

Public Health Goal for Chlordane in Drinking Water

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December 1997

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We thank the U.S. EPA's Office of Water, Office of Pollution Prevention and Toxic Substances, and National Center for Environmental Assessment for their peer review of the PHG documents, and the comments received from all interested parties.

PREFACE

Drinking Water Public Health Goal of the Office of Environmental Health Hazard Assessment

This Public Health Goal (PHG) technical support document provides information on health effects from contaminants in drinking water. The PHG describes concentrations of contaminants at which adverse health effects would not be expected to occur, even over a lifetime of exposure. PHGs are developed for chemical contaminants based on the best available toxicological data in the scientific literature. These documents and the analyses contained in them provide estimates of the levels of contaminants in drinking water that would pose no significant health risk to individuals consuming the water on a daily basis over a lifetime.

The California Safe Drinking Water Act of 1996 (amended Health and Safety Code, Section 116365) requires the Office of Environmental Health Hazard Assessment (OEHHA) to adopt PHGs for contaminants in drinking water based exclusively on public health considerations. The Act requires OEHHA to adopt PHGs that meet the following criteria:

1. PHGs for acutely toxic substances shall be set at levels at which scientific evidence indicates that no known or anticipated adverse effects on health will occur, plus an adequate margin-of-safety.
2. PHGs for carcinogens or other substances which can cause chronic disease shall be based solely on health effects without regard to cost impacts and shall be set at levels which OEHHA has determined do not pose any significant risk to health.
3. To the extent the information is available, OEHHA shall consider possible synergistic effects resulting from exposure to two or more contaminants.
4. OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult.
5. OEHHA shall consider the contaminant exposure and body burden levels that alter physiological function or structure in a manner that may significantly increase the risk of illness.
6. In cases of scientific ambiguity, OEHHA shall use criteria most protective of public health and shall incorporate uncertainty factors of noncarcinogenic substances for which scientific research indicates a safe dose-response threshold.
7. In cases where scientific evidence demonstrates that a safe dose-response threshold for a contaminant exists, then the PHG should be set at that threshold.
8. The PHG may be set at zero if necessary to satisfy the requirements listed above.
9. OEHHA shall consider exposure to contaminants in media other than drinking water, including food and air and the resulting body burden.
10. PHGs adopted by OEHHA shall be reviewed periodically and revised as necessary based on the availability of new scientific data.

PHGs adopted by OEHHA are for use by the California Department of Health Services (DHS) in establishing primary drinking water standards (State Maximum Contaminant Levels, or MCLs). Whereas PHGs are to be based solely on scientific and public health considerations without regard to economic cost considerations, drinking water standards adopted by DHS are to consider economic factors and technical feasibility. For this reason PHGs are only one part of the

information used by DHS for establishing drinking water standards. PHGs established by OEHHA exert no regulatory burden and represent only non-mandatory goals. By federal law, MCLs established by DHS must be at least as stringent as the federal MCL if one exists.

PHG documents are developed for technical assistance to DHS, but may also benefit federal, state and local public health officials. While the PHGs are calculated for single chemicals only, they may, if the information is available, address hazards associated with the interactions of contaminants in mixtures. Further, PHGs are derived for drinking water only and are not to be utilized as target levels for the contamination of environmental waters where additional concerns of bioaccumulation in fish and shellfish may pertain. Often environmental water contaminant criteria are more stringent than drinking water PHGs, to account for human exposures to a single chemical in multiple environmental media and from bioconcentration by plants and animals in the food chain.

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SUMMARY

A Public Health Goal (PHG) of 3×10^{-5} mg/L (0.03 ppb) is developed for chlordane in drinking water. Chlordane ($C_{10}H_6Cl_8$) is a broad spectrum insecticide mainly used to control termites and pests on field crops; all uses are now prohibited in the United States (U.S.). It is extraordinarily persistent and it bioconcentrates in the environment; trace levels are expected to occur in the environment for 10 or more years after last use. Animal studies in rats and mice provide sufficient evidence for the carcinogenicity of chlordane; incidences of liver carcinomas, adenomas and hemangiomas are increased. Epidemiological studies provide inadequate evidence due to methodological and other limitations. Chlordane is considered by the U.S. Environmental Protection Agency (U.S. EPA) as a probable human carcinogen (Group B2), whereas the International Agency on Research on Cancer (IARC) classifies it as possibly carcinogenic (Group 2B). On the basis of cancer risk calculations employing a cancer slope factor of $1.3 \text{ (mg/kg-day)}^{-1}$, and assuming the *de minimis* theoretical excess individual lifetime cancer risk level of one case per one million exposed persons (1×10^{-6}), a PHG of 3×10^{-5} mg/L (0.03 ppb) for chlordane is developed. The PHG value of 0.03 ppb is also considered to contain an adequate margin of safety (at least 6,700) for noncarcinogenic adverse effects including potential adverse effects on the developing endocrine system with low exposures.

