

## 2. RELEVANCE TO PUBLIC HEALTH

### 2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO HEXACHLOROCYCLOHEXANE IN THE UNITED STATES

Hexachlorocyclohexane (HCH) is a synthetic chemical consisting of eight isomers. Only four of these isomers— $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH, and  $\delta$ -HCH—are of commercial significance.  $\gamma$ -HCH, commonly referred to as lindane, is used as seed treatment for barley, corn, oats, rye, sorghum, and wheat. It is also used in very small quantities as a prescription medication for the treatment of scabies and head lice in humans. The FDA does not recommend the use of  $\gamma$ -HCH in infants or in children or adults weighing less than 50 kg. In the past,  $\gamma$ -HCH was used in veterinary products to control mites, lice, and other pests, but recent data suggest that no products are currently registered in the United States for this use. Other HCH isomers, as well as technical-grade HCH, are used either as fungicides or in the synthesis of other chemicals. Technical-grade HCH is comprised of 60–70%  $\alpha$ -HCH, 5–12%  $\beta$ -HCH, 10–15%  $\gamma$ -HCH, 6–10%  $\delta$ -HCH, and 3–4%  $\epsilon$ -HCH. Technical-grade HCH was banned for production and use in the United States in 1976, but still may be used in other countries in small quantities.

Monitoring data suggest that the general population is exposed to HCH through the inhalation of ambient air and the consumption of contaminated food and drinking water. The relatively high stability of the HCH isomers in the environment and their global use for many years has led to their continued detection in air, soil, surface water, groundwater, and drinking water. As worldwide use of HCH declines, however, the frequency of detection and the levels detected in the environment should continue to decrease. Very low levels of  $\alpha$ - and  $\gamma$ -HCH in air have been detected in a study conducted in the 1990s. The average air levels of  $\alpha$ -HCH at sites along Lake Michigan, Lake Superior, and Lake Erie were in the range of 0.110–0.140 ng/m<sup>3</sup> for samples collected during 1990–1997 and the average levels of  $\gamma$ -HCH were 0.024–0.062 ng/m<sup>3</sup> at the same sites. Similarly, fairly low levels of  $\gamma$ -HCH were detected in groundwater samples.  $\gamma$ -HCH was detected in two groundwater samples at levels of 0.028 and 0.032  $\mu$ g/L during a groundwater monitoring study conducted in the Ozark Plateaus Province of Arkansas, Kansas, Missouri, and Oklahoma from April to September 1993. The estimated average daily dietary intakes of  $\gamma$ - and  $\alpha$ -HCH were essentially the same in various adult age/sex groups in the United States, ranging from about 0.5 to 1.0 ng/kg/day for both isomers, whereas intake of  $\beta$ -HCH was <0.1 ng/kg/day (below the analytical detection limit in food).

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HCH can be detected in the blood and urine of exposed individuals. In humans, the concentration of  $\beta$ -HCH in adipose tissue is typically higher than other HCH isomers. It has been estimated that approximately 100% of the U.S. population had detectable levels of  $\beta$ -HCH in adipose tissue in 1970; in 1980, 80% of the population had detectable levels. In a U.S. biomonitoring study conducted in 1999–2000, less than 50% of the studied population had detectable levels of  $\beta$ -HCH in serum; the geometric mean serum concentration was 9.68 ng/g lipid (95% confidence interval of <4.8–10.4 ng/g lipid).  $\gamma$ -HCH was only detected in 1.7% of the population surveyed in 1999–2000; the geometric mean serum concentration was below the detection limit of 7.5 ng/g of lipid.

