CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Human studies of HCH isomers provide limited quantitative information on absorption, metabolism, distribution, and excretion. Toxicokinetics have been studied in rodents, with most quantitative information derived from studies conducted in mice and rats. An overview of these data is provided below.

- Absorption of HCH isomers has been demonstrated in humans by increased serum levels of the isomers following inhalation, oral, or dermal exposure.
- No animal data are available from the inhalation route to quantify the extent or rate of absorption. Technical-grade HCH has been shown to be well absorbed from the gastrointestinal tract of animals (>90% recovery).
- The distribution of HCH isomers in humans and animals is primarily to the adipose tissue but also to the brain, kidney, muscle, blood, and other tissues. β-HCH accumulates to a much greater extent than other HCH isomers.
- HCH isomers have been measured in the placenta and umbilical cord blood of humans, indicating that transplacental exposure to fetuses is likely to occur. HCH isomers have also been detected in breast milk.
- The primary urinary metabolites are chlorophenols and 1,2,4-trichlorocyclohexane-4,5-epoxide. The conversion occurs mainly by the action of hepatic CYP enzymes.
- HCH isomer metabolites are primarily excreted through the urine as conjugates of mercapturic acid, glucuronide, and sulfate.
- A rat physiologically based pharmacokinetic (PBPK) model simulated the toxicokinetics of γ-HCH. Predicted concentrations in blood, brain, muscle, and fat after a single intraperitoneal injection and chronic-duration oral dosing compared adequately well with experimental results; however, the model is not validated via biological evaluation of kinetic parameters.
- A human PBPK model was developed to simulate toxicokinetics of β-HCH in pregnant mothers and infants exposed during gestation and lactation. The model was validated by comparing predicted concentrations in breast milk, cord blood, and infant blood to concentrations measured in mothers and infants from a Canadian Inuit population. Correlations between model-predicted and measured values for β-HCH were relatively weak because measured concentrations were near the limit of detection.
- A human dermal PBPK model for γ-HCH was developed by modifying a flow-limited PBPK model to include a skin patch compartment for the exposure location. A comparison of model simulations in which the optimized diffusion constants were varied illustrated the importance of

considering protein binding of γ-HCH, when predicting the steady-state dermal permeability constant (K_p).

3.1.1 Absorption

α-, β-, γ-, and δ-HCH have been detected in the blood serum, adipose tissue, and semen of occupationally and environmentally exposed individuals, indicating that absorption takes place following inhalation exposure (Baumann et al. 1980; Czeglédi-Jankó and Avar 1970; Kashyap 1986; Nigam et al. 1986; Quintana et al. 2004; Saxena et al. 1980, 1981a, 1981b). Human case reports of accidental poisoning indicate that HCH is also absorbed following oral exposure. High blood concentrations of γ-HCH have been demonstrated following oral exposure in these cases (Berry et al. 1987; Harris et al. 1969; Khare et al. 1977; Munk and Nantel 1977; Nantel et al. 1977; Powell 1980; Starr and Clifford 1972).

Dermal absorption of γ-HCH has been demonstrated in several studies that examined absorption from anti-scabies lotion or head lice shampoo (EPA 2002; Feldmann and Maibach 1974; Franz et al. 1996; Ginsburg et al. 1977; Lange et al. 1981). Maximum serum levels in healthy volunteers and scabies patients were reported within 4–6 hours following whole-body application (Lange et al. 1981). However, the maximum serum levels of γ-HCH in scabies patients were greater than those reported for normal volunteers. Studies involving a single topical application of γ-HCH to the forearm, which was left for 24 hours before washing, indicate that at least 9% of the applied dose was absorbed; maximum absorption occurred during the first 12 hours after application of γ-HCH to the skin, but absorption continued for at least 5 days (Feldmann and Maibach 1974). In infants and children dermally treated with 1% γ-HCH lotion, maximum blood concentrations of γ-HCH were observed in 6 hours, and averaged 0.028 µg/mL for the group infested with scabies and 0.024 µg/mL for the non-infested group (Ginsburg et al. 1977). The maximum blood level measured in children aged 33–64 months treated with 1% topical γ-HCH lotion was 64 µg/L (EPA 2002). Children aged 3.5–18 years treated for head lice with 1% γ-HCH shampoo had a maximum γ-HCH blood level of 6.13 µg/L (EPA 2002). HCH isomers are bioavailable from soil and can be absorbed dermally (Duff and Kissel 1996). In an *in vitro* study using abdominal skin obtained from human cadavers, γ-HCH exhibited mean 24-hour dermal absorption values from 0.45 to 2.35% varying with different soil types and soil loadings of 1, 5, and 10 mg/cm^3.

The absorption of γ-HCH through the skin was studied following application of two different preparations to the forearm of volunteers (Dick et al. 1997a). The mean peak plasma concentrations of γ-HCH following exposure to 120 mg γ-HCH/mL acetone and a 3 mg γ-HCH/mL formulation containing white spirit (a petroleum-based solvent) were 0.91 and 0.47 ng/mL, respectively, although the preparation in

acetone contained a 40-fold higher concentration of γ-HCH. The proportion of the applied dose absorbed into the systemic circulation in 6 hours was 5% for the dose applied in acetone and 60% of the applied dose in white spirit-based formulation. Thus, the white spirit enhanced the absorption of γ-HCH relative to acetone as the vehicle. About 30% of the applied dose for the white-spirit based formulation was observed in the stratum corneum at 6 hours of exposure and decreased by 90% at 24 hours. Fifteen percent of the applied dose for the acetone-based application was in the stratum corneum. The absorption of γ-HCH through human skin was also assessed in an *in vitro* study (Dick et al. 1997b). γ-HCH absorption was reported to be 15–25% in 24 hours for the two formulations that contained white spirit as the predominant solvent, 3% in 24 hours from an aqueous spray dilution, and <1% in 24 hours for the acetone preparation.

