Technical HCH**
31	Rat (Wistar) 10 F, 10 M	107 weeks (F)	Males: 0, 0.7, 4, 7 Females: 0, 0.4, 0.9, 4, 9,	LE, BW, FI, GN, OW, HP	Resp	7 M 9 F			
					Cardio	7 M 9 F			
					Gastro	7 M 9 F			
					Hemato	7 M 9 F			
					Musc/skel	7 M 9 F			
					Hepatic	0.7 M 0.9 F	4		Very slight microscopic damage
					Renal	7 M 9 F			
					Endo	7 M 9 F			
					Repro	7 M 9 F			
Kashyap et al. 1979									**Technical HCH**
32	Mouse (Swiss) 30 M, 30 F	80 weeks (GO)	0, 10	CS, BW, FI, GN, HP	Neuro			10	Convulsions
					Cancer			10	CEL: hepatocellular carcinoma
Kashyap et al. 1979									**Technical HCH**
33	Mouse (Swiss) 30 M, 30 F	80 weeks (F)	0, 17	CS, BW, FI, GN, HP	Neuro			17	Convulsions
					Cancer			17	CEL: hepatocellular carcinoma

Table 2-5. Levels of Significant Exposure to δ- and Technical Hexachlorocyclohexane – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Munir et al. 1983									**Technical HCH**
34	Mouse (Swiss) 10–37 M	20 months (F)	0, 21.3, 42.5, 85	BW, OW, HP	Cancer			21.3	CEL: hepatocellular carcinoma

[a]The number corresponds to entries in Figure 2-9; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-9. Where such differences exist, only the levels of effect for the most sensitive sex are presented.

ALP = alkaline phosphatase; ALT= alanine aminotransferase; BC = serum (blood) chemistry; Bd wt or BW = body weight; BI = biochemical changes; CEL = cancer effect level; CS = clinical signs; Develop = developmental; DX = developmental toxicity; (F) = feed; F = female(s); FI = food intake; (G) = gavage; GABA = gamma-aminobutyric acid; GAD =glutamate decarboxylase; GD = gestation day; GN = gross necropsy; (GO) = gavage in oil; HCH = hexachlorocyclohexane; HE = hematology; Hemato = hematological; HP = histopathology; IX = immunotoxicity; LD_{50} = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = muscular/skeletal; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; NX = neurotoxicity; OF = organ function; OW = organ weight; PND = postnatal day; Repro = reproductive; (W) = drinking water

Figure 2-9. Levels of Significant Exposure to δ- and Technical Hexachlorocyclohexane (HCH) – Oral
Acute (≤14 days)

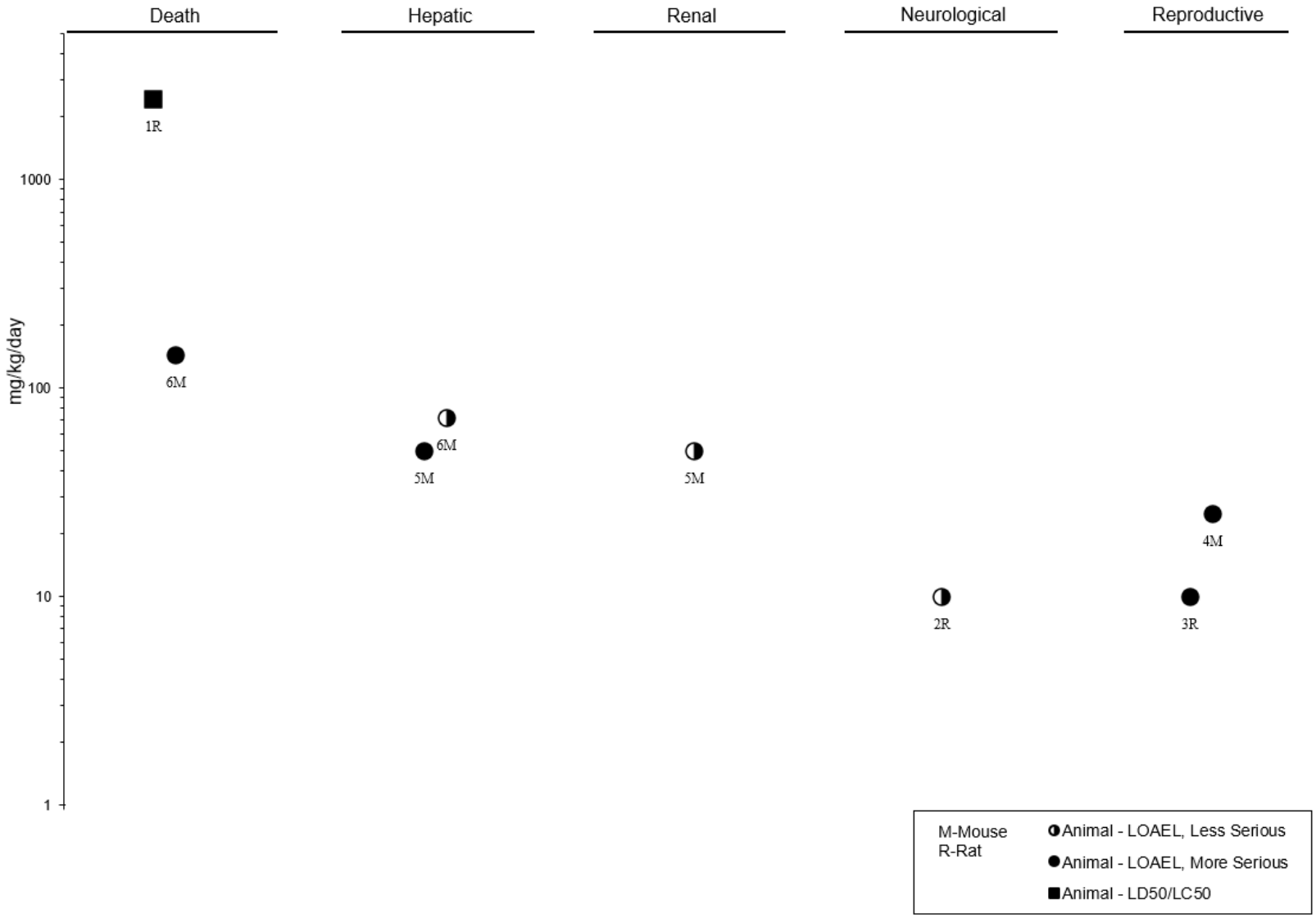


Figure 2-9. Levels of Significant Exposure to δ- and Technical Hexachlorocyclohexane (HCH) – Oral
Intermediate (15–364 days)

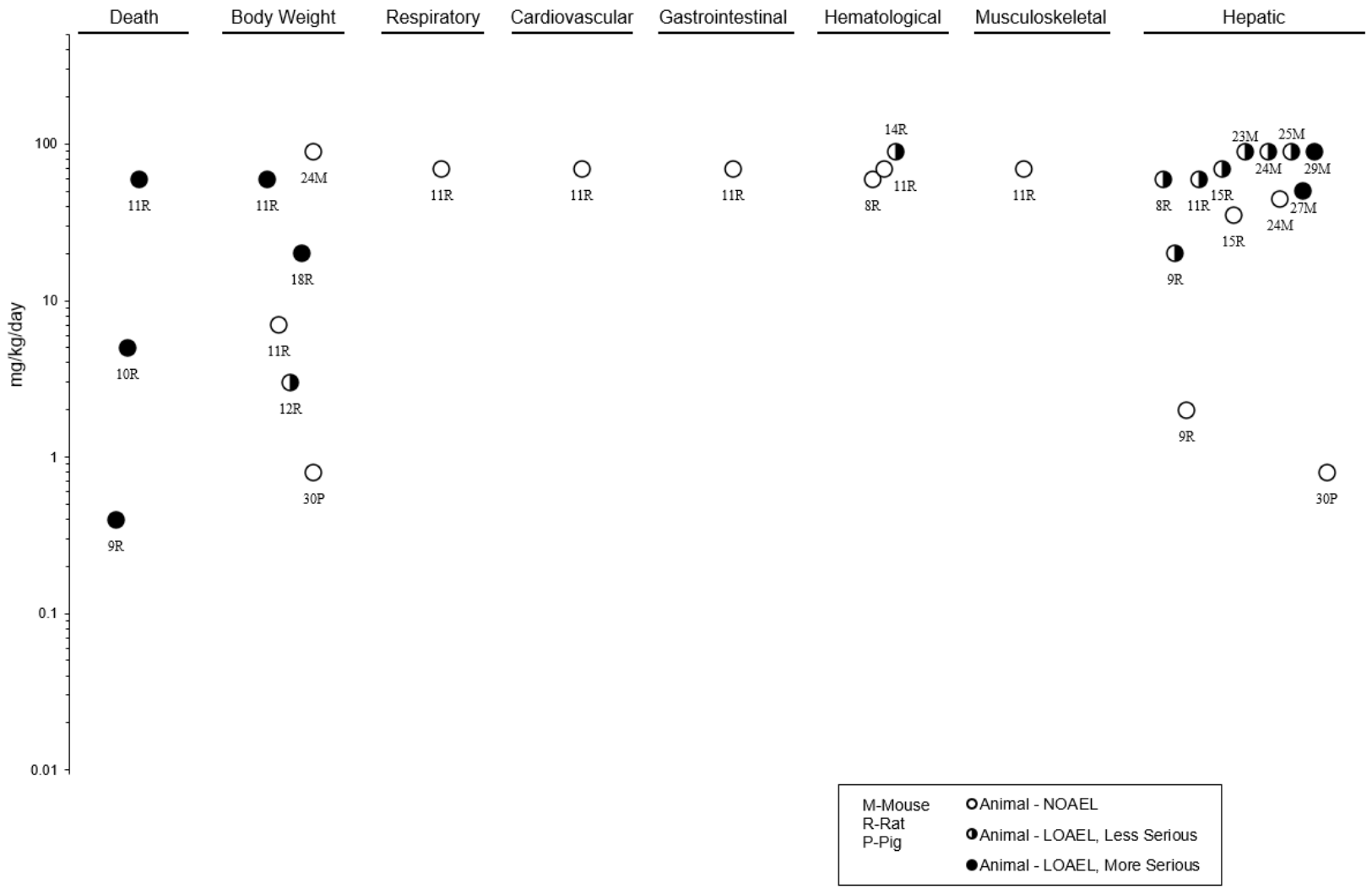


Figure 2-9. Levels of Significant Exposure to δ- and Technical Hexachlorocyclohexane (HCH) – Oral
Intermediate (15–364 days)

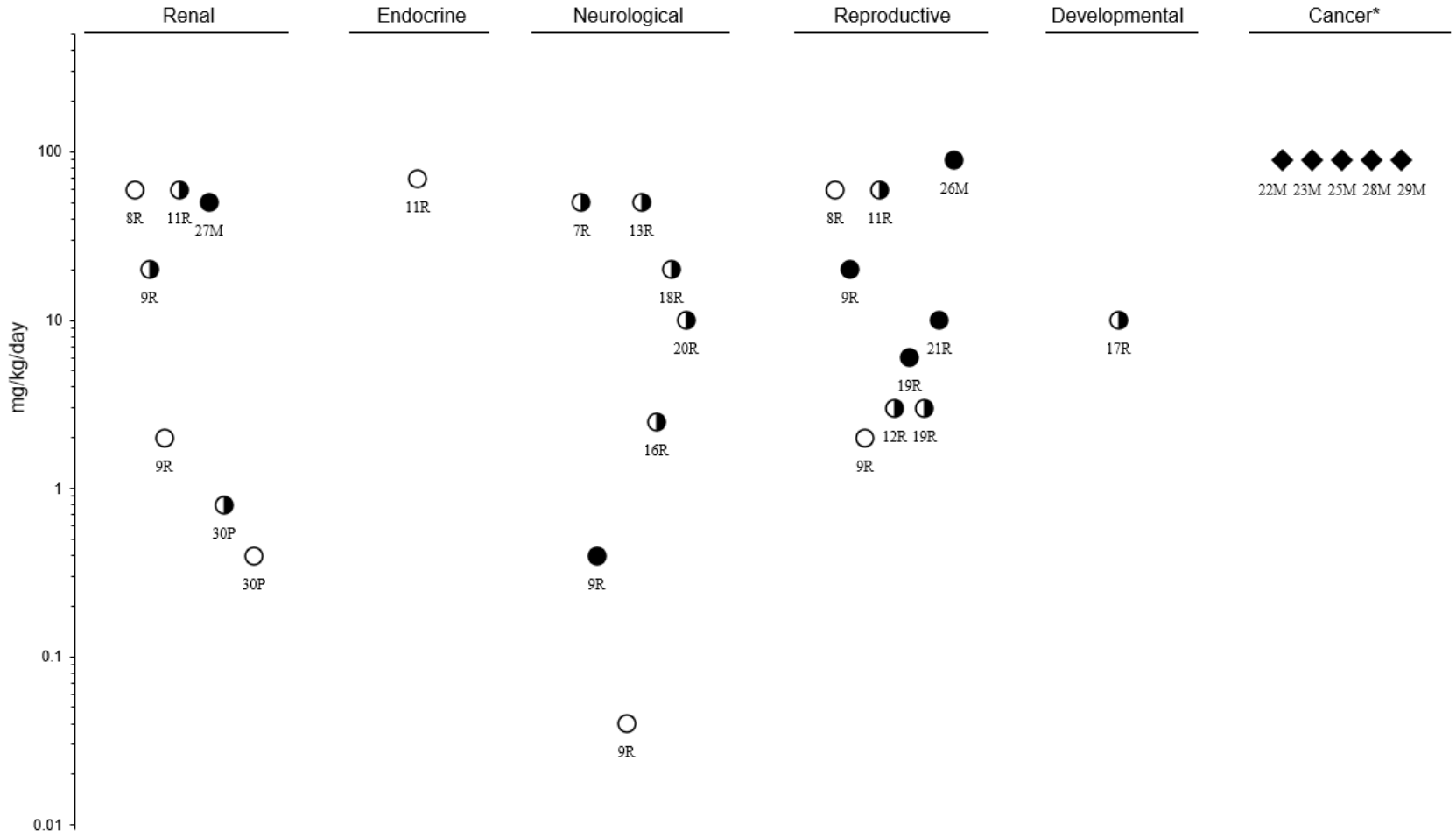


Figure 2-9. Levels of Significant Exposure to δ- and Technical Hexachlorocyclohexane (HCH) – Oral

Chronic (≥365 days)

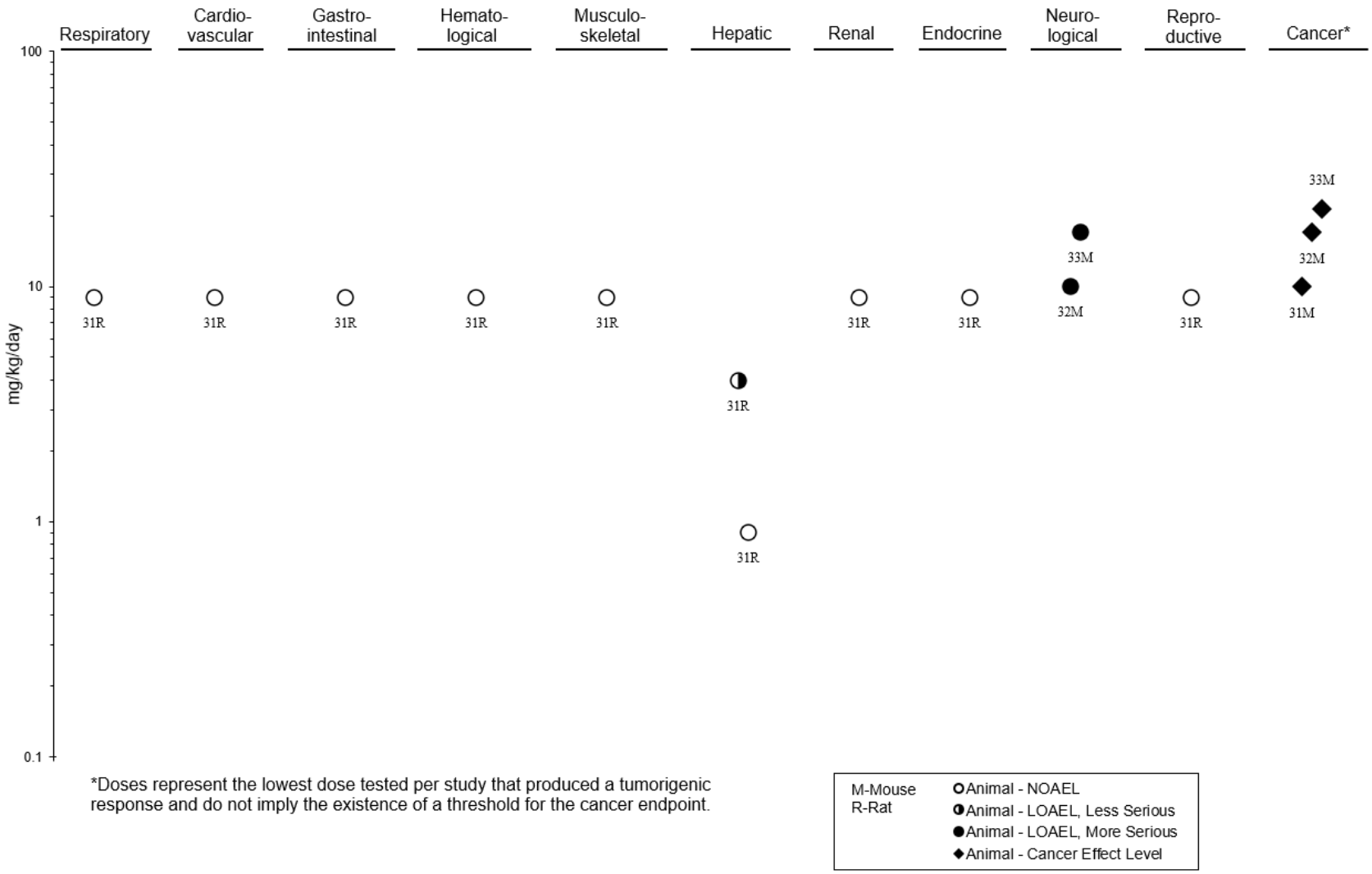


Table 2-6. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
ACUTE EXPOSURE								
Gaines 1960								
Rat (Sherman) 100 M, 70 F	Once (GO)	NS	LE, CS	Death			900 F	LD_{50}
							1,000 M	LD_{50}
Hanig et al. 1976								
Rabbit (New Zealand) 2–6 M	Once	0, 60 mg/kg	LE, CS	Death			60	Deaths among weanlings
				Neuro			60	Convulsions
Ullmann 1986a								
Rat (Wistar) 5 M, 5 F	24 hours once	0, 250, 600, 1,000, 2,000 mg/kg	LE, CS, BW, GN	Death			600	2/10 died at 600 mg/kg; LD_{50}=1,000 mg/kg
				Neuro	600	1,000	2,000 F	LOAEL: sedation, curved body position Serious LOAEL: severe sedation and spasms in one female
Ullmann 1986c								
Rabbit (New Zealand) 3 M, 3 F	Once	0, 40 mg/kg	LE, CS, BW	Ocular		40		Mild eye irritation
INTERMEDIATE EXPOSURE								
Dikshith et al. 1973								
Rat (I.T.R.C.) 30 F	25 days	0, 180 mg/kg/day	CS	Dermal		180		Mild dermatitis

Table 2-6. Levels of Significant Exposure to γ-Hexachlorocyclohexane – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effects
EPA 1988a								
Rat (Crl:(WI)BR) 13–23 M, 13–23 F	13 weeks 5 days/week 6 hours/day	0, 10, 60, 400 mg/kg/day	LE, CS, BW, FI, HE, BC, UR, GN, OF, HP, NX	Death			400 F	23 deaths out of 49
				Bd wt	400			
				Resp		10		Rapid respiration or wheezing
				Hemato	400			
				Hepatic	10	60		Centrilobular hypertrophy, increased absolute liver weight (8%) in females
				Renal	10 M 60 F	60 M		Basophilic tubules in males
				Neuro		10	60 F	LOAEL: hyperactivity Serious LOAEL: ataxia, tremors, convulsions

BC = serum (blood) chemistry; Bd wt or BW = body weight; CS = clinical signs; F = female(s); FI = food intake; GN = gross necropsy; HE = hematology; HP = histopathology; LE = lethality; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level;; M = male(s); Neuro = neurological; NOAEL = no-observed-adverse-effect level; NR = not reported; NS = not specified; NX = neurotoxicity; OF = organ function; Resp = respiratory; UR = urinalysis

Table 2-7. Levels of Significant Exposure to Technical Hexachlorocyclohexane – Dermal

Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
ACUTE EXPOSURE								
Dikshith et al. 1978								
Guinea pig (NS) 24 M	5–12 days	0, 100, 200, 500	LE	Death			200	24/24 deaths
INTERMEDIATE EXPOSURE								
Dikshith et al. 1991c								
Rat (Wistar) 6 F	15–30 days	0, 100	LE, BW, BC, BI, OW, HP	Death			100	Two deaths by day 15 and 2 by day 30
				Hepatic			100	Severe liver injury including hypertrophy, fatty degeneration, nuclear pyknosis of hepatocytes, diffuse and focal liver necrosis, and bile duct proliferation
				Renal			100	Mild to severe tubular epithelial cell necrosis and glomerular atrophy
				Dermal		100		Hyperkeratosis, epidermal cell vacuolization, thickening of collagen fibers
				Neuro			100	Tremors, degenerative changes in the cerebellum
Dikshith et al. 1989b								
Rabbit (NS) 8 M	30 days	0, 25	LE, CS, BW, BC, BI, UR, OW, HP	Death			25	6/24 deaths
				Hepatic		25		Hepatocyte degeneration, pycnotic nuclei, enlarged liver, altered ALT, AST, LDH, and ALP activities

Table 2-7. Levels of Significant Exposure to Technical Hexachlorocyclohexane – Dermal

Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
				Renal		25		Altered epithelial lining of proximal convoluted tubules, loss of brush borders of tubules, atrophy of glomerular capsules
				Dermal		25		Thickened epidermis, hyperkeratinization, and infiltration of mononuclear cells
				Neuro			25	Convulsions, tremor, and paralysis; changes in Purkinje cells of cerebellum including loss of dendrites and presence of deciduomatous cell body
				Repro			25	Severe effects on germinal cells of testes, including vacuolation, cytoplasmic changes, cell sloughing, and multinucleated giant cells
Dikshith et al. 1978								
Guinea pig (NS) 24 M	30 days	0, 100, 200, 500	BW, BC, BI, OW, HP	Hepatic		100		38% increase in liver weight, hepatic hypertrophy, pyknotic nuclei in cytoplasm, focal fatty inclusions, increased ALT and ALP activity
				Renal	100			
				Repro			100	Degeneration of seminiferous tubules, necrosed spermatogenic cells, multinucleated giant cells, and no active sperm in lumen

Table 2-7. Levels of Significant Exposure to Technical Hexachlorocyclohexane – Dermal

Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effects
Mathur et al. 1992								
Guinea pig (NS) 6 NS	30 days	0, 100	BI, HP	Hepatic		100		Increased enzyme activity and fatty and degenerative changes
				Renal		100		increased enzyme activity and histopathological changes
Mathur et al. 1993								
Guinea pig (NS) 6 M	15, 30 days	0, 100	BI, HP	Dermal		100		Dermal histopathology: hyperkeratinization, mononuclear infiltration, sloughing
CHRONIC EXPOSURE								
Kashyap et al. 1979								
Mouse (Swiss) 30 M, 30 F	80 weeks 2 days/week	0, 2.4	BW, FI, GN, HP, CS	Cancer			2.4	CEL: liver tumors
Prasad et al. 1995								
Rat (Wistar) 10 M	120 days	0, 50, 100	CS, BI, OF, RX	Repro			50	Decreased sperm count (57% relative to vehicle control) and motility (38%), increased percent abnormal sperm (>5-fold), alterations in testicular enzyme activity

ALP = alkaline phosphatase; ALT= alanine aminotransferase; AST = aspartate aminotransferase; BC = serum (blood) chemistry; BW = body weight; BI = biochemical changes; CEL = cancer effect level; CS = clinical signs; F = female(s); FI = food intake; GN = gross necropsy; HP = histopathology; LDH = lactate dehydrogenase; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OW = organ weight; Repro = reproductive; RX = reproductive toxicity; UR = urinalysis

2.2 DEATH

Human fatalities have been reported for γ-HCH but not for other isomers or for mixtures of isomers. Studies reporting deaths associated with γ-HCH are described below in the subsection on γ-HCH.

α-HCH. In a long-term study where Wistar rats were administered α-HCH in the diet, mean age of death was significantly decreased to 35.9 weeks in animals administered 60–70 mg/kg/day compared to 58.3 weeks in control animals (Fitzhugh et al. 1950). No inhalation or dermal studies of mortality in animals were identified for α-HCH.

β-HCH. Mortalities have occurred in rats and mice exposed to β-HCH in the diet for acute and intermediate exposure durations, often after showing signs of pronounced neurotoxicity. In the first week of a 30-day study, 80% of female mice receiving 200 mg/kg/day β-HCH in feed exhibited ataxia progressing to lateral recumbent position and were humanely sacrificed (Cornacoff et al. 1988). No deaths were observed in male rats exposed to 72 mg/kg/day β-HCH in food for 2 weeks (Srinivasan et al. 1984). However, maternal mortality occurred in a developmental study in rats (Srinivasan et al. 1991), in which all dams exposed to 80 mg/kg/day β-HCH in feed died within 3 weeks of treatment (during gestation). Similarly, in the first 2 weeks of a 13-week dietary study, two male and three female rats exposed to doses of 38 mg/kg/day exhibited ataxia and hypoactivity, progressing within 3 days to coma and leading to their humane sacrifice[1] (Van Velsen et al. 1986). Subsequently, five males and six females in this dose group became moribund and were euthanized later in the study (timing not reported) (Van Velsen et al. 1986). In a dietary study where Wistar rats were administered β-HCH, mean age of death for both males and females administered 60–70 mg/kg/day was 4.4 weeks compared to 58.3 weeks in control animals (Fitzhugh et al. 1950). All animals exposed to β-HCH at this dose died by 10 weeks of exposure (Fitzhugh et al. 1950). Finally, in a chronic study in which CF1 mice were administered 34 mg/kg/day β-HCH for up to 104 weeks, 12% of males and 25% of females died within the first 3 months (Thorpe and Walker 1973). Survival of the remaining animals did not differ from controls. No inhalation or dermal studies of mortality in animals were identified for β-HCH.

No information on the causes or mechanisms of death in animals exposed to β-HCH was located, although neurotoxicity preceded death in most instances. There appears to be substantial variability in susceptibility to the lethal effects of β-HCH, as demonstrated by the chronic study by Thorpe and Walker

[1]Because the deaths occurred after the end of 2 weeks, they were considered to occur as a result of intermediate-duration exposure.

(1973), in which animals that survived the first 3 months did not have any reductions in subsequent survival.

γ-HCH (Lindane). γ-HCH was once used in insecticide vaporizer and fumigator devices, resulting in human inhalation and dermal exposure to unspecified levels. Occasional deaths associated with the use of this product for several months or years have been reported, but it is not clear that γ-HCH was responsible for the deaths (Loge 1965). Two cases of pulmonary edema resulting in fatalities were reported in toddlers inhaling and ingesting unknown quantities of γ-HCH-containing pesticidal powder (McQueen et al. 1968). Following an accidental chemical spill from storage tanks in India, seven deaths occurred in the area, with the cause of death reported as asphyxia (Jain et al. 2022). Sampling near the spill revealed γ-HCH in water and soil at levels that exceeded permissible concentrations. All deaths were in individuals within 200 m of the site. Sixteen survivors exhibited symptoms including headache, nausea, and breathlessness.

Case reports of deaths in humans, often in children or suicidal adults, following ingestion of γ-HCH in tablets (doses unknown) intended for γ-HCH vaporizers have been reported (Storen 1955; Sunder Ram Rao et al. 1988). A single acute, whole-body dermal application of 1% γ-HCH in lotion to a 2-month-old infant for scabies treatment resulted in death (Davies et al. 1983). Autopsy identified pulmonary and epicardial petechiae and a concentration in the brain of 110 ppb γ-HCH. 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. A 66-year-old man died after being treated for scabies in a hospital with a 1% γ-HCH lotion applied dermally from neck to toes (Sudakin 2007). Eight hours after application, the man exhibited worsening of mental status and hypoxemia. Over the next 2 hours, his neurological symptoms increased in severity to include seizure and myoclonic jerks, and were accompanied by severe hypoxemia, tachycardia, diaphoresis, hypotension, and respiratory acidosis. The man remained in intensive care and subsequently died after 50 days in the hospital. The autopsy attributed the cause of death to hypoxic ischemic encephalopathy from γ-HCH poisoning. The dose of γ-HCH was not estimated, and neither blood nor tissue levels of γ-HCH were measured during hospitalization or at autopsy (Sudakin 2007).

In an acute animal study of rats exposed nose-only to γ-HCH aerosol for 4 hours, the LC_{50} was determined to be 1,560 mg/m^3 (Ullmann 1986b); the lowest concentration associated with lethality was 378 mg/m^3. No rats exposed whole body to γ-HCH for 4 hours up to a concentration of 603 mg/m^3 died

throughout the 14-day observation period (Oldiges et al. 1980). In the beginning of a 14-week study, 12/45 female and 2/45 male mice that were exposed to 10 mg/m^3 of γ-HCH dust aerosol via whole body (6 hours/day) died during the first week (Klonne and Kintigh 1988). In this study, the concentration was decreased from 10 to 5 mg/m^3 after the first week; additional deaths occurred at 5 mg/m^3 (two males and three females) and 1 mg/m^3 (one male and one female), but there were no deaths at 0.3 mg/m^3. One unexplained death in a control mouse was also reported (Klonne and Kintigh 1988).

Studies in laboratory animals have reported deaths after acute-duration oral administration of γ-HCH doses ≥20 mg/kg/day (Chadwick et al. 1988; Fitzhugh et al. 1950; Gaines 1960; Hassoun and Stohs 1996a; Liu and Morgan 1986; Martinez et al. 1991; Matsuura et al. 2005; Thorpe and Walker 1973; Tusell et al. 1988; Yuksel et al. 2009). In studies of rats administered a single dose of γ-HCH via gavage, LD_{50} values of 88 mg/kg in males and 91 mg/kg in females were obtained (Gaines 1960). One of seven male rats died following a single administration of γ-HCH at a dose of 60 mg/kg via gavage (Martinez et al. 1991), while 2/18 rats died within the third day of exposure to doses of 20 mg/kg/day (Tusell et al. 1988). Acute exposure-duration studies in mice showed similar lethal doses in some strains. Pregnant DBA/2J and C57BL/6J mice both exhibited mortality (14–25%) upon single gavage doses of 30 and 45 mg/kg, respectively, administered on gestation day (GD) 12 (Hassoun and Stohs 1996a). In another study, no mortality was reported in nonpregnant adult female C57BL/6 mice; however, six of six DBA/2 mice died after 20 mg γ-HCH/kg was administered by daily gavage for up to 10 days (Liu and Morgan 1986).

In intermediate- and chronic-duration studies, the doses inducing lethality were similar to those seen after acute-duration exposure. Two F0 female rats exposed to doses of 26.1 mg/kg/day in a 2-generation reproductive toxicity study died, but the times of death were not reported (Matsuura et al. 2005). In F344 rats administered γ-HCH for up to 15 weeks, 2/12 animals died at 20 mg/kg/day and 7/12 died at 40 mg/kg/day (Chadwick et al. 1988). When groups of 10 Sprague-Dawley rats were administered 20 or 40 mg/kg/day γ-HCH by gavage for 30 days, one animal in each group died during week 3 (Yuksel et al. 2009). The age at death in Wistar rats was significantly decreased to 39.7 weeks in animals administered 60–70 mg/kg/day, compared to 58.3 weeks in control animals (Fitzhugh et al. 1950). In a 2-year study with interim sacrifices, dietary administration of technical-grade γ-HCH at a dose of 32 mg/kg/day resulted in significantly decreased survival when compared with controls (Amyes 1990).