INTRODUCTION

This document represents an update of our earlier health risk assessment of chlordane in drinking water which provided part of the technical support for California's primary drinking water standard or Maximum Contaminant Level (MCL) (DHS, 1988). This document does not attempt to repeat all descriptions and information found in DHS (1988) but rather it focuses on new information and data, or new analyses or interpretations of earlier studies. As noted above the PHG is a stand alone drinking water goal as well as an initial step in setting or revising a California drinking water MCL.

The use of chlordane and related chlorinated cyclodiene insecticides such as heptachlor and metabolites or co-products such as *trans*-nonachlor, oxychlordane and heptachlor epoxide has resulted in environmental and human pollution. These compounds are very persistent in the environment and have been reported to remain biologically active for 30 years or more after application (U.S. EPA, 1987b). Although persistent and relatively immobile in soil, these compounds are volatile and some movement from soil to air will occur. Monitoring data have shown that chlorinated cyclodiene residues, especially their metabolites, are found throughout the food chain and in most human tissues analyzed (U.S. EPA, 1987b).

Chlordane was used as a broad-spectrum contact insecticide primarily for protection of structures, lawn and turf, ornamental trees and drainage ditches (WHO, 1984). The use of chlordane is now prohibited in a number of countries and restricted primarily to subterranean termite control uses. Prior exposures to chlordane and related cyclodienes have resulted primarily from food borne residues, although in recent years following cancellation of many agricultural uses residues and exposures appear to be gradually declining. Airborne exposures have also occurred via structural and even subterranean uses of chlordane as well as via contaminated well water. Levels of chlordane, oxychlordane and *trans*-nonachlor have been reported in the adipose tissues of the

general population in the U.S. and many other countries. The levels are usually in the 10 to 100 ng/g range (Kutz *et al.*, 1991).

While there has long been concern about the carcinogenic potential of chlordane and related cyclodienes based on liver cancer in rodents, more recently concerns have focused on chronic low-level exposures during development and how these might affect adult behavior and steroid mediated processes (Cassidy *et al.*, 1994). In addition, some case reports and epidemiological studies suggest causal associations between chlordane exposure and blood dyscrasia, leukemia and cancers of the lung, brain, skin, bladder and stomach (U. S. EPA, 1986).

CHEMICAL PROFILE

Identification

The term chlordane commonly refers to a complex mixture of chlordane isomers, other chlorinated hydrocarbons and by-products, and at least 26 different compounds (WHO, 1988). It was first described as an insecticide in 1945 by Kearns (WHO, 1984). Chlordane was first produced commercially in the U.S. in 1947 (IARC, 1979). For its commercial manufacture, hexachlorocyclopentadiene is condensed with cyclopentadiene to produce chlordene, which is then chlorinated to give chlordane (IARC, 1979).

Physical and Chemical Properties

Those properties most relevant to human exposure are listed in Table 1. The approximate composition of technical chlordane is as follows: *trans*-chlordane (γ -chlordane), 24%; chlordane isomers, 21.5%; *cis*-chlordane (α -chlordane), 19%; heptachlor, 10%; nonachlor, 7%; Diels-Alder adduct of cyclopentadiene and pentachlorocyclopentadiene, 2%; hexachlorocyclopentadiene, 1%; octachlorocyclopentene, 1%; miscellaneous constituents, 15.5% (IARC, 1979). Chlordane has been available in the U.S. in five basic formulations (IARC, 1979) including 5% granules, oil solutions containing chlordane at 2 to 200 g/L and emulsifiable concentrates containing chlordane at 400 to 800 g/L. The U.S. EPA has accepted voluntary cancellations of the registrations of certain chlordane pesticide products and imposed limitations on the continued sale, distribution and use of existing stocks of such products [52 FR 421145(11/03/87)].