No information is available on the absorption of α-, β-, γ-, and δ-HCH following inhalation exposure in experimental animals. γ-HCH is readily absorbed from the gastrointestinal tract of mice and rats (Ahdaya et al. 1981; Turner and Shanks 1980). Ahdaya et al. (1981) demonstrated that half of the administered dose was absorbed from the gastrointestinal tract of fasting mice approximately 14 minutes after administration of radiolabeled γ-HCH by stomach tube. Although this study demonstrates the rapid absorption of γ-HCH from the gastrointestinal tract, the use of fasted animals prevents an assessment of the effect of stomach contents on the rate of absorption. Turner and Shanks (1980) studied the rate of absorption of γ-HCH from the gastrointestinal tract and intestinal lymphatic system using rat intestinal loop preparations. Prepared loops were injected with γ-HCH, and the blood and lymph were sampled for 30 minutes. γ-HCH was readily absorbed from the intestine into the blood; however, only a small amount of γ-HCH entered the lymphatic system from the intestine. The extent of oral absorption of technical-grade HCH has been estimated to be 95.8% in rats within 4 days following the administration of single doses of the substance (Albro and Thomas 1974). Variation of the dosages from 30 to 125 mg/kg had no effect on the percent absorption. The overall degree of absorption of technical-grade HCH administered in the feed for 14 days was similar (94.9%), but the average absorption values of α-, β-, γ-, and δ-HCH were 97.4, 90.7, 99.4, and 91.9%, respectively (Albro and Thomas 1974). HCH isomers in contaminated soil were shown to be bioaccessible in an *in vitro* gastrointestinal model (Tao et al. 2009).

Dermal absorption of γ-HCH was demonstrated in rats and rabbits (Bosch 1987a, 1987b). Male rats treated dermally with radiolabeled γ-HCH (20% emulsifiable concentrate) on a 4.9 cm^2 shaved dorsal area exhibited absorption of radiolabel, which increased with time of exposure (Bosch 1987a). After 4 hours, 10.1, 5.3, and 2.0% were absorbed from doses of 0.06, 0.6, and 6 $mg/cm^2/kg$, respectively. After 24 hours, 27.7, 20.9, and 5.1% were absorbed from doses of 0.06, 0.6, and 6 $mg/cm^2/kg$, respectively.

Male rabbits treated dermally with radiolabeled γ-HCH (20% emulsifiable concentrate) in a 28.3-cm^2 shaved dorsal area absorbed, after 4 hours, 29.6, 18.3, and 7.3% radiolabel from doses of 0.005, 0.05, and 0.5 mg/cm^2/kg, respectively, and, after 24 hours, 55.7, 40.0, and 16.6% from the same respective doses (Bosch 1987b). In weanling rabbits, levels of γ-HCH in the blood after a single application of a 1% solution (60 mg γ-HCH/kg) were 1.67 and 2.48 μg/mL in two rabbits that had been shaved and depilated, then stripped to remove the keratin layer (Hanig et al. 1976). In contrast, a blood level of only 0.67 μg/mL was seen in a rabbit that had only been shaved and depilated, indicating that absorption increases with loss of skin integrity.

Dermal absorption of γ-HCH was evaluated in human skin grafted onto a nude mouse model and the results were compared to an *in vivo* rat model and *in vitro* rat and human models (Capt et al. 2007). The maximum percent absorbed, which included the amount directly absorbed and present in the skin and stratum corneum, was comparable for the human skin grafted onto a nude mouse (20.7% of applied dose) and the *in vitro* human skin model (24.5%). Data for the rat *in vivo* and *in vitro* models appeared to overestimate the potential human absorption. The maximum percent absorbed was 39.8% in the rat *in vivo* model and 62.5% in the rat *in vitro* model.

3.1.2 Distribution

Occupational studies provide information on the distribution of HCH isomers following inhalation exposure in humans. Air concentrations of α-HCH (0.002–1.99 mg/m^3), β-HCH (0.001–0.38 mg/m^3), and γ-HCH (0.004–0.15 mg/m^3) were associated with concurrent mean blood serum levels in workers of 69.6, 190.3, and 36.9 μg/L, respectively (Baumann et al. 1980). Serum total HCH concentrations of 0.14–0.60 ppm were found in workers with unknown levels of exposure to technical-grade HCH (Nigam et al. 1986). HCH isomers have also been detected in adipose tissues of occupational workers and the general population (Arrebola et al. 2013, 2014; Baumann et al. 1980; Kim et al. 2014; Mustieles et al. 2017; Pestana et al. 2011; Ploteau et al. 2017; Quintana et al. 2004; Siddiqui et al. 1981). Accumulation of β-HCH has been shown to increase approximately linearly with time of exposure (Baumann et al. 1980). In a national EPA survey, adipose tissue samples collected from surgical procedures or autopsies between 1969 and 1983 showed β-HCH concentrations >0.37 ppm lipid in the highest quartile (Quintana et al. 2004).

Case reports of poisoning confirm that γ-HCH is distributed to the central nervous system. γ-HCH was detected in the cerebrospinal fluid of a young boy following ingestion of an unknown quantity of γ-HCH

(Davies et al. 1983). γ-HCH was also detected in brain tissue (110 ppb) and heart blood (33.3 ppb) collected during the autopsy of an infant who was treated with a whole-body application of a 1% γ-HCH lotion after a hot bath (Davies et al. 1983).

HCH isomers have been measured in the placenta and/or umbilical cord blood of humans, indicating that transplacental exposure to fetuses is likely to occur (Alvarado-Hernandez et al. 2013; Anand and Taneja 2020; Anand and Taneja 2020; Anand et al. 2019; Dewan et al. 2013; Fukata et al. 2005; Hernik et al. 2016; Herrero-Mercado et al. 2010, 2011; Junque et al. 2020; Lopez-Espinosa et al. 2007; Morello-Frosch et al. 2016; Saxena et al. 1981a; Shen et al. 2007; Siddiqui et al. 2003; Vizcaino et al. 2011; Yin et al. 2019; Yu et al. 2013). Placental transfer of HCH isomers was analyzed using matched maternal serum, cord serum, and placenta samples in mother-infant pairs (Yin et al. 2019; Zhang et al. 2018). β-HCH was the predominant isomer measured in each sample type. An analysis of concentration ratios for all HCH isomers suggests that the rate of transfer from the placenta to cord blood is slower than for maternal serum to the placenta (Yin et al. 2019). Transfer data for the two enantiomers of α-HCH suggests that placental transfer may involve both simple diffusion and active transport (Yin et al. 2019). Experiments using an *in vitro* placenta model (human choriocarcinoma derived BeWo cells in a confluent, polarized monolayer) confirm that multiple mechanisms are likely involved in transplacental transfer of HCH isomers (Yin et al. 2020). Maternal serum concentrations of β-HCH were shown to increase between the first trimester of pregnancy and delivery, possibly due to mobilization of fat stores or changes in blood volume at different stages of pregnancy (Junque et al. 2020). Concentrations in cord blood serum were correlated with maternal serum concentrations at delivery in this study.

HCH isomers have also been detected in human breast milk (Bedi et al. 2013; Chen et al. 2018; Dewan et al. 2013; Dimitriadou et al. 2016; Elserougy et al. 2013; Fytianos et al. 1985; Hernik et al. 2016; Kao et al. 2019; Minh et al. 2004; Shen et al. 2007; Yalcin et al. 2015). α-, β-, and γ-HCH have been found to be bioconcentrated and excreted in breast milk of women who have been exposed to technical-grade HCH in pesticide residues (Nair et al. 1996). All four of the HCH isomers (α, β, γ, and δ) discussed in this profile have been detected in human semen following environmental exposure (Szymczynski and Waliszewski 1981).