Acute dermal exposure to γ-HCH resulted in death. Dermal LD_{50} values in rats exposed to γ-HCH once and observed for 10 days were 1,000 mg/kg in males and 900 mg/kg in females (Gaines 1960). In an

acute dermal study in which male and female rats were exposed to γ-HCH for 24 hours, mortality rates across both sexes were 0, 20, 40, and 30% at 250, 600, 1,000, and 2,000 mg/kg, respectively (Ullmann 1986a). 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/kg γ-HCH (Hanig et al. 1976). Significant mortality (47%) was seen in female rats, but not male rats, exposed dermally to γ-HCH at 400 mg/kg/day for 6 hours/day, 5 days/week, for 13 weeks (EPA 1988a). No studies regarding chronic dermal exposure to γ-HCH were located.

Technical HCH or Unspecified Isomers of HCH. Joseph et al. (1992a) reported an LD_{50} of 2,428 mg/kg in male rats administered a single gavage dose of technical-grade HCH (72.8% α-HCH, 12.6% γ-HCH, 7.95% δ-HCH, 5% β-HCH). Technical-grade HCH administered to rats for 90 days resulted in increased mortality: 6/12 males and 4/12 females exposed to 5 mg/kg/day died, and a 58% increase in mortality (incidence not reported) was observed at 25 mg/kg/day (Dikshith et al. 1991b). When Wistar rats were administered technical-HCH in the diet as part of a chronic study, age at death was significantly decreased to 32.9 weeks in animals administered 64 mg/kg/day compared to 58.3 weeks in control animals (Fitzhugh et al. 1950). 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).

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 dermal exposure to technical-grade HCH at 100 mg/kg/day for 15–30 days (Dikshith et al. 1991c). 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).

2.3 BODY WEIGHT

Epidemiological Studies. Studies of body weight effects in humans include three studies of β-HCH; these are summarized in Table 2-8. In a cohort of women residing in an agricultural area of California, serum levels of β-HCH were associated with increased body mass index (BMI), waist circumference, body fat percent, and obesity when measured over the 3 years after serum collection (Warner et al. 2018). A cross-sectional study of surgical patients in Spain provided support for an association between β-HCH (measured in serum or adipose tissue) and increased BMI (Arrebola et al. 2014). In another cross-

sectional study in adults from Seoul, South Korea, serum levels of β-HCH were associated with increased BMI (Seo et al. 2022).

Table 2-8. Summary of Epidemiological Studies of β-Hexachlorocyclohexane (β-HCH) Exposure and Body Weight Effects

Reference, study type, and population	Biomarker	Concentration	Outcome evaluated	Result
Arrebola et al. 2014 Cross-sectional, 298 noncancer surgical patients >16 years old, Spain	Serum or adipose	19.60±28.74 ng/g lipid (mean)	**BMI**	↑[a]
Warner et al. 2018 Cohort, 468 women >18 years old, residing in agricultural area of California, United States	Serum	>5.2 ng/g lipid (median)	**BMI**	↑
			Waist circumference	↑
			Body fat %	↑
			Obesity	↑
Seo et al. 2022 Cross-sectional, 880 adults, ages 20–80 years, South Korea	Serum	40.4 ng/g lipid	**BMI**	↑

[a]BMI exhibited a quadratic association with β-HCH, increasing at low concentrations and then decreasing at higher concentrations.

↑ = association with increase; BMI = body mass index

Data on body weight changes in animals exposed by inhalation or dermal contact were limited to γ-HCH.

α-HCH. Sumida et al. (2007) reported no body weight changes in male rats administered α-HCH via gavage at 20 mg/kg/day for up to 28 days. In rats exposed for 24 weeks to 45 mg/kg/day in feed, terminal body weight was decreased by 15% (Nagasaki et al. 1975); food intake was not reported. Significantly decreased body weight gain in the absence of changes in food intake was also seen in rats treated with 60–70 mg/kg/day of α-HCH in the diet for 6 months (Fitzhugh et al. 1950). In studies of several mouse strains given 90 mg/kg/day α-HCH in feed for 24 weeks, a significant (17%) decrease in terminal body weight was observed in male C57BL/6 mice, but not in other strains (dd, DDY, ICR, DBA/2, or C3H/He) or in females of any strain (Ito et al. 1973; Nagasaki et al. 1975). These authors conducted a similar experiment in male hamsters given 45 mg/kg/day in feed for 24 weeks. Terminal body weight was 14% lower than controls in the hamsters (Nagasaki et al. 1975).

β-HCH. Intermediate-duration oral studies of body weight effects after exposure to β-HCH exposure showed effects in rats, but not in mice. Body weight decreases of at least 10% were observed in Wistar

rats administered dietary β-HCH at a dose of 22.5 mg/kg/day in males or 25 mg/kg/day in females for 13 weeks; however, at this dose, half of the animals were sacrificed moribund prior to study termination (Van Velsen et al. 1986). No body weight effects were observed at doses up to 5 mg/kg/day in males or females (Van Velsen et al. 1986). After 6 months of β-HCH administration in the diet, body weight gain was decreased by 11% in female Wistar rats exposed to 9 mg/kg/day (Fitzhugh et al. 1950); body weight data for the lower dose group were not reported. No effect on body weight was observed in mice administered β-HCH at doses of 60 mg/kg/day for 30 days (Cornacoff et al. 1988) or 90 mg/kg/day for 24 weeks (Ito et al. 1973).

γ-HCH (Lindane). Limited information is available on body weight effects of γ-HCH in animals exposed by inhalation. In Wistar rats exposed for 4 hours to 603 mg/m^3 γ-HCH, females lost weight for the first post-exposure observation week. Neither mice nor rats exposed to γ-HCH aerosols at concentrations up to 5 mg/m^3 for 6 hours each day (5 or 7 days/week) for 13–14 weeks exhibited any change in body weight (Oldiges et al. 1983; Klonne and Kintigh 1988).

No effects on body weight were observed in rats administered acute-duration oral doses ranging from 8.8 to 15 mg/kg/day (Parmar et al. 2003; Sinha and Shukla 2003; Sumida et al. 2007) or in mice at doses ranging from 5.9 to 40 mg/kg/day (Di Consiglio et al. 2009; Hong and Boorman 1993; Maranghi et al. 2007; Serrano et al. 1990; Sinha and Shukla 2003). One study of Wistar rat dams exposed to ≥8 mg/kg/day exhibited decreased body weight gain (25% less than controls) on GDs 6–20; decreased food consumption was seen at the same dose (EPA 1999c). Body weights of pregnant mice administered 15–25 mg/kg/day γ-HCH via gavage during gestation were not affected (Maranghi et al. 2007; Traina et al. 2003). In a 7-week study of beagle dogs given γ-HCH in the diet, Rivett et al. (1978) reported suppression of body weight gain at 7 mg/kg/day; however, only two dogs per group were used in this study.

In intermediate-duration studies, there were no effects on body weight in rats administered γ-HCH via gavage at doses between 2.5 and 30 mg/kg/day for 15–28 days (Ahmed et al. 2008; Andrews and Gray 1990; Parmar et al. 2003; Sumida et al. 2007; Yang et al. 2014; Zhang et al. 2016) or up to 26.1 mg/kg/day for about 10 weeks in a 2-generation reproductive toxicity study (Matsuura et al. 2005). In another 2-generation reproductive toxicity study (EPA 1991a), body weight gain decreased, without changes in food intake, in high-dose (13.1 mg/kg/day) F0 parental females during gestation. Mice exposed to γ-HCH in feed for 24 weeks exhibited no body weight changes at doses up to 90 mg/kg/day (Ito et al. 1973).

Significantly decreased body weight gain was seen after 6 months in rats treated with 120–140 mg/kg/day γ-HCH in feed as part of a chronic-duration study, but this dose was also associated with significantly reduced survival (Fitzhugh et al. 1950). Beagle dogs given doses up to 2.92 mg/kg/day γ-HCH in feed displayed no changes in body weight over the 102-week exposure duration (Rivett et al. 1978).

Technical HCH or Unspecified Isomers of HCH. Wistar rats administered technical-grade HCH via gavage at doses up to 20 mg/kg/day for 7 days exhibited no effect on body weight (Samanta et al. 1999). Swiss albino mice had significantly decreased body weight after administration of a single dose of 100 mg/kg technical-grade HCH via gavage in oil (Dikshith et al. 1990). Significantly decreased body weight gain has been observed in rats treated orally with 3 or 20 mg/kg/day technical-grade HCH for up to 6 months (Gautam et al. 1989; Nagaraja and Desiraju 1994; Roy Chowdhury and Gautam 1990). Rats administered technical-grade HCH via gavage at a dose of 50 mg/kg/day for 30 days exhibited a 21% body weight loss (Khanna et al. 1990). In an 80-week study, Swiss mice administered technical-grade HCH at 17 mg/kg/day exhibited no changes in body weight or body weight gain, despite decreased food consumption (Kashyap et al. 1979). After 6 months of technical-grade HCH administration, no effect on body weight was observed at 7–9 mg/kg/day, but body weight was decreased by 16% in female rats exposed to 70 mg/kg/day and by 26% in males exposed to 60 mg/kg/day (Fitzhugh et al. 1950). No effect on body weight was observed in mice chronically administered technical-grade HCH at 10 mg/kg/day by gavage or 17 mg/kg/day in the diet (Kashyap et al. 1979).

2.4 RESPIRATORY

α-HCH. No studies were located regarding respiratory effects in humans after exposure to α-HCH. In rats exposed via diet to α-HCH doses up to 70 mg/kg/day for an average of 9 months or up to 9 mg/kg/day for 2 years, there were no histopathology findings in the lungs (Fitzhugh et al. 1950).

β-HCH. In a cross-sectional study in southern Ghana, vegetable farmers who reported pesticide use showed a significant, positive association between serum levels of β-HCH and respiratory symptoms of cough, phlegm production, and wheezing (Quansah et al. 2016). In another cross-sectional study in Ghana, children (up to 5 years old) of vegetable farmers who had accompanied their parents to the farm showed an association between urine concentrations of β-HCH and lower (but not upper) respiratory tract infections (Akyeampong et al. 2022). Rats given doses up to 70 mg/kg/day for up to 10 weeks or up to 9 mg/kg/day for 2 years exhibited no microscopic pathology in the lungs (Fitzhugh et al. 1950).

γ-HCH (Lindane). In a cross-sectional study of vegetable farmers in southern Ghana who used pesticides, no association was observed between serum levels of γ-HCH and respiratory symptoms (cough, phlegm production, and wheezing) (Quansah et al. 2016). 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. 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 (Davies et al. 1983).

No respiratory effects and no histopathology changes in the nasal cavities or lungs 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). Mononuclear cell infiltrates in peribronchial and perivascular regions of the lung were observed in male mice administered 0.25 mg/kg/day γ-HCH by gavage in groundnut oil for 61 days (Tewari et al. 2017). Bronchoalveolar lavage (BAL) from the treated mice contained increased total leukocyte counts and neutrophil percentages.

In rats, dietary exposure to γ-HCH at doses up to 140 mg/kg/day for ~10 months or 30 mg/kg/day for 2 years resulted in histopathology changes in the lungs (Fitzhugh et al. 1950). Slight dyspnea was reported in rats exposed dermally for 24 hours to 1,000 or 2,000 mg/kg γ-HCH 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 /kg/day γ-HCH for 13 weeks (EPA 1988a).

δ-HCH. No association between serum concentrations of δ-HCH and respiratory symptoms was observed in a cross-sectional study of vegetable farmers using pesticides in southern Ghana (Quansah et al. 2016). In another cross-sectional study in Ghana, children of vegetable farmers who had accompanied their parents to the farm showed a positive association between urine concentrations of δ-HCH and acute lower respiratory tract infections in children below the age of 5 years (Akyeampong et al. 2022). No association was observed with upper respiratory tract infections.

Technical HCH or Unspecified Isomers of HCH. Neither intermediate- nor chronic-duration dietary exposure to technical HCH resulted in lung histopathology changes in rats given doses up to 70 mg/kg/day for 6 months or 9 mg/kg/day for 2 years (Fitzhugh et al. 1950).

2.5 CARDIOVASCULAR

Epidemiological Studies. Epidemiological studies of associations between cardiovascular effects in humans and biomarkers of exposure to β-HCH have been conducted. Table 2-9 provides a summary of the epidemiological data pertaining to cardiovascular effects and exposure to β-HCH in humans. Arrebola et al. (2015a) observed an association between serum β-HCH concentrations and incident hypertension in a cohort of 297 surgical patients followed for 10 years. A positive association between serum β-HCH concentrations and hypertension was seen among surgical patients with a BMI >26.3, whereas no association was observed in those with a BMI <26.3 (Arrebola et al. 2015a). In cross-sectional studies, no association was reported between blood β-HCH concentrations and hypertension in a study of 1,615 Inuit adults in Greenland (Valera et al. 2013b), while an inverse association with hypertension was reported in a smaller study of Inuit adults in Quebec (Valera et al. 2013a). No association with peripheral artery disease was observed in a cross-sectional study of U.S. adult participants in the National Health and Nutrition Examination Survey (NHANES) (1999–2004) (Min et al. 2011). Epidemiological studies of cardiovascular effects and exposure to other HCH isomers were not located, but there are case reports of these effects in humans accidentally or intentionally exposed to γ-HCH.

Table 2-9. Summary of Epidemiological Studies of β-Hexachlorocyclohexane Exposure and Cardiovascular Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Mean concentration (unless otherwise noted)	Result
Arrebola et al. 2015a Cohort, 297 noncancer surgical patients >16 years old, Spain, follow-up 10 years	**Hypertension**	Serum	11.2 ng/g lipid (median) (BMI >26.3 kg/m^2)	↑
			6.6 (BMI <26.3kg/m^2)	↔
Valera et al. 2013a Cross-sectional, 315 Inuit ≥18 years, Quebec, Canada	**Hypertension**	Plasma	0.13 µg/L lipid (GM)	↓
Valera et al. 2013b Cross-sectional, 1,614 Inuit aged ≥18 years, Greenland	Hypertension	Plasma	27.0 µg/kg lipid (GM)	↔

Table 2-9. Summary of Epidemiological Studies of β-Hexachlorocyclohexane Exposure and Cardiovascular Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Mean concentration (unless otherwise noted)	Result
Min et al. 2011 Cross-sectional, 2,032 adults >40 years old, NHANES (1999–2004)	PAD	Serum	15.37 ng/g lipid (obese with PAD) 10.05 (non-obese with PAD) 10.90 (obese without PAD) 7.92 (non-obese without PAD)	↔

↑ = association with increase; ↓ = association with decrease (inverse association); ↔ = no association; GM = geometric mean; NHANES = National Health and Nutrition Examination Survey; PAD = peripheral artery disease

Data regarding cardiovascular effects in animals are limited to the γ-HCH isoform.

α-HCH. No histopathology changes were noted in the hearts of rats given α-HCH via the diet at doses up to 70 mg/kg/day for ~9 months or up to 9 mg/kg/day for 2 years (Fitzhugh et al. 1950).

β-HCH. There were no microscopic lesions in the heart when rats were exposed by dietary administration to β-HCH doses up to 70 mg/kg/day for up to 10 weeks or up to 9 mg/kg/day for 2 years (Fitzhugh et al. 1950).

γ-HCH (Lindane). Autopsy findings in a 2-month-old infant who expired after whole-body application of 1% γ-HCH lotion were minimal but revealed epicardial petechiae (Davies et al. 1983). In a suicide attempt, a 56-year-old man intentionally ingested approximately 12 ounces of an insecticide containing 20% γ-HCH (Wiles et al. 2015). Cardiac symptoms of premature atrial and ventricular contractions, and atrial fibrillation after 3–5 days were observed. The man died on day 12 by committing suicide by means unrelated to γ-HCH, and no cardiac abnormalities were noted at autopsy.

There were no treatment-related changes to cardiac histopathology findings in rats or mice exposed by inhalation to γ-HCH concentrations up to 5 mg/m^3 for 13–14 weeks (Klonne and Kintigh 1988; Oldiges et al. 1983). Evidence for cardiac effects comes from studies of animals exposed orally. Increased serum levels of lactate dehydrogenase (LDH) and creatine phosphokinase and cardiac histopathological changes including separated muscle fibers and inflammatory cells were observed in male rats administered 100 mg/kg/day γ-HCH by gavage in olive oil for 30 days (Vijaya Padma et al. 2013). 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 calcium-potassium-ATPase

activity. Electrocardiographic changes included increased ST segment and T-wave amplitude and reduced R-R interval and P-wave (Anand et al. 1995). Rats exposed to γ-HCH *in utero* exhibited alterations in cardiac electrophysiology and histopathology; see Section 2.17 for details. Fitzhugh et al. (1950) reported no histopathology findings in the hearts of rats given γ-HCH in feed at doses up to 140 mg/kg/day for 10 months or 30 mg/kg/day for 2 years.

Mechanisms. Oxidative stress may contribute to the cardiovascular effects of γ-HCH. Lipid peroxidation was increased and antioxidant enzyme activities (superoxide dismutase, catalase, glutathione peroxidase, and glutathione) were reduced in cardiac tissue from male rats treated with 8.8 mg/kg/day γ-HCH via gavage for 3 weeks (Kamal El-Dein et al. 2016). Serum lipid and creatine phosphokinase levels were also increased at this dose. Pre-treatment with the antioxidant α-lipoic acid attenuated the γ-HCH-induced effects on serum lipids, CPK, lipid peroxidation and antioxidant enzyme activities in the heart.

Technical HCH or Unspecified Isomers of 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. Technical HCH administered to rats in feed did not induce cardiac histopathology changes at doses up to 70 mg/kg/day for 6 months or 9 mg/kg/day for 2 years (Fitzhugh et al. 1950).

2.6 GASTROINTESTINAL

Data on gastrointestinal effects in humans were limited to γ-HCH. No studies were located regarding gastrointestinal effects in animals following dermal exposure to any of the HCH isomers.

α-HCH. There were no microscopic gastrointestinal lesions in rats given α-HCH via the diet at doses up to 70 mg/kg/day for ~9 months or up to 9 mg/kg/day for 2 years (Fitzhugh et al. 1950).

β-HCH. Dietary administration of β-HCH doses up to 70 mg/kg/day for up to 10 weeks or up to 9 mg/kg/day for 2 years did not result in histopathology changes in the gastrointestinal tracts of rats (Fitzhugh et al. 1950).

γ-HCH (Lindane) Lindane exposures reported to the Texas poison control network between 1998 and 2002 were reviewed by Forrester et al. (2004). Ingestion was the primary exposure route (79%), and reported symptoms included vomiting, nausea, and abdominal pain (doses were not specified). Vomiting

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

Rats exposed to 5 mg/m^3 γ-HCH aerosol for 6 hours/day, 7 days/week exhibited persistent diarrhea beginning after 2 weeks of exposure and continuing for nearly 3 weeks; exposure to 1 mg/m^3 did not induce diarrhea (Oldiges et al. 1983). No gross necropsy or histopathology changes were observed in the gastrointestinal tracts of these rats (Oldiges et al. 1983). In mice exposed to concentrations up to 5 mg/mg^3 for 14 weeks, no gastrointestinal effects were noted, and there were no gross or microscopic changes to the gastrointestinal tract at necropsy (Klonne and Kintigh 1988). Microscopic examination of the stomach, small intestine, and colon showed no treatment-related changes in rats given γ-HCH via feed for 10 months at up to 140 mg/kg/day or 2 years at up to 30 mg/kg/day (Fitzhugh et al. 1950).

Technical HCH or Unspecified Isomers of HCH. Fitzhugh et al. (1950) did not observe any effects of treatment on the gastrointestinal tract of rats given technical HCH via diet for 6 months (up to 70 mg/kg/day) or 2 years (up to 9 mg/kg/day).

2.7 HEMATOLOGICAL

Epidemiological Studies. In a cross-sectional study of adolescents and adults living near a former HCH production facility in Brazil, an increase in eosinophilia was associated with serum β-HCH levels (Freire et al. 2015). The results of a case-control study showed a positive association between serum levels of α-HCH and childhood aplastic anemia, but no association with serum β- or γ-HCH (Ahamed et al. 2006).

α-HCH. Dietary administration of α-HCH doses up to 70 mg/kg/day for up to 9 months or up to 9 mg/kg/day for 2 years did not result in histopathology changes in the spleen or bone marrow of rats (Fitzhugh et al. 1950).

β-HCH. Exposure to β-HCH at doses of 22.5–25 mg/kg/day in the diet for 13 weeks in rats resulted in statistically significant decreases in numbers of red blood cells and white blood cells, as well as reduced hemoglobin and packed cell volume values (Van Velsen et al. 1986). At this dose, 50% of the exposed animals were sacrificed moribund. Extramedullary hematopoiesis was observed in males and females surviving for 13 weeks at 23-26 mg/kg/day, but not in the early decedents. There were no histopathology findings in the spleen or bone marrow of rats given β-HCH in the diet for up to 10 weeks at 70 mg/kg/day

or 2 years at 9 mg/kg/day (Fitzhugh et al. 1950). No other data on hematological effects in animals exposed to β-HCH were located.

γ-HCH (Lindane). Hematological effects have been reported in case reports of humans following exposure to γ-HCH. 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. Aplastic anemia was also documented in a man who applied γ-HCH to his skin for 3 weeks for treatment of scabies (Rauch et al. 1990).

A woman who committed suicide by drinking γ-HCH was found to have disseminated intravascular coagulation (a condition where abnormal blood clotting occurs in blood vessels throughout the body) during the period when serum γ-HCH levels were elevated (Sunder Ram Rao et al. 1988). 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).

In an inhalation study of rats exposed to 5 mg/m^3 γ-HCH aerosol for 90 days, bone marrow myelogram changes were observed, including increased reticulocytes in males and females, increased stem cells and myeloblasts in males, and decreased lymphocytes in females (Oldiges et al. 1983). No changes to blood parameters were noted in this study (Oldiges et al. 1983). Mice exposed to concentrations of γ-HCH up to 5 mg/m^3 for 14 weeks exhibited no hematological changes or effects on bone marrow smears (Klonne and Kintigh 1988).

Hong and Boorman (1993) reported significant suppression in bone marrow cellularity, erythrocyte precursors, and granulocyte-macrophage progenitor cells, and residual progenitor cell damage in male B6C3F$_1$ mice given 20 or 40 mg γ-HCH/kg/day by gavage in corn oil for 3 days. In a similar experiment, dose-dependent decreases in bone marrow cellularity, granulocyte-macrophage progenitor cells, and pluripotent bone marrow stem cells were noted following 10 days of exposure to 10 or 20 mg γ-HCH/kg/day (Hong and Boorman 1993). No effects on blood leukocytes were reported in male mice

administered 0.25 mg/kg/day γ-HCH by gavage in oil for 61 days (Tewari et al. 2017). No hematological effects were noted in rats exposed to 10 mg γ-HCH/kg/day in the diet for 12 weeks (Suter 1983) or in beagle dogs exposed to 2.92 mg/kg/day γ-HCH in the diet for 104 weeks (Rivett et al. 1978). No histopathology changes were observed in the spleen or bone marrow of rats exposed to γ-HCH in feed for up to 10 months at 140 mg/kg/day or up to 2 years at 30 mg/kg/day (Fitzhugh et al. 1950).

Technical HCH or Unspecified Isomers of HCH. The results of a case-control study of childhood aplastic anemia indicated no association between this disease and serum levels of total-HCH (including α-, β-, γ-, and δ-HCH) (Ahamed et al. 2006). Hematological abnormalities, including alterations in polymorphonuclear leukocyte count, lymphocyte count, reticulocyte count, and prothrombin time have been reported following chronic human occupational exposure to γ-HCH (Brassow et al. 1981). Exposure concentrations were not specified in these studies and concomitant dermal exposure probably occurred. Granulocytopenia, aplastic anemia, and pancytopenia have been reported in several 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. 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 with a preparation that reportedly contained 2% HCH (Woodliff et al. 1966).

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). In a study evaluating the influence of vitamin A on the toxicity of technical-grade HCH, significant decreases in total white blood cell counts and clotting time were reported in rats fed vitamin A-deficient diets containing technical-grade HCH at a dose level of 90 mg/kg/day for 7 weeks (Joseph et al. 1992c). In a similar study, rats fed a vitamin A-supplemented diet containing the same dose level of technical-grade HCH for 7 weeks exhibited a significant reduction in total white blood cell count, but not red blood cell count (Joseph et al. 1992c). No treatment-related histopathology changes were seen in the spleen or bone marrow of rats after 6 months of dietary exposure to technical HCH at 70 mg/kg/day or 2 years of dietary exposure at 9 mg/kg/day (Fitzhugh et al. 1950).

2.8 MUSCULOSKELETAL

Data regarding musculoskeletal effects in humans were limited to oral exposure to γ-HCH (see γ-HCH subsection below).

α-HCH. There were no microscopic lesions in the leg muscles or bones of rats given α-HCH via the diet at doses up to 70 mg/kg/day for ~9 months or up to 9 mg/kg/day for 2 years (Fitzhugh et al. 1950).

β-HCH. Dietary administration of β-HCH at doses up to 70 mg/kg/day for up to 10 weeks or up to 9 mg/kg/day for 2 years did not induce histopathology changes in the leg muscles or bones of rats (Fitzhugh et al. 1950).

γ-HCH (Lindane) Ingestion of a single dose of approximately 15–30 mL γ-HCH powder (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). A suicidal 21-year-old male developed rhabdomyolysis as indicated by muscle pain, muscle tenderness, proteinuria, myoglobinuria, elevated serum levels of aspartate aminotransferase (AST), potassium, creatinine, and creatinine protein kinase (CPK) 1–3 days following ingestion of a single unknown dose of γ-HCH (Shah et al. 2013). The man recovered after 3 weeks of clinical care.

Microscopic examination of skeletal muscle in mice and rats (as well as femur in mice) exposed by inhalation to concentrations up to 5 mg/m^3 for 13–14 weeks did not show any treatment-related effects (Klonne and Kintigh 1988; Oldiges et al. 1983). Decreased medullary area in the femur bone was found in young rats treated with 20 mg/kg/day of γ-HCH by gavage for 10 weeks (Andrews and Gray 1990). Fitzhugh et al. (1950) reported no treatment-related histopathology changes in the leg muscles or bones of rats exposed via diet for 10 months at doses up to 140 mg/kg/day or for 2 years at 30 mg/kg/day.

Technical HCH or Unspecified Isomers of HCH. No microscopic lesions were observed in the leg muscles or bones of rats given dietary technical HCH doses up to 70 mg/kg/day for 6 months or 9 mg/kg/day for 2 years (Fitzhugh et al. 1950).

2.9 HEPATIC

Epidemiological Studies. Human epidemiological data pertaining to HCH exposure and hepatic effects (see Table 2-10) are limited to two cross-sectional studies of β-HCH (Arrebola et al. 2014; Freire et al. 2015). No association between serum lipids and serum or adipose concentrations of β-HCH was observed in a study of 298 non-surgical cancer patients in Spain (Arrebola et al. 2014). In a study of adolescents and adults residing near a former HCH production facility in Brazil, serum β-HCH was associated with increased risk of elevated total and indirect serum bilirubin in females, but not in males (Freire et al. 2015). There were no associations between serum β-HCH and risk of elevated direct serum bilirubin or serum enzymes (Freire et al. 2015).

Table 2-10. Summary of Epidemiological Studies of β-Hexachlorocyclohexane Exposure and Hepatic Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Mean concentration	Result
Arrebola et al. 2014 Cross-sectional, 298 noncancer surgical patients >16 years old, Spain	Measures of serum lipids (total triglycerides, HDL, LDL, and total cholesterol)	Serum or adipose	19.60±28.74 ng/g lipid (biomarker not reported)	↔
Freire et al. 2015 Cross-sectional, 339 males and 375 females, age >14 years residing near former HCH production facility, Brazil	**Elevated total and indirect serum bilirubin**	Serum	Males: 3.72 µg/g lipid Females: 3.09 µg/g lipid	M: ↔ F: ↑
	Elevated direct serum bilirubin			M: ↔ F: ↔
	Elevated serum AST, ALT, GGT			M: ↔ F: ↔

↑ = association with increase; ↔ = no association; ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = γ-glutamyl transferase; HCH = hexachlorocyclohexane; HDL = high-density lipoprotein; LDL = low density lipoprotein

α-HCH. Hepatic effects have been observed in rats, mice, and hamsters after intermediate- and chronic-duration oral exposures to α-HCH. Increases in absolute and relative liver weight, coupled with hepatocellular hypertrophy and/or hyperplasia, have been observed in F344 rats at doses of at least 20 mg/kg/day for 28 days (Sumida et al. 2007) and in Wistar rats at doses of 45 mg/kg/day for 24 weeks (Nagasaki et al. 1975) or 35 mg/kg/day for 48 weeks (Ito et al. 1975). Rats receiving 60–70 mg/kg/day α-HCH in the diet as part of a chronic study died early (mean survival 35.9 weeks compared with 58.3 weeks in controls); at necropsy, these animals exhibited 2-fold increases in liver weight and histopathology changes of moderate severity, including focal necrosis and fatty degeneration (Fitzhugh et

al. 1950). Hypertrophied liver cells were reported in mice fed 18 mg/kg/day α-HCH for 24 weeks (Ito et al. 1973). More severe effects, including hepatomegaly, bile duct proliferation, oval cells, nodular hyperplasia, megalocytosis, and a doubling of liver weight, were observed in several strains of mice (DDY, ICR, DBA/2, C57BL/6, C3H/He, and HPBC57BL) given feed containing 90 mg/kg/day for at least 21 weeks (Nagasaki et al. 1975; Tryphonas and Iverson 1983). This dose (90 mg/kg/day) yielded a significant increase in hepatocellular carcinomas in all but the C57BL/6 strain of mouse (Nagasaki et al. 1975; Tryphonas and Iverson 1983). Nagasaki et al. (1975) also conducted an experiment using male Syrian hamsters exposed to α-HCH in the diet for 24 weeks. In hamsters, a 38% increase in relative liver weight, with increased liver cell hypertrophy, was observed at 45 mg/kg/day.

Chronic (107 weeks) exposure to α-HCH in feed resulted in dose-related increases in the severity of liver histopathology changes in rats (Fitzhugh et al. 1950). Very slight to slight microscopic damage in the liver, along with ≥32% increase in relative liver weight, was seen at ≥4 mg/kg/day (Fitzhugh et al. 1950). The microscopic changes were described as "characteristic of certain chlorinated cyclic compounds" without further detail. Rats at the highest dose in this study (60–70 mg/kg/day) exhibited reduced survival (mean <1 year); these animals exhibited more severe liver effects, as described above with the intermediate-duration studies.

Both rats and mice exposed to α-HCH have developed liver cancers. Hepatocellular carcinomas were reported in rats administered 70 mg/kg/day in the diet for 48 or 72 weeks (Ito et al. 1975), and liver cancers were observed 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; Tryphonas and Iverson 1983; Tsukada et al. 1979) (see Section 2.19). Hamsters exposed to 45 mg/kg/day for 24 weeks did not develop liver tumors (Nagasaki et al. 1975).

Mechanisms: Little information is available on potential mechanisms of α-HCH-induced hepatotoxicity but is possible that oxidative stress and/or mitotic disturbances may be involved. Administration of 1.8 mg/kg/day α-HCH in the diet to rats for 15 or 30 days resulted in increases in lipid peroxidation and microsomal superoxide production in the liver (Barros et al. 1991). In male Donryu rats, a 3-week dietary exposure to α-HCH resulted in mitotic disturbances including an increased mitotic rate and an increased frequency of polyploid hepatic cells (Hitachi et al. 1975).

