

2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO HEXACHLOROCYCLOHEXANE IN THE UNITED STATES

Hexachlorocyclohexane (HCH) is essentially only produced for its pesticidal properties. HCH has been released to the environment during its formulation process and through its use. Technical-grade HCH consisted of 60–70% α -HCH, 5–12% β -HCH, 10–15% γ -HCH, 6–10% δ -HCH, and 3–4% ϵ -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. Although technical-grade HCH and its isomers are no longer manufactured in the United States, γ -HCH (lindane) is still imported into the United States and formulated into various products. Most of these formulated products are pesticides that can still be used as a seed treatment for barley, corn, oats, rye, sorghum, and wheat. γ -HCH is also used in very small quantities as a prescription medication for the treatment of scabies and head lice in humans. The FDA is revising the label for the treatment of scabies, which would effectively prohibit its use on infants and children weighing less than 60 kg. In the past, lindane 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. Laboratory studies have demonstrated that γ -HCH can be converted to α -, β -, and δ -HCH by microorganisms, but the amount converted in the environment is expected to be small.

The relatively high stability of the HCH isomers and their global use for many years has led to their continued detection in air, soil, surface water, groundwater, and drinking water. γ -HCH and the other isomers adsorb to soil, which attenuates their ability to leach into groundwater. The rate of degradation of HCH in air is relatively long, with half-lives of about 100 days or more depending upon the environmental conditions. Air monitoring data over southern Ontario, Canada, from July 1988 to July 1989 showed annual mean air concentrations for α -, β -, and γ -HCH to be 0.145, 0.0018, and 0.06 ng/m³, respectively, with a total HCH annual mean concentration of 0.21 ng/m³. The greatest HCH concentrations were observed during the summer months. The average levels of α -HCH at sites along Lake Michigan, Lake Superior, and Lake Erie were in the range of 0.110–0.140 ng/m³ for samples collected during 1990–1997 and the average levels of γ -HCH were 0.024–0.062 ng/m³ at the same sites. According to EPA's STORET database, γ -HCH was detected in 27% of 4,505 surface water samples collected in the United States at a median concentration of 0.020 μ g/L. γ -HCH was detected in two groundwater samples at levels of 0.028 and 0.032 μ g/L during a groundwater monitoring study conducted

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in the Ozark Plateaus Province of Arkansas, Kansas, Missouri, and Oklahoma from April to September 1993. α -, β -, γ - and δ -HCH were identified in at least 146, 157, 186, and 126 of the 1,636 hazardous waste sites that have been proposed for inclusion on the EPA NPL.

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. A 10-year FDA study conducted from 1982 to 1991 indicated that α -, β -, δ -, and γ -HCH were frequently detected at low levels in foods consumed in the United States. The average concentration of α -, β -, δ -, and γ -HCH in 234 ready-to-eat foods were 0.0010, 0.0027, 0.0030, and 0.0012 $\mu\text{g/g}$, respectively (see Chapter 6, Table 6-2). Estimated average daily dietary intakes of γ -HCH (lindane) and α -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 β -HCH was <0.1 ng/kg/day (below the analytical detection limit in food). As worldwide limitations on the use of HCH increase, the frequency of detection, and the levels detected in the environment should continue to decrease. Since γ -HCH is still available as a prescription medication for the treatment of head lice and scabies, a small percentage of the population can be directly exposed through this use. Prenatal exposure of children to HCH can occur. HCH has been detected in breast milk, maternal blood, and cord blood. Occupational exposure to γ -HCH can occur through inhalation and dermal contact with this compound at workplaces that formulate or use lindane as a seed treatment.

2.2 SUMMARY OF HEALTH EFFECTS

Health effects studies in humans and animals indicate that the nervous and immune systems, liver, and developing male reproductive tract are particularly sensitive targets of HCH toxicity, as specifically discussed by subsection below. HCH can also cause renal, hematological, and dermal effects. There is some evidence that lindane might act as an endocrine disruptor, but further investigation is necessary to ascertain the relevance and impact to public health. Carcinogenicity of HCH has been demonstrated in animals, but there are no conclusive data indicating that HCH is genotoxic. In general, effects of HCH are dependent on dose, duration of exposure, route of administration, and the isomer to which an individual is exposed. There is no clear evidence that children are more susceptible to HCH toxicity than adults.