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

of HCH or its isomers (Chand and Ramachandran 1980; Eichler et al. 1983; Srinivasan and Radhakrishnamurty 1983). γ- and β-HCH are primarily stored in the fat of rats acutely exposed for 5, 10, or 15 days (Srinivasan and Radhakrishnamurty 1983). The overall distribution of γ-HCH was greatest in fat, followed by brain, kidney, muscle, lungs, heart, spleen, liver, and blood. γ-HCH has also been found in the adrenal glands of rats (Lahiri et al. 1990; Sulik et al. 1988). In an experiment lasting 12 days, the accumulation of γ-HCH in the brain of rats dosed with 5 or 12 mg/kg/day by gavage began to decline after 8 days. This reduction was not observed in rats given 20 mg/kg/day (Tusell et al. 1988).

In the brain of rats, α-HCH has been found to accumulate preferentially in the white matter, an area containing lipid-rich myelin, as opposed to gray matter (Portig et al. 1989). However, the same brain distribution pattern was not noted for γ-HCH in mice, even though α- and γ-HCH are equally lipophilic. Differences in distribution of γ- and α-HCH are most likely due to stereospecificity, because only the +-enantiomer of α-HCH was shown to accumulate in white matter (Portig et al. 1989). A comparison of the enantiomeric fractions in the blood, liver, and brain of mice following administration of a single gavage dose of α-HCH, demonstrated enrichment of the +-enantiomer in the brain, but not in the liver or blood (Yang et al. 2010). Enantioselective transport across the blood-brain barrier was also demonstrated in rabbits exposed orally or dermally to α-HCH (Xue et al. 2010). Toxicokinetic modeling from this study suggested that enrichment of the +-enantiomer in blood was due to a faster elimination rate for the –-enantiomer in rabbits (Xue et al. 2010). In mice, gavage exposure to 5.9 mg/kg/day γ-HCH for 3 days was shown to increase the permeability of the blood-brain barrier, as measured by increased fluorescein dye uptake (Sinha and Shukla 2003). Similar effects were not observed in rats given 8.8 mg/kg/day for 3 days (Sinha and Shukla 2003).

The distribution pattern for β-HCH was found to be in the following order: fat > kidney > lungs > liver > muscle > heart > spleen > brain > blood. β-HCH accumulates in tissues to a greater degree than γ-HCH except in the brain, where the γ-HCH accumulates at a higher concentration (Srinivasan and Radhakrishnamurty 1983). This accumulation increases with increasing dose and treatment period for β-HCH more so than for γ-HCH. The greater accumulation of β-HCH in tissues is expected since this isomer is known to be metabolized more slowly. In addition, γ-HCH is known to induce the liver cytochrome P-450 mixed-function oxygenase system (CYP), and thus, self-induced metabolism is an important factor that minimizes the accumulation of γ-HCH residues in animal tissues.

The preferential accumulation of HCH in fatty tissues is also observed following intermediate- and chronic-duration exposure of rats to HCH isomers in the diet (overall distribution: fat > liver > serum)

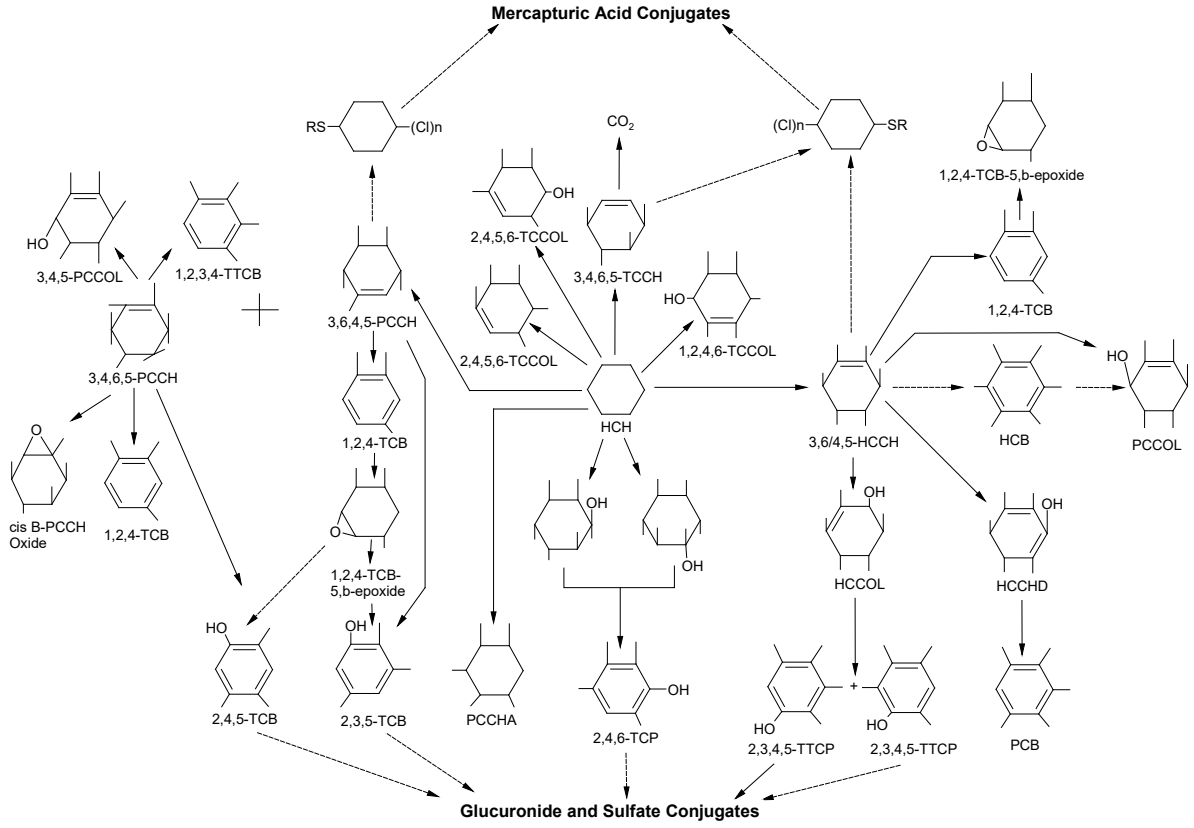
(Amyes 1990; Chand and Ramachandran 1980; Dikshith et al. 1991c; Fitzhugh et al. 1950) or exposure to α- or γ-HCH by gavage (overall distribution: fat > kidney > liver > brain > blood) (Eichler et al. 1983). HCH has been shown to accumulate in amniotic fluid, placenta, and fetal tissues after oral treatment of pregnant mice (Srivastava and Raizada 2000). In rats gavaged with γ-HCH on LDs 9 or 14, γ-HCH levels were higher in their milk than plasma (Dalsenter et al. 1997b). Levels of γ-HCH in the offspring of those rats were approximately twice as high in kidneys and liver than in brain and testes.