**2.2 SUMMARY OF HEALTH EFFECTS**

Information on the toxicity of HCH comes from human exposure reports and experimental studies in animals. Most of the information on the health effects of HCH in humans comes from studies of individuals involved in the production or use of HCH products, reports of exposures to domestic products containing HCH, and intentional ingestion of HCH. Most of these studies involve exposure to technical HCH or  $\gamma$ -HCH and exposure levels are not available. Except for skin rashes observed in humans following topical application of  $\gamma$ -HCH, there is no evidence that the toxicity of HCH is route-dependent. In humans and animals, the main target of acute exposure to high amounts of HCH is the nervous system, and the effects consist of hyperexcitability, seizures, and convulsions that eventually may lead to death. Although the available reports on humans describe a wide array of effects associated with exposure to HCH, it is difficult to define a clear target organ or system for HCH toxicity, largely because of limitations of the studies, such as lack of exposure data and simultaneous exposure to other chemicals in occupational settings, or exposure to lethal or near lethal amounts, which caused generalized non-specific toxicities. Yet, vomiting and nausea are usual manifestations of  $\gamma$ -HCH ingestion and also have been reported after dermal exposure to  $\gamma$ -HCH. There are also reports of adverse hematological effects in humans exposed to  $\gamma$ -HCH following inhalation and/or dermal exposure to domestic products containing  $\gamma$ -HCH and following chronic occupational exposure. There is no evidence that HCH alters immunocompetence in humans, even though there is a report of increased serum IgM levels in a small study of workers exposed to technical-grade HCH. A study of 54 men occupationally exposed to  $\gamma$ -HCH reported an increase in serum luteinizing hormone among the exposed subjects, but this sole finding is clearly insufficient to make any inference regarding reproductive effects of HCH in humans. Similarly, a single report of an association between women with serum levels of HCH isomers and babies with intra-uterine growth retardation is insufficient to draw any conclusion regarding developmental effects of HCH

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in humans, particularly since other organochlorine pesticides were also present. Studies of the cancer of HCH in humans have been inconclusive. Studies of the association between pesticide use and non-Hodgkin's lymphoma among U.S. farmers concluded that  $\gamma$ -HCH is not a major factor in the development of the disease, but may play some role. The majority of the studies of the general population have found no association between serum levels of HCH and breast cancer or breast tissue levels of HCH and breast cancer. In these studies, many other organochlorine chemicals were also detected. Results from studies of the genotoxic potential of HCH in humans have been inconclusive.

Studies in animals (mostly, but not exclusively, rats exposed orally) confirm the nervous system as a toxicity target for acute exposure to high amounts of HCH, regardless of the route of exposure. In addition to hyperexcitability and convulsions, treatment of animals with HCH has produced neurochemical alterations in the brain, behavioral alterations in adult animals, and in the offspring of animals exposed to HCH. Decreased numbers of red and white blood cells and hemoglobin have been reported in rats following repeated administration of  $\gamma$ -HCH or technical-grade HCH. Most HCH isomers were shown to increase cytochrome P-450 content and the activities of associated enzymes in rodents and also produced liver necrosis and degeneration with higher doses.  $\gamma$ - and  $\beta$ -HCH produced immunosuppression in intermediate-duration studies in rodents. HCH isomers have altered reproductive parameters in male and female animals including mink, rabbits, and rats. Effects included alterations in estrous cycle, embryotoxicity, and testicular and sperm alterations. Exposure of female rats to  $\gamma$ -HCH during lactation altered the development of the reproductive system of male offspring. Results of studies aimed to test whether  $\gamma$ -HCH and other HCH isomers are endocrine disruptors have yielded mixed results. Exposure to technical-grade HCH and  $\gamma$ -HCH during gestation caused fetotoxicity in mice. Teratogenicity of HCH has not been conclusively demonstrated. Numerous studies have examined the carcinogenicity of HCH in animals exposed orally.  $\alpha$ -HCH induced liver tumors in mice and rats,  $\beta$ -HCH induced liver tumors in mice, and technical grade HCH induced liver tumors in mice; inconclusive results have been obtained with  $\gamma$ - and  $\epsilon$ -HCH, and negative results were obtained with  $\delta$ -HCH. In genotoxicity assays, HCH isomers exhibited no genotoxic activity or weak activity at best.

A greater detailed discussion of HCH-induced hepatic, immunological, neurological, reproductive, and carcinogenic effects follows. The reader is referred to Section 3.2, Discussion of Health Effects by Route of Exposure, for additional information on other health effects.

**Hepatic Effects.** Hepatic effects, such as increased liver enzymes, have been reported in humans exposed to technical-grade HCH principally by inhalation in a pesticide formulating plant; but there are