β-HCH. Hepatic effects have been observed in intermediate-and chronic-duration studies of β-HCH in rats and mice exposed via the diet. Moderate to marked liver damage, including fatty degeneration and focal necrosis, was reported in rats that died prematurely (within 10 weeks of study initiation) after dietary exposure to doses of 60–70 mg/kg/day (Fitzhugh et al. 1950). In a comprehensive 13-week study of rats (Van Velsen et al. 1986), dose-related increases in hepatic effects were seen at all doses (≥0.18 mg/kg/day) in males. At 0.18 mg/kg/day, the effects consisted of hyalinization of centrilobular cells; at ≥4.5 mg/kg/day, increased mitoses in females, periportal fat accumulation in both sexes, and isolated instances of focal necrosis in males were observed. Relative liver weights were increased by 10% or more at 1.0 mg/kg/day in females and 4.5 mg/kg/day in males (Van Velsen et al. 1986). At the highest dose in this study (22.5–25 mg/kg/day), liver weights were doubled; at this dose, 50% of the animals were sacrificed moribund before the end of the study (Van Velsen et al. 1986). Liver cell hypertrophy was reported in rats fed 35 mg/kg/day in the diet for 48 weeks or 70 mg/kg/day for 24 weeks (Ito et al. 1975). In mice, exposure for 24 weeks to 18 mg/kg/day in the diet resulted in an 18% increase in liver weight, and liver cell hypertrophy at higher doses (≥45 mg/kg/day) (Ito et al. 1973). Mice exposed to 50–60 mg/kg/day in the diet for 32 weeks exhibited hepatic foci of degeneration (Hanada et al. 1973).

Chronic dietary exposure of rats to lower doses of β-HCH resulted in increased liver weight and dose-related histopathology changes in the liver (Fitzhugh et al. 1950). At 0.7–0.9 mg/kg/day, a 34% increase in liver weight and slight microscopic changes described as “characteristic of certain chlorinated cyclic compounds” were observed.

Liver tumors were not reported in mice exposed to β-HCH for 24–32 weeks (Hanada et al. 1973; Ito et al. 1973) or in rats exposed for 24–48 weeks (Ito et al. 1975); however, Thorpe and Walker (1973) reported liver cancer in mice fed 34 mg/kg/day for 26 months.

γ-HCH (Lindane). Two case reports of intentional ingestion of γ-HCH have documented increases in serum liver enzymes; neither report provided an estimate of the associated dose of γ-HCH. A 30-year-old male farmer from rural India ingested a single dose of approximately 50 mL of 2% γ-HCH solution in a suicide attempt (Paul et al. 2013). Six hours after the ingestion, the man went to the emergency department where initial examination and laboratory tests were normal. Nausea was noted on the second day, and abdominal tenderness and increased serum levels of bilirubin, AST, and alanine aminotransferase (ALT) were observed on day 5. Treatment with hemodialysis for acute kidney injury spontaneously reduced the hepatic enzymes and the man recovered after 3 weeks. A 56-year-old man

ingested approximately 12 ounces of an insecticide containing 20% γ-HCH intentionally in a suicide attempt (Wiles et al. 2015). Hepatic enzymes (AST, ALT, alkaline phosphatase [ALP], and γ-glutamyl transferase [GGT]) were increased after 3 days. The man died on day 12 after committing suicide by other means; at autopsy, no gross or microscopic abnormalities were noted.

Rats exposed to γ-HCH aerosol (5 mg/m^3 for 6 hours/day) exhibited increased hepatic cytochrome P450 concentration after 90 consecutive days, but this level returned to control values after a 4-week recovery period (Oldiges et al. 1983). Statistically significant, but modest, increases in absolute and relative liver weights (up to 12%) were observed at this exposure concentration, but there were no concomitant effects of treatment on serum chemistry or liver histopathology in the rats (Oldiges et al. 1983). Mice exposed to the same concentration 5 days/week for 14 weeks exhibited no changes in clinical chemistry, liver weights, or liver histology (Klonne and Kintigh 1988).

Hepatic effects have been documented in rats and mice exposed to γ-HCH via oral administration for acute, intermediate, and chronic durations. At lower doses, effects include increased serum enzymes, increased liver weight, and hepatocellular hypertrophy. Higher doses and/or longer exposure durations result in liver effects of increasing severity, including vacuolar degeneration, necrosis, and congestion.

Acute-duration oral studies in rats show increases in liver weight as well as histopathology changes in animals exposed to γ-HCH. In male Wistar rats, a single gavage dose of 60 mg/kg/day resulted in marked centrilobular hepatic necrosis (Singh and Sharma 2011), and fatty degeneration, vacuolation, and necrosis were observed after gavage doses of 5 mg/kg/day for 3 days (Hfaiedh et al. 2012). Ultrastructural changes observed in the liver of Sprague-Dawley rats (sex not specified) after 2 days of exposure to 30 mg/kg/day in feed were reduced number of cells per field; increased cell, nucleus, and nucleolus size; and slight cellular disorganization (Ali and Shakoori 1998). Although no histopathological examinations were performed, no significant increase in liver weight was noted in Sprague-Dawley rats exposed to 10 mg γ-HCH/kg/day for a minimum of 4 days (Joy et al. 1982). A significant increase (15% relative to controls) in absolute, but not relative, liver weight was observed in rats exposed to 15 mg γ-HCH/kg/day for 5 days (Parmar et al. 2003). A significant, but modest, increase (6% relative to controls) in relative liver weight was observed in a small group of male F344 rats given 10 mg/kg/day γ-HCH by gavage for 7 days, but not in a group similarly exposed for 14 days (Sumida et al. 2007). Histopathology evaluations of these animal were not reported.

Increases in serum enzymes indicative of hepatotoxicity have been reported in rats exposed once to 12 mg/kg/day (Attia et al. 2011) or for 3 days to 5 mg/kg/day (Hfaiedh et al. 2012). Male Wistar rats fed 13.5 mg γ-HCH/kg/day in their diet for 12 days exhibited decreased activities of liver enzymes (malic enzyme, glucose-6-phosphate dehydrogenase, phosphogluconate dehydrogenase, citrate cleavage enzyme, and fatty acid synthase) and increased levels of serum triglycerides (Boll et al. 1995). Significantly increased liver microsomal 7-ethoxycoumarin-O-dealkylase activity was found in Osborne-Mendel rats exposed to 11.2 mg/kg/day γ-HCH and in CF1 and B6C3F1 strain mice exposed to 23.6 and 50.5 mg/kg/day in the diet for 3 days (Oesch et al. 1982).

The most common histopathology finding in the livers of rats and mice exposed to oral doses of γ-HCH for intermediate durations is hepatocellular hypertrophy. 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). In multigeneration reproductive toxicity studies in rats, hepatocellular hypertrophy reportedly occurred at increased incidence in F0 and F1 male and female Sprague-Dawley rat parents exposed to 3–6 mg/kg/day (Matsuura et al. 2005) and in F1 male CD rat parents exposed to 1.7 mg/kg/day (EPA 1991a). In both studies, no hepatic effects were seen at a dose of about 1 mg/kg/day γ-HCH (EPA 1991a; Matsuura et al. 2005). Rats exposed to 35 mg/kg/day γ-HCH for 48 weeks in the diet exhibited hepatocellular hypertrophy (Ito et al. 1975). Similar findings were reported in a study of Wistar rats given ≥7–8 mg γ-HCH/kg/day in the diet for up to 52 weeks, in which a dose-related increase in periacinar hepatocytic hypertrophy was seen (Amyes 1990). In mice, administration of 90 mg γ-HCH/kg/day in the diet for 24 weeks was reported to result in centrilobular hypertrophy and a significant increase (33%) in relative liver weight (Ito et al. 1973).

In other intermediate-duration studies, more severe lesions have been noted in the liver. An early study (Ortega et al. 1957) reported the development of liver cell “lipospheres” in rats fed 2.5 mg γ-HCH/kg/day in the diet for 32 weeks; in older literature, these changes were described as spherical cytoplasmic inclusions of a fatty nature. Ali and Shakoori (1998) reported ultrastructural changes in the livers of Sprague-Dawley rats exposed for 15 days to a dose of 18 mg/kg/day in food. Findings observed in the treated animals included reduced number of cells per field; increased cell, nucleus, and nucleolus size; vacuoles in the cytoplasm; and apparent fatty degeneration. At a dose of 20 mg/kg/day γ-HCH administered by daily gavage for 30 days, liver histopathology changes in Sprague-Dawley rats included megalocytosis, vacuolar degeneration, venous and sinusoidal congestion, and lymphocytic infiltration (Fatih Fidan et al. 2008). At 100 mg/kg/day for the same duration, the livers of Wistar rats showed vacuolar degeneration of hepatocytes and marked degradation of the central vein (Vijaya Padma et al.

2011). 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).

Two intermediate-duration studies reported no liver effects in rats exposed to γ-HCH. Sumida et al. (2007) observed no treatment-related hepatic effects (clinical chemistry, liver weight, or histopathology) in F344 rats exposed for 28 days at 10 mg/kg/day via gavage. The lack of effect in this study may indicate a lower sensitivity of F344 rats to hepatic effects of this isomer, but also could be a reflection of the very small numbers of animals tested (four males per group). In an older study focused on neurotoxicity testing, groups of eight Wistar rats given 50 mg/kg/day for 40 days in feed exhibited increased liver weight, but normal liver function test results and histology (Desi 1974).

In addition to histopathology changes, intermediate-duration oral exposure to γ-HCH has resulted in increased liver weights. Rats exposed to 2.5 mg γ-HCH/kg/day for 21 days showed a significant increase (13% higher than controls) in absolute, but not relative, liver weight (Parmar et al. 2003). 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 significant increases in absolute and relative liver weights; histopathological examinations were not performed (Oesch et al. 1982). Increased absolute and relative liver weights occurred at doses (≥3–5 mg/kg/day) associated with increased incidences of hepatocellular hypertrophy in parental animals exposed to γ-HCH in the diet in a 2-generation reproduction toxicity study (Matsuura et al. 2005). Exposure of dd strain mice to dietary doses of 90 mg/kg/day for 24 weeks resulted in a 33% increase in relative liver weight (Ito et al. 1973).

Increased liver weights have also been reported in offspring of rats exposed to γ-HCH during gestation and/or lactation (Srinivasan et al. 1991). Additional details are provided in Section 2.17 (Developmental).

Increases in serum enzymes and lipids indicating hepatic effects have been observed in rats and rabbits exposed for intermediate durations to γ-HCH. Significant increases in serum AST, ALT, GGT, ALP, and/or LDH were observed in Wistar rats exposed to 100 mg/kg/day for 4 weeks (Etim et al. 2006; Vijaya Padma et al. 2011). Increased serum levels of AST, LDH, cholesterol, total triglycerides, free fatty acids, and total phospholipids were observed in male Sprague-Dawley rats administered 8.8 mg/kg/day γ-HCH by gavage in water for 3 weeks (Kamal El-Dein et al. 2016). Rabbits treated with 4.21 mg γ-HCH/kg/day by gavage for 28 days exhibited significant increases in plasma ALP and ALT activities immediately following initiation of dosing; these activities returned to control levels by day 14 (Cerón et al. 1995).

The plasma level of AST activity also increased immediately following dosing and remained elevated up to 7 days postexposure (day 35) (Cerón et al. 1995).

Oral exposure to γ-HCH for intermediate durations has resulted in induction of hepatic cytochrome P450 levels in rats and mice. Significant increases in hepatic microsomal cytochrome P450 levels were found in Wistar rats fed diets containing 1.8 mg/kg/day γ-HCH for 15 or 30 days (Barros et al. 1991). A dose- and time-dependent increase of P450 and P450-dependent enzyme levels was observed in the liver of rats exposed to γ-HCH (Parmar et al. 2003). P450 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 P450 content in rats exposed to <10 mg γ-HCH/kg/day for 5 days. Several P450-dependent enzymes, 7-ethoxyresorufin-O-deethylase (EROD), 7-pentoxyresorufin-O-dealkylase (PROD), and N-nitrosodimethylamine demethylase (NDMA-d), were significantly increased in rats exposed to 5 mg γ-HCH/kg/day for 5 days or 2.5 mg γ-HCH/kg/day for 15 and 21 days (Parmar et al. 2003). Increases in liver microsomal mixed-function oxidase activity were observed in rats exposed to ≥10.6 mg γ-HCH/kg/day and mice exposed to ≥21.1 mg/kg/day in the diet for 3 months (Oesch et al. 1982).

Hepatotoxicity has been documented in animals exposed by oral administration to γ-HCH for chronic durations. After Sprague-Dawley rats were exposed for 18 months to γ-HCH at a dose of 9 mg/kg/day in feed, the following microscopic findings were observed in the liver: increased cell, nucleus, and nucleolus size; extensive cytoplasmolysis; slight cytoplasmic degeneration; and increasing nuclear distortion (Ali and Shakoori 1998). Chronic exposure of rats to 7–9 mg/kg/day γ-HCH in the diet for 107 weeks resulted in increased liver weight (35% higher than controls) and very slight microscopic liver damage described as “characteristic of certain chlorinated cyclic compounds” (Fitzhugh et al. 1950). At higher doses, liver necrosis and fatty degeneration were observed (Fitzhugh et al. 1950). Male CD-1 mice exposed to 20.5 mg/kg/day γ-HCH via feed for 78 weeks exhibited centrilobular hepatocyte hypertrophy (EPA 2000a). No liver lesions were observed by light or electron microscopy in NMRI mice given ~8 mg/kg/day for 80 weeks (Herbst et al. 1975; Weisse and Herbst 1977). At gross necropsy, the livers of dogs exposed to 2.9 mg/kg/day for 104 weeks were noted to be dark, but no histopathology changes were reported (Rivett et al. 1978).

Increased incidences of liver tumors (hepatomas, hepatocellular carcinomas) have been observed in mice exposed via diet to γ-HCH for intermediate and chronic durations at γ-HCH doses as low as

13.6 mg/kg/day (Hanada et al. 1973; NCI 1977; Thorpe and Walker 1973; Wolff et al. 1987). These studies are discussed further in Section 2.19 (Cancer).

One study of dermal exposure to γ-HCH reported also reported liver effects. Centrilobular hepatocellular hypertrophy was reported in male and female rats exposed to γ-HCH ≥60 mg /kg/day by dermal application for 6 hours/day, 5 days/week, for 13 weeks (EPA 1988a).

Mechanisms. There is some evidence that oxidative stress may contribute to the hepatic effects of γ-HCH. Significant increases in hepatic microsomal superoxide anion production and cytoplasmic superoxide dismutase activity and lipid peroxidation were found in the livers of Wistar rats fed diets containing 1.8 mg/kg/day γ-HCH for 15 or 30 days (Barros et al. 1991). Groups of 10 male rats (strain not reported) were administered a single dose of γ-HCH (98% purity) in corn oil at a dose of 0 or 12 mg/kg and then sacrificed 24 hours later in a study aimed at evaluating the ameliorating effects of co-treatment with the antioxidants, nigella sativa oil and omega 3 fatty acids. Co-treatment with nigella sativa oil and omega 3 fatty acids attenuated the effects of γ-HCH on lipid parameters, clinical chemistry parameters, lipid peroxidation, and antioxidant enzyme activities (Attia et al. 2011). The mitigating effects of nigella sativa oil and omega 3 fatty acids could have resulted from their antioxidant activity or from effects on the absorption or metabolism of γ-HCH.

δ-HCH. Liver cell hypertrophy was observed in rats fed with 70 mg/kg/day of δ-HCH in the diet for 48 weeks (Ito et al. 1975). Similarly, mice exposed for 24 weeks to 90 mg/kg/day δ-HCH in feed exhibited a 23% increase in relative liver weight and centrilobular hypertrophy (Ito et al. 1973).

Unspecified HCH Isomers or Mixtures of HCH Isomers. In humans, statistically significant increases in the blood levels of the enzymes LDH (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) compared to a control group of workers. The HCH isomer concentrations in serum were 10-fold higher in the exposed group than in the control group of workers. Both inhalation and dermal exposure probably occurred.

Increases in liver weight, serum enzymes and lipids, and liver histopathology changes have been observed in animals exposed to technical-grade HCH by oral administration for acute durations. Technical-grade HCH was reported to cause increases in liver weight and serum enzyme activities (e.g., ALP, aminotransferases) in male Swiss mice given 72 mg/kg in the diet for 2 weeks (Ravinder et al. 1989).

Other effects seen in the mice included 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 activities of AST and LDH were observed in pregnant mice administered a single dose of technical-grade HCH (5 mg/kg) on GD 9 (Dikshith et al. 1990). Pregnant animals dosed with 25 mg/kg also experienced decreases in liver ALT and ALP activity (Dikshith et al. 1990). 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, and increases in liver ALP activity were observed in the virgin mice at doses ≥25 mg/kg. However, with the exception of decreased AST activity in pregnant mice, the dose-response relationships were questionable (Dikshith et al. 1990). There were also no corresponding pathological changes in the livers of the treated mice. However, at a higher dose (50 mg/kg/day) of technical-grade HCH administered to mice for 1, 5, or 15 days by oil gavage, congestion of hepatic portal vessels and central vein, swollen hepatic cells with vacuolar or parenchymatous degeneration, and fatty changes in periportal and centrilobular cells were observed (Philip et al. 1989).

Liver enzyme level changes were seen in male, but not female, rats given 5 or 25 mg/kg/day by gavage for 90 days; at these doses, there were significant mortalities (Dikshith et al. 1991b). A 65% decrease in liver weight, decreased liver AST and LDH activities, and increased ALP 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). 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). No adverse hepatic effects were seen in rats treated with 50 mg/kg/day technical-grade HCH for 30 days (Khanna et al. 1990) or in pigs exposed to 0.8 mg/kg/day for 90 days (Wang et al. 2006). 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). 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). Chronic dietary administration of technical-grade HCH to rats at a dose of 4 mg/kg/day resulted in slight microscopic liver damage, which the study authors described as “characteristic of certain chlorinated cyclic compounds” (Fitzhugh et al. 1950).

Technical-grade HCH was also reported to cause liver cancer in mice following exposure to 90 mg/kg/day in the diet for time periods ranging from 2 to 8 months (Bhatt and Bano 2009; Bhatt and Nagda 2012; Karnik et al. 1981; Thakore et al. 1981; Trivedi et al. 2007, 2009) or exposure to 10–50 mg/kg/day for 80–88 weeks (Kashyap et al. 1979; Munir et al. 1983) (see Section 2.19).

Hepatic effects have been seen after dermal exposure to technical-grade HCH. Alterations in liver histopathology, 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 by dermal application of 100 mg/kg/day technical-grade HCH for 30 days (Dikshith et al. 1978). The area 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 ALT and ALP (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 of changes, and the numbers of animals affected were not reported (Dikshith et al. 1991c).

2.10 RENAL

Epidemiological Studies. Very limited human epidemiological data on renal effects of HCH isomers are available, as shown in Table 2-11. In a cohort study of 1,545 male pesticide applicators in Iowa and North Carolina, no association was observed between self-reported pesticide exposure and chronic kidney disease (Shearer et al. 2021). A cohort study of 31,142 wives of pesticide applicators in Iowa and North Carolina followed for 15 years did not observe an association between self-reported pesticide exposure and end-stage renal disease (Lebov et al. 2015). Case-control studies of chronic kidney disease (Ghosh et al. 2017; Siddarth et al. 2014) did not observe associations with β-HCH in blood or serum, and results were mixed for α- and γ-HCH. Ghosh et al. (2017) reported an association between blood levels of γ-HCH and chronic kidney disease and no association for α-HCH, while Siddarth et al. (2014) reported the converse (association for serum α-HCH and no association for γ-HCH). No association between serum α-, β,- γ-, or δ-HCH and hyperuricemia (or increased serum uric acid) was noted in cross-sectional studies of 453 adults in Spain (Arrebola et al. 2019) or 880 adults in South Korea (Seo et al. 2022).

α-HCH. Fitzhugh et al. (1950) reported kidney damage (nephritis tubular dilatation, hyaline tubular casts, glomerular fibrosis or atrophy, pigment deposition) in rats fed 60–70 mg/kg/day α-HCH for an average of 35.9 weeks; no such effects were observed in rats fed up to 9 mg/kg/day for 107 weeks.

β-HCH. Renal effects have also been noted in rats exposed to β-HCH in the diet, often at doses associated with profound toxicity and/or death. Srinivasan et al. (1984) reported significantly increased

Table 2-11. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Renal Effects

Reference, study type, and population	Outcome evaluated	Isomer	Biomarker	Mean concentration (unless otherwise noted)	Result
Arrebola et al. 2019 Cross-sectional, 453 working adults, median age 35 years, Spain	Hyperuricemia	α-HCH	Serum	<LOQ ng/g lipid (all)	NA[a]
		β-HCH		<LOQ (median) 0.12 (95th percentile)	↔[b]
		γ-HCH		<LOQ (median) 0.05 (95th percentile)	↔[b]
Seo et al. 2022 Cross-sectional, 880 adults, ages 20–80 years, South Korea	Serum uric acid	β-HCH	Serum	40.4 ng/g lipid	↔
		α-HCH		1.24	↔
		γ-HCH		1.82	↔
		δ-HCH		0.17	↔
Shearer et al. 2021 Cohort, 1545 male pesticide applicators, age ≥50 years, Iowa and North Carolina, United States	CKD	γ-HCH	NA (self-reported pesticide exposure)	Ever used versus never used	↔
Ghosh et al. 2017 Case-control, 200 cases and 100 controls, ages 30–50 years, India	**CKD of known or unknown etiology**	α-HCH	Blood	1.26 ng/g (median) (CKD, known) 1.68 (CKD, unknown) 0.7 (controls)	↔
		β-HCH		2.49 (CKD, known) 2.15 (CKD, unknown) 1.7 (controls)	↔
		γ-HCH		2.15 (CKD, known) 2.03 (CKD, unknown) 2.6 (controls)	↑[c]
Siddarth et al. 2014 Case-control, 270 cases and 270 controls, mean ages 46 and 48 years (respectively), India	**CKD**	**α-HCH**	Serum	5.23 ng/mL (median, 3rd tertile) 0.87 (median, 1st tertile)	↑
		β-HCH		5.50 (3rd tertile) 0.20 (1st tertile)	↔
		γ-HCH		3.89 (3rd tertile) 1.86 (1st tertile)	↔
		Total HCH		12.51(3rd tertile) 3.63 (1st tertile)	↑

Table 2-11. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Renal Effects

Reference, study type, and population	Outcome evaluated	Isomer	Biomarker	Mean concentration (unless otherwise noted)	Result
Lebov et al. 2015 Cohort, 31,142 wives of pesticide applicators, ≥18 years at enrollment, Iowa and North Carolina, United States, mean follow-up 15.4 years	End-stage renal disease	γ-HCH	NA (self-reported pesticide exposure)	Ever used versus never used	↔

[a]Analysis was not performed because all samples were below the LOQ.
[b]Odds ratios comparing serum levels ≥ LOQ to samples < LOQ.
[c]Positive association associated with CKD of unknown etiology; no association with CKD of known etiology.

↑ = association with increase; ↓ = association with decrease (inverse association); ↔ = no association; CKD = chronic kidney disease; LOQ = limit of quantification; NA = not applicable

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/kg/day β-HCH for up to 2 weeks. Van Velsen et al. (1986) reported significantly increased kidney weights in female rats exposed to 0.2 mg β-HCH/kg/day for 13 weeks, but the change did not exhibit dose-dependence. In males, a significant increase in kidney weight was observed at 4.5 mg/kg/day. At the highest dose (22.5–25 mg/kg/day), a dose that was profoundly toxic and led to the humane sacrifice of half of the animals, males exhibited renal calcinosis in the outer medulla. The study authors noted that renal calcinosis is common in female rats but that this finding was unusual and therefore of significance in males (Van Velsen et al. 1986). Fitzhugh et al. (1950) examined the renal effects of exposure to β-HCH (60–70 mg/kg/day) in rats that died after an average of 4.4 weeks and found nephritis and basal vacuolation. At lower doses in this study, exposure to doses up to 9 mg/kg/day for up to 2 years did not induce renal histopathology changes (Fitzhugh et al. 1950).

γ-HCH (Lindane). Renal effects have been documented in case reports of accidental or intentional oral exposures to γ-HCH. In a suicide attempt, a 30-year-old male farmer from rural India ingested a single dose of approximately 50 mL of 2% γ-HCH solution (Paul et al. 2013). Six hours after the ingestion, the man went to the emergency department. On the second day symptoms of lethargy and reduced urinary output were noted, increasing in severity by day 5 to include additional symptoms of elevated pulse, increased serum levels of kidney enzymes (blood urea nitrogen [BUN] and creatinine), urinary white blood cells, red blood cells, and protein. An ultrasound of the kidneys indicated cortical echogenicity and mild ascites. The man was treated for acute kidney injury by hemodialysis. Urine output started to increase on day 10 and serum kidney enzymes recovered in 3 weeks, after which he was discharged in stable condition that persisted though a 3-month follow up (Paul et al. 2013).

A suicidal 21-year-old male ingested γ-HCH (dose unknown) (Shah et al. 2013). One day after ingestion, the man experienced reduced urine output, dark urine, pedal edema, and muscular pain lasting 2 days before he went to a clinic. Clinical evaluation at 3 days post-ingestion showed proteinuria, myoglobinuria, muscle tenderness, elevated serum levels of AST, potassium, creatinine, and CPK, and metabolic acidosis indicating acute kidney injury from rhabdomyolysis. Treatment included hemodialysis and supportive care and the man recovered after 3 weeks (Shah et al. 2013). 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). Myoglobin release resulting from muscle lysis in this case led to kidney shutdown, which was the ultimate cause of death.

Small (<10%) increases in kidney weights, in the absence of clinical chemistry, urinalysis, and histopathology changes, were observed in female rats exposed to 5 mg/m^3 γ-HCH aerosol 6 hours/day for 90 consecutive days (Oldiges et al. 1983). Male rats at this concentration exhibited significantly increased kidney weights. Dose-related increases in the incidences of kidney lesions (dilated tubules with protein-containing contents; proliferated tubules) were observed in males, but not females, at concentrations of 0.5 and 5 mg/m^3 (Oldiges et al. 1983). No renal effects (including clinical chemistry, organ weight, and histopathology) were seen in mice exposed up to 5 mg/m^3 γ-HCH aerosol 6 hours/day, 5 days/week for 14 weeks (Klonne and Kintigh 1988).

In studies of animals exposed orally to γ-HCH, renal effects have largely been limited to male rats, although many studies did not test females, and two studies reported histopathology changes in females exposed to higher doses (Suter 1983; Vijaya Padma et al. 2011). In an acute study, male Fischer-344 rats receiving gavage doses of 10 mg/kg/day of γ-HCH for 4 days showed histopathological changes in the proximal tubule epithelial cells including accumulation of protein droplets, hypertrophy, necrosis, pyknotic nuclei, cellular exfoliation, and regenerative epithelium (Dietrich and Swenberg 1990, 1991). Significantly increased excretion of glucose in urine, and histological changes consisting of hypertrophy and degeneration of the renal tubular epithelia, were observed in male Wistar rats exposed to 72 mg/kg/day of γ-HCH for up to 2 weeks (Srinivasan and Radhakrishnamurty 1988; Srinivasan et al. 1984).

Male Sprague-Dawley rats exposed for 30 days to γ-HCH via gavage at doses ≥20 mg/kg/day exhibited medullary and cortical hemorrhage, and degeneration and vacuolation of proximal convoluted tubules (Fatih Fidan et al. 2008). Similarly, after 30 days of exposure to 20 mg/kg/day, male Wistar rats showed intertubular hemorrhage, tubular degeneration and desquamation of tubular epithelium, cystic dilatation, mononuclear cell infiltrate, and necrosis (Prasad et al. 2016). No renal effects other than significantly increased kidney weight were observed in rats exposed to γ-HCH doses up to 5–50 mg /kg/day in the diet for up to 40 days (Desi 1974); histological examination of the kidney did not reveal any changes. In female Wistar rats exposed for 30 days to daily gavage doses of 100 mg/kg/day, glomerular degeneration and shrinkage and degeneration of the proximal and distal tubules were observed (Vijaya Padma et al. 2011).

Increased kidney weight, hyaline droplet accumulation, and tubular regeneration were observed in male Long-Evans rats exposed for 10 weeks to 10 mg/kg/day γ-HCH via gavage (Andrews and Gray 1990). In male rats treated with 0.4–10 mg γ-HCH/kg/day in their diets for 12 weeks, dose-dependent renal effects

of increasing severity were seen, including basophilic proximal tubules and proximal tubular distention with cell debris, as well as hyaline droplet formation, and minimal to moderate interstitial nephritis (Suter 1983). Both male and female rats exposed to doses ≥2 mg/kg/day exhibited minimal to slight epithelial cell necrosis in the proximal tubules (Suter 1983). In a 2-generation reproductive toxicity study, no renal effects were observed in female adult Crj:CD(SD)IGS rats exposed to doses up to 26.1 mg/kg/day (Matsuura et al. 2005). In contrast, doses ≥0.56 mg/kg/day resulted in increased incidences and severity of basophilic tubules and hyaline droplets in the proximal tubules of F0 and F1 parental males (Matsuura et al. 2005). EPA (1991a) also observed renal histopathological changes characteristic of alpha-2μ-globulin accumulation in F0 and F1 male CD rats at ≥1.7 mg/kg/day in a 2-generation reproduction study with γ-HCH. No gross or histopathological changes were observed in kidneys of females in either generation.

Male Wistar rats exposed for up to 52 weeks to γ-HCH in their diet exhibited hyaline droplets in the renal proximal tubules, interstitial chronic nephritis, and regeneration in proximal tubules at doses ≥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). In contrast, no renal effects were seen in females at doses up to 32 mg/kg/day in this study (Amyes 1990). Very slight microscopic kidney damage (not further specified) was reported in Wistar rats exposed to 7–9 mg γ-HCH/kg/day for up to 104 weeks (Fitzhugh et al. 1950). The histopathology findings were not reported by sex, so it is not clear whether the effects were limited to males.

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 turbidity, proteinuria, and hematuria (EPA 1988a). Females in the same study exhibited a slight increase in the incidence of tubular basophilia at 60 mg/kg/day.

Mechanisms. Available data suggest that the renal effects of γ-HCH may result from at least two possible mechanisms: (1) alpha-2μ-globulin accumulation in male rats; and (2) increased oxidative stress in both male and female rats. Dietrich and Swenberg (1990, 1991) demonstrated α-2μ-globulin staining in the kidney cortex of male F-344 rats exposed for 4 days to 10 mg/kg/day of γ-HCH. No α-2μ-globulin staining was detected in the kidneys of F-344 male controls, F-344 control or exposed female rats, or exposed male NBR rats (a strain that does not synthesize α-2μ-globulin). Matsuura et al. (2005) used immunohistochemistry staining to examine kidneys of male parental rats in a 2-generation reproductive

toxicity study, and observed that smaller hyaline droplets stained positive for α-2μ-globulin, while larger ones did not.

Renal effects seen only in male rats that are attributable to alpha-2μ-globulin accumulation are not relevant to human health (EPA 1991b). However, kidney effects have also been seen in female rats (Suter 1983; Vijaya Padma et al. 2011), and other mechanisms, such as induction of oxidative stress, may play a role in these effects. Increases in lipid peroxidation and nitric oxide, as well as depletion of antioxidant enzyme activities (superoxide dismutase, catalase, and glutathione peroxidase) and reduced glutathione, have been observed in the kidneys of male rats exposed by gavage to doses of ≥20 mg/kg/day γ-HCH in intermediate-duration studies (Fatih Fidan et al. 2008; Prasad et al. 2016; Vijaya Padma et al. 2011).

Technical HCH or Unspecified Isomers of HCH. Oral exposure to technical HCH has been shown to induce kidney effects in mice, rats, and pigs. Mice treated daily with 50 mg/kg/day technical-grade HCH for 1, 5, or 15 days by oil gavage exhibited renal changes including 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 the kidneys of male rats treated with 50 or 60 mg/kg/day technical-grade HCH for 30 days (Dikshith et al. 1989a; Khanna et al. 1990). Wang et al. (2006) observed a 24% increase in kidney weights in pigs given technical-grade HCH at a dose of 0.8 mg/kg/day for 90 days; renal histopathology was not evaluated. Nephritis, pigmentation, and basal vacuolation were observed in kidneys of rats (sex not specified) fed 60–70 mg/kg/day technical-grade 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 were seen in male rats exposed for 360 days to 20 mg/kg/day of technical-grade HCH (Dikshith et al. 1991a).