Some information on the distribution of γ-HCH is available from studies in which laboratory animals were exposed by dermal application (Bosch 1987a, 1987b; Hanig et al. 1976; Solomon et al. 1977a, 1977b). A study on the distribution of γ-HCH in guinea pigs following acute dermal exposure indicates that accumulation of γ-HCH in the brain is greater than in the blood after single and multiple topical applications (Solomon et al. 1977a, 1977b); the levels in both tissues increased with the number of applications. Following dermal treatment of rats with 50 or 100 mg/kg/day technical-grade HCH for 120 days, α-, β-, γ-, and δ-HCH were accumulated in testicular tissue and sperm in a dose-related manner (Prasad et al. 1995). β-HCH was present at the highest concentration in testicular tissue and sperm.

3.1.3 Metabolism

The metabolism of γ-HCH is illustrated in Figure 3-1. Angerer et al. (1983) determined that chlorophenols were the primary urinary metabolites of γ-HCH excreted by workers involved in γ-HCH production. In the study, glucuronides and sulfates of chlorophenols were cleaved by acidic hydrolysis of urine samples. The metabolites 2,3,5-, 2,4,6-, and 2,4,5-trichlorophenol accounted for almost 57.7% of the γ-HCH metabolites identified in the urine collected during the last 2 hours of the workers' shifts. Other urinary metabolites identified included other trichlorophenols, dichlorophenols, tetrachlorophenols, and dihydroxychlorobenzenes. Pentachlorophenol has also been identified as a urinary metabolite in humans following occupational exposure (Engst et al. 1979). *In vitro* investigations indicate that human liver microsomes convert γ-HCH by dechlorination, dehydrogenation, dehydrochlorination, and hydroxylation to five primary metabolites: 3,6/4,5-hexachlorocyclohexene, pentachlorocyclohexene, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, and pentachlorobenzene (Fitzloff et al. 1982). Similar *in vitro* studies have demonstrated that an epoxide forms during the metabolism of pentachlorocyclohexene. This stable halogenated hydrocarbon epoxide metabolite may be responsible for the mutagenic and carcinogenic effects of γ-HCH (Fitzloff and Pan 1984).

Figure 3-1. The Proposed Metabolism of Hexachlorocyclohexane



3,6/4,5-HCCH = 3,6/4,5-hexachlorocyclohexene; HCB = hexachlorobenzene; HCCHD = hexachlorocyclohexadiene; HCCOL = hexachlorocyclohexenol; HCH = hexachlorocyclohexane; PCB = pentachlorobenzene; PCCH = pentachlorocyclohexene; PCCHA = pentachlorocyclohexane; PCCOL = pentachlorocyclohexenol; TCB = trichlorobenzene; TCCH = tetrachlorobenzene; TCCOL = tetrachlorocyclohexenol; TCP = trichlorophenol; TTCP = tetrachlorophenol

Sources: Chadwick et al. 1985; Fitzloff and Pan 1984; Fitzloff et al. 1982

In animals, γ-HCH appears to be transformed by hepatic enzymes to form chlorophenols, chlorobenzenes, chlorocyclohexanes, chlorocyclohexenes, chlorocyclohexenols, and conjugates of mercapturic acid, glucuronide, and sulfate (Chadwick and Freal 1972a; Chadwick et al. 1978a; Engst et al. 1979; Kujawa et al. 1977). These metabolites have been identified in various tissues and in the urine of laboratory animals. Metabolites found in the liver of rats following intermediate exposure to γ-HCH via gavage or diet include di-, tri-, tetra-, and pentachlorobenzenes; pentachlorocyclohexenes; and pentachloro-2-cyclohexen-1-ol (Chadwick and Freal 1972a; Kujawa et al. 1977). Metabolites identified in the blood of these rats include di-, tri-, tetra-, and pentachlorophenols and pentachloro-2-cyclohexen-1-ol (Kujawa et al. 1977). Di-, tri-, and tetrachlorophenols; pentachlorocyclohexenes; and pentachloro-2-cyclohexen-1-ol have been identified in samples of kidney, spleen, heart, and brain tissue from rats fed γ-HCH

(Kujawa et al. 1977). Metabolites found in the urine include tri-, tetra-, and pentachlorophenol; pentachloro-2-cyclohexen-1-ol; and isomers of tetrachloro-2-cyclohexen-1-ol (Chadwick and Freal 1972a; Chadwick et al. 1978c; Kujawa et al. 1977). The metabolism of γ-HCH in the intestine was reported to be very minor, or the metabolites were completely absorbed. No metabolites were detected in the feces or in the adrenal gland (Kujawa et al. 1977). *In vitro* preparations using rat liver slices have also found that γ-HCH is converted to hexachlorobenzene (Gopalaswamy and Aiyar 1984). However, these findings have not been confirmed in *in vivo* experiments.

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

The toxicity of γ-HCH appears to be dependent on CYP enzymes. Intermediate exposure to γ-HCH resulted in greater toxicity in DBA/2 (D2) mice than in C57BL/6 (B6) mice; the DBA/2 (D2) mice are considered unresponsive to microsomal enzyme induction by aromatic hydrocarbons (Liu and Morgan 1986). Increased toxicity was associated with higher blood and brain concentrations in D2 mice than in B6 mice at the time of sacrifice. In addition, D2 mice were found to have more 2,4,6-trichlorophenol in the liver, kidney, and spleen than the less-susceptible B6 mice. The inability of D2 mice to undergo enzyme induction to increase the rate of detoxification led to γ-HCH's enhanced toxicity in this strain. Other investigators have demonstrated the importance of the hepatic microsomal enzymes in the toxicity of γ-HCH (Baker et al. 1985; Chadwick and Freal 1972a; Chadwick et al. 1981; Chand and Ramachandran 1980; Tanaka et al. 1979). Chadwick et al. (1981) demonstrated that pretreatment of rats with inducers of hepatic enzymes significantly influenced the metabolism and excretion of γ-HCH and its metabolites by altering specific metabolic pathways; excretion of γ-HCH metabolites in the urine increased nearly 4-fold following pretreatment with Aroclor 1254 or phenobarbital. Following pretreatment with Aroclor 1254, a 7-fold increase in expired metabolites was observed. Naphthoflavone had no effect on the excretion rate.