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no liver data reported for individuals who ingested HCH or applied  $\gamma$ -HCH to their skin. An increase in cytochrome P-450 concentration has been reported in rats following inhalation exposure. Animal studies have also reported that ingestion of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -HCH isomers, individually or as technical-grade HCH, has resulted in some degree of liver toxicity including increased microsomal activity, increased liver weight, mild-to-moderate liver necrosis and fatty degeneration, and liver cancer. Biochemical or gross hepatic changes often were not accompanied by histopathological changes. Hepatic effects in animals following dermal exposure to  $\gamma$ -HCH or technical-grade HCH were similar to those observed with oral exposure. Although available human data are limited, effects on liver enzymes following exposure to technical-grade HCH were similar to those observed in animal studies. The observation of serious hepatic effects in animals (e.g., fatty degeneration and necrosis) suggests that the same results could potentially occur in workers following prolonged occupational exposure. Liver toxicity was used as the basis for an intermediate-duration oral MRL for  $\beta$ -HCH and a chronic-duration oral MRL for  $\alpha$ -HCH. As detailed in Section 2.3 and Appendix A, the intermediate oral MRL for  $\beta$ -HCH is based on a lowest-observed-adverse-effect level (LOAEL) of 0.18 mg/kg/day for liver effects in rats (centrilobular hyalinization, with periportal fatty changes and focal necrosis at  $\geq 4.5$  mg/kg/day) exposed for 13 weeks. The chronic oral MRL for  $\alpha$ -HCH is based on a hepatic no-observed-adverse-effect level (NOAEL) of 0.8 mg/kg/day in rats exposed for up to 107 weeks. Liver effects at higher doses of  $\alpha$ -HCH progressed from slight histological changes at 3.5–4 mg/kg/day to hepatic cell atrophy, fatty degeneration, and focal necrosis at 56–64 mg/kg/day.

**Immunological Effects.** A significant increase in the level of IgM was observed in workers exposed to technical-grade HCH. Although there is no evidence of an increase in immunoglobulins in animals, antibody response has been reported to be depressed in rats, rabbits, and mice exposed to  $\gamma$ -HCH. Biphasic effects on immunosuppression were reported in mice fed  $\gamma$ -HCH. This is suggestive evidence that HCH may affect the human immune system.

Immunotoxicity was used as the basis for an intermediate-duration MRL for oral exposure to  $\gamma$ -HCH. As detailed in Section 2.3 and Appendix A, the intermediate oral MRL for  $\gamma$ -HCH is based on a LOAEL of 0.012 mg/kg/day for immunological effects in mice exposed for up to 24 weeks. Effects observed at  $\geq 0.012$  mg/kg/day included changes in delayed-type hypersensitivity reaction to sheep red blood cells (SRBC), response of IgM antibody forming cells in spleen to SRBC or lipopolysaccharide, and post-treatment histology of the spleen (reductions in lymphoid follicles and overall cellularity), lymph nodes (reduced lymphocyte population and size of medullary cords), and thymus (necrosis in the medulla).

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**Neurological Effects.** In humans, neurological effects, including paresthesia of the face and extremities, headaches, vertigo, abnormal EEG patterns, and often seizures and convulsions, have been reported in individuals occupationally exposed to  $\gamma$ -HCH or in individuals exposed accidentally or intentionally to large amounts of  $\gamma$ -HCH by ingestion or dermal application. Acute- and intermediate-duration exposure of animals to high oral or dermal doses of  $\gamma$ - or  $\beta$ -HCH affects the central nervous system as evidenced by behavior disorders, decreased nerve conduction velocity, neurochemical changes, convulsions, seizures, and coma. Results of acute, intermediate, and developmental neurotoxicity test batteries in rats found that  $\gamma$ -HCH caused effects such as decreased motor activity, decreased habituation, and increased forelimb grip strength at lower doses and hypersensitivity to touch, hunched posture, tremors, and convulsions at higher doses. There is evidence that exposure to  $\gamma$ -HCH caused functional impairment (reduced permeability) of the developing blood brain barrier in young rats. The effects in humans and animals suggest that exposure of humans to high air concentrations or large oral doses could potentially result in neurotoxic effects. An effect level for neurotoxicity in rats was used as the basis for an acute-duration oral MRL for  $\beta$ -HCH, as detailed in Section 2.3 and Appendix A.

**Reproductive Effects.** Information on the potential reproductive toxicity of HCH in humans is limited. An increase in serum luteinizing hormone levels was observed in male workers, but other reproductive hormone levels were not significantly altered. Additionally, increased blood levels of  $\gamma$ -HCH and total HCH isomers were detected in women experiencing spontaneous abortion or premature delivery. Because the women were exposed to multiple organochlorine pesticides, it is difficult to establish a causal relationship between HCH exposure and adverse reproductive outcomes.