Renal changes have been reported in animals exposed to technical-grade HCH by dermal application. 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 for 30 days (Dikshith et al. 1989b). In both of these studies, mortalities were seen at the doses associated with renal effects.

2.11 DERMAL

γ-HCH (Lindane). A 10-year-old boy who was being treated for scabies was exposed to a 1% γ-HCH solution by dermal application from the neck down for 8 hours/day on 3 consecutive days (Juan et al. 2004). Following the initial application, erythema developed on the neck, extending to the trunk and axillae after 2 days. On the third day, pustules were present on his neck, trunk, arms, and thighs. He was examined in a clinic, where symptoms of leukocytosis were noted. A biopsy of a skin lesion showed sub-corneal and intraepidermal pustule formation with neutrophilic infiltrations and scattered eosinophils in the dermis, suggesting acute generalized exanthematous pustulosis. After discontinuation of the γ-HCH solution, the boy recovered within a week (Juan et al. 2004).

A 57-year-old man was admitted to the hospital and diagnosed with scabies for which he was treated with γ-HCH lotion (concentration not reported) by dermal application to the whole body for 8 hours/day for 3 consecutive days (Yu et al. 2015). Instructions included washing/removal of the lotion after 8 hours, but the man was only partially washed for unknown reasons. One week after exposure, multiple scattered and coalescent polymorphic ulcerations with hemorrhagic spots and black burn-like crusted edges developed. A skin biopsy revealed papillary edema, and acute and chronic inflammation indicating ulcerative irritant contact dermatitis. Patch testing was performed but the results were unavailable, as the patient died from sepsis. The time elapsed between exposure and death was not reported and it was not clear whether the ulceration caused the sepsis (Yu et al. 2015).

In a summary of γ-HCH poisoning cases reported to the Texas poison control network (Forrester et al. 2004), commonly reported symptoms of exposure included erythema and dermal irritation or pain; the authors did not describe symptoms by routes of exposure, which included oral and dermal. An itchy red rash was observed in a 10-month-old boy after 7 days of twice-daily application of 1% γ-HCH for scabies treatment (Bhalla and Thami 2004). 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 over a period up to 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).

Technical HCH or Unspecified Isomers of HCH. 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).

2.12 OCULAR

No studies were located regarding ocular effects in humans following exposure to HCH isomers.

γ-HCH (Lindane). In a survey of γ-HCH exposures reported to the Texas poison control network, Forrester et al. (2004) reported that ocular irritation and pain were common symptoms. The authors did not distinguish between effects seen after oral and dermal exposures, both of which were considered in the survey.

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), and histopathology of the eyes showed no changes in these mice or in rats exposed similarly (Oldiges et al. 1983). Mild eye irritation was seen in rabbits exposed to 40 mg/kg γ-HCH in the conjunctival sac for up to 72 hours. The irritation level was given a primary irritation score of 0.6 out of a maximum possible cumulative score of 16 (Ullmann 1986c).

2.13 ENDOCRINE

Epidemiological Studies. Several epidemiological studies have suggested that exposure to HCH may be associated with changes in thyroid function (see Table 2-12). In a cohort study of 21,788 male pesticide applicators, occupational exposure to γ-HCH was associated with increased odds of hypothyroid disease (Goldner et al. 2013). In other studies, there was suggestive evidence for associations between β-HCH in blood and alterations in serum thyroid hormone levels, but the direction of change and affected hormones were not consistent. A case-control study in Chinese subjects with thyroid disease found no association between serum levels of β-HCH and thyroid disease; however, serum β-HCH levels were associated with decreased total and free thyroxine (T4) in males and increased levels of free T4 in females (Han et al. 2019). Levels of total T4 in females was not related to serum β-HCH, nor were levels of total triiodothyronine (T3), free T3, and thyroid-stimulating hormone (TSH) in either sex (Han et al. 2019).

Table 2-12. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Endocrine Effects

Reference, study type, and population	Isomer	Concentration in serum (unless otherwise noted)	Outcome evaluated	Result
Han et al. 2019 Case-control, 186 cases of thyroid disease and 186 controls, mean ages 46 and 44 years, China	**β-HCH**	78.96 ng/g lipid (median) (cases) 65.58 (controls)	Thyroid disease	↔
			Serum total T4	M: ↓ F: ↔
			Serum free T4	M: ↓ F: ↑
			Serum total T3, free T3, and TSH	↔
Freire et al. 2013 Cross-sectional, 303 males and 305 females >14 years old, Brazil	**β-HCH**	6.00 ng/mL (median) (males) 6.98 (females)	Serum total T3	↔
			Serum Free T4	M: ↓ F: ↔
			Serum TSH	M: ↑ F: ↔
			Serum anti-thyroperoxidase	↔
	α-HCH	2.52 (males) 2.60 (females)	Serum total T3, free T4, TSH, and anti-thyroperoxidase	↔
	γ-HCH	0.95 (males) 0.97 (females)	Serum total T3, free T4, TSH, and anti-thyroperoxidase	↔
Alvarez-Pedrerol et al. 2009 Cross-sectional, 1,090 pregnant women, Spain	**β-HCH**	32.3 ng/mL (median) (Sabadell [S]) <LOD (median); 22.1 (75^{th} percentile) (Gipuzkoa [G])	**Serum total T3**	S: ↓ G: ↔
			Serum free T4	S: ↔ G: ↑
Dallaire et al. 2009 Cross-sectional, 623 Inuit adults ≥18 years old, Canada	**β-HCH**	8.33 µg/kg lipid (plasma) (mean)	**Serum total T3**	↓
			Serum thyroxine binding globulin	↓
			Serum free T4, TSH	↔

Table 2-12. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Endocrine Effects

Reference, study type, and population	Isomer	Concentration in serum (unless otherwise noted)	Outcome evaluated	Result
Freire et al. 2012 Cross-sectional, 193 children <15 years old, Brazil	**β-HCH**	479 ng/mL (mean)	**Serum total T3**	↑
			Serum free T4, TSH	↔
	α-HCH	300	**Serum total T3**	↑
			Serum free T4, TSH	↔
	γ-HCH	77.5	**Serum total T3**	↑
			Serum free T4, TSH	↔
Seo et al. 2022 Cross-sectional, 880 adults, ages 20–80 years, South Korea	**β-HCH**	40.4 ng/g lipid (mean)	**Serum free T4**	↓
			Serum TSH	↔
	γ-HCH	1.82	Serum TSH and free T4	↔
	α-HCH	1.24		↔
	δ-HCH	0.17		↔
Kim et al. 2013 Cross-sectional, 105 pregnant women 22–46 years old, South Korea	β-HCH	7.58 ng/g lipid (median)	Serum total T3, free T3, total T4, free T4, TSH	↔
Piccoli et al. 2016 Cross-sectional, 275 farmers and farm residents, 18–69 years old, Brazil	**β-HCH**	<LOD ng/g (median) 77.87 (95^{th} percentile)	**Serum total T3**	↑
			Serum free T4, TSH	↔
	γ-HCH	3.71 (median) 24.35 (95^{th} percentile)	Serum total T3, free T4, TSH	↔
	α-HCH	<LOD (median) 21.8 (95^{th} percentile)	Serum total T3, free T4, TSH	↔
Yamazaki et al. 2020 Cross-sectional, 333 pregnant women 17–48 years old, Japan	β-HCH	235.6 pg/g (late gestation or post-partum) (75^{th} percentile)	Serum TSH (early gestation)	↔
			Serum free T4 (early gestation)	↔

Table 2-12. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Endocrine Effects

Reference, study type, and population	Isomer	Concentration in serum (unless otherwise noted)	Outcome evaluated	Result
Goldner et al. 2013 Cohort, 21,788 male private pesticide applicators, Iowa and North Carolina, United States	**Lindane**	NA (occupational, ever use)	**Hypothyroid disease**	↑

↑ = association with increase; ↓ = association with decrease (inverse association); ↔ = no association; F= female; LOD = limit of detection; M = male; NA = not applicable; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone

Several cross-sectional studies evaluated effects of β-HCH on thyroid hormone levels. Decreased total T3 and thyroxine binding globulin in serum were associated with higher plasma β-HCH levels in Canadian Inuit adults, while there were no associations with free T4 or TSH (Dallaire et al. 2009). In a study of pregnant women in Spain, there was an association between serum β-HCH and decreased total T3 levels in women in the city of Sabadell, but no association was seen in women from the city of Gipuzkoa (Alvarez-Pedrerol et al. 2009). In contrast, free T4 levels were not associated with serum β-HCH in Sabadell women, while there was an increased association in Gipuzkoa women (Alvarez-Pedrerol et al. 2009). No relationship between serum β-HCH levels and total T3, free T3, total T4, free T4, or TSH was observed in pregnant women or adults of both sexes in South Korea (Kim et al. 2013) or serum TSH and free T4 levels in pregnant women in Japan (Yamazaki et al. 2020). A cross-sectional study in adults from South Korea observed an inverse association between serum levels of β-HCH and serum free T4 (Seo et al. 2022). No association was observed between serum levels of β-HCH and TSH, or between serum α-, γ-, or δ-HCH concentrations and serum TSH or free T4.

A population-based survey of residents living near an HCH production factory (operating from late 1940s to 1955) in Brazil examined associations of serum levels of α-, β-, and γ-HCH and thyroid function in 193 children <15 years old (Freire et al. 2012) and in adolescent and adult (>14 years old) males (n=303) and females (n=305) (Freire et al. 2013). In children, serum levels of α-, β-, and γ-HCH were associated with increased serum total T3 levels, while no association was observed for serum free T4 or TSH levels (Freire et al. 2012). In adults, serum levels of β-HCH were associated with decreased free T4 and increased TSH levels in men, while no association was determined in women for either parameter. Further, there were no associations between serum β-HCH and total T3 or anti-thyroperoxidase levels and there were no associations between α-HCH or γ-HCH and total T3, free T4, TSH, and anti-thyroperoxidase (Freire et al. 2013). Increased total T3 levels corresponded to serum β-HCH levels in 275 adult Brazilian farmers and farm residents, while no relationship was found with α- or γ-HCH (Piccoli et al. 2016). In this study, there was also no association between serum α-, β-, or γ-HCH and free T4 or TSH levels (Piccoli et al. 2016).

α-HCH. No histopathology changes were noted in the adrenal glands or thyroids of rats given α-HCH via the diet at doses up to 70 mg/kg/day for ~9 months or up to 9 mg/kg/day for 2 years (Fitzhugh et al. 1950).

β-HCH. There were no microscopic lesions in the thyroid or adrenal glands when rats were exposed by dietary administration to β-HCH doses up to 70 mg/kg/day for up to 10 weeks or up to 9 mg/kg/day for 2 years (Fitzhugh et al. 1950).

γ-HCH (Lindane). In 13- and 14-week inhalation studies of rats and mice (respectively) exposed to concentrations up to 5 mg/m^3 for 6 hours/day, there were no effects of treatment on the histology of the pancreas, thyroid, or adrenal glands (Klonne and Kintigh 1988; Oldiges et al. 1983) or on the histology of the pituitary in the mice (Klonne and Kintigh 1988). Wistar rats administered 50 mg/kg/day γ-HCH in drinking water for 30 days had 84% higher serum levels of free T4 and 74% lower serum TSH compared to controls (Hfaiedh et al. 2011). In a 2-generation reproductive toxicity study, F0 and F1 male and/or female parental rats exposed via feed to doses of 17.2–26.1 mg/kg/day exhibited endocrine effects including decreased absolute and relative pituitary weights (F0 and F1 females), altered serum thyroid hormone levels, and increased incidences of thyroid follicular cell hypertrophy (F0 females and F1 males) (Matsuura et al. 2005). Fitzhugh et al. (1950) reported no histopathology findings in the thyroid or adrenal glands of rats given γ-HCH in feed at doses up to 140 mg/kg/day for 10 months or 30 mg/kg/day for 2 years. No effect on adrenal gland weights or adrenal, thyroid, or parathyroid histopathology findings in CD-1 mice given γ-HCH in the diet at doses up to 26.8 mg/kg/day for 78 weeks (EPA 2000a).

Technical HCH or Unspecified Isomers of HCH. Technical HCH administered to rats in feed did not induce thyroid or adrenal gland histopathology changes at doses up to 70 mg/kg/day for 6 months or 9 mg/kg/day for 2 years (Fitzhugh et al. 1950).

2.14 IMMUNOLOGICAL

Epidemiological Studies. Few studies of immune system effects in humans exposed to HCH isomers were located, and the available studies examined limited endpoints. Table 2-13 provides an overview of the epidemiological studies. Landgren et al. (2009) followed a cohort of 678 male pesticide applicators in the United States for 9 years and evaluated the risk of monoclonal gammopathy of undetermined significance (MGUS). MGUS is a condition in which an abnormal protein (monoclonal or M protein) accumulates in the blood; this condition sometimes progresses to lymphoma or multiple myeloma. There was no increase in risk for MGUS among applicators who reported use of γ-HCH compared with those who had never used γ-HCH (Landgren et al. 2009). Ryu et al. (2018) reported a positive association between serum levels of β-HCH and specific T-lymphocyte frequencies (CD8+ CD57+ and CD8+

Table 2-13. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Immune Effects

Reference, study type, and population	Outcome evaluated	Isomer	Biomarker	Mean concentration (unless otherwise noted)	Result
Meng et al. 2016 Case-control, 124 cases of asthma, 109 controls, children ages 3–6 years, China	**Asthma**	**α-HCH**	Plasma	Cases: 40.73±36.01 ng/g lipid Controls: 12.52±16.03	↑
		β-HCH		Cases: 111.11±70.1 Controls: 31.49±74.02	↑
		γ-HCH		Cases: 27.5±12.13 Controls: 9.34±20.21	↑
	Severe asthma[a]	α-HCH		See above	↔
		β-HCH		See above	↔
		γ-HCH		See above	↔
Landgren et al. 2009 Cohort, 678 male pesticide applicators followed for at least 9 years, Iowa and North Carolina, United States	Monoclonal gammopathy of undetermined significance	γ-HCH	None (occupational)	Ever versus never used	↔
Ryu et al. 2018 Cross-sectional, 95 healthy adults, age 30–69 years, Korea	T-lymphocyte frequencies: **CD8+ CD57+**	**β-HCH**	Serum	10.7 ng/g lipid (median of 4th quartile)	↑
	CD8+ CD28-				↑
	CD4+ CD57+				↔
	CD4+ CD28-				↔

Table 2-13. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Immune Effects

Reference, study type, and population	Outcome evaluated	Isomer	Biomarker	Mean concentration (unless otherwise noted)	Result
Seth et al. 2005 Case-control, 20 patients hospitalized with lindane poisoning and 20 unexposed age- and sex-matched healthy subjects, India	Serum immunoglobulins and cytokines:	γ-HCH	None (exposure assessed based on clinical symptoms, history, and AChE activity)	Exposed versus unexposed	
	IgG, IgM, IgA, and IgE				↔
	IL-2,IL-4, and TNF-α				↑
	IFN-γ				↓
Wang et al. 2021a Cross sectional, 10 women (5 from rural and 5 from urban areas), mean age 37 years, China	IL-8, MCP-1	α-HCH	Estimated dietary intake	0.471 ng/kg/day	↔
		β-HCH		0.185	
		γ-HCH		0.043	
		δ-HCH		0.104	

[a]Severe asthma was defined as “asthma attacks more than 10 times over the year with repeated episodes (more than 3 times) during the last month, or if severe oxygen deficiency occurred in one attack.”

↑ = association with increase; ↓ = association with decrease (inverse association); ↔ = no association; AChE = acetylcholinesterase; Ig = immunoglobulin; IL = interleukin; TNF = tumor necrosis factor; IFN = interferon; MCP = monocyte chemotactic protein.

CD28-) in a cross-sectional study of healthy adults over 30 years of age. In a case-control study of children 3–6 years old, associations between asthma and increased plasma α-, β-, and γ-HCH levels were reported (Meng et al. 2016); however, in this study, exposure (blood level) was measured after the outcome (asthma) occurred. A small cross-sectional study of 10 women in China found no association between any of the HCHs and serum interleukin-8 (IL-8) or monocyte chemotactic protein-1 (MCP-1) (Wang et al. 2021a).

β-HCH. 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 (Cornacoff et al. 1988). Cortical atrophy of the thymus and depletion of splenic lymphoid tissue were observed in rats fed 22.5–25 mg/kg/day β-HCH in the diet (Van Velsen et al. 1986). In this 13-week study (Van Velsen et al. 1986), 50% of the rats exposed at this dose were sacrificed humanely before study termination (as early as the first 3 weeks) due to moribund condition.

γ-HCH (Lindane) Immune system parameters in blood were evaluated in a group of 20 patients seen in a hospital in India with γ-HCH poisoning and compared with results in a group of age- and sex-matched controls without pesticide exposure (Seth et al. 2005). The dose, route, nature, and timing of γ-HCH exposures were not reported, and there was no effort to adjust for potential confounders. No differences in serum immunoglobulin levels (IgG, IgM, IgA, or IgE) were seen; however, several serum cytokine levels, including interleukin-2 (IL-2), interleukin-4 (IL-4), and tumor necrosis factor-alpha (TNF-α) were higher in the poisoning victims, and serum IFN-γ levels were lower (Seth et al. 2005).

A 14-day exposure to 10 mg/kg/day γ-HCH in male rats previously sensitized to Keyhole Limpet Hemocyanin (KLH) resulted in a reduction in delayed-type hypersensitivity response (measured as a 43% decrease in foot pad thickness in response to KLH challenge) (Mediratta et al. 2008). Decreased relative thymus weight (28% less than controls) was observed in mice gavaged with 20 mg/kg/day γ-HCH for 3 days; at 40 mg/kg/day, atrophy of the thymic cortex was seen (Hong and Boorman 1993). Another experiment by these authors showed significant decreases in relative weights of thymus (≥7% decrements) and spleen (≥17% decrements) in mice exposed to 10–20 mg/kg/day γ-HCH for 10 days (Hong and Boorman 1993).

Immunosuppression, as measured by decreased antibody titers against typhoid vaccine and *Salmonella* vaccine, was reported in rats exposed by gavage to doses of ≥6.25 mg/kg/day γ-HCH 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 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 (response after repeat exposure) 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 (Banerjee et al. 1996). A biphasic, dose-dependent immunological effect of γ-HCH on components of cell- and humoral-mediated immunity, characterized by initial stimulation followed by immuno-suppression, was reported in mice fed 0.012, 0.12, or 1.2 mg γ-HCH/kg/day for 24 weeks (Meera et al. 1992). Histological examinations in these animals revealed decreased lymphocyte populations in the thymus and lymph nodes, 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 dinitrofluorobenzene antigen, was suppressed in sheep that were exposed to 1.25 ppm γ-HCH in the diet for 6 months (Khurana et al. 1999).

Technical HCH or Unspecified Isomers of HCH. 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.

2.15 NEUROLOGICAL

Epidemiological Studies. Epidemiological studies of neurological effects in humans exposed to HCH isomers are summarized in Table 2-14. Most of the studies used serum or blood levels of β-HCH to assess exposure; only Singh et al. (2012, 2013, 2014) and Xu et al. (2022) measured α- γ-, and/or δ-HCH levels as well. A cohort study of 669 Canadian adults at least 65 years old who were followed for 10 years showed no association between blood levels of β-HCH and dementia, Alzheimer's disease, or cognitive deficits (Medehouenou et al. 2019). In contrast, case-control studies reported increased risks of Parkinson's disease, Alzheimer's disease, and cognitive deficits with higher β-HCH levels in blood or serum (Kim et al. 2015; Petersen et al. 2008; Richardson et al. 2009, 2011; Singh et al. 2012, 2013, 2014; Xu et al. 2022). The study by Xu et al. (2022) also reported increased risk of Parkinson's disease

associated with increased serum levels of α- and δ-HCH. However, in the case-control studies, exposures were measured after the outcome occurred, so there is no clear temporal relationship between exposure and effect. A small cross-sectional study that suffered from the same limitation reported no association between β-HCH in serum and tremors at rest or cognitive deficits (Steenland et al. 2014).

Some studies have reported impairments in sensory function associated with HCH exposure. Serum levels of α-HCH were associated with hearing loss in a small case-control study (87 pairs) in China (Zhang et al. 2021), but the exposure was measured after the outcome in this study. Shrestha et al. (2021) observed an association between γ-HCH lifetime days of use and self-reported olfactory impairment 20 years after enrollment in a very large cohort of pesticide applicators in the United States. The odds of olfactory impairment increased with intensity-weighted days of γ-HCH use in this cohort. The absence of an objective measure of olfactory impairment limits the conclusions that can be drawn from this study.

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. No other data on neurological effects of α-HCH were located.

β-HCH. Clinical signs of neurotoxicity have often preceded death in rats and mice exposed to β-HCH via oral administration. Mice treated with 60 or 200 mg/kg/day β-HCH in the diet in a 30-day study developed ataxia within the first week of treatment (Cornacoff et al. 1988). The animals receiving 60 mg/kg/day recovered within a few days, while those receiving 200 mg/kg/day became markedly worse, leading to humane sacrifice of 80% of the animals in this group (Cornacoff et al. 1988). In the first 2 weeks of a 13-week study, male and female rats exposed to 38 mg/kg/day in the diet exhibited ataxia and hypoactivity, progressing to coma within 3 days (Van Velsen et al. 1986). The animals were humanely sacrificed, as were five additional males and six additional females that showed similar signs later in the study. A single study of electrophysiology was located; in this study, 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. No comprehensive tests of sensitive neurotoxicity endpoints other than electrophysiology in animals exposed to β-HCH were located.

γ-HCH (Lindane). Neurological effects have been seen in humans and animals exposed to γ-HCH by inhalation, oral, and dermal routes. The effects range in severity from subtle neurobehavioral changes and altered neurotransmitter levels to tremors, convulsions, and ultrastructural changes in the brain.

Table 2-14. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Neurological Effects

Reference, study type, and population	Outcome evaluated	Isomer	Exposure Biomarker	Mean concentration (unless otherwise noted)	Result
Richardson et al. 2009, 2011 Case-control, 149 cases of Parkinson's disease, 134 controls, four sites in Texas and Georgia, United States	**Parkinson's disease**	**β-HCH**	Serum	10.77–79.43 ng/mg cholesterol (range of medians among cases across four sites)	↑
Petersen et al. 2008 Case-control, 79 cases of Parkinson's disease, 154 controls, mean ages 74 and 75 years, respectively, Faroe Islands	**Parkinson's disease**	**β-HCH**	Serum	0.06 µg/g lipid (GM) (cases) 0.04 (controls	↑
Xu et al. 2022 Case-control, 90 cases of Parkinson's disease, 90 controls who were spouses of the cases, mean ages 65.76 and 64.23, respectively, China	**Parkinson's disease**	**α-HCH**	Serum	80.19 ng/g lipid (cases) 1.79 ng/g lipid (controls)	↑
		β-HCH		1126.4 ng/g lipid (cases) 349.88 ng/g lipid (controls)	↑
		γ-HCH		583.30 ng/g lipid (cases) 39.62 ng/g lipid (controls)	↔
		δ-HCH		12.09 ng/g lipid (cases) 5.82 ng/g lipid (controls)	↑
Singh et al. 2012, 2013, 2014 Case-control, 100 patients with Alzheimer's disease, 100 age-matched controls, Delhi, India	**Alzheimer's disease**	**β-HCH**	Serum	4.42±0.54 ng/mL (in cases)	↑
		α-HCH	Serum	0.37±0.11 ng/mL	↔
		γ-HCH	Serum	0.78±0.23 ng/mL	↔
Kim et al. 2015 Cross-sectional, 633 adults aged 60–85 years, NHANES 1999–2002, United States	**Cognitive deficit**	**β-HCH**	Blood	89.6 ng/g lipid (median of 4th quartile)	↑
Medehouenou et al. 2019 Cohort, 669 adults ≥65 years old, Canadian Study of Health and Aging (CSHA), Canada	Dementia, Alzheimer's disease, cognitive deficit	β-HCH	Blood	0.13 µg/L (median) (cases) 0.12 (controls)	↔
Steenland et al. 2014 Cross-sectional, 89 adults >65 years of age, Costa Rica	Movement disorder (tremor at rest)	β-HCH	Serum	≥0.88 ng/mL (4th quartile cutoff)	↔
	Cognitive deficit	β-HCH	Serum	≥0.88 ng/mL (4th quartile cutoff)	↔

Table 2-14. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Neurological Effects

Reference, study type, and population	Outcome evaluated	Isomer	Exposure Biomarker	Mean concentration (unless otherwise noted)	Result
Sullivan et al. 2018 Cohort, 159 Gulf War veterans who had worked in pest control, mean age 48 years, United States	**Depression, fatigue scores on mood test**	**γ-HCH**	NA (self-reported exposure)	(high exposure versus low exposure)	↑
	Anger, confusion, tension scores on mood test				↔
Zhang et al. 2021 Case-control, 87 cases and 87 age- and gender-matched controls, mean age 51 years, China	**Hearing loss**	β-HCH	Serum	27.0 ng/g lipid (cases) (geometric mean) 29.6 ng/g lipid (controls)	↔
		α-HCH		14.4 ng/g lipid (cases) 12.2 ng/ml (controls)	↑
		δ-HCH		8.98 ng/g lipid (cases) 9.54 ng/g lipid (controls)	↔
Shrestha et al. 2021 Cohort, 20,409 adult, mostly male pesticide applicators, Iowa and North Carolina, United States	**Olfactory impairment**	**γ-HCH**	NA (self-reported exposure)	Quartiles of intensity-weighted lifetime days of use	↑

↑ = association with increase; ↔ = no association; GM = geometric mean; NA = not applicable; NHANES = National Health and Nutrition Examination Survey

Studies in animals exposed *in utero* and/or during lactation show that γ-HCH can also elicit these neurological effects in offspring of exposed parents; neurotoxic effects in animals exposed during development are discussed in Section 2.17.

Abnormal electroencephalographic (EEG) patterns (increased variation in the frequency and amplitude of wave pattern or more serious changes without specific EEG signs) were recorded in 16 of 37 workers following exposure to γ-HCH for 0.5–2 years in a fertilizer plant (Czeglédi-Jankó and Avar 1970). Exposure concentrations were not reported; however, these EEG changes were found to correlate with blood levels of γ-HCH. Effects on mood were examined in a cohort of 159 Gulf War veterans who had been engaged in pesticide application (Sullivan et al. 2018). In this group, higher self-reported exposure to γ-HCH was associated with higher scores for depression and fatigue in mood tests, while no association was seen with anger, confusion, or tension scores on the tests (Sullivan et al. 2018).

Seizures and convulsions have been observed in individuals who accidentally or intentionally ingested γ-HCH in insecticide pellets, liquid scabicide, or contaminated food (Davies et al. 1983; Forrester et al. 2004; Harris et al. 1969; Munk and Nantel 1977; Nordt and Chew 2000; Powell 1980; Ramabhatta et al. 2014; Starr and Clifford 1972; Storen 1955; Wiles et al. 2015). In most cases, the amount of γ-HCH ingested could not be determined. A 56-year-old man intentionally ingested approximately 12 ounces of an insecticide containing 20% γ-HCH in a suicide attempt (Wiles et al. 2015). Thirty minutes later, he developed a progressive decline in consciousness and multiple seizures ensued between 3 and 14 hours later. After 18 hours, the man was conscious and responsive, but neurological symptoms were noted 6 days later, including ataxia, slurred speech, paranoia, depression, and defects in higher mental functioning. The man committed suicide 12 days later by other means. At autopsy, γ-HCH levels in blood and adipose tissue were 0.248 μg/mL and 132.83 μg/g, respectively.

Several case studies of acute γ-HCH exposure to children ingesting liquid scabicide reported similar neurological effects, including tremors and tonic/clonic seizures (Aks et al. 1995; CDC 2005; Lifshitz and Gavrilov 2002; Wheeler 1977). One hour after a 3-year-old boy ingested approximately one teaspoon of a 1% γ-HCH shampoo, the boy had a tonic-clonic seizure for a duration of 4–5 minutes, despite the mother's efforts to induce vomiting (CDC 2005). Three hours later, the boy's condition was stable, and he was discharged from the hospital emergency room. Ramabhatta et al. (2014) reported two cases of neurological effects in children after accidental γ-HCH ingestion. In the first case, a 3-year-old boy was orally administered a single dose of 10 mL of a γ-HCH lotion (due to a mix-up of prescribed oral and dermal medications). The child had convulsions 1 hour after ingestion and recovered in 24 hours with

supportive measures. In the second case, a 6-year-old girl ingested a γ-HCH lotion (amount and concentration not reported) and had a generalized seizure lasting 10–15 minutes. The child recovered 24 hours after clinic admission (Ramabhatta et al. 2014).

There have been many reports of human intoxication involving seizures or convulsions in adults and children after excessive topical application of γ-HCH (Boffa et al. 1995; Fischer 1994; Hall and Hall 1999; Lee and Groth 1977; Matsuoka 1981; Ramchander et al. 1991; Solomon et al. 1995; Sudakin 2007; Telch and Jarvis 1982; Tenenbein 1991); exposure levels were generally not quantified. Central nervous systems symptoms of severe γ-HCH poisoning, including uncontrollable 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 anti-scabies 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 et al. 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 10-month-old boy exposed to 1% γ-HCH by repeated dermal application to the whole body 2 times/day for the treatment of scabies developed jerky movements, listlessness, and loss of consciousness after 7 days (Bhalla and Thami 2004). Upon examination by a medical professional after 10 days, the boy exhibited apathy, semi-consciousness, absence of superficial reflexes, reduced response to touch, pain, and pressure, and tremor in tongue and limbs. Use of γ-HCH was immediately discontinued and the infant regained normal consciousness and interaction with environmental stimuli over the subsequent 2 weeks (Bhalla and Thami 2004). The study authors reported that the boy showed evidence of anemia and malnutrition, which were described as risk factors for γ-HCH-induced neurotoxicity. A 7-year-old boy exposed to γ-HCH by dermal application 3 times in 4 days (dose not reported) exhibited ataxia, weakness, and burning paresthesia, and following the third application, the boy had myoclonic jerks and tonic-clonic seizures, whereupon he was brought to the hospital (Daud et al. 2010). No details of the extent of exposure were reported. After treatment to control the seizures, induction of diuresis, and frequent bathing and changing of clothing, the boy was discharged after 2 days (Daud et al. 2010).

Rats exposed to various concentrations of γ-HCH aerosol via nose-only inhalation for 4 hours exhibited concentration-related neurological effects (Oldiges et al. 1980; 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 ≥273 mg/m^3; and spasms were also noted at the highest concentration (2,104 mg/m^3). Concentrations ≥378 mg/m^3 were also associated with mortality in one study (Ullmann 1986b) but not in the other even at concentrations up to 603 mg/m^3 (Oldiges et al. 1980). Rats exposed to 0.02–5 mg/m^3 γ-HCH aerosol for 90 consecutive days exhibited a "slightly disturbed general condition" (not further characterized) within 2 weeks (Oldiges et al. 1983). At the end of the 90-day exposure, there were no treatment-related changes in brain weight or histology, or on histology of the sciatic or optic nerves. Mice were exposed to similar concentrations (0.3–5 mg/m^3) for 14 weeks (5 days/week) and exhibited no clinical signs of neurotoxicity (Klonne and Kintigh 1988).