3.1.4 Excretion

Humans excrete HCH isomers and their metabolites in urine, breast milk, sweat, and semen (Angerer et al. 1981; Genuis et al. 2016). Analysis of urine from humans occupationally exposed to HCH showed the presence of chlorinated phenols and all isomers of di-, tri-, and tetrachlorophenol (Angerer et al. 1981). In another study, the elimination of β-HCH (a byproduct of γ-HCH production studied due to its long

half-life in humans) was investigated in a group of 40 former workers of a γ-HCH-producing plant by analyzing at least two blood specimens from different time points between 1952 and 1980. The median half-life of β-HCH was 7.2 years, calculated by concentrations in whole blood, and 7.6 years, calculated by concentrations in extractable lipids (Jung et al. 1997), assuming first-order kinetics for excretion.

Nonmetabolized γ-HCH was excreted in the urine and feces of healthy volunteers and scabies patients acutely exposed to a 0.3% γ-HCH emulsion by whole-body application. The cumulative excretion of nonmetabolized γ-HCH was almost the same in the healthy volunteers and the scabies patients (Zesch et al. 1982). The elimination of γ-HCH was studied following application of two different preparations to the forearm of volunteers (Dick et al. 1997a). The elimination half-life was between 50 and 111 hours for the acetone-based application, and 25–58 hours for the white-spirit based formulation. Absorbed γ-HCH was excreted in the urine as conjugates of 2,4,6-, 2,3,5-, and 2,4,5-trichlorophenol. Only 0.01–0.15% of the dose was excreted in the urine in 72 hours following dermal exposure for 6 hours. In a study in which children infested with scabies and their noninfested siblings were treated dermally with 1% γ-HCH lotion, the blood level was found to diminish rapidly after application, with a half-life of 17.9 hours in infested children and 21.4 hours in noninfested children (Ginsburg et al. 1977).

The excretion kinetics of β-HCH into breast milk were studied by monitoring breast milk concentrations in lactating mothers after birth (Song et al. 2018; Waliszewski et al. 2009). In 40 lactating women, breast milk concentrations of β-HCH decreased by approximately 30%, from 95 µg/kg fat on the 4th day after birth to 66 µg/kg fat on PND 30 (Waliszewski et al. 2009). Song et al. (2018) monitored monthly breast milk concentrations in 40 lactating women during the first 6 months after birth. The average breast milk concentrations of β-HCH decreased from 127 µg/kg lipid 1 month after birth to 84.8 µg/kg lipid 6 months after birth, representing a 32% reduction over this time period. The excretion profile for β-HCH in milk lipids followed zero-order kinetics and the mean excretion rate was approximately 7% per month.

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

Very little γ-HCH is excreted unaltered. Various phenylmercapturic acid derivatives have been detected in the urine of rats, formed by the conjugation of γ-HCH metabolites with glutathione subsequent to dechlorinations and dehydrochlorinations (Allsup and Walsh 1982; Kurihara et al. 1979). *In vitro* investigations using rat liver cells indicate that glutathione conjugation is lower for β-HCH compared to γ- and α-HCH, which are readily conjugated (Fitzloff and Pan 1984; Fitzloff et al. 1982). Γ-HCH metabolites are excreted in the form of phenylmercapturic acids and glucuronide and sulfate conjugates (Chadwick et al. 1978a).

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

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

Models are simplified representations of a system with the intent of reproducing or simulating its structure, function, and behavior. PBPK models are more firmly grounded in principles of biology and biochemistry. They use mathematical descriptions of the processes determining uptake and disposition of chemical substances as a function of their physicochemical, biochemical, and physiological characteristics (Andersen and Krishnan 1994; Clewell 1995; Mumtaz et al. 2012a; Sweeney and Gearhart 2020). PBPK models have been developed for both organic and inorganic pollutants (Ruiz et al. 2011) and are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Mumtaz et al. 2012b; Ruiz et al. 2011; Sweeney and Gearhart 2020; Tan et al. 2020). PBPK models can also be used to more accurately extrapolate from animal to human, high dose to low dose, route to route, and various exposure scenarios and to study pollutant mixtures (El-Masri et al. 2004). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints (Clewell 1995).

DeJongh and Blaauboer (1997) simulated the toxicokinetics of γ-HCH in rats with a PBPK model. A five-compartment model was constructed: (1) the liver, serving as the metabolizing organ; (2) blood;

(3) fat; (4) brain; and (5) a lumped compartment representing all other tissues, consisting mainly of muscle tissue. Values for the physiological parameters and tissue-blood partition coefficients were obtained from the literature. The model was calibrated on a dataset from the literature on the disposition of γ-HCH from blood *in vivo* after single oral dosage and first-order biotransformation and gastrointestinal absorption constants for γ-HCH were obtained.

The model was validated by simulating the disposition of γ-HCH *in vivo* after single intraperitoneal and chronic-duration oral dosing and comparing these simulations with experimental results. Simulated γ-HCH concentrations in fat, brain, and muscle compared well with measured values obtained after single intraperitoneal exposure in rats. Simulated levels in blood were slightly higher than measured levels after oral and intraperitoneal exposure.

A human PBPK model was developed to assess pre- and postnatal exposure to β-HCH and other neutral persistent organic pollutants (Verner et al. 2009). The infant portion of the model was added to a previously published maternal model (Verner et al. 2008) that consisted of nine compartments (liver, brain, adipose tissue, richly perfused tissues, poorly perfused tissues, mammary tissue, uterus, placenta, and fetus). B-HCH was assumed to be completely absorbed from contaminated food and absorption was entered as a direct input to the maternal liver. Excretion into breast milk was modeled as an output from mammary tissue. The infant portion of the model consisted of five compartments, including liver, brain, adipose tissue, richly perfused tissues, and poorly perfused tissues. The infant liver was modeled as receiving β-HCH directly from breast milk for the first year of life (100% absorption was assumed) with subsequent first-pass metabolism. The brain was included as a potential target organ of toxicity and adipose tissue was considered the primary site for β-HCH storage. The initial body burden in the infant was equivalent to lipid-adjusted levels of β-HCH in maternal blood at delivery. Distribution between compartments in both the maternal and infant models was derived using blood flow and tissue:blood partition coefficients. Metabolism was parameterized by transforming a published physiological half-life of 7.6 years into a liver volume-adjusted intrinsic clearance value. Intrinsic clearance values based on hepatic metabolism were assumed to be the same in mothers and infants. Model parameters described by Verner et al. (2008) for the maternal model were adjusted for blood lipids during pregnancy, breast milk lipid content, and excreted volume in breast milk. Parameters for the infant model described infant physiology as a function of sex, age, body weight, and body height, and included sex-specific organ volumes and blood flows and tissue:blood and milk:blood partition coefficients.