Evidence of kidney dysfunction has not been observed in humans exposed to HCH, although renal effects including increased kidney weight, glucosuria, calcification, and nephritis have been reported in animals exposed to technical-grade HCH and α -, β -, and γ -HCH in the diet. Studies indicate that the mechanism

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for renal toxicity of γ -HCH in Fischer-344 male rats may be based on rat α -2 μ -globulin, a protein that does not occur in humans, female rats, or other species of animals. However, renal toxicity was shown in female rats treated dermally with technical-grade HCH, indicating that all renal effects of HCH cannot be attributed to α -2 μ -globulin nephropathy.

Blood disorders, including anemia, leukopenia, leukocytosis, granulocytopenia, granulocytosis, eosinophilia, monocytosis, pancytopenia, and thrombocytopenia, have been observed in people exposed to γ -HCH at work or in homes where HCH vaporizers were operated. Dermal exposure to γ -HCH via its direct (human) or indirect (pet) use in the treatment of mites resulted in anemia, bone marrow hyperplasia, and reduction of blood cell precursors in bone marrow in humans. Although human studies suggest that γ -HCH has the potential to induce adverse hematological effects, a causal relationship has not been conclusively established due to a lack of personal exposure data. Oral exposure to γ -HCH had no effect on hematological parameters in dogs and rats, but caused a reduction in bone marrow precursor cells in mice. Oral exposure to β -HCH resulted in reduced erythrocyte and leukocyte numbers, hemoglobin concentration, and packed cell volumes in rats, and ingestion of technical-grade HCH caused decreased white blood cell counts in rats. Animals appear to be less sensitive to γ -HCH than humans, but comparison between humans and animals is difficult because of limitations in the available data.

Dermal exposure to HCH has the potential to cause skin irritation. The use of shampoo containing γ -HCH resulted in skin rashes in humans. Dermatitis was observed in rats after daily dermal exposure to γ -HCH/kg/day for 15–25 days and in rabbits dermally exposed to technical-grade HCH for 30 days had hyperkeratinization of the epidermal layer and swollen collagen fibers in the dermis.

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 γ -HCH or in individuals exposed accidentally or intentionally to large amounts of γ -HCH by ingestion or dermal application. Acute- and intermediate-duration exposure of animals to high oral or dermal doses of γ - or β -HCH affects the central nervous system as evidenced by behavior disorders, decreased nerve velocity, convulsions, seizures, and coma. Results of acute, intermediate, and developmental neurotoxicity test batteries in rats found that lindane 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 lindane caused functional impairment (reduced permeability) of the developing blood brain barrier in young rats. The effects in humans and animals suggest that

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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 minimal risk level (MRL) for oral exposure to β -HCH, as detailed in Section 2.3 and Appendix A.

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 no liver data reported for individuals who ingested HCH or applied γ -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 α -, β -, and γ -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 lindane 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 β -HCH and a chronic-duration oral MRL for α -HCH. As detailed in Section 2.3 and Appendix A, the intermediate oral MRL for β -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 ≥ 4.5 mg/kg/day) exposed for 13 weeks. The chronic oral MRL for α -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 α -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 and Lymphoreticular 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 γ -HCH. Biphasic effects on immunosuppression were reported in mice fed γ -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 γ -HCH. As detailed in Section 2.3 and Appendix A, the intermediate oral MRL for γ -HCH is based on a LOAEL of

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0.012 mg/kg/day for immunological/lymphoreticular effects in mice exposed for up to 24 weeks. Effects observed at ≥ 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).