Adverse reproductive effects have been observed in male and female laboratory animals orally exposed to  $\gamma$ -,  $\beta$ -, or technical-grade HCH. In male rats, exposure to  $>1$  mg/kg/day  $\gamma$ -HCH resulted in decreases in the number of sperm and/or spermatids. This effect was observed following exposure of mature animals and in animals exposed during gestation or lactation. A decrease in sperm count was also observed in rats exposed to technical-grade HCH. At higher doses of  $\gamma$ -,  $\beta$ -, or technical-grade HCH, degeneration of the seminiferous tubules or testicular atrophy were also observed in rats and mice. An acute-duration oral MRL for  $\gamma$ -HCH is based on the reproductive effects observed in the offspring of rats exposed to  $\gamma$ -HCH during lactation, as detailed in Section 2.3 and Appendix A.

Effects in female rats, mice, and rabbits exposed to  $\gamma$ - or  $\beta$ -HCH include ovarian atrophy, increased length of estrous cycle, disruption of ovarian cycling, and decreased ovulation rate. In general, the effects in the females occurred at higher doses than in the males. Although a number of reproductive effects have been

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observed in male and female rats, two multigeneration studies did not find alterations in fertility following exposure to 13.1 mg/kg/day  $\gamma$ -HCH or 32 mg/kg/day technical-grade HCH.

**Cancer.** Use of  $\gamma$ -HCH pesticides by farmers was associated with a 50% increased risk of non-Hodgkin's lymphoma. However, a causal relationship could not be determined due to confounding effects such as use of other pesticides. Several studies have examined the possible relationship between elevated blood levels of HCH and risk of breast cancer; one study found an association and three studies did not find associations. With oral exposure,  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and technical-grade HCH have been found to be carcinogenic in mice following long-term exposure. Hepatocellular carcinoma is the most frequently reported tumor type, although in many studies, the liver was the only organ under investigation. Benign lung adenomas were also increased in mice following chronic exposure to  $\gamma$ -HCH. In general, mice appear to be more susceptible to the carcinogenic effects of HCH isomers, even though some strains have a high background level of liver tumors; and rats generally developed cancer following longer exposure or exposure to higher doses. In addition, a study reported that  $\alpha$ -,  $\beta$ -, and  $\gamma$ -HCH promoted tumor development in rats exposed to a single dose of *N*-nitrosomorpholine. A metabolite of  $\gamma$ -HCH, 2,4,6-trichlorophenol, accounts for 10–20% of  $\gamma$ -HCH-derived excretion products; this metabolite is carcinogenic in animals and might account for some or all of the carcinogenic activity observed in animals. A stable halogenated epoxide of another  $\gamma$ -HCH metabolite, pentachlorocyclohexene, could also contribute to the hepatocarcinogenicity of  $\gamma$ -HCH.

The available animal data suggest that liver cancer may be of potential concern to individuals exposed to HCH isomers for prolonged periods of time. The Department of Health and Human Services (DHHS) has determined that  $\gamma$ -HCH and other HCH isomers may reasonably be anticipated to cause cancer in humans. The International Agency for Research on Cancer (IARC) has determined that HCH is possibly carcinogenic to humans. The Environmental Protection Agency (EPA) has classified technical HCH and  $\alpha$ -HCH as probable human carcinogens,  $\beta$ -HCH as a possible human carcinogen, and  $\delta$ - and  $\epsilon$ -HCH as not classifiable as to human carcinogenicity. The EPA has additionally classified lindane ( $\gamma$ -HCH) as having suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential.

### 2.3 MINIMAL RISK LEVELS

The general population is predominantly exposed to HCH by consumption of contaminated food, with minor exposures occurring from drinking water and ambient air. Average daily dietary intakes of HCH isomers in the U.S. adult population have been estimated to be in the range of 0.5–1.0 ng/kg/day for

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$\alpha$ -HCH, 0.5–1.0 ng/kg/day for  $\gamma$ -HCH, and <0.1 ng/kg/day for  $\beta$ -HCH (Gunderson 1995b). Inhalation and dermal exposure to  $\gamma$ -HCH can also occur through occupational contact or at workplaces that formulate or use  $\gamma$ -HCH as a seed treatment. Additionally, a small percentage of the population can be dermally exposed to  $\gamma$ -HCH through pharmaceutical use, since this isomer is still available as a prescription lotion, cream, or shampoo medication for the treatment of head lice and mites.

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for HCH. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Sufficient health effects data are available to derive oral MRLs for the  $\alpha$ -,  $\beta$ -, and  $\gamma$ -HCH isomers. Technical-grade HCH and the  $\alpha$ - and  $\beta$ -HCH isomers are currently unavailable in the United States; therefore, exposure to these isomers is likely to occur only in or near hazardous waste sites at which technical-grade HCH was disposed. No MRLs were derived for technical-grade HCH. HCH is not found in the environment as technical-grade HCH, and analytical methods do not detect or measure technical-grade HCH, but rather, the individual isomers. When technical-grade HCH enters the environment, individual isomers partition into various media at different rates depending on the physical characteristics of each isomer. Some isomers may be more mobile in soil or water than others. Differences in partitioning and degradation would result in a different proportion of isomers than when initially spilled. Therefore, the development of an MRL(s) for technical grade HCH would not be relevant.