The model was validated by comparing predicted concentrations in breast milk, cord blood, and infant blood to concentrations measured in mothers and infants from a Canadian Inuit population. Correlations between model predictions and measured values for β-HCH were relatively weak (r=0.35 for cord blood; r=0.7 for breast milk; r=0.62 for infant blood) because measured concentrations were near the limit of detection. A sensitivity analysis was performed using Monte Carlo simulations based on variability in breast milk consumption, fraction of lipids in breast milk, and fraction of lipids in infant adipose tissue for PCB-153 and *p,p'*-DDT. Variability was estimated to be approximately 2.5-fold between the 5th and 95th percentiles. Predicted blood concentrations were highly variable for β-HCH, with only 66% of individual values falling within the 2.5-fold range of variation.

A human dermal PBPK model for γ-HCH was developed by modifying a flow-limited PBPK model to include a skin patch compartment for the exposure location (Sawyer et al. 2016). Optimized dermal absorption parameters were calculated for γ-HCH by adjusting diffusion equations for binding to protein and lipids and these parameters were included in the PBPK model to describe *in vivo* toxicokinetics. Model simulations were run using the time course data from Dick et al. (1997a) where volunteers were exposed to a 3 mg γ-HCH/mL formulation containing white-spirit for 6 hours and blood samples were analyzed for γ-HCH for up to 80 hours after exposure. A comparison of model simulations in which the optimized diffusion constants were varied illustrated the importance of considering protein binding of γ-HCH, when predicting the steady-state dermal permeability constant (K_p).

3.1.6 Animal-to-Human Extrapolations

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

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

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

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels of HCH are discussed in Section 5.7, Populations with Potentially High Exposures.

Several human studies suggest that the developing fetus may be susceptible to health effects of prenatal exposure to HCH isomers, with reports of decreased birth weight or increased risk of fetal growth restriction associated with higher maternal or fetal concentrations of HCH isomers (see Section 2.17). Individuals with genetic polymorphisms that alter the metabolism and excretion of HCH isomers, may be at increased risk of these effects. For example, polymorphism of glutathione S-transferase mu 1 (GSTM1) was shown to contribute to the risk of preterm birth or fetal growth restriction following

exposure to β-HCH (Mustafa et al. 2013; Sharma et al. 2012). A significant interaction was also observed between polymorphism of the CYP17 gene (A1A2) and γ-HCH in maternal blood and the risk of preterm birth (Sharma et al. 2013).

In human studies, serum cord blood levels of β-HCH were associated with increased serum levels of TSH (see Section 2.17 and Table 2-16); altered thyroid hormone status may affect neurodevelopment of infants. In addition, adverse neurodevelopmental outcomes have been associated with elevated concentrations of total HCH or specific HCH isomers in maternal breast milk or children's blood (Lenters et al. 2019; Sisto et al. 2015).

Serious neurological effects and occasional deaths have been reported in children following exposure to γ-HCH by accidental ingestion or by topical application (see Sections 2.2 and 2.15).

Studies of animals exposed to γ-HCH by oral administration demonstrate that the developing organism is exquisitely sensitive to the toxic effects of this isomer. Developmental effects of γ-HCH observed in studies of rats, mice, and mink 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 (see Section 2.17). Little to no data are available for the other isomers of HCH.

Few studies have compared effects in young and aged animals exposed by the same regimen to HCH isomers. Weanling rabbits were more sensitive to γ-HCH treatment than young adults, as seen by higher 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). As discussed in Section 2.17, there is evidence that γ-HCH causes functional impairment of the developing blood brain barrier in young rats (Gupta et al. 1999). The brain uptake of fluorescein was significantly increased in 10-day-old pups treated with a single 2 mg/kg dose, as well as in those treated with 2 mg/kg/day for 8 days. 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, and there was no effect on brain permeability at a higher dose of 4 mg/kg/day when administered for 3 days to adults (Gupta et al. 1999).

Following intraperitoneal dosing of dams with γ-HCH on GDs 12–17, GABAA receptors in rat fetuses were studied with radiolabeled t-butylbicyclophosphorothionate (TBPS), a ligand that binds to the GABAA receptor (Brannen et al. 1998). Treatment with γ-HCH significantly reduced the TBPS binding

affinity in fetal brainstems and it was concluded that the effect could potentially lead to abnormal brain activity, increased susceptibility to seizures, and behavioral effects. TBPS binding in brains of fetuses was reduced when compared to adults (Brannen et al. 1998).

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. Male Wistar rats treated with γ-HCH beginning at 9 weeks of age exhibited significantly decreased serum testosterone and growth hormone and increased serum LH and FSH (Agrahari et al. 2019). Similar results were observed in another group exposed 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 (Agrahari et al. 2019).

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

Although it is unknown whether the ability to metabolize HCH specifically differs between children and adults, some enzymes, which belong to the enzyme superfamilies involved in phase II HCH metabolism, are developmentally regulated in humans. The development of uridine 5'-diphospho-glucuronosyl-transferase (UDP-glucuronosyltransferase; responsible for glucuronide conjugation) depends on the enzyme isoform, but, in general, adult activity is attained by 6–18 months of age (Leeder and Kearns 1997). Development of sulfotransferase (responsible for sulfate conjugates) activity is also substrate-specific and is usually earlier than UDP-glucuronosyltransferase. In fact, levels of some sulfotransferases may be greater during infancy and early childhood than during adulthood (Leeder and Kearns 1997). A series of enzymes are involved in the production of mercapturic acid conjugates: γ-glutamyltrans-peptidase, glutathione S-transferase, cysteinyl glycinase, and N-acetyl transferase (Sipes and Gandolfi 1991). There are two superfamilies of N-acetyltransferase, and the N-acetyltransferase 2 superfamily has members that are developmentally regulated in humans. There is some N-acetyltransferase 2 activity in fetuses by 16 weeks of gestation. Infants up to 2 months of age have the slow metabolizer phenotype of this gene; the adult distribution of slow and fast metabolizer phenotypes is reached by 4–6 months of age and full adult activity is achieved at 1–3 years of age (Leeder and Kearns 1997).