Reproductive and Developmental Effects. Alterations in reproductive hormones and increased blood levels of γ -HCH and total-HCH isomers have been detected in women who have undergone spontaneous abortion and premature delivery and have been reported in men occupationally exposed to γ -HCH and total-HCH isomers as well as to other organochlorine pesticides. Study results suggest that pregnant women exposed to organochlorine pesticides, including γ -HCH, were at a greater risk for premature labor and/or abortion. The biological significance of altered hormonal levels in humans is difficult to assess, although the data do suggest that HCH may potentially affect reproductive capability. Similar reproductive hormonal effects have not been reported in animals. Acute preovulatory exposure to lindane caused embryotoxic effects in mice. Histological effects on the testes and uterus, as well as increases in sperm abnormalities and decreases in sperm counts, have been observed in rats orally exposed to generally high doses of β -HCH, γ -HCH, or technical-grade HCH. Reductions in testicular and epididymis weights, spermatid and sperm numbers, and serum testosterone level were found in male rats exposed to relatively low doses of γ -HCH during lactation and evaluated at puberty and adulthood. The LOAEL for these developmental/reproductive effects in male rats, 1 mg/kg/day, was used as the basis for an acute-duration MRL for oral exposure to γ -HCH, as detailed in Section 2.3 and Appendix A. Altered testicular histology (e.g., necrotic changes and reduced Leydig cell numbers and spermatogenesis) was observed in the rats at 6 mg/kg/day, and similar effects on testicular histology and sperm numbers occurred in adult male offspring of mice that were orally exposed to lindane during gestation. Results of single and multigeneration reproduction studies in rats and mink indicate that exposure to γ -HCH or technical HCH caused effects, such as decreased numbers of offspring at birth, reduced neonatal viability, and delayed maturation of pups. These effects were primarily results of prenatal and/or postnatal developmental toxicity occurring at doses of 13–16 mg/kg/day in rats and 1 mg/kg/day in mink.

There are no data regarding developmental effects in humans via any route of exposure. Developmental effects of HCH have been observed in animals at levels of oral exposure that generally also caused maternal toxicity. A dose of 30 mg/kg γ -HCH administered to mice on day 12 of gestation caused decreases in fetal weight, fetal thymic weight, and placental weight. The only consistent finding is for

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extra ribs, which is considered a normal variation and not a toxic effect. However, dams that were exposed to 20 mg/kg/day β -HCH had pups that died within 5 days of birth, and γ - and technical-grade HCH have altered neurotransmitter levels in rat offsprings. As indicated above, oral exposure of rats and mice to γ -HCH during gestation or lactation caused developmental/reproductive effects in their adult male offspring that included adverse changes in testicular weight and histology, sperm numbers, and serum testosterone levels, and the LOAEL for male developmental/reproductive effects in rats was used as the basis for an acute-duration oral MRL for γ -HCH.

Cancer. Use of γ -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. With oral exposure, α -HCH, β -HCH, γ -HCH, 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 γ -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, it has been reported that α -, β -, and γ -HCH promoted tumor development in rats exposed to a single dose of *N*-nitrosomorpholine. A metabolite of γ -HCH, 2,4,6-trichlorophenol, accounts for 10–20% of γ -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 γ -HCH metabolite, pentachlorocyclohexene, could also contribute to the hepatocarcinogenicity of γ -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 γ -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 α -HCH as probable human carcinogens, β -HCH as a possible human carcinogen, and δ - and ϵ -HCH as not classifiable as to human carcinogenicity. The EPA has additionally classified lindane (γ -HCH) as having suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential.

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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 α -HCH, 0.5–1.0 ng/kg/day for γ -HCH, and <0.1 ng/kg/day for β -HCH (Gunderson 1995b). Inhalation and dermal exposure to γ -HCH can also occur through occupational contact or at workplaces that formulate or use lindane as a seed treatment. Additionally, a small percentage of the population can be dermally exposed to γ -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 (Minimal Risk Levels or 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.

Sufficient health effects data are available to derive oral MRLs for the α -, β -, and γ -HCH isomers. Technical-grade HCH and the α - and β -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.