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***Inhalation MRLs***

No inhalation MRLs could be developed for isomers of HCH due to insufficient data (Table 2-1).

Information on health effects following acute inhalation of  $\gamma$ -HCH in animals (Klonne and Kintigh 1988; Oldiges et al. 1980; Ullmann 1986b) is limited. Neurological effects following acute inhalation exposure to  $\gamma$ -HCH have included excitation, sedation, ataxia, and spasms (Ullmann 1986b). Acute inhalation studies for the other HCH isomers and technical-grade HCH are not available. Intermediate-duration inhalation studies of  $\gamma$ -HCH have been performed in rats with mortality reported (Klonne and Kintigh 1988). Inhalation of 5 mg/m<sup>3</sup> of  $\gamma$ -HCH for 90 days has not resulted in adverse respiratory, hematological, hepatic, or renal effects in rats (Oldiges et al. 1983), but the data are insufficient for developing an intermediate-duration inhalation MRL. No chronic-duration inhalation studies in animals are available for any HCH isomer. Due to the limitations of the database, additional information is needed on thresholds, dose-response relationships, and sensitive target organs for determining levels of significant human exposure to HCH and associated health effects following inhalation.

***Oral MRLs***

Five oral MRLs have been derived for  $\alpha$ -,  $\beta$ -, and  $\gamma$ -HCH isomers of HCH, as discussed below, detailed in Appendix A, and summarized in Table 2-1.

 ***$\alpha$ -HCH***

- An MRL of 0.008 mg/kg/day has been derived for chronic-duration (365 days and longer) oral exposure to  $\alpha$ -HCH.

The chronic oral MRL for  $\alpha$ -HCH is based on a NOAEL of 0.8 mg/kg/day and LOAEL of 3.5 mg/kg/day for liver effects in rats (Fitzhugh et al. 1950) and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

The critical NOAEL was identified in a chronic toxicity study in which groups of 10 Wistar rats of each sex were exposed to  $\alpha$ -HCH in the diet for up to 107 weeks at estimated doses of 0, 0.7, 3.5, 7, or 56 mg/kg/day in males and 0, 0.8, 4, 8, or 64 mg/kg/day in females (Fitzhugh et al. 1950). End points included clinical signs, body weight, food consumption, organ weights, gross pathology, and



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**Table 2-1. MRL Values for Hexachlorocyclohexane (HCH)**

Isomer	Inhalation MRLs			Oral MRLs (mg/kg/day)		
	Acute	Intermediate	Chronic	Acute	Intermediate	Chronic
$\alpha$ -HCH	—	—	—	—	—	0.008
$\beta$ -HCH	—	—	—	0.05	0.0006	—
$\gamma$ -HCH	—	—	—	0.003	0.00001	—
$\delta$ -HCH	—	—	—	—	—	—
$\epsilon$ -HCH	—	—	—	—	—	—
Technical HCH	—	—	—	—	—	—

— Insufficient data

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histopathology. No exposure-related changes occurred at the low dose in either sex, indicating that the highest NOAEL is 0.8 mg/kg/day in females. Liver effects were qualitatively described in both sexes at higher doses, progressing from very slight histological changes with no gross liver pathology at 3.5–4 mg/kg/day, slight histological changes with no gross pathology at 7–8 mg/kg/day, and moderate histological damage accompanied by moderate gross pathology at 56–64 mg/kg/day. The hepatic histopathological changes classified as moderate included hepatic cell atrophy, fatty degeneration, and focal necrosis. Non-hepatic effects included decreased body weight gain, slight kidney histopathology (focal nephritis), and reduced lifespan at 56–64 mg/kg/day.

***β-HCH***

- An MRL of 0.05 mg/kg/day has been derived for acute-duration (14 days or less) oral exposure to β-HCH.