Analytical Methods

Determination of chlordane is difficult because of the complex nature of the components and the fact that each component degrades independently (WHO, 1984). Extraction from crops, other plant products, dairy products, plants and oils was achieved with an 80 to 110% efficiency using acetonitrile for extraction, petroleum ether for partitioning and clean-up on a Florisil column (Canada NRC, 1974). The principal method for the qualitative and quantitative estimation of chlordane isomers is gas-liquid chromatography with electron capture detection (WHO, 1984). The method sensitivity is 0.001 to 0.010 $\mu\text{g/L}$ for a single component pesticide and 0.050 to 1.0 $\mu\text{g/L}$ for multiple component pesticides when analyzing one liter samples.

Table 1. Physical and Chemical Properties (U.S. EPA, 1985; Simpson *et al.*, 1995.)

Description:	Viscous, amber-colored liquid
Relative molecular mass:	409.78
Boiling point:	175 °C at 2 mm (decomposes)
Melting point:	106-107 °C (<i>cis</i> -isomer) 104-105 °C (<i>trans</i> -isomer)
Specific gravity:	1.59-1.63 at 25 °C
Solubility:	9 µg/L at 25 °C in water for technical grade and 56 µg/L for <i>cis:trans</i> (75:25) chlordane; miscible with aliphatic and aromatic hydrocarbon solvents, kerosene, cyclohexanone, propan-2-ol, trichloroethylene; soluble in petroleum hydrocarbons and petroleum solvents
Conversion Factors:	1 mg/L = 59.7 ppm 1 ppm = 16.76 mg/m ³ at 25°C; 760 mm Hg
Henry's Law Coefficient:	0.0006 atm·m ³ /mole
Log P	6.16 (P = octanol/water partition coefficient)
Molecular formula:	C ₁₀ H ₆ Cl ₈
Molecular weight:	409.8
CAS No.:	57-74-9
Chemical name:	1,2,4,5,6,7,8,8-octachlor-2,3,3a,4,7,7a-hexahydro-4,7-methano-1 <i>H</i> -indene
Common trade names:	Aspon, Belt, CD68, Chlorindan, Chlor Kil, Chlordane, Corodane, Cortilan-nea, Chlortox, Dowchlor, Dow-Klor., Gold Crest, HCS 3260, Intox, Kypchlor, M410, Niran, Octa-Klor, Octaterr, Ortho-Klor, Shell sd-5532, Synklor, Syndane, Tat Chlor 4, Termi-ded, Topiclor 20, Topichlor 20, Toxichlor, Velsicol 1068
U. S. and Foreign Producer:	Velsicol Chemical Corporation

Production and Use

In 1974, production in the U.S. amounted to 9.5 million kilograms (IARC, 1991). According to U.S. EPA (1987b), about 3.5 to 4.0 million pounds of chlordane were distributed annually in the U.S. About 200,000 pounds of chlordane are used in the manufacture of home-use termiticide products. Chlordane has been used as an insecticide for more than 35 years. The main uses for chlordane have been in the control of cutworms, ants, root weevils, rose beetles, grasshoppers, grubs and termites. It has been used mainly in agriculture (25%), home use such as turf treatment, garden and household use (25%) and soil treatment around buildings (50%). Since July 1, 1983, the only use of chlordane approved in the U.S. was for the control of underground termites (IARC, 1991). New limitations on the sale of chlordane have been imposed by U.S. EPA (Occup. Health & Safety Letter, 1987). From December 1, 1987 to April 15, 1988, existing stocks could be sold, distributed and used; only certified applicators or those under their direct supervision could apply the products. All use of currently existing stocks of chlordane was prohibited after April 15, 1988.

ENVIRONMENTAL OCCURRENCE AND HUMAN EXPOSURE

Chlordane is not known to occur as a natural product. Because of its long history of use in a wide variety of applications and its persistence, residues of chlordane and its oxidation products are found in food, soils, sediments, wildlife and air.

Air

During 1970 and 1972, two national ambient air monitoring surveys sampled pesticides at a number of sites across the U.S. (U.S. EPA, 1986). Chlordane concentrations as high as 72 ppm have been found in house dust for up to four years after fumigation treatment. Chlordane was found in 10/13 air samples taken around Bermuda and between Bermuda and Rhode Island during February to June 1973; levels ranged from less than 0.005 to 0.90 mg/m³ (Bidleman & Olney, 1974). More recently a sampling of nine homes in North Carolina with years of construction ranging from 1930 to 1989 found the highest levels in and around a 1962 home. Chlordane levels were 2.8 µg/g in carpet dust and 0.36 µg/m³ in indoor air. Mean levels of 1.8 µg/g for 5/9 homes and 0.12 µg/m³ for 7/8 homes were observed (Lewis *et al.*, 1994).