Neurotoxic effects have been reported in several species of animals exposed to γ-HCH. The most serious effects were seizures and/or convulsions following intragastric administration of approximately 15–60 mg/kg for ≥1 day in rats (Amyes 1990; EPA 1999a; Fitzhugh et al. 1950; Gilbert and Mack 1995; Johri et al. 2008; Joy et al. 1982; Martinez and Martinez-Conde 1995; Martinez et al. 1991; Matsuura et al. 2005; Parmar et al. 2003; Tusell et al. 1988; Vendrell et al. 1992a, 1992b; Woolley and Griffith 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 γ-HCH poisoning. A single oral dose of 5–20 mg/kg γ-HCH to either naïve or rats previously kindled by electrical stimulus produced myoclonic jerks and clonic seizures, which increased in a dose-dependent manner and were increased in kindled animals (Gilbert and Mack 1995). Enhanced susceptibility to kindled seizures brought on by electrical stimulation was seen in rats exposed for 10 weeks to 10 mg/kg/day γ-HCH, 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). 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 (Hulth et al. 1978).

Two studies of rats showed that oral administration of γ-HCH can alter neurotransmitter levels in the brain. Decreased levels of brain serotonin were reported in rats exposed for 6 days to a dose of 3 mg/kg/day γ-HCH (Attia et al. 1991), while 10 doses totaling 60 mg/kg γ-HCH over a period of 30 days resulted in decreased brain dopamine levels (Martinez and Martinez-Conde 1995).

Acute and subchronic neurotoxicity screening bioassays including functional observational battery, motor activity assessments, and neuropathology were reported in unpublished Confidential Business Information (CBI) submissions summarized by EPA (1999a, 1999b). In the acute neurotoxicity screening study, exposure to γ-HCH caused decreased motor activity 3 hours after gavage dosing of female rats with ≥20 mg/kg and males at 60 mg/kg (EPA 1999a). 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 (EPA 1999a). A 13-week neurotoxicity screening study in Crl:CDBR rats by the same author (EPA 1999b) showed neurological effects in both sexes at the highest dose (28.1–30.2 mg/mg/day), including clinical signs (e.g., piloerection, abnormal grooming behavior), increased rearing, walking on tiptoes, hypersensitivity to touch, hunched posture, and several deaths. There were no effects on forelimb or hindlimb grip strength, hindlimb splay, motor activity, or neuropathology (EPA 1999b).

Neurobehavioral testing in rats exposed for acute and intermediate durations have shown effects on activity, cognition, and memory. Increased anxiety (Llorens et al. 1990) and decreased motor activity (EPA 1999a) were reported in rats following a single gavage dose of 20 mg/kg, and increased spontaneous motor behavior was observed at 10 mg/kg (Llorens et al. 1989). Avoidance response latency was significantly increased in rats administered a single dose of 15 mg/kg by gavage (Tilson et al. 1987). Impaired neurocognition, measured as decreased step-down latency in passive avoidance test and prolonged transfer latency in the elevated plus maze test, occurred in rats exposed for 6 weeks to a γ-HCH dose of 15 mg/kg/day (Sahaya et al. 2007). Srivastava et al. (2019) observed behavioral changes (reduced locomotor activity and impaired spatial memory) in rats exposed to 2.5 mg/kg/day for 21 days. A longer exposure (40 days) at this dose (2.5 mg/kg/day) resulted in significantly altered Skinner box behavior (operant conditioning) in a small number of rats (Desi 1974).

At a γ-HCH dose (2.5 mg/kg/day for 21 days) that induced changes in locomotor activity and spatial memory, Srivastava et al. (2019) detected ultrastructural changes in the hippocampus and substantia nigra of rats. Changes in the brain included swollen mitochondria with disintegrated cristae, shortened fuzzy synapse, disintegrated myelin layer, and autophagosomes (Srivastava et al. 2019). Peripheral nerve effects were seen in one oral study of γ-HCH. Significantly decreased nerve conduction velocity was measured in rats exposed to 25.4 mg/kg/day for 30 days (Muller et al. 1981).

While data are more limited, neurotoxicity has been documented in rats and rabbits exposed to γ-HCH via dermal application. Clinical signs such as excitability, seizures, and convulsions were observed in rabbits following a single topical application of 60 mg/kg γ-HCH 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). One female exposed to 2,000 mg/kg in this study exhibited severe sedation and spasms (Ullmann 1986a). Aggressiveness or hyperactivity were noted in rats exposed dermally for 13 weeks to ≥10 mg γ-HCH/kg/day, while ataxia, tremors, and convulsions were seen in females at 60 mg/kg/day (EPA 1988a).

Mechanisms. 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). 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 CYP1A1/1A2, had no significant effect in the incidence of convulsions induced by γ-HCH. However, induction of CYP 2B1/2B2 (by pretreatment with phenobarbital) or CYP2E1 (by pretreatment with ethanol) significantly increased the incidence of convulsions caused by γ-HCH, as did blocking of cytochrome P450-mediated metabolism with cobalt chloride (Parmar et al. 2003). Taken together, the results suggest that the convulsive activity is due to γ-HCH *per se* and/or to metabolites formed by phenobarbital- or ethanol-inducible P450 isoenzymes.

Decreased myelin was observed in rats exposed to 5 mg/kg/day by gavage for 3 days (Serrano et al. 1990). These authors also detected a significant decrease in 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP) activity in treated animals, although no dose-response was seen. This enzyme is myelin-specific, but its exact function in normal myelin in unknown (Serrano et al. 1990).

Increased lipid peroxidation (thiobarbituric acid reactive substances [TBARS]) and decreases in antioxidant enzyme activities (superoxide dismutase, catalase, and glutathione peroxidase) were measured in the brains of Wistar rats after three daily doses of 5 mg/kg/day γ-HCH; neurological endpoints were not evaluated in these animals (Hfaiedh et al. 2012). Fatih Fidan et al. (2008) reported increased malondialdehyde and decreased levels of reduced glutathione in the brains of rats exposed to γ-HCH (≥10 mg/kg/day) by daily gavage for 30 days. Similarly, a 30-day exposure to doses of 50 mg/kg/day induced lipid peroxidation (measured as TBARS) and depletion of antioxidant enzymes (glutathione peroxidase and catalase) in the brains of rats exposed by drinking water (Hfaiedh et al. 2011). After

6 weeks of daily exposures to 15 mg/kg/day, rats exhibited increased malondialdehyde and non-protein thiols in the brain (Sahaya et al. 2007). In contrast, 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 (Arisi et al. 1994).

Technical HCH or Unspecified Isomers of HCH. Paresthesia of the face and extremities, headache, and vertigo were 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 exposures were possible. Heiberg and Wright (1955) reported convulsions in a woman who had treated cattle with an insecticide containing 11% γ-HCH and 16% other HCH isomers.

In animals exposed to technical-grade HCH by oral administration, effects like those seen with β- and γ-HCH were seen. 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 (Sahoo et al. 1999). Mudawal et al. (2018) observed impairments in learning (conditioned avoidance response and Y-maze continuous alternation test) and increased spontaneous locomotor activity in rats given technical-grade HCH for 21 days beginning at 3, 18, or 48 weeks of age. The most pronounced effects were observed in the aged rats. After exposure that began at 48 weeks of age, the animals also exhibited ultrastructural changes in the hippocampus and substantia nigra when examined by transmission electron microscopy; in contrast, similar exposure beginning at 3 or 18 weeks of age did not result in ultrastructural changes (Mudawal et al. 2018). Increased 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. 1991). 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 and 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 for 80 weeks (Kashyap et al. 1979). 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).

Increased levels of brain catecholamines, particularly norepinephrine and dopamine, and associated signs of toxicity such as mild tremor, lacrimation, salivation, and dyspnea 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, an enzyme that oxidizes monoamine neurotransmitters) in the cerebrum showed a marginal decrease, while significant increases and decreases were observed in the cerebellum and spinal cord, respectively (Raizada et al. 1993). 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).

Mechanisms. As with γ-HCH, there is some evidence that oxidative stress may contribute to the neurotoxic effects of technical-HCH. Mudawal et al. (2018) observed increased lipid peroxidation and decreases in both antioxidant enzyme activities (superoxide dismutase and catalase) and reduced glutathione in the hippocampus and substantia nigra of rats given 2.5 mg/kg/day technical HCH for 21 days. These changes correlated with neurobehavioral effects, as discussed above.

2.16 REPRODUCTIVE

Epidemiological Studies. Few epidemiological studies on the reproductive effects of HCH isomers were located; the available studies are summarized in Table 2-15. With one exception (Freire et al. 2014), the studies were conducted in populations without known sources of exposure to HCH; in these populations, consumption of contaminated food is expected to be the primary exposure route. All these studies measured HCH isomers in serum, fat, or follicular fluid as biomarkers of exposure, and exposure was measured simultaneously with outcome assessment or after the outcome occurred.

Freire et al. (2014) conducted a cross-sectional study of reproductive hormone and HCH levels in the serum of 604 people residing near a former HCH manufacturing facility in Brazil. In this population, an inverse association between serum testosterone concentrations and serum α- and β-HCH concentrations was observed in men. In women, increases in serum β-HCH were associated with increased serum

Table 2-15. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Reproductive Effects

Reference, study type, and population	Outcome evaluated	Isomer	Biomarker	Mean concentration (unless otherwise noted)	Result
Akkina et al. 2004 Cross-sectional, 219 menopausal women, Hispanic Health and Nutrition Examination survey (HHANES), United States	**Age at menopause**	**β-HCH**	Serum	>2.09 ng/g (> median)	↓
Buck Louis et al. 2012 Matched cohort, operative cohort: 473 women undergoing laparoscopy or laparotomy; population-based cohort: 127 women matched on age and residence, 18–44 years old, United States	**Endometriosis**	**β-HCH**	Serum	Operative cohort, medians: 0.0063 ng/g (cases) 0.0063 (non-cases)	↔
				Population cohort, medians: 0.0066 (cases) 0.0063 (non-cases)	↑
		γ-HCH	Omental fat	Operative cohort, medians: 0.1991 ng/g fat (cases) 0.1200 (non-cases)	↑
Ploteau et al. 2017 Case-control, 55 cases of deep infiltrating endometriosis and 44 controls, 18–45 years old, France	Deep infiltrating endometriosis	**β-HCH**	Adipose tissue	13.62 ng/g lipid (median) (cases) 14.33 (controls)	↔
	Deep infiltrating endometriosis with ovarian endometrioma			21.61(cases) 14.33 (controls)	↑
Upson et al. 2013 Case-control, 248 cases of endometriosis and 538 population-based controls, Washington, United States	**Endometriosis**	**β-HCH**	Serum	>43.06 pg/g (3rd quartile)	↑
		γ-HCH		>13.89 (4th quartile)	↔
		Sum HCH		>0.29 mol/g (4th quartile)	↔
	Ovarian endometriosis	**β-HCH**	Serum	>43.06 pg/g (3rd quartile)	↑
		γ-HCH		>13.89 (4th quartile)	↔
		Sum HCH		>0.29 mol/g (4th quartile)	↔
Al-Hussaini et al. 2018 Cross-sectional, 94 women in infertile couples undergoing intracytoplasmic sperm injection, 20–38 years old, Egypt	**Endometrial thickness**	**γ-HCH**	Follicular fluid	418.6±171.4 µg/L (mean±SD)	↑
	Implantation rate				↓

Table 2-15. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Reproductive Effects

Reference, study type, and population	Outcome evaluated	Isomer	Biomarker	Mean concentration (unless otherwise noted)	Result
Pollack et al. 2021 Operative cohort, 339 women undergoing laparoscopy or laparotomy, age 18-44 years, United States	**Endometriosis**	**β-HCH**	Omental fat	0.14 ng/g (median)	↑
			Serum	0.013	↔
			Adipose to serum ratio	Not reported	↑
		γ-HCH	Omental fat	0.137	↑
			Serum	0.019	↔
			Adipose to serum ratio	Not reported	↑
Génard-Walton et al. 2023 Case-control, 138 cases of diminished ovarian reserve and 151 controls, mean age 33.8 years for cases, 32.4 years for controls, France	Diminished ovarian reserve	**β-HCH**	Serum	4.1 ng/g lipid (cases) (median) 4.5 (controls)	↓
Abou Ghayda et al. 2020 Prospective cohort, 152 males enrolled in the Russian children's study at 8–9 years of age and followed up at 18–23 years of age, Russia	Semen volume	**β-HCH**	Serum	172 ng/g lipid (median)	↓
	Sperm concentration				↔
	Total sperm count				↔
	Progressive motility				↔
Madrigal et al. 2021 Cross-sectional, 748 men aged ≥20 years, NHANES 1999–2004, United States	Serum testosterone	**β-HCH**	Serum	22.56-1200.0 ng/g lipid (4th quartile)	↔
	Serum estradiol				↔
	Serum sex hormone binding globulin				↔
	Serum androstanediol glucuronide				↔

Table 2-15. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Reproductive Effects

Reference, study type, and population	Outcome evaluated	Isomer	Biomarker	Mean concentration (unless otherwise noted)	Result
Miao et al. 2022 Cross-sectional, 387 men seeking semen analysis, mean age 30.5 years, China	Sperm motility	α-HCH	Serum	0.08 ug/L	↔
		β-HCH		0.85	↔
		γ-HCH		0.12	↓
		δ-HCH		0.70	↔
	Sperm concentration, sperm count	α-HCH		See above	↔
		β-HCH		See above	↔
		γ-HCH		See above	↓
		δ-HCH		See above	↔
Zeng et al. 2022 Cross-sectional, 421 men seeking semen analysis, mean age 30.6 years, China	Serum testosterone	α-HCH	Serum	0.10 ug/L (75th percentile)	↔
		β-HCH		1.03	↔
		γ-HCH		0.12	↔
		δ-HCH		0.89	↔
Freire et al. 2014 Cross-sectional, 604 persons 15–94 years old, residing near former HCH manufacturing facility, Brazil	In men:				
	Serum testosterone	**α-HCH**	Serum	2.52 ng/mL (median)	↓[a]
		β-HCH		6.00	↓
		γ-HCH		0.95	↔
	In premenopausal women:				
	Serum estradiol, progesterone, prolactin, LH, and FSH	α-HCH	Serum	2.77 ng/mL (median)	↔
		β-HCH		6.32	↔
		γ-HCH		0.89	↔

Table 2-15. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Reproductive Effects

Reference, study type, and population	Outcome evaluated	Isomer	Biomarker	Mean concentration (unless otherwise noted)	Result
	In peri- or post-menopausal women:				
	Serum estradiol	α-HCH	Serum	2.43 ng/mL (median)	↔
		β-HCH		11.72	↑[a]
		γ-HCH		1.07	↔
	Serum LH	α-HCH	Serum	See above	↔
		β-HCH		See above	↓
		γ-HCH		See above	↔
	Serum progesterone, prolactin, and FSH	α-HCH	Serum	See above	↔
		β-HCH		See above	↔
		γ-HCH		See above	↔

[a]Borderline significant.

↑ = association with increase; ↓ = association with decrease (inverse association); ↔ = no association; FSH = follicle-stimulating hormone; LH = luteinizing hormone; NHANES = National Health and Nutrition Examination Survey; SD = standard deviation

estradiol and decreased serum luteinizing hormone (LH) levels among peri- and post-menopausal women, but not premenopausal women. No associations were seen between any HCH isomer and serum progesterone, prolactin, or follicle-stimulating hormone (FSH) in women. Serum α- and γ-HCH levels showed no association with reproductive hormone levels in women (Freire et al. 2014).

There were no observed associations between serum levels of HCHs and serum testosterone in two cross-sectional studies (Madrigal et al. 2021; Zeng et al. 2022), and no association between serum β-HCH and serum estradiol, sex hormone binding globulin, or androstanediol glucuronide in one of the studies (Madrigal et al. 2021). A decrease in semen volume at adulthood was associated with pre-pubescent serum levels of β-HCH in a prospective cohort study of 152 Russian men (Abou-Ghayad et al. 2020). Miao et al. (2022) observed an association between γ-HCH levels in serum and decreased sperm motility in a cross-sectional study in China. Neither γ-HCH nor other HCH isomers in serum were associated with changes in sperm count or concentration in this study.

In a matched cohort study of 473 women undergoing laparoscopy or laparotomy (operative cohort) and 127 women matched on age and residence (population-based cohort), both β-HCH levels in serum and γ-HCH levels in omental fat were associated with increased risk of endometriosis (Buck Louis et al. 2012). Three case-control studies also reported associations between endometriosis and biomarkers of β-HCH exposure. An association between deep infiltrating endometriosis with ovarian endometrioma and β-HCH in adipose tissue was seen in a study of 99 adult women in France (55 cases and 44 controls) (Ploteau et al. 2017). Upson et al. (2013) observed associations between serum levels of β-HCH and both endometriosis and ovarian endometriosis in a larger study in the United States (248 cases and 538 controls). Serum concentrations of γ-HCH were not associated with endometriosis in this study (Upson et al. 2013). Pollack et al. (2021) observed an association between incident endometriosis and β- and γ-HCH concentrations in adipose tissue and adipose to serum concentration ratios; however, no association was seen with β- or γ-HCH concentrations in serum. Measurements of γ-HCH in follicular fluid from 94 women undergoing intracytoplasmic sperm injection were associated with increased endometrial thickness and decreased implantation rate (Al-Hussaini et al. 2018).

A case-control study in France observed an inverse association between diminished ovarian reserve and serum levels of β-HCH (Génard-Walton et al. 2023). There were no other studies of this endpoint.

A small cross-sectional study (Akkina et al. 2004) of Hispanic women in the United States reported a decrease in the age at menopause associated with higher serum levels of β-HCH; no other studies of this endpoint were located.

α-HCH. No treatment-related histopathology findings were noted in the testes or uterus and ovaries of rats given α-HCH in feed at doses up to 70 mg/kg/day for an average of 6 months or 9 mg/kg/day for 2 years (Fitzhugh et al. 1950).

β-HCH. 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/kg/day β-HCH in the diet (Van Velsen et al. 1986). Half of the animals in this group showed significant clinical signs of neurotoxicity and were humanely sacrificed before the end of the 13-week study; abnormal reproductive organ pathology was seen in both survivors and early decedents (Van Velsen et al. 1986). While no effects were seen upon microscopic examination of the testes, uteri, and ovaries of rats given β-HCH in the diet at doses up to 70 mg/kg/day for up to 10 weeks, slight testicular atrophy was seen after 2 years of exposure to 7 mg/kg/day (Fitzhugh et al. 1950).

γ-HCH (Lindane). Statistically significant increases in the levels of serum LH 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 FSH was increased and testosterone was decreased, these differences were not statistically significant compared to mean values determined in a control group.

Studies of reproductive effects in animals exposed by inhalation are limited to two intermediate-duration studies focused on systemic toxicity endpoints. Histopathology evaluation of the testes, prostate, ovaries, and uterus of rats and mice exposed by inhalation to 5 mg/m^3 γ-HCH for 13–14 weeks showed no effects of treatment (Klonne and Kintigh 1988; Oldiges et al. 1983).

In animals exposed orally for acute and intermediate durations, γ-HCH induced effects on the male reproductive system, female reproductive system, and on mating, fertility, and early gestation endpoints, as discussed below.

Effects on male reproductive system. The male reproductive system appears to be sensitive to the toxic effects of orally administered γ-HCH. Rats and mice exposed to this isomer have exhibited effects on spermatogenesis, reproductive organ weight changes, and histopathology changes in the testes, while altered sexual behavior was reported in sheep. The lowest dose associated with effects on the male reproductive tract in acute-duration studies is 6 mg/kg/day (Dalsenter et al. 1996); for intermediate-duration studies, it is 1 mg/kg/day in mink (Beard and Rawlings 1998).

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 sperm 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 (Dalsenter et al. 1996). Sharma and Singh (2010) administered γ-HCH (30 mg/kg/day) by gavage to Wistar rats for 14 and 28 days for evaluation of effects on the male reproductive tract. After 14 days, the rats had markedly decreased epididymis (27%) and testes (68%) weights. In addition, substantial and persistent reductions (≥85% less than controls) in sperm head count, motility, and percent live sperm, and marked and persistent increases (4-fold) in percent abnormal sperm were observed (Sharma and Singh 2010). After 28 days at this dose, the effects on epididymis and testes weights were more pronounced, as were the changes in sperm parameters: decreases of ≥89% compared to controls were seen in sperm head count, motility, and percent live sperm, as well as a 4-fold increase in percent abnormal sperm (Sharma and Singh 2010). Similar results were seen in Wistar rats given 50 mg/kg/day γ-HCH in water for 30 days (Hfaiedh et al. 2011). The weights of the testes, epididymides, and prostate gland were decreased by 42–52% relative to controls, and the seminal vesicle weight was reduced by 5%. Compared to control values, sperm count was diminished by 56% and sperm motility by 37% at this dose (Hfaiedh et al. 2011). In a 45-day exposure study, Saradha and Mathur (2006) observed decreased sperm count (~7%) and motility (~15%) in male Wistar rats administered doses of 1 mg/kg/day by gavage. At the higher dose of 5 mg/kg/day in this study, the effects were more severe, with a 27% decrease in sperm count and ~25% decrease in sperm motility (Saradha and Mathur 2006). In a 2-generation reproductive toxicity study in Crj:CD(SD)IGS rats given dietary doses up to 23.3 mg/kg/day, no statistically significant treatment-related effects on sperm count, motility, or percent abnormal sperm were noted in F0 or F1 males (Matsuura et al. 2005). Histology of the parental male reproductive organs was also normal (Matsuura et al. 2005). Dietary exposure to up to 120 mg/day γ-HCH for 10 months or 30 mg/kg/day for 2 years or did not result in histopathology changes in the testes of rats (Fitzhugh et al. 1950).

Exposure to γ-HCH has also induced altered levels of reproductive hormones in male rats. When male Wistar rats were exposed to 50 mg/kg/day γ-HCH in water for 30 days, serum FSH levels were decreased by 74% relative to controls (Hfaiedh et al. 2011). Effects of γ-HCH on the levels of reproductive hormones in blood of male animals appear to be more significant in younger animals compared with older animals. Groups of 30 male Wistar rats were administered 5 mg/kg/day γ-HCH for 5 days beginning at 9, 18, or 27 weeks of age (Agrahari et al. 2019). Animals treated with γ-HCH beginning at 9 weeks of age exhibited significantly decreased serum testosterone (39%) and growth hormone (29%), and increased serum LH (42%) and FSH (31%), compared to controls. Similar results were observed in the group treated at 18 weeks of age, but treatment at 27 weeks of age resulted in smaller decreases in serum testosterone and growth hormone, and no significant effect on serum LH or FSH. No dose-related effects on serum hormone levels were observed in F0 or F1 male parents in a 2-generation study of Crl:CD(SD)IGS rats at doses of 17.2–26.1 mg/kg/day in diet for ~10 weeks (Matsuura et al. 2005).

One study reported male reproductive tract effects in mice exposed to γ-HCH. Nagda and Bhatt (2011) exposed Swiss mice by gavage (40 mg/kg/day) for 60 days. At sacrifice at the end of exposure, the mice exhibited a 10% decrease in testes weight as well as histopathology changes in the testes, including shrunken and distorted seminiferous tubules, sparse Leydig cells, and oligospermia (Nagda and Bhatt 2011). The male reproductive effects of γ-HCH were also studied in young rams given 1 mg/kg/day in treated feed from conception to sexual maturity (Beard et al. 1999a). The subjectively-scored sexual behavior in the rams was significantly reduced in treated animals presented with estrous ewes (Beard et al. 1999a).

Effects on female reproductive system. Studies examining female reproductive effects in animals exposed to γ-HCH are more limited, but suggest effects on estrous cycling and other endpoints in a variety of species. Oral administration of γ-HCH for acute and intermediate durations has resulted in alterations in estrous cycling, sexual behavior, and uterine histopathology. In acute-duration studies, the lowest dose associated with these effects was 25 mg/kg/day (Uphouse and Williams 1989); in intermediate-duration studies, the lowest dose associated with these effects was 1 mg/kg/day (Beard and Rawlings 1999). 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). 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). Histopathological changes were observed in the uteri of female Sprague-Dawley rats given γ-HCH by gavage at a dose of 8 mg/kg/day for 4 weeks. The uterine changes were described as low columnar endometrial glandular epithelial cells (Yang et al. 2014; Zhang et al. 2016). Delayed vaginal

opening and disrupted ovarian cycling in female F344 rats given ≥10 mg/kg/day by gavage for 15 weeks beginning at weaning (Chadwick et al. 1988). Finally, in estrus synchronized ewe lambs dosed with γ-HCH in feed at 1 mg/kg/day from conception to sexual maturity, significantly shorter estrous cycle length and reduced number and total volume of corpus lutea were observed (Beard and Rawlings 1999). No other detrimental fertility effects were observed. No effects on maternal female reproductive organ weights or histology were noted in CD mice exposed to 15 mg/kg/day via gavage on GDs 9–16 (Maranghi et al. 2007). In a 2-generation reproductive toxicity study in Crj:CD(SD)IGS rats, doses of 28.0 mg/kg/day in diet for ~10 weeks resulted in a significantly decreased estrus cycle length (4 days versus 4.45 days in controls) in F1, but not F0 female adults (Matsuura et al. 2005). No effects were observed on ovarian follicle counts at any dose in F1 females, and no changes in reproductive organ weights or histology were observed at sacrifice of F0 or F1 female parental animals (Matsuura et al. 2005). Fitzhugh et al. (1950) observed no effects on the histopathology of the uterus or ovaries of rats given γ-HCH via the diet for up to 140 mg/kg/day for 10 months or up to 30 mg/kg/day for 2 years.

In female Wistar rats dosed with γ-HCH by daily gavage for 4 weeks, significantly decreased serum levels of estradiol (20 and 26%) and testosterone (28 and 37%) were observed at 4 and 8 mg/kg/day γ-HCH, respectively (Zhang et al. 2016; Yang et al. 2014). Significant, dose-related increases in serum LH were seen at all doses (23–44% relative to controls at doses from 0.95 to 28 mg/kg/day) in F1 females during proestrus in a 2-generation study of Crj:CD(SD)IGS rats (Matsuura et al. 2005). Serum hormone levels in F0 female parents were not impacted by exposure in this study. Female mink exposed to 1 mg/kg/day γ-HCH before mating and through mating, gestation, and lactation exhibited no effects on serum estradiol or progesterone when evaluated at weaning of their kits (Beard et al. 1997). Sheep exposed on a similar schedule to the same dose did not show changes in serum LH or FSH (Beard et al. 1999b)

Effects on mating, fertility, and early gestation. In a multigeneration reproduction 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 2 generations (EPA 1991a). No treatment-related effects on mating, fertility, gestation survival, liveborn indices, or mean litter sizes occurred in either generation, although 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 F2 pups (see Section 2.17). Similar findings were noted in the 2-generation study reported by Matsuura et al. (2005). No effects on mating, fertility, gestation length, birth index, or gestation index were seen in Crj:CD(SD)IGS rats of either generation at doses up to 28 mg/kg/day, but there were developmental effects on offspring body weight, viability, and sexual maturation (see

Section 2.17). Female rabbits dosed with 0.8 mg γ-HCH/kg/day, 3 days/week for 12 weeks followed by artificial insemination exhibited no effects on the fertilization rate or on pre- or post-implantation losses (Seiler et al. 1994).

Mice and mink appear to be more sensitive than rats to the effects of γ-HCH on fertility and early gestation. When mouse dams were treated with γ-HCH (6.2 mg/kg) during GDs 6–12, all fetuses were resorbed (Sircar and Lahiri 1989). In another experiment by these authors, pregnant mice exposed to 10.8 mg/kg/day on GDs 1–4 exhibited no implantation sites. When pregnant mice were exposed to 3.6 mg/kg/day on GDs 14–19, all pups died (Sircar and Lahiri 1989). Acute preovulatory exposure to γ-HCH caused embryonic effects in mice (Scascitelli 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-mating treatment.

Reproductive toxicity studies in mink showed effects on sexual receptivity, whelping rate, and embryo mortality. A 2-generation reproduction study of γ-HCH was conducted in mink that were exposed to dietary doses of 0 or 1 mg/kg/day (Beard and Rawlings 1998). The parental (P0) generation was exposed from 3 weeks before breeding until weaning of the offspring. Following weaning, the F1 females were exposed throughout growth and mating (to untreated males), and subsequently throughout pregnancy and lactation until 3 months post-lactation. The F2 females were exposed until they reached full adult body size at 30 weeks of age. The F1 and F2 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, testosterone) and histology of male and female reproductive tissues 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 in F2 males. In a single-generation study, 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.

Mechanisms. 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). 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 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.

In vitro studies have not shown binding of γ-HCH to the estrogen receptor, but one study showed that this isomer could inhibit the activity of aromatase (the enzyme that forms estrogen in mammals) in human placental and embryonic kidney cells transfected with the associated gene (reviewed by IARC 2018).

Technical or Unspecified HCH. Studies of reproductive toxicity in animals exposed orally to technical-grade HCH have shown effects on the male reproductive tract of rats and mice. Dermal exposure of rats and guinea pigs induced similar changes.

Immature (15-day-old) and mature (90-day-old) rats 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). 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. Shivanandappa and Krishnakumari (1983) reported testicular atrophy, degeneration of seminiferous tubules, and disruption of spermatogenesis in male rats fed technical-grade HCH at 75 mg/kg/day for 90 days. After 180 days of exposure to 3 mg/kg/day technical-grade HCH, male Charles Foster rats exhibited significant decreases in testes and vas deferens

weights, as well as decreases in seminiferous tubule diameter and degeneration of muscle tissue in the vas deferens (Gautam et al. 1989; Roy Chowdhury and Gautam 1990). At a higher dose of 6 mg/kg/day, complete degeneration of testicular tissue, post-meiotic spermatogenic arrest, and degeneration of spermatogenic cells were seen (Roy Chowdhury and Gautam 1990). 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). Fitzhugh et al. (1950) observed no effects on the histopathology of the testes, uterus, or ovaries of rats given technical HCH via the diet for up to 9 mg/kg/day for 2 years. Moderate testicular atrophy was observed in rats given technical HCH in the diet for 6 months at a dose of 60 mg/kg/day (Fitzhugh et al. 1950). 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).

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 technical-HCH throughout 3 generations (Srivastava and Raizada 2000). There were no exposure-related effects on reproduction in any of the 3 parental generations, and no morphological or teratological changes in any of the offspring generations (F1b, F2b, or F3b).

In studies of dermal exposure, 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-glucuronidase. 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. 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.

Mechanisms. There is evidence that oxidative stress may contribute to the effects of technical-grade HCH on the male reproductive system. 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). Endpoints 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). 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.

2.17 DEVELOPMENTAL

Developmental effects of HCH isomers have been evaluated in human populations and in animals. These studies are discussed below in the individual isomer subsections. However, the epidemiological studies of all isomers share limitations that render the reported associations uncertain, especially when considered without any supporting animal data. Epidemiological studies of developmental effects were conducted in the general population (without occupational exposure), generally using measurements of HCH isomers in physiological fluids or tissues of mothers and infants. In the general population, the route(s) of exposure is unknown. In the studies discussed herein, other organochlorine compounds (such as hexachlorobenzene, aldrin, heptachlor and its epoxide, DDT and its metabolites, polychlorinated biphenyls, and/or polychlorinated dioxins and furans) were also present in the blood. Few of the studies controlled for these co-exposures; thus, the role of HCH isomers in the observed effects, if any, cannot be ascertained. In addition, the case-control studies examined levels of HCH isomers in blood and tissues after the outcome was established, rendering the temporal association between exposure and outcome uncertain.