The acute oral MRL for β-HCH is based on a NOAEL of 4.5 mg/kg/day and LOAEL of 22.5 mg/kg/day for clinical signs of ataxia in rats (Van Velsen et al. 1986) and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

The principal study, Van Velsen et al. (1986), is a 13-week toxicity study in which groups of 10 Wistar rats of each sex were exposed to estimated dietary doses of 0, 0.18, 0.9, 4.5, or 22.5 mg/kg/day in males, or 0, 0.2, 1.0, 5, or 25 mg/kg/day in females. At week 2 of the study, two male and two female rats receiving the highest dose (22.5 and 25 mg/kg/day, respectively) exhibited clinical signs of ataxia and became progressively inactive. Within 3 days of the first signs of ataxia, the animals became comatose and were sacrificed. The investigators did not report adverse clinical signs at the other dose levels; thus, the 4.5 mg/kg/day (in males and 5 mg/kg/day in females) dose is considered a NOAEL.

Similar neurotoxic effects were observed in an immunotoxicity study in which groups of six female B6C3F<sub>1</sub> mice were exposed to β-HCH in the diet at estimated doses of 0, 19, 57, or 190 mg/kg/day for up to 30 days (Cornacoff et al. 1988). Mice receiving 57 or 190 mg/kg/day showed signs of ataxia within the first week of exposure. The signs resolved in a few days in the 57 mg/kg/day group, whereas approximately 80% of the 190 mg/kg/day mice became laterally recumbent and moribund. No ataxia or other signs of neurotoxicity occurred at 19 mg/kg/day. Other effects in this study included immunological alterations at 57 mg/kg/day (e.g., decreased lymphoproliferative responses to T-cell mitogens and decreased natural killer cell activity), but these end points were only evaluated after 30 days and are therefore not considered to be consequences of acute duration exposure. Support for neuro-

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toxicity as the critical effect for acute oral exposure to  $\beta$ -HCH is provided by the Cornacoff et al. (1988) study reporting ataxia after 1 week of exposure to 57 mg/kg/day and a study by Muller et al. (1981) reporting a significant reduction in tail nerve motor conduction velocity in rats exposed to 66 mg/kg/day  $\beta$ -HCH for 30 days.

- An MRL of 0.0006 mg/kg/day has been derived for intermediate-duration oral exposure to  $\beta$ -HCH.

The intermediate oral MRL for  $\beta$ -HCH is based on a LOAEL of 0.18 mg/kg/day for liver effects in rats (Van Velsen et al. 1986) and an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

The critical LOAEL was identified in a 13-week subchronic toxicity study in which groups of 10 Wistar rats of each sex were exposed to estimated dietary doses of 0, 0.18, 0.9, 4.5, or 22.5 mg/kg/day in males, or 0, 0.2, 1.0, 5, or 25 mg/kg/day in females (Van Velsen et al. 1986). End points that were examined included body weight, food consumption, hematology, blood biochemistry, organ weights, gross pathology, and histopathology. Hepatic effects were observed that included hyalinization of centrilobular cells in males at  $\geq 0.18$  mg/kg/day and females at 25 mg/kg/day; increased absolute and relative liver weight in both sexes at  $\geq 0.9$  mg/kg/day in males and  $\geq 1.0$  mg/kg/day in females; periportal fat accumulation, increased mitosis, and/or focal liver cell necrosis in males at  $\geq 4.5$  mg/kg/day and females at  $\geq 5$  mg/kg/day; and centrilobular hepatocytic hypertrophy, proliferation of smooth endoplasmic reticulum, increased microsomal activity, and/or increased glycogen content in males at 22.5 mg/kg/day and females at 25 mg/kg/day. Other systemic effects included increased absolute and/or kidney weight in females at  $\geq 2.0$  mg/kg/day and males at  $\geq 4.5$  mg/kg/day; renal medulla calcinosis in males at 22.5 mg/kg/day; and clinical signs (ataxia progressing to inactivity and coma), hematologic and splenic changes indicative of anemia (decreased red blood cells and hemoglobin, increased extramedullary hematopoiesis), and reduced body weight in males at 22.5 mg/kg/day and females at 25 mg/kg/day.

Due to the dose-related nature and progression in severity of the hepatic effects, and the mild, reversible nature of the changes at the lowest dose level, 0.18 mg/kg/day is considered to be a minimal LOAEL based on hyalinization of centrilobular cells. The liver is an established target of  $\beta$ -HCH in other subchronic and chronic studies in rats and mice (Fitzhugh et al. 1950; Ikegami et al. 1991a, 1991b; Ito et al. 1973; Schoter et al. 1987).

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*γ-HCH (lindane)*

- An MRL of 0.003 mg/kg/day has been derived for acute-duration oral exposure to  $\gamma$ -HCH.