Inhalation MRLs

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

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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 |
| α -HCH | — | — | — | — | — | 0.008 |
| β -HCH | — | — | — | 0.2 | 0.006 | — |
| γ -HCH | — | — | — | 0.003 | 0.00001 | — |
| δ -HCH | — | — | — | — | — | — |
| ϵ -HCH | — | — | — | — | — | — |
| Technical HCH | — | — | — | — | — | — |

— Insufficient data

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Information on health effects following acute inhalation of γ -HCH in animals (Klonne and Kintigh 1988; Oldiges et al. 1980; Ullmann 1986b) is limited. Neurological effects following acute inhalation exposure to γ -HCH 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 γ -HCH have been performed in rats with mortality reported (Klonne and Kintigh 1988). Inhalation of 5 mg/m³ of γ -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 the α -, β -, and γ -HCH isomers of HCH, as discussed below, detailed in Appendix A, and summarized in Table 2-1.

 α -HCH

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

The chronic oral MRL for α -HCH is based on a NOAEL of 0.8 mg/kg/day for liver effects in rats and uses 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 α -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 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

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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 0.2 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 19 mg/kg/day for neurotoxicity in mice and uses an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

The critical NOAEL was identified in an immunotoxicity study in which groups of six female B6C3F₁ 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 neurotoxicity as the critical effect for acute oral exposure to β-HCH is provided by other studies of this isomer identifying the nervous system as a target of toxicity. Rats exposed to 12.5 mg/kg/day of β-HCH in food for 13 weeks underwent early autopsy due to progressive clinical signs (e.g., ataxia followed by coma) (Van Velsen et al. 1986), and tail nerve motor conduction velocity was significantly reduced in rats exposed to 66 mg/kg/day of β-HCH in food for 30 days (Muller et al. 1981).

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

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

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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 ≥ 0.18 mg/kg/day and females at 25 mg/kg/day; increased absolute and relative liver weight in both sexes at ≥ 0.9 mg/kg/day in males and ≥ 1.0 mg/kg/day in females; periportal fat accumulation, increased mitosis, and/or focal liver cell necrosis in males at ≥ 4.5 mg/kg/day and females at ≥ 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 ≥ 2.0 mg/kg/day and males at ≥ 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 β -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).

 γ -HCH (lindane)

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

The acute oral MRL for γ -HCH is based on a LOAEL of 1 mg/kg/day for developmental/reproductive effects in rats and uses an uncertainty factor of 300 (10 for extrapolation of a LOAEL to a NOAEL, 10 for extrapolation from animals to humans, and 3 for human variability). An uncertainty factor of 3 for human variability was used instead of 10 because the critical effect was identified in a sensitive population (offspring exposed during lactation).

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 lindane in peanut oil by gavage as a single 6 mg/kg dose on day 9 or day 14 of lactation, or

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as daily 1 mg/kg/day doses on days 9–14 of lactation. 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. The testicular histological examinations of the 6 mg/kg/day offspring showed large areas of normal tissue, although some areas had distinct changes ranging from small alterations to a pronounced effect. 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 lindane in doses ≥ 15 mg/kg/day (lower doses not tested) on gestation days 9–16 (Traina et al. 2003). Additionally, intermediate-duration studies of lindane showed that testicular and other reproductive effects occurred in mink exposed to 1 mg/kg/day. Female mink treated with 1 mg/kg/day lindane 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 lindane (Beard and Rawlings 1998). Acute exposure to γ -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 γ -HCH

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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 CrI:CD BR rats were administered a single dose of lindane 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 ≥ 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 γ -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 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 γ -HCH.

The intermediate oral MRL for γ -HCH is based on a LOAEL of 0.012 mg γ -HCH/kg/day for immunological/lymphoreticular effects in mice and uses 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 γ -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

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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 ≥ 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 ≥ 0.12 mg/kg/day and decreased at 8–24 weeks at ≥ 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 ≥ 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. Histo-pathology 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 γ -HCH. Immunosuppression in the form of reduced antibody responses to *Salmonella* and typhoid vaccines occurred in rats exposed to 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).