α-HCH. Data on the developmental effects of α-HCH are limited to a small number of human epidemiological studies; there are no animal studies of developmental toxicity for this isomer. Table 2-16 provides a summary of human epidemiological data on developmental effects of α-HCH. As the table shows, the epidemiology data suggest possible associations between growth retardation and maternal or placental levels of α-HCH. Increased risks of fetal growth restriction (defined as $<10^{th}$ percentile of birth weight for gestational age, and also termed ‘intrauterine growth retardation’ in some studies) were associated with maternal blood levels of α-HCH in two small case-control studies in India (Sharma et al. 2012; Siddiqui et al. 2003). Reduced birth weight was associated with higher levels of α-HCH in placenta in a small cross-sectional study in India (Anand and Taneja 2020), but not with maternal serum levels of α-HCH in a cross-sectional study in an Arctic population in Russia (Bravo et al. 2019) or with cord serum levels in a large cross-sectional analysis of 1,028 mother-infant pairs in China (Fang et al. 2019a, 2019b). No association between cord serum α-HCH and infant BMI through age 24 months was observed in a

Table 2-16. Summary of Epidemiological Studies of α-Hexachlorocyclohexane (HCH) Exposure and Developmental Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Mean concentration (unless otherwise noted)	Result
Siddiqui et al. 2003 Case-control, 30 mothers of infants with intra-uterine growth retardation, 24 mothers of normal weight infants, India	**Intra-uterine growth retardation**	Maternal blood	5.82±3.22 ng/g (mean±SD) (cases) 3.79±3.14 (controls)	↑
		Placenta	9.91±3.89 (cases) 8.88±5.17 (controls)	NR
		Cord blood	9.84±5.12 (cases) 6.74±7.83 (controls)	NR
Fang et al. 2019a, 2019b Cross-sectional, 1,028 pregnant mother-infant pairs, China	Birth weight	Cord serum	≥0.718 ng/g lipid (3rd tertile)	↔
	Birth length			↔
	Ponderal index			↔
	Gestational age			↓ (among term births)
Anand and Taneja 2020 Cross-sectional, 90 mother-infant pairs, India	**Birth weight**	Placenta tissue	1.09–211.43 µg/L (range)	↓
	Birth length			↔
	Head circumference			↔
	Ponderal index			↔
Bravo et al. 2019 Cross-sectional, 247 mother-child pairs, Russia	Gestational age	Maternal serum (last week of pregnancy)	3.3 ng/g lipid (median)	↔
	Birth weight			↔
	Birth length			↔
	Head circumference			↔
Yang et al. 2021a Birth cohort, 1,039 mother-infant pairs followed for 2 years after birth, China	Infant BMI at birth and ages 6, 12, and 24 months; risk of overweight status	Cord serum	0.35 ng/g lipid (median)	↔

Table 2-16. Summary of Epidemiological Studies of α-Hexachlorocyclohexane (HCH) Exposure and Developmental Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Mean concentration (unless otherwise noted)	Result
Mustafa et al. 2013 Case-control, 156 mothers with preterm births and 150 mothers with term births, India	**Preterm birth**	Maternal blood	4.04±2.63 ng/g (mean±SD) (cases) 2.93±2.59 (controls)	↑
		Cord blood	1.91±2.03 (cases) 1.69±2.25 (controls)	↔
Sharma et al. 2012 Case-control, 50 cases delivering babies with fetal growth restriction and 50 women with healthy term infants, mean ages 23–24 years, India	**Fetal growth restriction**	Maternal blood	4.55±3.2 ng/g (mean±SD) (cases) 2.92±2.7 (controls)	↑
		Cord blood	2.01±1.6 (cases) 1.90±2.3 (controls)	↔
Yin et al. 2021 Case-control, 119 mothers delivering infants or electively terminating pregnancies with neural tube defects and 119 controls, China	Neural tube defects	Cord tissue	0.23 ng/g (cases) (median) 0.13 (controls)	↔

↑ = association with increase; ↓ = association with decrease (inverse association); ↔ = no association; SD = standard deviation

birth cohort of 1,039 mother-infant pairs in China (Yang et al. 2021a). None of the available studies suggested associations between biomarkers of α-HCH exposure in maternal and infant tissues and birth length, head circumference, or ponderal index (Anand and Taneja 2020; Bravo et al. 2019; Fang et al. 2019a, 2019b).

In cross-sectional studies, a negative association between gestational age among term births and α-HCH in cord serum was observed in a study of 1,028 mother-infant pairs in China (Fang et al. 2019a, 2019b), but no association was seen in a smaller group of 247 mother-infant pairs in Russia (Bravo et al. 2019). Increased risk of preterm birth was associated with higher maternal blood levels of α-HCH in a case-control study in India (Mustafa et al. 2013).

A small case-control study in China did not observe an association between α-HCH in umbilical cord tissue and neural tube defects (Yin et al. 2021).

β-HCH. Epidemiological studies of developmental endpoints in humans exposed to β-HCH are summarized in Table 2-17. As the table shows, most of the studies examined metrics pertaining to fetal growth and gestational age. The studies provide suggestive evidence for an association between β-HCH concentrations in maternal or umbilical cord blood and reduced birth weight.

In birth cohorts of mother-infant pairs in California, Lebanon, and China, birth weight showed no association with β-HCH in maternal serum sampled during the second trimester or maternal or cord serum at delivery (Fenster et al. 2006, Wang et al. 2022a). An association between increased infant BMI at 1 and 2 years of age and cord serum levels of β-HCH was observed in another birth cohort in China; this study reported no association with infant BMI at birth or 6 years of age (Yang et al. 2021a). Case-control studies of fetal growth restriction (<10^{th} percentile weight for gestational age) reported conflicting findings. Siddiqui et al. (2003) reported no association of fetal growth restriction with maternal, cord blood, or placental concentrations of β-HCH in a small (30 cases and 24 controls) study conducted in India. However, a slightly larger study of 50 cases and 50 controls in India showed a positive association between increased risk of fetal growth restriction and β-HCH in maternal blood (but not cord blood) (Sharma et al. 2012).

Associations between reduced birth weight and increased maternal plasma, umbilical cord blood, or placental tissue concentrations of β-HCH were reported in cross-sectional studies in Australia (Callan et al. 2016), Spain (Lopez-Espinosa et al. 2011), China (Fang et al. 2019a, 2019b; Guo et al. 2014; Yang et

Table 2-17. Summary of Epidemiological Studies of β-Hexachlorocyclohexane Exposure and Developmental Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Concentration	Result
Birth outcomes				
Siddiqui et al. 2003 Case-control, 30 mothers of infants with intra-uterine growth retardation, 24 mothers of normal weight infants, India	Fetal growth restriction (intrauterine growth retardation)	Maternal blood	7.95±11.43 ng/g (mean±SD) (cases) 6.55±5.43 (controls)	↔
		Placenta	7.30±10.92 (cases) 7.00±7.14 (controls)	↔
		Cord blood	3.03±5.22 (cases) 2.96±3.62 (controls)	↔
Callan et al. 2016 Cross-sectional, 161 mother-infant pairs, Australia	**Birth weight, proportion of optimal birth weight**	Maternal plasma (2 weeks prior to birth)	0.18 µg/L (mean)	↓ (boys) ↔ (girls)
	Ponderal index			↔
Hjermitslev et al. 2020 Cross-sectional, 468 mother-infant pairs, Greenland	Birth weight	Maternal serum	3.6 µg/kg lipid (median)	↔
	Gestational age			↓
Fenster et al. 2006 Cohort, 385 mother-infant pairs, California, United States	Length of gestation	Maternal serum (2^{nd} trimester or at delivery)	37.2 ng/g lipid (median)	↔
	Birth weight		See above	↔
	Crown-heel length		See above	↔
Khanjani and Sim 2006 Cross-sectional, 815 mother-infant pairs, Australia	Prematurity	Breast milk	0.0098±0.0286 mg/kg milk fat (mean±SD)	↔
	Previous miscarriage or still birth			↔
	Low birth weight			↔
	Small for gestation age			↔
	Head circumference			↔
	Sex ratio			↔
Gladen et al. 2003 Cross-sectional, 197 mother-infant pairs, Ukraine	Birth weight	Breast milk	860 ng/g milk fat (3^{rd} tertile)	↔

Table 2-17. Summary of Epidemiological Studies of β-Hexachlorocyclohexane Exposure and Developmental Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Concentration	Result
Lopez-Espinosa et al. 2011 Cross-sectional, 494 mothers-infant pairs, Spain	**Birth weight**	Cord serum	0.085 ng/mL (median)	↓ (marginal)
	Birth length			↔
	Head circumference			↔
Fang et al. 2019a, 2019b Cross-sectional, 1,028 pregnant mother-infant pairs, China	**Birth weight**	Cord serum	≥12.64 ng/g lipid (3rd tertile)	↓ (boys)
	Birth length			↔
	Ponderal index			↓ (boys)
	Gestational age			↔
Wang et al. 2022a Cohort, 1,522 mother-child pairs, China	Birth weight	Cord serum	0.65 ug/L (mean)	↔
	Birth length			↔
	Head circumference			↓
Yang et al. 2021a Birth cohort, 1,039 mother-infant pairs followed for 2 years after birth, China	**Infant BMI at ages 12 and 24 months**	Cord serum	8. 42 ng/g lipid (median)	↑
	Infant BMI at birth and age 6 months			↔
	Risk of overweight status			↑ (girls) ↔ (boys)
Guo et al. 2014 Cross-sectional, 81 mother-infant pairs, China	**Birth weight**	Maternal serum at birth	73.96 (median)	↓
		Cord serum	35.29 (median)	↓
Anand and Taneja 2020 Cross-sectional, 90 mother-infant pairs, India	**Birth weight**	Placenta tissue	1.10–678.74 µg/L (range)	↓
	Birth length			↔
	Head circumference			↔
	Ponderal index			↔
Mustafa et al. 2013 Case-control, 156 mothers with preterm births and 150 mothers with term births, India	Preterm birth	Maternal blood	5.07±3.40 ng/g (mean±SD) (cases) 4.03±3.40 (controls)	↔
		Cord blood	2.10±1.83 (cases) 1.84±2.10 (controls)	↔

Table 2-17. Summary of Epidemiological Studies of β-Hexachlorocyclohexane Exposure and Developmental Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Concentration	Result
Tan et al. 2009 Cross-sectional, 41 mother-infant pairs, Singapore	Birth weight	Cord blood	85.4±173 ng/g lipid (mean±SD)	↔
	Birth length			↑
	Head circumference			↑
	Gender			↔
Tyagi et al. 2016 Cross-sectional, 30 mothers with preterm births and 30 mothers with term births, India	**Preterm birth**	Maternal blood	6.42±2.158 ng/mL (mean±SD) (cases) 3.06±2.05 (controls)	↑
Bravo et al. 2019 Cross-sectional, 247 mother-child pairs, Russia	Gestational age	Maternal serum (last week of pregnancy)	38 ng/g lipid (median)	↔
				↔
	Birth weight			↔
	Birth length			↔
	Head circumference			↔
Yang et al. 2020 Cohort, 102 healthy pregnant women, mean age 28 years, China	**Birth weight**	Maternal serum	7.44 ng/mL (mean)	↓
Yang et al. 2021b Case-control, 89 infants with orofacial clefts and 129 controls, China	Orofacial cleft	Cord tissue	0.74 ng/g dry weight (cases) 0.66 (controls)	↔
Sharma et al. 2012 Case-control, 50 cases delivering babies with fetal growth restriction and 50 women with healthy term infants, mean ages 23–24 years, India	**Fetal growth restriction**	Maternal blood	3.97±3.9 ng/g (mean±SD) (cases)	↑
		Cord blood	2.67±2.4 (cases)	↔
Torres-Arreola et al. 2003 Case-cohort, 100 mothers with preterm births, 133 controls with full-term births, Mexico	Preterm birth (<37 weeks)	Maternal serum at birth	>76.53 ng/g (3rd tertile)	↔

Table 2-17. Summary of Epidemiological Studies of β-Hexachlorocyclohexane Exposure and Developmental Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Concentration	Result
Pierik et al. 2007 Case-control nested in birth cohort, 219 mothers of children with cryptorchidism, 564 controls, United States	Cryptorchidism within first year of life	Maternal serum (3rd trimester)	>3.41 µg/L (90th percentile)	↔
Desalegn et al. 2021 Case-cohort, 641 mother-male infant pairs, Norway	**Cryptorchidism**	Breast milk	4.43 ng/g (median)	↑
Warembourg et al. 2016 Cross-sectional, 282 newborns, France	Umbilical cord blood free testosterone, sex hormone binding globulin, anti-Müllerian hormone, estradiol, aromatase index	Cord blood	11.27 ng/g lipid (median)	↔
Debost-Legrand et al. 2016 Cross-sectional, 268 mother-infant pairs, France	Umbilical cord serum insulin, adiponectin	Cord serum	>0.061µg/L (4th quartile)	↔
Yin et al. 2021 Case-control, 119 mothers delivering infants or electively terminating pregnancies with neural tube defects and 119 controls, China	**Neural tube defects**	Cord tissue	1.81 ng/g dry weight (cases) (median) 0.93 (controls)	↑
Postnatal development				
Namulanda et al. 2016 Case-control, 218 girls with early menarche (<11.5 years of age), 230 controls, England	Early menarche	Maternal serum	47.4 ng/g lipid (median)	↔
Marks et al. 2021 Nested case-control, 218 cases of early menarche (<11.5 years of age), 230 controls, United Kingdom	Early menarche	Maternal serum	45.3 ng/g lipid (cases) (median) 47.5 (controls)	↔
Lam et al. 2014, 2015 Cohort, 350 boys 8–9 years old, followed for 8 years, Russia	Age of pubertal onset **Age of sexual maturity**	Serum at cohort entry	1.3–14 ng/g (4th quartile)	↔ ↑

Table 2-17. Summary of Epidemiological Studies of β-Hexachlorocyclohexane Exposure and Developmental Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Concentration	Result
Garcia-Villarino et al. 2022 Birth cohort, 201 mother-child pairs with children followed at 8 years of age, Spain	**Anogenital index at 8 years of age**	Maternal serum	27.23 ng/g lipid (Median)	↓ **(**boys) ↔ (girls)
Cupul-Uicab et al. 2013 Birth cohort, 1,915 children followed until age 7 years, United States	Childhood obesity or overweight and obese	Maternal serum (3rd trimester)	≥2.12 µg/L (4th quartile)	↔
Lauritzen et al. 2018 Cohort, 412 mother-child pairs followed until child age of 5 years, Norway and Sweden	Childhood obesity at 5 years old (BMI, triceps skinfold, subscapular skinfold, overweight)	Maternal serum (2nd trimester)	Norway: 21.2 ng/g lipid (median) Sweden: 25 ng/g lipid (median)	↔
Mendez et al. 2011 Birth cohort, 518 mother-infant pairs followed for 14 months, Spain	Rapid infant growth during first 6 months; elevated BMI at 14 months	Maternal serum (1st trimester)	≥47.28 ng/g lipid (4th quartile)	↔
Salo et al. 2019 Case-control, 40 cases with autoantibodies and 11 control children up to 6 years old, Finland	Diabetes-associated autoantibodies	Cord plasma	>LOQ (not specified)	↔
Alvarez-Pedrerol et al. 2008a Cross-sectional, 21 newborn infants, Spain	**Neonatal plasma TSH (3 days postpartum)**	Cord serum	0.48 ng/mL (geometric mean) (group with TSH ≥10 mU/L) 0.24 (group with TSH <10 mU/L)	↑
Alvarez-Pedrerol et al. 2008b Cross-sectional, 259 children 4 years old, Spain	Serum free T4, TSH at age 4 years	Serum (child)	≥0.305 ng/mL (4th quartile)	↔
	Serum total T3 at age 4 years		≥0.191 ng/mL (3rd quartile)	↓
Ribas-Fito et al. 2003 Cross-sectional, 98 newborn infants, Spain	**Neonatal plasma TSH ≥10 mU/l**	Cord serum	0.54 ng/mL (median)	↑
Lopez-Espinosa et al. 2010 Cross-sectional, 453 newborn infants, Spain	**Postpartum (≥2 days) neonatal serum TSH**	Cord serum	>104 ng/g lipid (90th percentile)	↑

Table 2-17. Summary of Epidemiological Studies of β-Hexachlorocyclohexane Exposure and Developmental Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Concentration	Result
Yamazaki et al. 2020 Cross-sectional, 333 mother-child pairs, Japan	Serum free T4 or TSH in infants 7–43 days old	Maternal serum	235.6 pg/g (75th percentile)	↔
Li et al. 2014 Cross-sectional, 247 pregnant women, Yanchen City, China	Umbilical cord serum free T3, free T4, TSH	Cord serum	13.336 ng/g (median)	↔
Wang et al. 2022b Birth cohort, 228 mother-child pairs, China	Total or free T3, total or free T4, TSH in cord serum	Cord serum	0.48 ug/L (median)	↔
Sunyer et al. 2008 Cross-sectional, 52 children, 4 years old, Spain	Urinary porphyrins (child)	Serum (child)	>0.37 ng/mL	↔
Neurodevelopmental endpoints				
Lenters et al. 2019 Birth cohort, 1,199 mother-child pairs, Norway	ADHD by 13 years of age	Breast milk	4.367 ng/g lipid (median)	↑
Kokroko et al. 2020 Cohort, 256 mother child pairs, United States	IQ at 7 years old (Wechsler Intelligence Scale for Children)	Maternal serum during pregnancy	33.3 ng/g lipid (geometric mean)	↔[a]
Braun et al. 2014 Cohort, 175 mother-child pairs, Ohio, United States	Social responsiveness scale score, children aged 4 and 5 years	Maternal serum	<LOD (median) 1.9 ng/g lipids (75th percentile)	↔[b]
Jeddy et al. 2018 Cohort, 400 mother-daughter pairs, England	Communication development: nonverbal communication, social development, verbal comprehension, vocabulary comprehension in daughters at 15 and 38 months	Maternal serum	>56.15 ng/g lipid (3rd tertile)	↔

Table 2-17. Summary of Epidemiological Studies of β-Hexachlorocyclohexane Exposure and Developmental Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Concentration	Result
Wang et al. 2021b Birth cohort, 242 mother-child pairs, China	Language development at 18 months of age	Cord blood	0.51 ug/L (median)	↔
	Motor development at 18 months of age			↓
	Cognitive development at 18 months of age			↔
Lee et al. 2007 Cross-sectional, 278 children aged 12–15 years, NHANES, United States	Learning disability	Serum (child)	17.9 ng/g lipid (median)	↔
	Attention deficit disorder			↔
Fabisiková et al. 2012 Cross-sectional, 143 mother-infant pairs, Slovakia	Bayley mental development index at 10 months of age	Serum (child)	0.01–82.9 ng/g lipid (range)	↔
	Bayley psychomotor development index at 10 months of age			↔
Sisto et al. 2015 Cohort, 351 infants enrolled at birth, Slovakia	**Cochlear deficits measured as altered distortion product otoacoustic emissions at 45 months of age**	Cord blood	9.84±8.09 ng/g lipid	↑
		Serum at 6 months old	12.24±12.9	↓
		Serum at 16 months old	13.36±16.06	↓
		Serum at 45 months old	7.70±9.21	↓
Kornvig et al. 2021 Birth cohort, 102 mother-child pairs, Greenland	Problematic child behavior at 3–5 years of age	Maternal serum	3.50 ug/kg lipid (median)	↔
	Abnormal hyperactivity at 3–5 years of age			↔

Table 2-17. Summary of Epidemiological Studies of β-Hexachlorocyclohexane Exposure and Developmental Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Concentration	Result
Cheslack-Postava et al. 2022 Nested case-control, 359 cases of ADHD and 359 sex-, age- and birthplace-matched controls, Finland	**ADHD**	Maternal serum	NR	↑

[a]A significant increase in working memory IQ score was observed but is not shown here as it is not considered adverse.
[b]A significant decrease in SRS score, corresponding to lower autism-related behaviors, was observed but is shown here as it not considered adverse.

↑ = association with increase; ↓ = association with decrease (inverse association); ↔ = no association; ADHD = attention deficit hyperactivity disorder; BMI = body mass index; IQ = intelligence quotient; LOD = limit of detection; NHANES = National Health and Nutrition Examination Survey; SD = standard deviation; T3 = triiodothyronine, T4 = thyroxine; TSH = thyroid-stimulating hormone

al. 2020), and India (Anand and Taneja 2020), but not in cross-sectional studies in Greenland (Hjermitslev et al. 2020) or Russia (Bravo et al. 2019).

Studies evaluating whether β-HCH concentrations in maternal or infant blood were associated with preterm birth or gestational age have yielded mixed results. Tyagi et al. (2016) reported a positive association between risk of preterm birth and β-HCH in maternal blood in a small cross-sectional study in India, but no association was reported in a case-control study in India (Mustafa et al. 2013) or in a case-cohort study in Mexico (Torres-Arreola et al. 2003). In 468 mother-infant pairs in Greenland, decreased gestational age was associated with maternal serum levels of β-HCH (Hjermitslev et al. 2020), but no association was seen in cross-sectional studies in China (Fang et al. 2019a, 2019b) or Russia (Bravo et al. 2019) or between gestation length and β-HCH in maternal blood in a cohort study in the United States (Fenster et al. 2006).

No association between umbilical cord levels of β-HCH and orofacial clefts were observed in a case-control study in China (Yang et al. 2021b). A case-control study in China identified a positive association between umbilical cord tissue levels of β-HCH and neural tube defects (Yin et al. 2021).

A case-control study nested in a birth cohort in the United States observed no association between maternal serum concentrations of β-HCH and cryptorchidism or hypospadias (Pierik et al. 2007), but a case-cohort study of 641 mother-male infant pairs in Norway showed a positive association between breast milk concentrations of β-HCH and cryptorchidism (Desalegn et al. 2021). Cross-sectional studies in France reported no association between β-HCH in umbilical cord blood and reproductive hormone levels (Warembourg et al. 2016), or insulin and adiponectin concentrations in umbilical cord (Debost-Legrand et al. 2016).

As shown in Table 2-17, serum and cord blood levels of β-HCH were associated with increased serum levels of TSH in cross-sectional studies conducted in Spain (Alvarez-Pedrerol et al. 2008a, 2008b; Lopez-Espinosa et al. 2010; Ribas-Fito et al. 2003) but not in a similar study in China (Li et al. 2014). No association between maternal serum levels of β-HCH and concentrations of free T4 or TSH in infants from 1 to 6 weeks old (Yamazaki et al. 2020), or between cord serum β-HCH and concentrations of T4, T3, or TSH in cord serum in a birth cohort in China (Wang et al. 2022b).

In British case-control studies nested in birth cohorts, there was no association between maternal serum levels of β-HCH and early menarche (Namulanda et al. 2016, Marks et al. 2021). A cohort of 332 mother-

child pairs found an inverse association between β-HCH levels in serum and anogenital index in boys at 8 years of age (Garcia-Villarino et al. 2022). In a cohort study of 350 boys in Russia, serum levels of β-HCH were associated with a higher age at sexual maturity (Lam et al. 2014, 2015).

Studies examining neurodevelopmental effects in relationship to maternal or cord serum, breast milk, or infant or child blood levels of β-HCH are shown in Table 2-17. In general, these studies showed no associations with IQ, problematic behavior, social responsiveness, communication, or mental and psychomotor development (see Table 2-17). A birth cohort study in China reported diminished motor development at 18 months of age with higher β-HCH in cord blood, but no effect on language or cognitive development (Wang et al. 2021b). Lenters et al. (2019) showed an association between breast milk concentrations of β-HCH and diagnosis of attention deficit hyperactivity disorder (ADHD) at approximately 13 years of age. Similarly, β-HCH in maternal serum was associated with ADHD in a nested case-control study in Finland (Cheslack-Postava et al. 2022).

Sisto et al. (2015) reported an association between serum concentrations of β-HCH in children (obtained at birth [cord serum] and 6, 16, and 45 months of age) and cochlear deficits (measured as altered distortion product otoacoustic emissions [DPOAEs] measured at 45 months of age). DPOAEs have been established as an objective diagnostic tool for assessing the function of cochlear outer hair cells. In this study, the investigators controlled for co-exposure confounding by evaluating the association between β-HCH and cochlear function both with and without potentially ototoxic co-exposures. Cord serum β-HCH concentration was *positively* associated with the amplitude of otoacoustic emissions across a range of f2 frequencies, while analyses using β-HCH concentrations from serum samples at 6, 16, and 45 months of age yielded *inverse* associations with the amplitude of otoacoustic emissions and at inconsistent frequencies. Significant inverse associations with β-HCH concentrations were seen at low frequencies in analyses of 6-month blood samples, and at higher frequencies in analyses of 16-month blood samples. In analyses using the 45-month blood samples, a significant inverse association between β-HCH concentration and amplitude of otoacoustic emissions was seen for only 1 of 11 frequencies tested. The study authors suggested that increased exposure during lactation might account for the change from positive to inverse association between birth and 6 months of age, and indeed, the maximum serum concentration of β-HCH almost doubled between these two measurements. Sisto et al. (2015) also assessed tonotopicity (specificity of the effect on different regions of the basal membrane in the organ of Corti, which correspond to effects on different frequencies), observing that the strength of the association with each compound varied by noise frequency.

Limited data are available on developmental effects of β-HCH in animals. Dietary exposure of pregnant rats to 20 or 25 mg/kg/day of β-HCH during gestation caused increased perinatal mortality, with deaths of 48 or 100% of the pups within 5 days of birth; exposure to 5 mg/kg/day did not influence perinatal survival (Srinivasan et al. 1991). In another experiment by these authors, a dose 5 mg/kg/day of β-HCH during gestation and lactation or during lactation only resulted in increased liver weights of pups when measured at 28 days of age (Srinivasan et al. 1991).

γ-HCH (Lindane). Epidemiological studies of developmental endpoints in humans exposed to γ-HCH are summarized in Table 2-18. Two case-control studies in India reported associations between maternal and cord blood levels of γ-HCH and fetal growth restriction (Sharma et al. 2012; Siddiqui et al. 2003). In a birth cohort of 385 mother-infant pairs in California, birth weight and length were not associated with γ-HCH concentration in maternal serum collected during the second trimester and at delivery (Fenster et al. 2006). Birth weight, birth length, and ponderal index showed no association with γ-HCH in cord serum in a cross-sectional study of 1,028 infants in China (Fang et al. 2019a, 2019b). Higher BMI in 6-month-old infants was associated with increased γ-HCH in cord serum in a cohort in China; however, BMI at 12 and 24 months of age was not associated with γ-HCH levels (Yang et al. 2021a).

Gestation length was not associated with γ-HCH concentration in maternal serum in a birth cohort of 385 mother-infant pairs in an agricultural area of California (Fenster et al. 2006). Gestational age was inversely associated with γ-HCH in cord serum in a cross-sectional study of 1,028 infants in China (Fang et al. 2019a, 2019b). A case-control study in India observed an increased risk of preterm birth associated with maternal, but not cord blood levels of γ-HCH (Mustafa et al. 2013).

The concentration of γ-HCH in the placenta was associated with an increased risk of male reproductive tract abnormalities (cryptorchidism or hypospadias observed at birth and 1 month of age) in a case-control study nested within a birth cohort in Spain (Fernandez et al. 2007). No association between γ-HCH in maternal serum and anogenital index at age 8 years was observed in a birth cohort of 201 mother-child pairs in Spain (Garcia-Villarino et al. 2022).

No associations between levels of γ-HCH in umbilical cord tissue and orofacial cleft or neural tube defects were observed in case-control studies in China (Yang et al. 2021b; Yin et al. 2021).

A cross-sectional study of 220 male newborns in Spain showed no association between placental γ-HCH and TSH levels in umbilical cord serum (Freire et al. 2011).

Table 2-18. Summary of Epidemiological Studies of γ-Hexachlorocyclohexane Exposure and Developmental Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Concentration	Result
Siddiqui et al. 2003 Case-control, 30 mothers of infants with intrauterine growth retardation, 24 mothers of normal weight infants, India	**Intrauterine growth retardation**	Maternal blood	6.30±7.51 ng/g (mean±SD) (cases) 2.65±2.15 (controls)	↑
		Placenta	8.71±4.57 (cases) 6.86±4.46 (controls	↔
		Cord blood	9.23±10.31 (cases) 4.23±4.59 (controls)	↑
Fenster et al. 2006 Cohort, 385 mother-infant pairs, California, United States	Length of gestation	Maternal serum (2nd trimester and at delivery)	1.0 ng/g lipid (median)	↔
	Birth weight			↔
	Crown-heel length			↔
Fang et al. 2019a, 2019b Cross-sectional, 1,028 pregnant mother-infant pairs, China	Birth weight	Cord serum	≥1.125 ng/g lipid (3rd tertile)	↔
	Birth length			↔
	Ponderal index			↔
	Gestational age			↓
Yang et al. 2021a Birth cohort, 1,039 mother-infant pairs followed for 2 years after birth, China	Infant BMI at age 6 months	Cord serum	0.70 ng/g lipid (median)	↑
	Infant BMI at birth and ages 12 and 24 months; risk of overweight status			↔
Mustafa et al. 2013 Case-control, 156 mothers with preterm births and 150 mothers with term births, India	**Preterm birth**	Maternal blood	2.63±2.46 ng/g (mean±SD) (cases) 1.52±1.83 (controls)	↑
		Cord blood	0.988±1.31 (cases) 0.887±1.24 (controls)	↔

Table 2-18. Summary of Epidemiological Studies of γ-Hexachlorocyclohexane Exposure and Developmental Effects

Reference, study type, and population	Outcome evaluated	Biomarker	Concentration	Result
Sharma et al. 2012 Case-control, 50 cases delivering babies with fetal growth restriction and 50 women with healthy term infants, mean ages 23–24 years, India	**Fetal growth restriction**	Maternal blood	7.06±6.7 ng/g (mean±SD) (cases) 2.58±3.9 (controls)	↑
		Cord blood	3.59±3.8 (cases) 1.44±2.1 (controls)	↑
Yang et al. 2021b Case-control, 89 infants with orofacial clefts and 129 controls, China	Orofacial cleft	Cord tissue	0.01 ng/g dry weight (cases) (median) 0.15 (controls)	↔
Yin et al. 2021 Case-control, 119 mothers delivering infants or electively terminating pregnancies with neural tube defects and 119 controls, China	Neural tube defects	Cord tissue	9.92 ng/g dry weight (cases) (median) 7.19 (controls)	↔
Fernandez et al. 2007 Case-control (nested), 50 newborn boys with cryptorchidism or hypospadias, 114 boys without malformations, Spain	**Cryptorchidism or hypospadias**	Placenta	0.9±0.8 ng/g lipid (mean±SD) (cases) 0.7±1.0 (controls)	↑
Garcia-Villarino et al. 2022 Birth cohort, 201 mother-child pairs with children followed to 8 years of age, Spain	Anogenital index at 8 years of age	Maternal serum	2.49 ng/g lipid (median)	↔
Freire et al. 2011 Cross-sectional, 220 mother-infant son pairs, Spain	Umbilical cord serum TSH	Placenta	0.25 ng/g placenta (median)	↔

↑ = association with increase; ↓ = association with decrease (inverse association); ↔ = no association; SD = standard deviation; TSH = thyroid-stimulating hormone

The effects of oral exposure to γ-HCH on development in animals have been extensively studied, primarily in rats and mice. Developmental effects of γ-HCH in these species include reduced viability and pup body weight; perturbation of male and female reproductive tract development; alterations in the developing liver, thymus, spleen, and heart; and developmental neurotoxicity.

Birth outcomes. Effects of γ-HCH administered orally to animals include stillbirths, reduced viability, and decreased body weight; in addition, some studies have suggested delays in developmental milestones. There is no evidence for teratogenic effects in animals exposed orally to γ-HCH.

In Wistar rats exposed from GD 6 through lactation to doses of 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, effects observed at 8.0 to 19.1 mg/kg/day included increased stillbirths (live birth index of 77% compared to 99% in controls), and increased neonatal mortality (postnatal day [PND] 4 viability index of 71% compared to 89% in controls) (EPA 1999c). In a 2-generation reproductive toxicity study of rats, a dose of 13.1 mg/kg/day γ-HCH in food resulted in significant reductions in the pup survival on lactation day (LD) 4; for the F1 and F2 pups, survival was 81 and 85%, respectively, compared with ≥96% for the controls (EPA 1991a). In another 2-generation rat study, F0 dams exhibited normal lactation and maternal behavior, but six F1 dams exposed to 26–28 mg/kg/day γ-HCH showed abnormal lactation and retrieving behavior, leading to death of nearly all their offspring by PND 4, and a significant (49%) reduction in the F2 PND 0–4 viability index for this group (Matsuura et al. 2005). In rats, dietary exposure to 25 mg/kg/day of γ-HCH during gestation (GDs 0–21) did not result in changes in numbers of litter or pup survivals (Srinivasan et al. 1991). 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.