Case-control studies have evaluated the interactive effect of exposure to HCH isomers and genetic polymorphisms on the increased risk of cancer or diabetes (Li et al. 2013, 2016; McCready et al. 2004; Sharma et al. 2013, 2019). The risk of breast cancer associated with higher serum levels of HCH isomers was increased by the presence of a polymorphism in the GSTM1 gene (GSTM1 null) (Li et al. 2013; McCready et al. 2004). Genetic polymorphisms of CYP1A1 did not influence the breast cancer risk associated with β-HCH exposure (McCready et al. 2004). A significant interaction was demonstrated between the GSTM1 null polymorphism and β-HCH blood levels on risk of urinary bladder cancer (Sharma et al. 2013). Mortazavi et al. (2019) did not show a similar correlation between GSTM1 or GSTT1 polymorphisms and bladder cancer risk associated with HCH isomers. Sharma et al. (2019) evaluated the influence of CYP1A1, GSTM1, and GSTT1 polymorphisms on the increased risk of epithelial ovarian cancer risk associated with HCH isomers. Significant interactions between β-HCH blood levels and CYP1A1m1 and GSTM1 and GSTT1 null genotypes were observed for increased risk of ovarian cancer (measured as increased cancer antigen-125 or CA-125 levels). The risk of type 2 diabetes associated with exposure to β-HCH was elevated in individuals carrying a single-nucleotide polymorphism in the gene encoding adiponectin (*ADIPOQ*) (Li et al. 2016). Serum adiponectin levels were reduced in individuals with this polymorphism.

People with lowered convulsion thresholds due to epilepsy (treated or untreated), cerebrovascular accidents, or head injuries may be at greater risk of the central nervous system effects of γ-HCH toxicity and may suffer increased risk of or severity of seizures (Kramer et al. 1980; Matsuoka 1981). Exposure to β-HCH may increase the risk of hypertension in individuals with an elevated BMI (Arrebola et al. 2015a).

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 2006).

The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for HCH from this report are discussed in Section 5.6, General Population Exposure.

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 2006). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to HCH are discussed in Section 3.3.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 2006). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by HCH are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

HCH isomers measured in human serum are generally normalized by total lipid content, based on total cholesterol and triglycerides (e.g., ng/g lipid) (Bradman et al. 2007; Curren et al. 2014; Everett and Matheson 2010; Kaur et al. 2020; Mørck et al. 2014). Porta et al. (2009) suggested that adjustment of serum concentrations by total cholesterol may be more appropriate than total lipid in studies of patients with severe disease (e.g., pancreatic cancer). Concentrations of HCH isomers have also been measured using whole blood (Sexton and Ryan 2012; Sexton et al. 2011).

Urinary concentrations of 2,4,5- and 2,4,6-trichlorophenol (in units of µg/g creatinine) were measured as indicators of γ-HCH exposure in the NHANES III survey (1998–2004) (Allen et al. 2006). Pentachlorophenol was also included as a γ-HCH metabolite in some studies (Naeher et al. 2009). The use of these phenolic urinary metabolites as exposure biomarkers is limited because these are not specific

to γ-HCH and may result from exposure to other chlorinated benzenes or phenols (Angerer et al. 1981; Naeher et al. 2009).

Measurement of HCH isomers in hair has also been used as an exposure biomarker and was suggested to be a better measure of chronic exposure than blood or serum concentrations (Michalakis et al. 2012; Tsatsakis et al. 2008). A linear relationship between exposure level and hair concentration was observed in a 90-day gavage study in rats exposed to a mixture of pesticide including γ- and β-HCH (Appenzeller et al. 2017).

HCH isomers have been detected in adipose tissue samples taken by biopsy or following surgical procedures (Aulakh et al. 2007; Ociepa-Zawal et al. 2010).

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

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

There is also a direct correlation between HCH levels in the blood and human adipose tissue and semen (Baumann et al. 1980; Radomski et al. 1971a, 1971b; Szymczynski and Waliszewski 1981); concentrations of β-HCH in subcutaneous adipose tissues were found to be 300 times higher than blood levels (Baumann et al. 1980). Levels of β-HCH detected in skin lipids correlated with those found in

human adipose tissue (Sasaki et al. 1991). Although exposure levels were not known, the results of this study indicate that measuring β-HCH in skin lipids can be an easy means of determining relative levels or times of individual exposure. The method of collecting the skin lipid samples was noninvasive, involving washing the face with soap and wiping 3–4 hours later with fat-free cotton soaked in 70% ethanol. B- and γ-HCH have also been found in samples of human maternal adipose tissue, maternal blood, cord blood, and breast milk in women who were exposed to unknown levels of various organochlorine pesticides in Kenya (Kanja et al. 1992).

3.3.2 Biomarkers of Effect

No biomarkers of effect, specific for HCH isomers, have been identified in the literature. Several studies have demonstrated increases in lipid peroxidation and depletion of antioxidants in the central nervous system, liver, kidney, male reproductive tract, and maternal or fetal tissues in animals exposed to γ-HCH; however, these are nonspecific effects induced by a wide range of compounds.

3.4 INTERACTIONS WITH OTHER CHEMICALS

Toxicokinetics. The metabolism of γ-HCH can be altered by exposure to other chlorinated hydrocarbon insecticides such as DDT. Exposure to various chlorinated hydrocarbon insecticides, including γ-HCH, is thought to produce generalized nonspecific induction of microsomal enzymes, including cytochrome P450s. Induction of these enzymes could affect the toxicokinetics of a variety of xenobiotics that are metabolized through microsomal oxidation. Induction of mixed-function oxidase activity by other chlorinated hydrocarbon insecticides stimulates the oxidative degradation of γ-HCH to the tetrachlorophenols and enhances its elimination in the urine (Chadwick and Freal 1972b). Guinea pigs maintained on diets deficient in vitamin C and protein showed altered γ-HCH metabolism and excretion. Vitamin C deficiency decreased the amount of γ-HCH and its metabolites excreted in the urine and increased the amount stored in the kidney (Chadwick et al. 1972). Cadmium, which is known to inhibit hepatic drug-metabolizing enzymes in mammals, also inhibited the metabolism of γ-HCH in adult male Wistar rats exposed to the compound after short- and long-term pretreatment with cadmium (Chadwick et al. 1978b). Cadmium may inhibit γ-HCH metabolism indirectly by increasing levels of zinc and reducing levels of copper in the liver (Chadwick et al. 1978b). The addition of cadmium to the diet also increased the concentration of γ-HCH measured in the plasma and liver (Khanna et al. 1988).

Liver Effects. Pretreatment with γ-HCH reduced the clearance and exacerbated the liver toxicity (i.e., increased ALT and AST levels) of a single oral dose of 500 mg/kg acetaminophen in rats (Akhlaq et al. 2006). Acute exposure to ethanol was shown to increase the hepatotoxicity of γ-HCH in an intraperitoneal injection study as measured by increased ALT and AST activity (Radosavljević et al. 2008). The increase in liver weight produced by oral subchronic exposure to commercial HCH was exacerbated by concurrent exposure to phenobarbital or carbon tetrachloride (Khanna et al. 2002).