Water

Based upon the use of chlordane as an insecticide injected into the soil and its persistence, it is believed to have the potential to contaminate ground water, particularly when it is applied over or near existing wells. Analyses of ground water in California as mandated by the Pesticide Contamination Prevention Act of 1985 has revealed only a few detections of chlordane, heptachlor or heptachlor epoxide in thousands of sampled wells in the period 1989 to 1996 (DPR, 1997). Another potential source is rain water. However, in two studies chlordane levels did not exceed 2 to 3 µg/L in rain water (Bevenue *et al.*, 1972; WHO, 1984).

One important aspect of chlordane residues is that they accumulate in sediment (WHO, 1984). The fate and behavior of chlordane was investigated in an isolated fresh water lake, previously free from pesticide residues (Oloffs *et al.*, 1978). The lake was treated with technical chlordane at 10 µg/L, and sediment samples were analyzed for chlordane residues 7, 24, 52, 279 and 421 days after treatment. The concentration in water was 4-5.5 µg/L after 7 days and 0.008-0.011 µg/L after 412 days.. It was observed that chlordane residues moved quickly to the bottom sediment and persisted there. The concentration in the lake sediments reached 20-30 µg/kg during the first 279 days and 10 µg/kg after 421 days. Chlordane was found in concentrations from 4.3 to 800 µg/kg in bottom material from 36 of 39 (92%) streams tributary to San Francisco Bay (Law & Goerlitz, 1974) and in 70 of 214 (32.7%) sediment samples from surface waters in southern Florida (Matraw, 1975).

Harrington *et al.* (1978) reported that a section of the public water system of Chattanooga, Tennessee was contaminated with chlordane in 1976. Chlordane concentrations in the tap water of affected houses ranged from less than 0.1 to 92,500 ppb. In 23 houses, the concentrations exceeded 100 ppb; 11 of these had concentrations greater than 1,000 ppb.

Soil

In 1970, as part of the National Soils Monitoring Program, data on soil and crop residues were collected from 1,506 cropland sites in 35 states; chlordane was detected 165 times, in a range of 0.01 to 13.34 µg/kg (Crockett *et al.*, 1974). Monitoring of the corn belt region in the U.S. (12 states) in 1970 showed chlordane to be one of the most commonly detected insecticides with values from nondetectable to 0.20 mg/kg (Carey *et al.*, 1973). Residues of chlordane were found in 16 to 64% of 400 soil samples taken from eight cities, at levels of 0.02 to 20.48 mg/kg (IARC, 1979), and in 7.4 to 42.3% of 356 soil samples taken from 14 cities, at levels of 0.04 to 13.9 mg/kg (Carey *et al.*, 1976). The half-life of chlordane in soil when used at agricultural rates is approximately one year (IARC, 1979). In a study in 1970 (WHO, 1984), it was found that 10 years after application of chlordane at 8.5 kg/hectare, approximately 18 to 20% remained.

Food

Food residues of chlordane and related cyclodienes have declined considerably in the past 10 to 20 years. The U.S. Department of Health, Education and Welfare found chlordane in a trace amounts in one composite sample of garden fruits collected at retail outlets in the period July 1972 to July 1973 (U.S. EPA, 1985), and in one composite sample of grains and cereals collected at retail outlets in the period August 1973 to July 1974 (U.S. EPA, 1985). *Trans*-Chlordane was found in 78% of chicken eggs, at an average concentration of 2 µg/kg, and *cis*-chlordane was found in 81% of eggs, at an average concentration of 1 µg/kg (Mes *et al.*, 1974). Of 200 cow's milk samples analyzed in the U.S., 87% were positive for chlordane, with levels ranging from 0.02 to 0.06 mg/L (IARC, 1979). One-third of small oysters sampled in Hawaii contained *cis*-chlordane in a concentration range of 1.34 to 57.64 µg/kg (18.64 average), and 13% contained *trans*-chlordane in an average concentration of 8.17 µg/kg. All of the large oysters sampled contained *cis*-chlordane, in a concentration range of 1.58 to 22.99 µg/kg (8.28 average), and 64% contained *trans*-chlordane in a concentration range of 1.35 to 23.38-µg/kg (7.86 average) (IARC, 1979).