The acute oral MRL for  $\gamma$ -HCH is based on a minimal LOAEL of 1 mg/kg/day for developmental/reproductive effects in rats (Dalsenter et al. 1997b) and an uncertainty factor of 300 (3 for extrapolation from a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

The critical LOAEL was identified in a study that assessed reproductive toxicity in male offspring of rats that were exposed during lactation (Dalsenter et al. 1997b). Groups of nine Bor:spf females were administered  $\gamma$ -HCH in peanut oil by gavage as a single 6 mg/kg dose on day 9 or day 14 of lactation, or as daily 1 mg/kg/day doses on days 9–14 of lactation. Actual doses to the offspring were not determined. The control group was administered the oil vehicle alone on days 9–14 of lactation. Male offspring (10 or 20/group) were terminated on postnatal day (pnd) 65 (puberty) or 140 (adulthood) and evaluated for the following end points: testis and epididymis weights, spermatid and sperm numbers, serum testosterone level, sexual behavior at 130 days of age during 1:1 mating with unexposed females (mount latency, intromission and ejaculatory latency, number and frequency of intromissions), mating index (number sperm positive females/number males mated x100), pregnancy index (number of males that made females pregnant/number of males that made females sperm-positive x100), fertility index (number of days elapsed until males fertilized their female partner), pregnancy end points (numbers of litters, implantations/litters, fetuses/litter, resorptions), and testicular histology (6 mg/kg offspring only). Effects observed in the 1 mg/kg/day offspring included statistically significant ( $p < 0.05$ ) reductions in relative testicular weight at pnd 140 (6.4% less than controls), relative epididymis weight at pnd 65 (7.1%), spermatid number at pnd 65 and 140 (29.0 and 12.8%, respectively), sperm number at pnd 140 (13.2%), serum testosterone at pnd 65 (30.0%), and increased number of intromissions per minute up to ejaculation at pnd 130 (45%). Effects were generally similar in type and magnitude in the 6 mg/kg offspring exposed on gestation day 9 or 14, including significantly reduced relative testicular weight at pnd 65 and 140 (~10%), spermatid and sperm numbers at pnd 140 (~8–10%), and serum testosterone at pnd 140 (~50%). There were no significant effects on sexual behavior or fertility in the 1 mg/kg/day or 6 mg/kg offspring as shown by the mating, pregnancy, and fertility indices or other pregnancy end points. Because no significant alterations in fertility were observed, the significant changes observed for relative organ weights, sperm number, hormone levels, and intromission incidence are considered minimally adverse. The testicular histological examinations of the 6 mg/kg/day offspring showed large areas of normal tissue, although some areas had distinct changes ranging from small alterations to a pronounced

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effect. The most affected areas were the tubules in which the effects included necrotic changes and reductions in Leydig cell numbers and spermatogenesis.

Similar effects on testicular histology and sperm numbers occurred in adult male offspring of mice that were orally exposed to  $\gamma$ -HCH in doses  $\geq 15$  mg/kg/day (lower doses not tested) on gestation days 9–16 (Traina et al. 2003). Additionally, intermediate-duration studies of  $\gamma$ -HCH showed that testicular and other reproductive effects occurred in mink exposed to 1 mg/kg/day. Female mink treated with 1 mg/kg/day  $\gamma$ -HCH in their diet from 3–6 weeks before mating until weaning at 8–10 weeks of age showed effects on reproductive efficiency that included reduced receptivity to mating and reduced whelping rate (Beard et al. 1997). The decreased fertility was primarily due to embryo mortality after implantation. Reductions in whelping rate, litter size, and testicular size were observed in a three-generation study of mink exposed to 1 mg/kg/day dietary  $\gamma$ -HCH (Beard and Rawlings 1998). Acute exposure to  $\gamma$ -HCH caused effects on neurological and other systemic end points at oral doses higher than the 1 mg/kg/day LOAEL for developmental/reproductive toxicity. Neurological effects of  $\gamma$ -HCH included enhanced susceptibility to kindling (induction of seizures by repeated subthreshold electrical stimulation of the brain) following a single 5-mg/kg dose (Gilbert and Mack 1995) or 3 mg/kg/day for 4 days (Joy et al. 1982), reduced brain serotonin level following 3 mg/kg/day for 6 days (Attia et al. 1991), and reduced brain barrier permeability in 10-day-old pups exposed to 2 mg/kg as a single dose or 8 daily doses (Gupta et al. 1999). The toxicological relevance of these effects is unclear because there were no concurrent tests of neurobehavioral function (as well as the unnatural method of seizure induction). A comprehensive neurotoxicity screening study was conducted in which groups of 10 male and 10 female Crl:CD BR rats were administered a single dose of  $\gamma$ -HCH by gavage at levels of 0, 6, 20, or 60 mg/kg (Hughes 1999a). This study is an unpublished Confidential Business Information (CBI) submission summarized by EPA (2000). End points included functional observational battery (FOB) and motor activity (MA) tests performed prior to treatment, within 3 hours of dosing, and on post-exposure days 7 and 14, as well as histopathology of nervous system tissues at study termination. No clinical signs or any other effects were observed at 6 mg/kg. Motor activity was decreased in females at  $\geq 20$  mg/kg and males at 60 mg/kg. Females also had increased forelimb grip strength and decreased grooming behavior at 20 mg/kg, as well as 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.