A low-protein diet potentiated the effects of γ-HCH on reducing the weights of various organs in male rats (Khanna et al. 1990). Serum and liver lipid content and cholesterol levels were increased in animals fed low-protein diets. The low-protein diet increased the levels of γ-HCH found in the various organ tissues. Histopathological changes in the liver, kidneys, and muscles following dietary exposure to a pesticide mixture containing monocrotophos, endosulfan, and HCH were exacerbated in protein-malnourished and diabetic rats (Benjamin et al. 2006).

Natural plant extracts (i.e., ajwain extract, *Hyrtios aff. Erectus* sponge extract) have been shown to reduce rodent liver toxicity of HCH isomers administered individually (Anilakumar et al. 2009) or as a mixture with other organochlorine compounds (Abd El-Moneam et al. 2017). The mechanisms by which these plant extracts mitigate HCH toxicity may include antioxidant activity and/or alterations in HCH absorption, distribution, metabolism, or elimination. Oral administration of aloe vera extract prevented the liver toxicity of γ-HCH in rats (measured by serum enzymes), when administered concurrently for 4 weeks (Etim et al. 2006). Intravenous administration of gadolinium chloride to hyperthyroid rats resulted in Kupffer cell depletion and a reduction in oxidative stress and liver injury following intraperitoneal injection of γ-HCH (Simon-Giavarotti et al. 2002). Administration of the antioxidant plant extract andrographolide (from *Andrographis paniculate)* was shown to exert a hepatoprotective effect in mice chronically exposed to technical-grade HCH (Trivedi et al. 2007, 2009). Liver toxicity measured by serum enzymes and histopathology and liver tumor formation occurring in mice exposed to HCH in the diet were not seen in mice given the combined exposure to andrographolide and HCH. Measures of oxidative stress in the mouse liver associated with HCH were also ameliorated by the combined treatment (Trivedi et al. 2007).

Γ-HCH was shown to be an aryl hydrocarbon receptor (AhR) antagonist in rat and human hepatoma cells (DR-H4IIE and DR-Hep-G2, respectively) and mammary gland carcinoma DR-T47-D cells. When administered as a mixture with other organochlorine compounds, an additive response on AhR antagonism was observed (Doan et al. 2019).

Gupta et al. (2011) showed that γ-HCH promoted the formation of preneoplastic lesions (glutathione-S-transferase placental [GST-P] positive foci) in the rat liver following initiation by diethylnitrosamine. Simultaneous treatment with the dietary flavonoid quercetin appeared to reverse this promotion resulting in decreased apoptosis, reduced incidence of GST-P positive foci and lower expression of p53.

Immunological Effects. Γ-HCH produced apoptosis and necrotic cell death in isolated mouse thymocytes *in vitro* and a greater-than-additive effect was observed when γ-HCH was administered in combination with malathion or permethrin (Olgun et al. 2004). Each of these pesticides produced oxidative stress in mouse thymocytes (increased superoxide anion and hydrogen peroxide) with a greater-than-additive effect on superoxide anion production observed when γ-HCH and malathion were administered together (Olgun and Misra 2006). Hydrogen peroxide production was not significantly higher if pesticides were given in combination. Γ-HCH given in combination with malathion or permethrin increased superoxide dismutase activity and decreased the activity of glutathione-peroxidase and glutathione-reductase in mouse thymocytes, suggesting a role for oxidative stress in cytotoxicity (Olgun and Misra 2006).

Ocimum sanctum seed oil (OSSO) antagonized the immunotoxic effects of γ-HCH on humoral immunity (i.e., anti-SRBC response) and delayed-type hypersensitivity (i.e., footpad thickness) (Mediratta et al. 2008).

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

Γ-HCH and dieldrin, given in combination, produced a greater-than-additive effect on reactive oxygen species generation, caspase activation, reduced mitochondrial membrane potential, and enhanced cytotoxicity in immortalized rat dopaminergic neuronal cells *in vitro* (Sharma et al. 2010). Pretreatment with an antioxidant plant extract (*Decalepis hamiltonii*) for 7 days was shown to prevent lipid peroxidation, glutathione depletion, and altered activity of antioxidant enzymes in major rat brain regions

induced by a single dose of technical-grade HCH (Srivastava and Shivanandappa 2014). The natural product Chaetoglobosin K (ChK) was shown to prevent or reverse the γ-HCH-induced inhibition of gap junction-mediated communication in rat RG-2 astroglial cells *in vitro* (Sidorova and Matesic 2008). Ethanol acted as an antagonist to γ-HCH in the central nervous system by decreasing the seizure incidence and intensity of convulsions and prolonging the duration of the latency period in rats (Mladenović et al. 2007). Anticonvulsant medications (e.g., diazepam, clonazepam, phenobarbital) were also effective in reducing seizures and lethality induced by γ-HCH in mice (Tochman et al. 2000).

Reproductive Effects. Vitamin A supplements decreased HCH-induced toxicity in the rat testes, while deficiencies in vitamin A potentiated the toxicity (Pius et al. 1990). Combined antioxidant treatment with vitamin C, vitamin E, and α-lipoic acid was shown to reduce γ-HCH -induced testicular toxicity in mice (i.e., testes weight and histopathology) (Nagda and Bhatt 2011). Daily injection of garlic extracts following oral exposure to γ-HCH reversed the observed male reproductive toxicity of γ-HCH alone in rats (decreased weights of testes, epididymis, seminal vesicles and prostate, sperm effects, and altered serum hormone levels). Injection of garlic extracts also reduced measures of oxidative stress in the rat testes and brain (Hfaiedh et al. 2011). Oral administration of the antioxidant, curcumin, either before, concurrently, or after oral γ-HCH dosing for 14 or 28 days, protected against male reproductive toxicity including decreased testes and epididymis weight and effects on sperm count, morphology, and motility (Sharma and Singh 2010). Curcumin also reduced the testicular levels of superoxide dismutase, catalase, and glutathione-transferase; however, testicular glutathione content was not affected.

Soy isoflavones in the diet have been shown to alter uterine morphology (i.e., increased hyperplasia) and expression of Erα in the rat (Yang et al. 2014; Zhang et al. 2016). These effects were reduced by simultaneous gavage administration of γ-HCH.

Developmental Effects. Cadmium interacts with γ-HCH to cause significant embryotoxic and teratogenic effects in the developing rat fetus when administered together at a dosage that, for either toxin alone, is insufficient to cause any deleterious effects on development (Saxena et al. 1986).