Reduced pup body weights have been reported in rats and mice exposed to γ-HCH during gestation and/or lactation. Sauviat et al. (2005) observed a 21% reduction in body weight on PND 42 in rats exposed to 0.0003 mg/kg/day γ-HCH in drinking water during gestation, lactation, and growth; body weight was not affected at 0.00015 mg/kg/day. Exposure of female Wistar rats from GD 6 through lactation to doses ≥4.2 mg/kg/day resulted in decreased pup body weights (up to 18% less than controls at the mid-dose and up to 20% less than controls at the high dose), and body weight gains (16–24% less than controls at the mid-dose, and up to 40% less than controls at the high dose) in both sexes during LDs 1–11 (EPA 1999c). Similarly, body weights of the pups of both generations were significantly lower than controls in 2-generation studies of γ-HCH in rats exposed via feed at doses of 13.1 mg/kg/day (EPA 1991a) and 26.1 mg/kg/day (Matsuura et al. 2005). In the latter study, F2 female offspring also exhibited a 10%

reduction in body weight at 5.6 mg/kg/day (Matsuura et al. 2005). Administration of 30 mg/kg to pregnant C57BL/6J mice and 45 mg/kg to pregnant DBA/2J mice on GD 12 resulted in significant decreases in fetal and placental weights (Hassoun and Stohs 1996a).

No malformations were observed in the fetuses of pregnant C57BL/6J or DBA/2J mice dosed with 30 and 45 mg/kg/day γ-HCH by gastric intubation on GD 12, even though both doses caused maternal deaths (Hassoun and Stohs 1996a). 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 GDs 6–15; statistical significance was attained only at 20 mg/kg (Palmer et al. 1978). The incidence of fetuses with an extra 13th rib was statistically increased in rabbits exposed to 20 mg/kg γ-HCH by gavage during GDs 6–18 (Palmer et al. 1978). 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) (Palmer et al. 1978). No effects on embryonic development were seen in rabbits treated by gavage with 0.8 mg/kg/day γ-HCH 3 times/week for 12–15 weeks before artificial insemination (at week 15) and throughout gestation (Seiler et al. 1994).

In a 2-generation study of γ-HCH in CD rats exposed via feed to a dose of 13.1 mg/kg/day, the onset and completion of tooth eruption and completion of hair growth were delayed by 10.5, 11.6, and 24% in the high-dose F2 pups, respectively, compared to controls (EPA 1991a). In contrast, doses up to 26–28 mg/kg/day in a 2-generation reproductive toxicity study of Crj:CD(SD)IGS rats did not influence offspring developmental landmarks (pinna unfolding, incisor eruption, or eye opening) (Matsuura et al. 2005).

Male reproductive system development. Studies in rats and mice exposed to γ-HCH via oral administration during gestation and lactation show effects on the developing male reproductive system, including effects on serum hormone levels, spermatogenesis, reproductive organ weights, and testicular histopathology; effects on sexual behavior and fertility have not been seen in these studies. Serum testosterone was reduced in 7-month-old male rats exposed to 30 mg/kg/day γ-HCH on GD 15 (Dalsenter et al. 1997a) and in PND 65 male rats exposed to 6 mg/kg/day on LD 9 or 14 or 1 mg/kg/day on LDs 9–14 (Dalsenter et al. 1997b).

Lactational exposure of rats to 6 mg/kg/day on LD9 or 14 resulted in significantly reduced spermatid and sperm counts (~8–10%) at PND 140 (Dalsenter et al. 1997b). In rats exposed to 1 mg/kg/day on LDs 9–14, reduced spermatid numbers at PNDs 65 and 140 (29 and 12.8% less than controls, respectively) and reduced sperm number at PND 140 (13.2%) were observed (Dalsenter et al. 1997b). Similarly, CD-1 mice that were administered 15 or 25 mg/kg/day doses of γ-HCH in olive oil by gavage on GDs 9–16 showed reduced sperm count at ≥15 mg/kg/day on PND 60 and reduced sperm concentration at 25 mg/kg/day on PNDs 60–69 and 100 (Traina et al. 2003; Di Consiglio et al. 2009). La Sala et al. (2009) reported decreased numbers of primordial germ cells in male CD1 mouse embryos exposed to ≥15 mg/kg/day on days 8.5–11.5 post-coitum and collected on day 12.5.

Exposure of rats to 1 mg/kg/day on LDs 9–14 resulted in statistically significant reductions in relative testicular weight at PND 140 and relative epididymis weight at PND 65 (Dalsenter et al. 1997b). In another experiment by these authors, a single dose of 6 mg/kg/day on LD 9 or 14 resulted in 10% reductions in relative testicular weights on PNDs 66 and 140 (Dalsenter et al. 1997b). Traina et al. (2003) observed reduced absolute (8%) and relative (10%) testicular weights in male mouse offspring of dams exposed to 15 mg/kg/day during GDs 9–16, but not at 25 mg/kg/day; in addition, no change in testes weight was evident in 25 mg/kg/day F1 males sacrificed on PND 100. No change in testis weight was observed in PND 50 or 100 mouse offspring exposed *in utero* to 25 mg/kg/day γ-HCH on GDs 9–16 (Di Consiglio et al. 2009).

Gestational or lactational exposure to γ-HCH also resulted in histopathology changes in male reproductive organs. Dalsenter et al. (1997b) reported that microscopic examination of the testes in male rat pups exposed to 6 mg/kg/day on LD 9 or 14 showed large areas of normal tissue, but some areas had distinct changes ranging from small alterations to a pronounced effect, including necrotic changes and reductions in Leydig cell numbers and spermatogenesis. However, there were no significant effects on sexual behavior or fertility in the group exposed to 1 mg/kg/day on LDs 9–14 or to 6 mg/kg on LD 9 or 14 (Dalsenter et al. 1997b). In CD-1 mice administered 15 or 25 mg/kg/day doses of γ-HCH by gavage on GDs 9–16, testicular histological alterations (increased number and size of Leydig cells) were seen along with increased number of epididymal sperm with chromatin abnormalities at ≥15 mg/kg/day on PND 60 and altered testicular germ cell distribution at 25 mg/kg/day on PNDs 60 and 100 (Traina et al. 2003). 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).

Agrahari et al. (2019) showed that gestational exposure to γ-HCH could influence reproductive hormone levels in male offspring. Pregnant Wistar rats were administered γ-HCH by gavage in corn oil at doses of 0 or 0.25 mg/kg/day on GDs 5–21. Serum hormone levels were evaluated in male offspring at 9, 18, and 27 weeks of age. In offspring evaluated at 9 weeks of age, decreased serum levels of testosterone (16% compared to controls) and growth hormone (15%) were observed, while serum LH (22%) and FSH (18%) levels were significantly increased. Offspring evaluated at 18 and 27 weeks of age exhibited no statistically significant changes in serum hormone levels (Agrahari et al. 2019). In other groups of male offspring that were exposed similarly during gestation and then treated by gavage with 5 mg/kg/day for 5 days at 9, 18, or 27 weeks of age, significant hormone level changes were seen in all groups, including decreased testosterone and growth hormone, and increased LH and FSH (Agrahari et al. 2019).

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 developmental 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, but 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.

A multigeneration reproduction toxicity study in Crj:CD(SD)IGS rats exposed to γ-HCH via diet showed a delay of 1.5 days in preputial separation in F1 animals exposed to 26.1 mg/kg/day (Matsuura et al. 2005). In contrast, the mean day of preputial separation was not affected by treatment in Wistar rat pups exposed to doses up to 10.5–19.1 mg/kg/day from GD 6 through lactation (EPA 1999c) or in mouse pups exposed to doses up to 25 mg/kg/day during GDs 9–16 (Traina et al. 2003).

Female reproductive system development. There are few data on the effects of γ-HCH on development of the female reproductive tract, but available studies show effects in mice exposed during gestation to oral doses of 15 mg/kg/day. La Sala et al. (2009) exposed CD-1 mice to 15 or 30 mg/kg/day γ-HCH by gavage for 3 days during gestation (days 8.5–11.5 post-coitum) and collected embryos 1 day after the last dose. The female embryos exhibited reduced numbers of primordial germ cells in the ovaries at both doses (La Sala et al. 2009). In female pups of CD mice exposed to 15 mg/kg/day γ-HCH on GDs 9–16 and sacrificed on PND 22, significant increases in uterine weight (13–17% higher than controls) were

observed, while those pups sacrificed on PND 60 exhibited decreased oocyte diameter (21%) in primary follicles (Maranghi et al. 2007). Exposed female pups also developed vaginal patency earlier than controls; by PND 33, 93% of female pups exposed to 15 mg/kg/day and 36% of control females exhibited complete vaginal opening (Maranghi et al. 2007). No effect on vaginal opening was observed in Wistar rat pups exposed to doses as high as 10.5–19.1 mg/kg/day from GD 6 through lactation (EPA 1999c) or in CD-1 mouse pups exposed on GDs 9–16 to doses up to 25 mg/kg/day (Traina et al. 2003). In contrast, vaginal opening was delayed by 1.4 days in Crj:CD(SD)IGS rats exposed to 26.1 mg/kg/day γ-HCH via diet in a multigeneration reproductive toxicity study (Matsuura et al. 2005).

Systemic development. Oral exposure to γ-HCH during gestation and/or lactation has resulted in significant effects on liver, thymus, and spleen weights in the offspring, and on cardiac development. Srinivasan et al. (1991) observed significant increases in pup relative liver weight when rat dams were exposed to 25 mg/kg/day during gestation and lactation or during lactation only (LDs 0–28). When administered by gavage on GD 12 to pregnant C57BL/6J mice, a dose of 30 mg/kg γ-HCH resulted in significant decreases in fetal thymic weight; fetal body weight was also reduced (Hassoun et al. 1996). At doses of 26–28 mg/kg/day administered in feed to rats through 2 generations, treatment-related decreases in absolute and relative thymus (13–31%) and spleen (13–32%) weights were observed in both generations (Matsuura et al. 2005).

Development of the heart was examined in a study of female Sprague-Dawley rats exposed to very low doses (0.000076, 0.00015, or 0.0003 mg/kg/day) of γ-HCH in drinking water (0.5, 1, and 2 µg/L, respectively) prior to mating; during mating, gestation, and lactation; and for 3 weeks post-weaning (Sauviat et al. 2005). The pups were sacrificed at 6 weeks of age for evaluation of heart weight, morphometry, and lipid content and electrophysiology of dissected left ventricular papillary muscles. Heart weights and lipid content of exposed rats did not differ from control. Morphometry analysis showed that hearts of pups in the 0.0003 mg/kg/day group had a 9% increase in heart width (relative to controls), but no significant change in length, with a corresponding 9% decrease in length-to-width ratio. At this dose, offspring heart morphology was described as rounder and "cherry like." The study authors reported that hearts of treated offspring showed hypertrophied areas with thinning of the left ventricular wall and few developed papillary muscles. Histopathological examination in offspring exposed to 0.0003 mg/kg/day showed that the heart tissue muscle bundles and layers were unorganized and dissociated, with large hemorrhagic interspaces and dispersion of cell nuclei, destruction of fibroblasts, and dispersion and disorganization of collagen bundles, compared to control heart muscle. Histopathology was not assessed in the groups exposed to 0.000076 or 0.00015 mg/kg/day.

Electrophysiology changes were also evident in the dissected left ventricular muscles of exposed pups. Action potential durations were unchanged at 0.000076 mg/kg/day, but the plateau was shortened moderately at 0.00015 mg/kg/day, and significantly shortened at 0.0003 mg/kg/day. At 0.0003 mg/kg/day, the slow repolarizing phase was also significantly shortened. The effects of γ-HCH on action potential durations were mitigated by addition of quinidine or E-4031 (blockers of the rapid delayed rectifier potassium current or I_{Kr}) to the test solution, indicating that γ-HCH may act directly on the I_{Kr}. Sauviat et al. (2005) noted that the I_{Kr} channel is involved in long QT syndrome (a disorder that increases the risk of cardiac arrhythmias).

These authors conducted a related study examining whether the cardiac effects could be induced by paternal exposure to γ-HCH in drinking water (Sauviat et al. 2007). In this study, male rats were exposed to a concentration of 2 μg/L for an unspecified "chronic" duration prior to mating with untreated females. The lack of information on exposure duration in the males precluded estimation of doses. In offspring sacrificed at 6 weeks of age, there were no effects on heart weight or shape or electrophysiology, but there were histopathology changes in the hearts similar to those reported by Sauviat et al. (2005) at the same water concentration.

Developmental neurotoxicity. Convulsions and seizures have been observed in offspring of rats exposed to γ-HCH during gestation and/or lactation. Johri et al. (2008) observed convulsions in 8/10 male rat pups that had been exposed during gestation (GDs 5–21) to 0.25 mg/kg/day γ-HCH and then received a single gavage dose of 30 mg/kg on PND 45. In pups exposed only during gestation, no convulsions were observed (Johri et al. 2008). Epileptiform seizures were reported in male rats fed milk from dams that were gavaged with 20 mg/kg/day γ-HCH on PNDs 3–15 (Albertson et al. 1985).

In a developmental neurotoxicity study, Han Wistar rats were exposed to 0, 10, 50 or 120 ppm γ-HCH in the diet from GD 6 through LD 10 (EPA 1999c). 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 F1 offspring were evaluated for functional observational battery, motor activity, auditory startle response, learning and memory, and brain endpoints (weight, histology, and morphometrics) on postpartum days 11 and 65. The offspring showed increased motor activity (both sexes) and decreased habituation of motor activity (females) at the two highest dose levels. Reduced auditory startle response habituation was observed at the high dose in both sexes on day 28 and day 60 postpartum (EPA 1999c). No significant changes in brain weight, morphometry, or histology were detected in the pups. This study was classified as an unacceptable developmental neurotoxicity study by EPA (2000a) because there was

no laboratory validation of the neurobehavioral tests and the number of animals (six per dose level) was insufficient.

Increased motor activity was also reported in rat pups exposed during gestation only. Exposure on GDs 5–21 to doses ≥0.25 mg/kg/day resulted in increased locomotor activity (increases in distance traveled, ambulatory time, horizontal count, stereotypic time, and stereotypic movement burst) in 3-week-old rat pups, and some of the changes (increased distance traveled) persisted through 9 weeks of age (Johri et al. 2007). At a dose of 0.125 mg/kg/day, distance traveled was increased at 3 weeks of age, but not at subsequent evaluations (Johri et al. 2007). Srivastava et al. (2019) observed no significant changes in spontaneous locomotor activity or spatial memory (Y-maze activity) in 12-week-old male rat pups whose mothers were exposed by gavage from GD 5 through GD 21 to 0.25 mg/kg/day γ-HCH. However, other groups of pups similarly exposed *in utero* and then rechallenged at 12 weeks of age with 21 daily gavage doses of 2.5 mg/kg/day γ-HCH exhibited significantly reduced spontaneous locomotor activity (reductions in distance travelled, moving time, numbers of rearings, and stereotypic counts, along with significantly increased resting time) and spatial memory effects (significant reduction in percent alterations in Y-maze testing) (Srivastava et al. 2019). Acquisition of a passive avoidance task was improved in 15-day-old rat pups that were treated with γ-HCH by gavage as either a single 20 mg/kg dose or 7-day repeated 10 mg/kg/day doses (Rivera et al. 1998). Exposure to the single 20 mg/kg dose resulted in a decrease in motor activity, while repeated exposure to the lower dose increased motor activity in this study (Rivera et al. 1998).

Neurobehavioral testing of F1 offspring exposed to doses up to 26–28 mg/kg/day in a 2-generation reproductive toxicity study showed no effects in tests of reflex, sensory function, surface righting reflex, corneal reflex, startle response, pain response, or mid-air righting reflex at 4–6 weeks of age (Matsuura et al. 2005). In addition, no effects were observed in the open field test, rotarod test, or pole climbing test (Matsuura et al. 2005).

Studies of neurotransmitter levels in rats exposed to γ-HCH showed that the effects depended on the treatment schedule and brain region (Rivera et al. 1991, 1998). In suckling Wistar rats treated once with 20 mg/kg γ-HCH by gavage at PNDs 8, 15, 22, or 29, regional changes in brain noradrenaline, serotonin, and the dopamine metabolite, 3,4-dihydroxyphenyl- acetic acid (DOPAC) levels were seen, with differential effects depending on age at the time of exposure (Rivera et al. 1991). Similarly, Rivera et al. (1998) observed different patterns (e.g., ratios of 5-HIAA/serotonin and DOPAC/dopamine) in brain monoaminergic levels in rat pups after exposure on PND 15 to a single 20 mg/kg dose or 7 consecutive

daily doses of 10 mg/kg/day γ-HCH. The patterns suggested that monoaminergic turnover was increased by the single dose but decreased by repeated exposure at lower doses (Rivera et al. 1998).

A study of rats exposed to γ-HCH in drinking water at very low doses (0.000055–0.00011 mg/kg/day) for 12 days prior to mating and through gestation and lactation examined effects of treatment on wake-sleep cycle in male offspring at 14 weeks of age (Breton et al. 2005). Sleep cycle was analyzed by EEG in three phases: wakefulness, slow wave sleep, and paradoxical sleep, and there were no treatment-related changes in the sleep cycle. Spectral electrocorticographic analysis showed slight changes in brain activity relative to controls (increased relative energy in the 11–15 Hz frequency during wakefulness and slow wave sleep, and increased activity in the 7–15 Hz range in all sleep phases) in both exposure groups, but the changes did not show dose-dependence. No histopathological effects were reported in the brain at any dose (Breton et al. 2005).

Srivastava et al. (2019) examined transmission electron ultrastructural microscopy of the brains in 15-week old male rat pups whose mothers were exposed by gavage from GD 5 through GD 21 to 0.25 mg/kg/day γ-HCH. Although neurobehavioral changes were not seen in these pups, ultrastructural changes were detected in the hippocampus and substantia nigra of exposed pups, including moderately distorted mitochondria, demyelinated neurons, and autophagic vesicles with damaged cytoplasmic contents (Srivastava et al. 2019). In another experiment by these authors, electron microscopy of the hippocampus and substantia nigra showed loss of mitochondrial integrity (loss of cristae and number), severe loss of synaptic structure, severe demyelination, and highly condensed nuclei with cytoplasmic content showing necrotic effects after similar prenatal exposure followed by a rechallenge exposure at 12 weeks of age (21 days at 2.5 mg/kg/day) (Srivastava et al. 2019).

Mechanisms. Superoxide production, lipid peroxidation, and deoxyribonucleic acid (DNA) single-strand breaks were increased in fetal and placental tissues, and lipid peroxidation markers were increased in maternal sera and amniotic fluid 48 hours after administration of a single dose of 30 mg/kg γ-HCH to pregnant mice on GD 12 (Hassoun and Stohs 1996b; Hassoun et al. 1996). Significant increases in lipid peroxidation also occurred in fetal livers collected on GD 18. 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 mice.

There is evidence that the developmental neurotoxicity effects of γ-HCH may be mediated by metabolites and involve alterations in the blood-brain barrier. Johri et al. (2007) detected dose-dependent increases in brain cytochrome P450 (CYP1A1, 1A2, 2B1, 2B2, and 2E1) protein expression and mRNA, and CYP-

dependent enzyme (EROD, PROD, and NDMA-d) activities in F1 offspring exposed to 0.0625–0.25 mg/kg/day γ-HCH. The enzyme changes persisted longer at the high dose, correlating with the effects on locomotor activity and suggesting that metabolites of γ-HCH may be responsible for this effect (Johri et al. 2008). γ-HCH exposure causes functional impairment of the developing blood brain barrier in young rats (Gupta et al. 1999). The integrity (impermeability) of the blood brain barrier 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).

δ-HCH. Epidemiological studies that have examined relationships between δ-HCH in maternal or fetal blood or tissues and developmental outcomes are shown in Table 2-19. A small case-control study in India reported positive associations between fetal growth restriction and higher δ-HCH concentrations in maternal and umbilical cord blood, but not with concentrations in placenta (Siddiqui et al. 2003). No association between infant birth size and δ-HCH in placental tissue was observed in a cross-sectional study in India (Anand and Taneja 2020). No studies of developmental outcomes in animals exposed to δ-HCH by any exposure route were located.

Technical HCH or Unspecified Isomers of HCH. 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 GD 9, but fetal development was normal (Dikshith et al. 1990). Srivastava and Raizada (2000) further studied the prenatal effect of orally administered technical-grade HCH. While mice exposed to HCH during the preimplantation period (GDs 2–6) did not show fetolethality, exposure during the post-implantation period (GDs 6–12) 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 GDs 6–15 failed to produce teratogenic effects in rats (Khera et al. 1979). 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 with 10 mg technical-grade HCH/kg/day for 60 days beginning at birth (Nagaraja and Desiraju 1994).

Table 2-19. Summary of Epidemiological Studies of δ-HCH and Total HCH Exposure and Developmental Effects

Reference, study type, and population	Outcome evaluated	Isomer	Biomarker	Mean concentration (unless otherwise noted)	Result
Siddiqui et al. 2003 Case-control, 30 mothers of infants with fetal growth restriction (intrauterine growth retardation), 24 mothers of normal weight infants, India	**Fetal growth restriction**	**δ-HCH**	Maternal blood	2.14±1.94	↑
			Placenta	2.92±3.99	↔
			Cord blood	4.51±4.63	↑
Anand and Taneja 2020 Cross-sectional, 90 mother-infant pairs, India	Birth weight	δ-HCH	Placenta tissue	1.18–24.4µg/L (range)	↔
	Birth length				↔
	Head circumference				↔
	Ponderal index				↔
Yin et al. 2021 Case-control, 119 mothers delivering infants or electively terminating pregnancies with neural tube defects and 119 controls, China	Neural tube defects	δ-HCH	Cord tissue	0.81 ng/g dry weight (cases) (median) 0.57 (controls)	↔

↑ = association with increase; ↔ = no association; HCH = hexachlorocyclohexane

2.18 OTHER NONCANCER

Epidemiology Studies. Several case-control studies (Berg et al. 2021; Charles et al. 2022; Daniels et al. 2018; Han et al. 2020; Li et al. 2016; Rylander et al. 2015; Tawar et al. 2022; Tyagi et al. 2021; Zong et al. 2018) and cross-sectional studies (Arrebola et al. 2013; Everett and Matheson 2010; Gasull et al. 2012; Schwarz et al. 2021; Ukropec et al. 2010) evaluated associations between diabetes and exposure to HCH isomers (see Table 2-20). There were no associations with serum levels of α-, γ-, or δ-HCH isomers and type 2 diabetes reported in a case-control study of 723 cases of Chinese type 2 diabetes patients compared to control subjects, while higher mean serum levels of β-HCH and with total HCH levels were associated with type 2 diabetes (Li et al. 2016); significant interactions with several ADIPOQ (gene encoding adiponectin) genotypes were reported for the β-HCH isomer. No association was observed with type 2 diabetes and serum levels of β-HCH or γ -HCH in case-control studies in Norway (Berg et al. 2021; Charles et al. 2022) or in a case-cohort study in France (Magliano et al. 2021). However, nested case-control studies reported an association between serum and plasma β-HCH levels and increased incidences of type 2 diabetes in American nurses (Zong et al. 2018), Norwegian women (Rylander et al. 2015), and in Chinese adults (Han et al. 2020). One nested case-control study comparing South Asians and European whites living in London determined a significant association between plasma concentrations of β-HCH and increased prevalence of diabetes in a Tamil-descent population, while an elevated, but not significant, association was reported in a Telugu-descent population with much higher plasma concentrations of β-HCH (Daniels et al. 2018). Tawar et al. (2022) conducted a case-control study in India and reported a positive association between levels of δ- HCH in adipose tissue and type 2 diabetes, but no association between α-, β-, or γ-HCH and type 2 diabetes was observed.

In cross-sectional studies evaluating β-HCH, there was no association between the prevalence of diabetes and levels in adipose tissue (Arrebola et al. 2013), and no association between diabetes and pre-diabetes and serum levels (Everett and Matheson 2010; Gasull et al. 2012; Schwarz et al. 2021). Another cross-sectional study similarly found no association with diabetes; however, an association was seen between serum β-HCH and increased incidence of pre-diabetes (Ukropec et al. 2010). Additionally, a cross-sectional study using NHANES data reported an association with serum β-HCH and increased incidence of diabetes without nephropathy, but no association in adults with both diabetes and nephropathy (Everett and Thompson 2015). There was no relationship between serum β-HCH and insulin sensitivity and secretion indicators (Lee et al. 2017), nor were there associations with leptin and insulin levels and insulin resistance (Burns et al. 2014).

Table 2-20. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Other Noncancer Effects

Reference, study type, and population	Outcome evaluated	Isomer	Biomarker	Concentration	Result
Arrebola et al. 2013 Cross-sectional, 386 patients undergoing noncancer-related surgery, Spain	Type 2 diabetes	β-HCH	Adipose	>16.81 ng/g lipid	↔
Berg et al. 2021 Case-control, 44 cases of Type 2 diabetes and 44 age-matched controls, mean age 52 years, Norway	Type 2 diabetes	β-HCH	Serum	12.8 ng/g lipid (cases) (median, prediagnostic sample) 13.9 (controls)	↔
		γ-HCH		3.36 (cases) 4.39 (controls)	↓
Charles et al. 2022 Nested case-control, 116 cases of Type 2 diabetes, 139 controls, mean ages 48 and 45 years, respectively, Norway	Type 2 diabetes	β-HCH	Serum	14.0-36.7 ng/g lipid (cases) (mean, prediagnostic samples) 12.9-31.8 (controls)	↔
Han et al. 2020 Case-control, 158 cases of Type 2 diabetes, 158 controls, China	**Type 2 diabetes**	β-HCH	Serum	1351 pg/mL (cases) (GM) 695 (controls)	↑
Magliano et al. 2021 Case-cohort, 200 cases of type 2 diabetes and 553 controls, mean ages 51 and 47 years, respectively, France	Type 2 diabetes	β-HCH	Serum	811 ng/L (cases) (median) 513 (controls)	↔
		γ-HCH		18.4 ng/L (cases) (median) 14.7 (controls)	↔
Li et al. 2016 Case-control, 723 cases and 723 controls, mean age 62 years, China	**Type 2 diabetes**	α-HCH	Serum	0.012 ng/mL (cases) (GM) 0.011 (controls)	↔
		β-HCH		0.575 (cases) 0.266 (controls)	↑[a]
		γ-HCH		0.020 (cases) 0.018 (controls)	↔
		δ-HCH		0.068 (cases) 0.060 (controls)	↔
		Total HCH		NR	↑

Table 2-20. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Other Noncancer Effects

Reference, study type, and population	Outcome evaluated	Isomer	Biomarker	Concentration	Result
Zong et al. 2018 Nested case-control, 793 cases and 793 controls participating in Nurse Health Study II, age 32–52 years, United States	**Type 2 diabetes**	**β-HCH**	Serum	14.3 ng/g lipid (median) (cases) 9.84 (controls)	↑ (trend)
Rylander et al. 2015 Nested case-control, 106 cases and 106 control women, age 30–70 years, Norway	**Type 2 diabetes**	**β-HCH**	Plasma	20.3 ng/g lipid (mean) (cases) 10.0 (controls)	↑
Daniels et al. 2018 Nested case-control, 73 adults of Tamil descent and 47 adults of Telugu descent, >21 years old, United Kingdom	**Diabetes in Tamil population**	**β-HCH**	Plasma	≥50.58 ng/g lipid	↑
	Diabetes in Telugu population			≥369.30 ng/g lipid	↔
Everett and Matheson 2010 Cross-sectional, 3,414 adults ≥20 years old, NHANES, United States	Diabetes	β-HCH	Serum	>9.36 ng/g lipid	↔
	Pre-diabetes				↔
Gasull et al. 2012 Cross-sectional, 886 adults 18–74 years, Spain	Diabetes	β-HCH	Serum	>1.547 ng/mL (4^{th} quartile cutoff)	↔
	Pre-diabetes				↔
Ukropec et al. 2010 Cross-sectional, 2,047 adults 21–75 years old, Slovakia	Diabetes	**β-HCH**	Serum	83–781 ng/g lipid (5th quintile)	↔
	Pre-diabetes				↑
Schwarz et al. 2021 Cross-sectional, 200 adults 75–76 years old, Germany	Known diabetes	β-HCH	Serum	0.12 ug/L (median)	↔
	Newly diagnosed diabetes			0.10	↔
	Pre-diabetes			0.08	↔
Everett and Thompson 2015 Cross-sectional, 2,992 adults ≥20 years old, NHANES (1999–2004), United States	**Diabetes without nephropathy**	**β-HCH**	Serum	≥0.1018 ng/g	↑
	Diabetes with nephropathy	β-HCH	Serum	≥0.1018 ng/g	↔

Table 2-20. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Other Noncancer Effects

Reference, study type, and population	Outcome evaluated	Isomer	Biomarker	Concentration	Result
Tawar et al. 2022 Case-control, 70 cases of type 2 diabetes and 70 age-, gender-, and BMI- matched controls, mean ages 43.65 (control) and 44.9 (cases) years, India	**Type 2 diabetes**	α-HCH	Adipose tissue	0.90 ng/g (cases) 0.41 (controls)	↔
		β-HCH		1.25 (cases) 0.89 (controls)	↔
		γ-HCH		1.17 (cases) 0.86 (controls)	↔
		δ-HCH		2.70 (cases) 0.79 (controls)	↑
Lee et al. 2017 Cross-sectional, 200 adults >30 years old, Korea	Insulin sensitivity and secretion indicators	β-HCH	Serum	157.97 pg/mL (4th quartile cutoff)	↔
Burns et al. 2014 Cohort, 318 boys, age 8–9 years, Russia	Leptin	β-HCH	Serum	165 ng/g lipid (median)	↔
	Insulin				↔
	Insulin resistance				↔
Gasull et al. 2018 Cross-sectional, 860 adults 18–74 years old, Spain	**Unhealthy metabolic phenotype**[b]	**β-HCH**	Serum	≥0.671 ng/mL (4th quartile)	↑
Reina-Pérez et al. 2023 Cross-sectional, 117 adult surgical patients, median age 44 years, Spain	**Metabolic syndrome**	**γ-HCH**	Adipose tissue	11.44 ng/g (median)	↑
	Elevated waist circumference				↑
	Elevated blood pressure				↑
	Elevated fasting blood glucose, triglycerides, or HDL cholesterol				↔

Table 2-20. Summary of Epidemiological Studies of Hexachlorocyclohexane (HCH) Exposure and Other Noncancer Effects

Reference, study type, and population	Outcome evaluated	Isomer	Biomarker	Concentration	Result
Zhang et al. 2023 Cross-sectional, 1,996 adult residents of Wuhan, mean age 44.8 years, China	**Metabolic syndrome**	**β-HCH**	Serum	34.5 ng/g lipid (median)	↑
Lee et al. 2016 Cohort, 214 children 7–9 years old, Korea	Measures of metabolic syndrome	β-HCH	Serum	6.13 ng/g lipid (median)	↔
Mustieles et al. 2017 Cross-sectional and 10-year longitudinal, 387 noncancer surgical patients (at baseline), median age 52 years; 154 without metabolic disease at baseline followed for 10 years, median age 42 years, Spain	**Metabolically compromised[c] at baseline**	**β-HCH**	Adipose tissue	10.6 ng/g lipid (median)	↑
	Metabolically compromised[c] at follow up			6.9	↑
Wang et al. 2021a Cross sectional, 10 women, mean age 37 years, China	Serum lipoprotein a, total cholesterol, triglycerides, high- and low-density lipoprotein cholesterol	α-HCH	Estimated dietary intake	0.471 ng/kg/day	↔
		β-HCH		0.185	↔
		γ-HCH		0.043	↔
		δ-HCH		0.104	↔

[a]Significant interactions with several ADIPOQ genotypes.
[b]Unhealthy metabolic phenotype was defined as exhibiting two or more of the following: hypertension, hypertriglyceridemia, low HDL cholesterol, hyperglycemia, insulin resistance, or systemic inflammation.
[c]Metabolically compromised was defined as exhibiting one or more of the following: type 2 diabetes, hypertension, hypertriglyceridemia, or low HDL cholesterol.