Other acute oral effects of  $\gamma$ -HCH included hematological and immunological changes in mice at 10–20 mg/kg/day (Hong and Boorman 1993), developmental changes in rats and mice at 20–45 mg/kg/day in

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rats and mice (Dalsenter et al. 1997b; Hassoun and Stohs 1996a; Rivera et al. 1991), and liver and kidney changes in mice at 72 mg/kg/day (Srinivasan and Radhakrishnamurty 1988; Srinivasan et al. 1984).

- An MRL of 0.00001 mg/kg/day has been derived for intermediate-duration oral exposure to  $\gamma$ -HCH.

The intermediate oral MRL for  $\gamma$ -HCH is based on a LOAEL of 0.012 mg  $\gamma$ -HCH/kg/day for immunological/lymphoreticular effects in mice (Meera et al. 1992) and an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).

The critical LOAEL was identified in an immunotoxicity study in which groups of six female Swiss mice were exposed to  $\gamma$ -HCH in measured dietary doses of 0, 0.012, 0.12, or 1.2 mg/kg/day for up to 24 weeks (Meera et al. 1992). End points that were evaluated throughout the study included delayed-type hypersensitivity reaction to sheep red blood cells (SRBC), lymphoproliferative response to mitogenic stimulation by concavalin A, mixed lymphocyte reactions, response of IgM antibody forming cells in spleen (plaque formation) to SRBC or lipopolysaccharide (LPS), and peritoneal macrophage phagocytic activity in response to LPS or *Staphylococcus aureus*. Histology of the thymus, peripheral lymph nodes, and spleen was evaluated at 4, 12, and 24 weeks post-treatment. Both the cell-mediated and humoral components of the immune system showed a biphasic response, characterized initially by stimulation followed by suppression in a dose-dependent manner at all dose levels, indicating that a NOAEL was not identified. Effects observed at  $\geq 0.012$  mg/kg/day included biphasic changes in delayed-type hypersensitivity reaction to SRBC (increased at 4–12 weeks and decreased at 12–24 weeks), IgM plaque formation to SRBC (increased at 4–8 weeks and decreased at 12–24 weeks), and plaque formation to LPS-SRBC (increased at 4 weeks at  $\geq 0.12$  mg/kg/day and decreased at 8–24 weeks at  $\geq 0.012$  mg/kg/day). Histological changes occurred in lymphoid organs of treated animals and were consistent with the biphasic immunomodulatory responses. Effects were observed in the spleen at  $\geq 0.12$  mg/kg/day, including no significant reaction except for active proliferation of megakaryocytes at 4 weeks post-treatment, an apparent reduction in lymphoid follicles at 12 weeks post-treatment, and considerable reduction in the overall cellularity of red pulp and white pulp areas at 24 weeks post-treatment. Histopathology at 1.2 mg/kg/day included effects in lymph nodes (reduced lymphocyte population and size of medullary cords) and thymus (necrosis in the medulla) at 12–24 weeks post-treatment at 1.2 mg/kg/day.

Immunotoxic effects have been observed in other oral studies of  $\gamma$ -HCH. Immunosuppression in the form of reduced antibody responses to *Salmonella* and typhoid vaccines occurred in rats exposed to

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6.25 mg/kg/day for up to 5 weeks (Dewan et al. 1980). Exposure to 10 mg/kg/day for 10 days caused residual bone marrow damage and suppressed granulocyte-macrophage progenitor cells in mice, and atrophy of the thymus was observed in mice following 40 mg/kg/day for 3 days (Hong and Boorman 1993). Serum antibody response to SRBC was suppressed in rats exposed to 3.6 mg/kg/day for 8 weeks (Koner et al. 1998).