↑ = association with increase; ↓ = association with decrease (inverse association); ↔ = no association; GM = geometric mean; HDL = high-density lipoprotein; NHANES = National Health and Nutrition Examination Survey

Gasull et al. (2018) reported a positive association between unhealthy metabolic phenotypes (defined as exhibiting at least two of the following: hypertension, hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol, hyperglycemia, insulin resistance, or systemic inflammation) and serum β-HCH levels. A study of adult noncancer surgical patients also showed an association between β-HCH levels in adipose tissue and increased likelihood of being metabolically compromised (exhibiting type 2 diabetes, hypertension, hypertriglyceridemia, or low HDL cholesterol) at baseline and at a 10-year follow-up (Mustieles et al. 2017). A cross-sectional study of 1,996 adults in China reported a positive association between β-HCH in serum and metabolic syndrome (Zhang et al. 2023). There was no association between serum β-HCH levels and measures of metabolic syndrome in a cohort of 214 Korean children between 7 and 9 years of age (Lee et al. 2016).

In a cross-sectional study of surgical patients in Spain, associations between metabolic syndrome, elevated waist circumference, and elevated blood pressure with γ-HCH levels in adipose tissue were observed (Reina-Pérez et al. 2023). In a small cross-sectional study of 10 women in China, Wang et al. (2021) reported no association between any of the HCH isomers and serum levels of lipids.

2.19 CANCER

Epidemiological Studies. Epidemiological studies of HCH isomers and cancer are shown in Table 2-21. Studies shown in the table include only those that accounted for at least one potential confounding variable (i.e., studies reporting only univariate analyses were excluded). In addition, only the most recent analysis of a given cohort or case-control population is shown in the table.

Table 2-21. Summary of Epidemiological Studies Evaluating Possible Associations between Hexachlorocyclohexane Exposure and Risk of Selected Cancer Types

Cancer type	Isomer	Association[a]	No association[b]
Non-Hodgkin's lymphoma	Beta (β)	Bassig et al. 2020; Viel et al. 2011	Brauner et al. 2012; Cantor et al. 2003; Cocco et al. 2008
	Gamma (γ)	Alavanja et al. 2014[c]; Kachuri et al. 2020	Cocco et al. 2008; De Roos et al. 2021; Viel et al. 2011
Multiple myeloma	Beta (β)	Weber et al. 2018	
	Gamma (γ)		Presutti et al. 2016

Table 2-21. Summary of Epidemiological Studies Evaluating Possible Associations between Hexachlorocyclohexane Exposure and Risk of Selected Cancer Types

Cancer type	Isomer	Association[a]	No association[b]
Leukemia	Alpha (α)	Rafeeinia et al. 2023;	
	Beta (β)	Rafeeinia et al. 2023;	Cocco et al. 2008
	Gamma (γ)	Purdue et al. 2007; Rafeeinia et al. 2023	
Colon/colorectal	Alpha (α)		Howsam et al. 2004
	Beta (β)	Lee et al. 2018a	Howsam et al. 2004; Park et al. 2021
	Gamma (γ)		Howsam et al. 2004; Purdue et al. 2007
Female breast cancer	Alpha (α)	Miao et al. 2021	
	Beta (β)	Arrebola et al. 2015b Miao et al. 2021; Waliszewski et al. 2005	Hoyer et al. 1998; Lopez-Carrillo et al. 2002; McCready et al. 2004; Raaschou-Nielsen et al. 2005[d]; Ward et al. 2000; Wielsoe et al. 2017; Xu et al. 2010
	Gamma (γ)	Ibarluzea et al. 2004[e]	Hoyer et al. 1998; Miao et al. 2021
	Delta (δ)	Miao et al. 2021	
Prostate cancer	Alpha (α)		Pi et al. 2016[d]
	Beta (β)	Kumar et al. 2010; Xu et al. 2010	Aronson et al. 2010; Lim et al. 2017; Pi et al. 2016; Sawada et al. 2010
	Gamma (γ)	Band et al. 2011	Barry et al. 2011; Koutros et al. 2011; Pi et al. 2016
Lung cancer	Gamma (γ)	Purdue et al. 2007	
Hepatocellular carcinoma	Beta (β)	Zhao et al. 2012	
Bladder cancer	Gamma (γ)		Purdue et al. 2007
Endometrial cancer	Beta (β)		Sturgeon et al. 1998
Melanoma	Gamma (γ)		Purdue et al. 2007
Soft tissue sarcoma	Gamma (γ)		Pahwa et al. 2011
Pancreatic cancer	Beta (β)		Porta et al. 2022
Brain cancer	Alpha (α)		Yousefi et al. 2022
	Beta (β)	Yousefi et al. 2022	
	Gamma (γ)	Yousefi et al. 2022	
Testicular germ cell tumors	Beta (β)		McGlynn et al. 2008

Table 2-21. Summary of Epidemiological Studies Evaluating Possible Associations between Hexachlorocyclohexane Exposure and Risk of Selected Cancer Types

Cancer type	Isomer	Association[a]	No association[b]
Thyroid cancer	Alpha (α)	Salimi et al. 2023	
	Beta (β)	Salimi et al. 2023	Deziel et al. 2021; Lerro et al. 2018
	Gamma (γ)	Lerro et al. 2021; Salimi et al. 2023	

[a]Statistically significant positive association.
[b]No statistically significant positive association.
[c]Follicular B-cell subtype.
[d]Statistically significant inverse association.
[e]Among post-menopausal women only.

The available studies provide evidence for an association between exposure to HCH and NHL, and suggestive evidence for associations with some other cancer types (see Table 2-21). The strongest evidence for an association between HCH exposure and NHL comes from a prospective cohort study of 54,306 pesticide applicators (the Agricultural Health Study) in Iowa and North Carolina (Alavanja et al. 2014). Cohort members were enrolled between 1993 and 1997 and followed through 2011. At enrollment and 5 years later, the subjects filled out questionnaires about use of specific pesticides, including frequency and duration of γ-HCH use. A total of 523 incident cases of NHL were observed over 803,140 person-years of follow up. The risk of incident NHL was increased with total days of γ-HCH exposure and with intensity-weighted total days of exposure after adjustment for confounders identified in the NHL literature and for herbicide use. Analysis by subtype of NHL showed the increased risk with HCH exposure to be limited to the follicular B-cell subtype (Alavanja et al. 2014).

Three case-control studies nested within prospective cohort studies of populations without known sources of HCH exposure examined associations between NHL and prediagnostic blood or adipose tissue levels. In an analysis of 167 cases and 167 controls from three prospective cohort studies (>150,000 subjects) in Shanghai and Singapore, Bassig et al. (2020) observed a positive association between NHL and blood levels of β-HCH measured approximately 7 years prior to diagnosis. In contrast, Cantor et al. (2003) observed no association between NHL and exposure among 74 cases and 157 controls from a large cohort (25,802 participants) in Maryland. In this study, serum concentrations of β-HCH were measured in 1974, and cases were identified through 1994. Importantly, the blood concentrations of β-HCH were markedly higher in the study by Bassig et al. (2020) (median among cases was 5,670 ng/g lipid) than in the study by Cantor et al. (2003) (mean among cases was 139.9 ng/g lipid). Finally, Brauner et al. (2012) did not

observe a significant association between NHL and β-HCH in adipose tissue among 256 cases and 256 referents in a cohort of 57,053 participants in Denmark. Adipose samples were collected at enrollment between 1993 and 1997 and cases were identified through 2008; the median adipose concentration of β-HCH among cases was 59 ng/g. A large, pooled case-control study provides support for the association between NHL and HCH exposure. Kachuri et al. (2020) pooled data across three population-based, case-control studies in the United States and Canada (North American Pooled Project). The odds of NHL were increased with self-reported exposure to γ-HCH in analyses of 1,690 cases and 5,131 controls (Kachuri et al. 2020). In contrast, another pooled case-control study that included 4,373 cases and 4,373 controls from studies in the United States, Canada, and Italy, found no association between self-reported exposure to γ-HCH and increased odds of NHL or any individual subtype (De Roos et al. 2021).

Other epidemiological studies have reported positive associations between β- or γ-HCH in blood or qualitative exposure to γ-HCH and multiple myeloma, leukemia, colorectal cancer, female breast cancer, prostate cancer, lung cancer, thyroid cancer, brain cancer, and hepatocellular carcinoma (see Table 2-21). However, the evidence for these cancer types is relatively weak. In an earlier analysis of cancer incidence in the Agricultural Health Study (cohort of 57,311 pesticide applicators), increased risks of incident leukemia and lung cancer were observed among subjects with any self-reported use of γ-HCH compared with those who never used it (Purdue et al. 2007). In a case-control study of children in Iran, Rafeeinia et al. (2023) reported an association between serum levels of α-, β-, and γ-HCH and acute lymphoblastic leukemia (ALL). Lerro et al. (2021) examined incident thyroid cancer in subjects in the Agricultural Health Study cohort and observed an increase in the hazard ratio among subjects who reported ever use of lindane (compared with those reporting no lindane use) after a relatively brief follow-up period of 5 years after enrollment. Band et al. (2011) reported an association between γ-HCH exposure assessed through a job-exposure matrix and increased risk of prostate cancer in a study of 1,153 cases and 3,999 controls. Many of the studies reporting positive associations between multiple myeloma, colorectal cancer, hepatocellular carcinoma, breast cancer, prostate cancer, or thyroid cancer and HCH exposures (Arrebola et al. 2015b; Ibarluzea et al. 2004; Kumar et al. 2010; Lee et al. 2018a; Miao et al. 2021; Salimi et al. 2023; Waliszewski et al. 2005; Weber et al. 2018; Xu et al. 2010; Zhao et al. 2012) are case-control studies in which exposure was assessed using concentrations of HCH in blood or adipose samples collected after disease onset. The lack of temporal relationship between exposure and outcome render these studies of uncertain utility for hazard identification.

Epidemiological studies published to date have not shown any associations between exposure to HCH and cancers of the bladder, endometrium, pancreas, and testicular germ cells, or melanoma and soft tissue sarcoma (see Table 2-21).

α-HCH. Increased incidences of neoplastic nodules in the liver, hepatomas, and/or hepatocellular carcinomas were reported in several strains of mice exposed to doses between 13 and 95 mg/kg/day for 16–36 weeks (Hanada et al. 1973; Ito et al. 1973, 1976; Nagasaki et al. 1975; Tryphonas and Iverson 1983; Tsukada et al. 1979). No evidence of liver carcinogenicity was reported in Wistar rats exposed to 45 mg/kg/day α-HCH in the diet for 24 weeks (Nagasaki et al. 1975), but a dose of 70 mg/kg/day for 48 weeks resulted in liver tumors (Ito et al. 1975). Ito et al. (1975) also reported an increased incidence of hepatocellular carcinomas in male rats exposed to α-HCH in the diet at ≥70 mg/kg/day for 72 weeks.

In studies of α-HCH tumor promotion, mixed results were observed. In rats, 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). A study of γ-glutamyltranspeptidase-positive liver foci in rats pretreated with the tumor initiator *N*-nitrosomorpholine showed that administration of α-HCH at 20 mg/kg/day in food for 49 weeks increased the volume fraction of positive foci, largely by reducing apoptosis (Luebeck et al. 1995). Schröter et al. (1987) reported significant increases in the number and areas of preneoplastic hepatic foci in female Wistar rats treated with doses ≥2 mg/kg/day in the diet.

β-HCH. Animal studies of β-HCH carcinogenicity are limited by short duration of exposure, concurrent mortality, and/or reporting limitations. β-HCH did not increase liver tumor incidences in Wistar rats exposed to 35 or 70 mg/kg/day in the diet for 24 or 48 weeks (Ito et al. 1975), but this study was hampered by significant mortality. No increase in liver tumor incidence was noted 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, in a longer study, Thorpe and Walker (1973) reported an increased incidence of hepatocellular carcinomas in male CF1 mice and a significant increase in other (unspecified) tumors in female CF1 mice exposed to 34 mg/kg/day in the diet for 104 weeks. In this study, significant mortality occurred early in the study (12% of males and 25% of females died within 3 months).

β-HCH, at a single oral dose of 100 mg/kg/day, did not induce an increase in the number or size of preneoplastic hepatic foci in a two-stage study using female Wistar rats dosed with phenobarbital as a promoting agent (Schröter et al. 1987). However, a significant increase in preneoplastic hepatic foci was

noted in rats exposed for 20 weeks to doses ≥3 mg/kg/day β-HCH in the diet after initiation with N-nitrosomorpholine (Schröter et al. 1987).

γ-HCH (Lindane). In Wistar rats, exposure to 25 mg γ-HCH/kg/day in the diet for 24 or 48 weeks did not result in any liver tumors (Ito et al. 1975); however, the abbreviated exposure duration and high mortality in the control and treatment groups preclude conclusions as to carcinogenicity 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 increased tumor incidences when compared to controls (Ito et al. 1973). 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). In that study, survival to study end at the carcinogenic dose was very low, so the magnitude of the effect may be underestimated.

Chronic-duration studies have shown increased incidences of tumors in mice, but not rats, exposed to γ-HCH. 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) or 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. Liver tumors have been reported in CF1 and B6C3F1 mice exposed to 13.6–68 mg/kg/day in the diet for 80 to 104 weeks (NCI 1977; Thorpe and Walker 1973). In contrast, EPA (2000a) did not observe an increase in liver tumor incidence in CD-1 mice exposed to doses up to 26.8 mg/kg/day for 78 weeks. Female mice, but not male mice, in this study exhibited increased incidences of lung adenomas at the high dose (26.8 mg/kg/day) (EPA 2000a). Increased incidences of hepatocellular adenomas and carcinomas were also observed in obese mottled yellow A^{vy}/a and lean pseudoagouti A^{vy}/a (YSxVY) F1 mice exposed to 27.2 mg/kg/day in the diet for 96 weeks, but not in lean black *a/a* (YSxVY) F1 mice (Wolff et al. 1987). The obese mottled yellow and lean pseudoagouti strains have a dominant mutation at the agouti locus (Avy) that increases their susceptibility to strain-specific neoplasms. Incidences of benign lung adenomas were also increased in female obese mottled yellow A^{vy}/a and lean pseudoagouti A^{vy}/a (YSxVY)F1 mice exposed to 27.2 mg/kg/day for 24 months (Wolff et al. 1987).

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). Limitations of this study include less-than-lifetime exposure and study duration, testing of only one dose, and potential for ingestion of some of the compound from the skin.

δ-HCH. δ-HCH did not induce a significant increase in liver tumors in male Wistar rats exposed to doses up to 70 mg/kg/day in the diet for 48 weeks (Ito et al. 1975) or in male dd mice exposed to doses up to 90 mg/kg/day in the diet for 24 weeks (Ito et al. 1973). However, these studies were of relatively short exposure durations, and organs other than the liver were not evaluated for histopathology.

Technical HCH or Unspecified Isomers of HCH. Ito et al. (1973) examined the carcinogenicity of pairs of HCH isomers in dd mice exposed to 45 mg/kg/day of each isomer in the diet (total HCH dosage of 90 mg/kg/day) for 24 weeks. Exposure to β-HCH with γ- or δ-HCH, or to γ-HCH together with δ-HCH did not result in hepatocellular carcinomas. However, when any of these isomers was administered with α-HCH, an increased incidence of hepatocellular carcinomas was observed.

Thakore et al. (1981) reported the appearance of neoplastic nodules in the livers of Swiss mice following dietary exposure to technical-grade HCH at 90 mg/kg/day for 6 months. Increased incidences of hepatocellular carcinoma were reported in Swiss mice exposed to 90 mg/kg/day in the diet for 6–8 months (Bhatt and Bano 2009; Bhatt and Nagda 2012; Trivedi et al. 2007, 2009); to 21.3–85 mg/kg/day in the diet for 20 months (Munir et al. 1983); and to 10 or 17 mg/kg/day through gavage or the diet, respectively, for 80 weeks (Kashyap et al. 1979). Dermal application of 2.4 mg technical-grade HCH/kg/day by skin painting on Swiss mice for 80 weeks resulted in nonsignificant increases in the incidences of hyperplastic and preneoplastic areas in the liver and hepatic tumors (Kashyap et al. 1979).

The EPA (IRIS 1987a) listed α-HCH as a probable human carcinogen based on sufficient evidence of carcinogenicity in animals and inadequate data in humans. IRIS (1987b) lists β-HCH as a possible human carcinogen based on evidence for benign liver tumors in exposed mice and inadequate data in humans. Data on δ- and ε-HCH were considered inadequate to classify the potential human carcinogenicity (IRIS 1987d, 1987e). Although the IRIS (1987c) program did not evaluate the carcinogenicity of γ-HCH, EPA's Office of Pesticide Programs (EPA 2001, 2002) classified γ-HCH into the category "suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential."

The HHS NTP determined that γ-HCH and other HCH isomers may reasonably be anticipated to cause cancer in humans (NTP 2021). In 2018, IARC determined that there was sufficient evidence in both humans and animals for the carcinogenicity of γ-HCH, assigning it to Group 1 (carcinogenic to humans). IARC (2018) concluded that γ-HCH causes NHL in humans.

Mechanisms. IARC (2018) conducted an extensive review of the available data on mechanisms of HCH carcinogenicity using the 10 key characteristics of carcinogens (Smith et al. 2016) as a framework. Their analysis noted that the metabolism of γ-HCH yields several intermediates and metabolites, but that to date, those involved in carcinogenesis have not yet been identified. *In vitro* data in rat liver microsomes have shown the formation of a stable epoxide, indicating that γ-HCH can form electrophilic metabolites (reviewed by IARC 2018). Based on the available *in vivo* and *in vitro* data, IARC (2018) concluded that there was strong evidence that γ-HCH induces immunosuppression and oxidative stress, and moderate evidence for genotoxicity and modulation of receptor-mediated effects. Section 2.14 provides details on the *in vivo* evidence for immunosuppression in animals exposed orally to γ-HCH; little to no data are available for immune system effects of other isomers. Several *in vivo* studies reported increased measures of oxidative stress in the heart, liver, kidney, central nervous system, testes, and maternal or fetal tissues after oral exposure to γ-HCH; these studies are described in the *Mechanisms* subsections of Sections 2.5, 2.9, 2.10, 2.15, 2.16, and 2.17. *In vivo* studies of estrogen-mediated effects are described under *Mechanisms* in Section 2.16. Genotoxicity studies of HCH isomers are summarized in Section 2.20.

2.20 GENOTOXICITY

Numerous *in vivo* and *in vitro* studies have assessed the genotoxic potential of HCH and its isomers (α-, β-, and γ-HCH). Genotoxicity testing results for HCH isomers are summarized below. Results of *in vivo* and *in vitro* genotoxicity studies are presented in Tables 2-22 and 2-23, respectively.

Table 2-22. Genotoxicity of Hexachlorocyclohexane Isomers *In Vivo*

Species (test system)	Endpoint	Results	Isomer	Reference
Mammalian cells				
Human (peripheral blood)	DNA damage	–	Alpha, beta	Varona-Uribe et al. 2016
Human (peripheral blood)	Comet assay	–	Alpha, beta	Varona-Uribe et al. 2016
Human (peripheral lymphocytes)	Micronucleus test	–	Alpha, beta, and gamma	Jonnalagadda et al. 2012
Mouse (bone marrow)	Micronucleus test	+	Gamma	Yaduvanshi et al. 2012
Human (peripheral lymphocytes)	Chromosomal aberrations	+	Alpha, beta, and gamma	Jonnalagadda et al. 2012
Rat (bone marrow)	Chromosomal aberrations	+	Beta	Shimazu et al. 1972
Human (peripheral lymphocytes)	Chromosomal aberrations	–	Gamma	Kiraly et al. 1979
Syrian hamster (bone marrow)	Chromosomal aberrations	–	Gamma	Dzwonkowska and Hubner 1986

Table 2-22. Genotoxicity of Hexachlorocyclohexane Isomers *In Vivo*

Species (test system)	Endpoint	Results	Isomer	Reference
Mouse	Micronuclei	–	Gamma	Jenssen and Ramel 1980
Mouse (bone marrow)	Chromosomal aberrations	+	Gamma	Kumar et al. 1995
Rat (liver)	Mitotic disturbances	+	Alpha	Hitachi et al. 1975
Mouse (liver)	DNA binding	(+)	Alpha/ gamma	Iverson et al. 1984
Mouse (germ cells)	Dominant lethal	+	Technical	Lakkad et al. 1982
Human (MGMT tumor suppressor gene in colorectal cancer cells)	Hypermethylation	–	Alpha, beta, and gamma	Abolhassani et al. 2019
Human (P16 tumor suppressor gene in colorectal cancer cells)	Hypermethylation	–	Alpha and beta	Abolhassani et al. 2019
Human (P16 tumor suppressor gene in colorectal cancer cells)	Hypermethylation	+	Gamma	Abolhassani et al. 2019
Human (Leukocyte DNA)	DNA hypomethylation	+	Beta	Itoh et al. 2014
Human (tumor suppressor gene E-cadherin [CDH1] in peripheral blood mononuclear cells)	Methylation	(+)	Beta	Lee et al. 2018b

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

Table 2-23. Genotoxicity of Hexachlorocyclohexane Isomers *In Vitro*

		Results			
Species (test system)	Endpoint	With activation	Without activation	Isomer	Reference
Prokaryotic organisms					
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538 (reversion assay)	Gene mutation	–	–	Gamma	Moriya et al. 1983
S. typhimurium TA98, TA100, TA102 *(Salmonella*/microsome mutagenicity assay)	Gene mutation	–	–	Gamma	Yaduvanshi et al. 2012
Escherichia coli (WP2/spot test)	Gene mutation	NT	–	Gamma	Nagy et al. 1975
E. coli (WP2 *hcr*) (reversion assay*)*	Gene mutation	–	–	Gamma	Moriya et al. 1983
Bacillus subtilis (rec assay)	DNA damage	NT	–	Gamma	Shirasu et al. 1976

Table 2-23. Genotoxicity of Hexachlorocyclohexane Isomers *In Vitro*

Species (test system)	Endpoint	Results		Isomer	Reference
		With activation	Without activation		
Eukaryotic organisms					
Fungi and plant cells:					
Saccharomyces cerevisiae	Gene mutation	–	–	Gamma	Shahin and von Borstel 1977
S. cerevisiae (transformed reporter strain HLYRGI)	DNA damage	NT	+	Gamma	Schmitt et al. 2005
Nostoc muscorum	Gene mutation	NT	–	Gamma	Kar and Singh 1979a
Allium cepa	Mitotic disturbances	NT	+	Gamma	Nybom and Knutsson 1947
Mammalian cells					
Human (peripheral lymphocytes)	Micronuclei	NT	–	Alpha	Ennaceur 2017
Human (peripheral lymphocytes)	Micronuclei	NT	+	Beta	Ennaceur 2017
Human (peripheral lymphocytes)	Micronuclei	NT	+	Gamma	Ennaceur 2017
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 (SV-40 fibroblasts)	Unscheduled DNA synthesis	–	–	Gamma	Ahmed et al. 1977
Human (peripheral lymphocytes)	Unscheduled DNA synthesis	NT	+	Gamma	Rocchi et al. 1980
Rat (primary hepatocytes)	Unscheduled DNA synthesis	NT	–	Gamma	Cifone 1990
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 (ovary surface epithelial cells)	DNA damage	NT	+	Beta	Shah et al. 2020
Human hepatocytes	DNA fragmentation	NT	+	Alpha	Mattioli et al. 1996
Rat (primary cultures)	DNA fragmentation	NT	+	Alpha	Mattioli et al. 1996
Mouse (hepatocytes)	DNA fragmentation	NT	–	Alpha	Mattioli et al. 1996
Chinese hamster lung (CHL) cells	Chromosomal aberrations	NT	(+)	Gamma	Ishidate and Odashima 1977
Chinese hamster ovary (CHO) cells	Chromosomal aberrations	NT	–	Gamma	NTP 1984

Table 2-23. Genotoxicity of Hexachlorocyclohexane Isomers *In Vitro*

Species (test system)	Endpoint	Results		Isomer	Reference
		With activation	Without activation		
CHO cells	Sister chromatid exchange	NT	–	Gamma	NTP 1984
CHO cells	Chromosomal aberrations	–	–	Gamma	Murli 1990
Human (peripheral lymphocytes)	Sister chromatid exchange	NT	+	Technical	Rupa et al. 1989d
Human (peripheral lymphocytes)	Chromosomal aberrations	NT	+	Technical	Rupa et al. 1989d
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

Several studies in humans evaluated the genotoxicity of HCH. Several studies examined the genotoxicity of hexachlorocyclohexane in agricultural workers exposed to mixtures of pesticides. In rice field workers exposed to pesticide mixtures, there was no association between peripheral blood levels of α-HCH or β-HCH and DNA damage measured by comet assay (Varona-Uribe et al. 2016). The frequency of micronuclei in peripheral lymphocytes in agricultural workers exposed to a complex mixture of pesticides including HCH was not significantly different compared to unexposed workers, while the frequency of chromosomal aberrations was significantly increased in exposed workers (Jonnalagadda et al. 2012). In addition, a correlation between chromosomal aberrations per cell and HCH level was reported (Jonnalagadda et al. 2012).

In workers occupationally exposed primarily to γ-HCH by inhalation in a pesticide production factory, no appreciable increase in the frequency of chromosome aberrations was observed compared to the factory employee control group (Kiraly et al. 1979). In colorectal cancer patients, serum levels of the α-HCH isomer were not associated with changes in the methylation status of CpG islands of MGMT and P16 tumor suppressor genes in colorectal cancer cells (Abolhassani et al. 2019). γ-HCH was not associated with methylation status of the MGMT tumor suppression gene; however, hypermethylation was found in the P16 tumor suppressor gene (Abolhassani et al. 2019). There was no significant association between serum levels of β-HCH and methylation status of MGMT and P16 tumor suppressor genes (Abolhassani et al. 2019); however, serum β-HCH was associated with a slight increase in methylation of the tumor suppressor gene E-cadherin [CDH1] in peripheral blood mononuclear cells of

healthy Korean subjects (Lee et al. 2018b). There was a decreased level of global methylation associated with β-HCH serum levels in human leukocyte DNA of Japanese women (Itoh et al. 2014).

Other studies are available regarding genotoxic effects (chromosomal aberrations, sister chromatid exchanges) in humans exposed to a wide variety of pesticides, including 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 other exposures, are not evident from these studies.

α-HCH. Both *in vivo* and *in vitro* assays for genotoxicity of α-HCH are available. α-HCH was observed to bind to liver DNA in HPB mice following intraperitoneal administration (Iverson et al. 1984).

In human peripheral lymphocytes, *in vitro* exposure to α-HCH did not increase the frequency of micronuclei in a cytokinesis-block micronucleus assay (Ennaceur 2017). Exposure to α-HCH produced DNA fragmentation in primary cultures of rat and human hepatocytes, but not in mouse hepatocytes; DNA repair was not induced in hepatocytes from all three species tested (Mattioli et al. 1996). α-HCH was observed to bind to calf thymus DNA with metabolic activation (Iverson et al. 1984).

β-HCH. Limited *in vivo* and *in vitro* assays for the genotoxicity of β-HCH are available. In animals, chromosomal aberrations were induced in bone marrow cells of Long-Evans rats following intraperitoneal exposure to β-HCH in a study reported only as an abstract (Shimazu et al. 1972). *In vitro* exposure to β-HCH increased the frequency of micronuclei at cytotoxic concentrations in human peripheral lymphocytes in a cytokinesis-block micronucleus assay (Ennaceur 2017). β-HCH also induced DNA damage in ovary surface epithelial cells (Shah et al. 2020).

γ-HCH (Lindane). γ-HCH has been tested in several *in vivo* and *in vitro* genotoxicity assays. The incidence of chromosomal abnormalities (breaks and gaps with or without acentric fragments) 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). In a mouse bone marrow micronucleus test, the frequency of micronucleated-polychromatic erythrocytes was increased, and the frequency of polychromatic erythrocytes was decreased in bone marrow cells (Yaduvanshi et al. 2012). γ-HCH was negative in a micronucleus assay in CBA mice (Jenssen and Ramel 1980). Intraperitoneal exposure of Syrian hamsters did not induce chromosome aberrations in bone marrow cells (Dzwonkowska and Hubner 1986). γ-HCH was observed to bind to liver DNA in mice following intraperitoneal administration (Iverson et al. 1984).

γ-HCH did not induce gene mutations in *Salmonella typhimurium* (TA100, TA98, TA1535, TA1537, and TA1538) or *Escherichia coli* (WP2) with or without a metabolic activation system (Moriya et al. 1983) or in *E. coli* without metabolic activation in a WP2 spot test (Nagy et al. 1975). γ-HCH was also negative in an Ames *Salmonella*/microsome mutagenicity assay in *S. typhimurium* (TA98, TA100, TA102) with and without metabolic activation (Yaduvanshi et al. 2012). Exposure to γ-HCH did not produce DNA damage in *Bacillus subtilis* in a rec assay, although a mammalian metabolic activation system was not present (Shirasu et al. 1976). γ-HCH was not mutagenic in *Nostoc muscorum* 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 yeast, exposure to γ-HCH did not induce mutations in *Saccharomyces cerevisiae* (XV185-14C) in a reversion study (Shahin and von Borstel 1977) but did induce DNA damage in transformed reporter strain HLYRGI (Schmitt et al. 2005).

In mammalian cells, γ-HCH induced a marginal increase in the frequency of chromosome aberrations (including chromosomal gaps) in Chinese hamster ovary (CHO) cells (without metabolic activation), which was interpreted by the authors of the study as providing suggestive, but not conclusive, evidence of an effect (Ishidate and Odashima 1977). Another study reported negative results in cytogenetic tests (chromosomal aberrations and sister chromatid exchange) in CHO cells exposed to γ-HCH without metabolic activation (NTP 1984). In addition, γ-HCH was reported to be negative for chromosomal aberrations in CHO cells with and without metabolic activation (Murli 1990).

In a cytokinesis-block micronucleus assay, exposure of human peripheral lymphocytes to γ-HCH induced micronuclei and binucleated cells with micronucleus (BNMN) at a concentration of 100 μg/L, with significant cytotoxicity at that concentration (Ennaceur 2017). γ-HCH exposure in human mammary carcinoma MCF-7 and human prostate carcinoma PC-3 cell lines increased the frequency of micronuclei in both cell lines in the absence of DNA damage or cytotoxicity, suggesting a clastogenic effect (Kalantzi et al. 2004). In a microgel single cell assay, DNA damage was observed in cultures of rat nasal and gastric mucosa cells and human nasal mucosa cells following exposure to γ-HCH (Pool-Zobel et al. 1994). γ-HCH was found to induce unscheduled DNA synthesis in human peripheral lymphocytes without metabolic activation (Rocchi et al. 1980), while it was inactive for inducing unscheduled DNA synthesis in human SV-40 fibroblasts, both with and without activation (Ahmed et al. 1977). In rat primary hepatocytes tested without metabolic activation mammalian cells, γ-HCH did not induce unscheduled DNA synthesis (Cifone 1990). γ-HCH was observed to bind to calf thymus DNA when tested with exogenous metabolic activation (Iverson et al. 1984).

Technical HCH or Unspecified HCH Isomers. Technical-grade HCH has been tested for genotoxic effects in one *in vivo* and one *in vitro* study. When male Swiss mice were exposed to technical-grade HCH prior to mating, dominant-lethal mutations were induced, as evidenced by the number of dead implantations per pregnant female (Lakkad et al. 1982). Cultured human lymphocytes showed a dose-dependent increase in chromosomal aberrations (gaps, breaks, and fragments) with significant increases at 0.1 µg/mL technical-grade HCH for 48-hour treatment and at 0.05 and 0.1 µg/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 µg/mL) producing the only significant result. These results suggest mild mutagenic activity at high doses in humans (Rupa et al. 1989d).