The U.S. Food and Drug Administration (FDA's) analysis of dietary intake of pesticides based on the total diet study of 1982 to 1984 indicated only a 7% incidence of detectable residues of heptachlor epoxide or octachlor epoxide. Estimates of daily intake for chlordane and related cyclodienes were generally less than 3 ng/kg-day for all age groups except 6 to 11 months (5 ng/kg-day) (Gunderson, 1988). FDA's estimates of total chlordane intake in 1987 ranged from 1.5 to 1.8 ng/kg and in 1988 from 0.7 to 1.0 ng/kg (FDA, 1987; FDA, 1988). More recent FDA annual reports of pesticide residue monitoring show relatively few detections of chlordane or heptachlor (e.g., FDA, 1996). Similarly, significant declines in chlordane concentrations in California mussels were observed over the period 1977 to 1992 (Stephenson *et al.*, 1995).

Residue tolerances in food for chlordane have been established at the following levels: European Economic Community, 0.02 to 0.05 mg/kg; Australia, 0.02 to 0.2 mg/kg; Canada, 0.1 mg/kg; Sweden, 0.01 to 0.1; U.K., 0.002 to 0.05 mg/kg; and U.S., 0.1 to 0.3 mg/kg (IARC, 1991).

Human Exposure and Tissue Residues

Technical chlordane is a mixture of chlorinated hydrocarbons and contains heptachlor. Humans may be exposed to technical chlordane from a variety of sources. Drinking water, food and air are

pathways considered to be sources common to all individuals (U.S. EPA, 1985). It must be recognized that individual exposure will vary widely based on where one lives, works and travels, what one eats and physiologic characteristics related to age, gender and health status. Individuals uniquely at risk are breast-fed infants who obtain a large portion of their total fluid intake from milk.

Data from several studies indicate that contamination of water with chlordane is not a widespread problem (WHO, 1984), probably because of the extreme lipophilicity of this compound. Levels of chlordane monitored in the New Orleans water supply (U.S. EPA, 1975a) ranged from "not detected" to less than 0.1 µg/L with a mean concentration of less than 0.03 µg/L. In a study in Hawaii in 1970 to 71, chlordane was found in drinking water in 9% of samples at a mean level of 1.0 ng/L. Levels of chlordane in water vary from one location to another. For example, samples of finished water were collected from 83 utilities in U.S. EPA Region V; only one of these finished drinking water samples contained chlordane and it was found at a level of 0.004 µg/L (U.S. EPA, 1975b). In one study, chlordane contamination of a municipal water system was reported, where concentrations of chlordane accidentally rose to 1.2 µg/L (Harrington *et al.*, 1978). Data are limited on the dietary intake of chlordane and oxychlordane in the U.S. The average total intakes of chlordane for adults, infants and toddlers over the years studied were 0.0009, 0.0010 and 0.0067 µg/kg-day, respectively. The average daily intakes of oxychlordane for adults, infants and toddlers were 0.0023, 0.0016 and 0.0053 µg/kg-day, respectively (U.S. EPA, 1985).

During 1970 and 1972, two national ambient air monitoring surveys sampled pesticides at a number of sites across the U.S. Heptachlor, chlordane, heptachlor epoxide and oxychlordane were detected (U.S. EPA, 1986). Chlordane concentrations as high as 72 ppm have been found in house dust up to four years after pesticide treatment. An estimated mean level of chlordane in ambient air, based on information in Kutz *et al.* (1976), is 0.1 ng/m³. Estimated respiratory intake is 0.0021 µg/day assuming 70-kg men inhale 23 m³ of air/day (U.S. EPA, 1985).

The data available for residues in human tissue are more extensive and reliable than the data for food levels. One of the indices of human exposure to environmental pollutants is the presence of foreign residues in human adipose tissue. The source of data has been the National Human Monitoring Program (U.S. EPA, 1986). Oxychlordane was found in 97% of 5,000 samples, and about 98% of 755 samples showed residues of *trans*-nonachlor. The geometric mean levels of oxychlordane and nonachlor are about 0.11 ppm for both. Generally, *trans*-nonachlor consists of some 7% of technical chlordane (Sovocool and Lewis, 1975). Chlordane is readily metabolized by the body to oxychlordane, while *trans*-nonachlor is apparently more resistant to metabolic change. The mean adipose tissue level of oxychlordane in ten autopsy samples from Florida was 0.19 ppm (Barquet *et al.*, 1981). The widespread occurrence of chlordane-related compounds in the tissues of non-occupationally exposed persons suggests extensive and persistent environmental contamination by this pesticide.

A route of chlordane or oxychlordane excretion in human females is lactation. Breast-fed infants may be exposed to higher levels of chlordane and its metabolites. Savage *et al.* (1981) found detectable levels of oxychlordane in 74% of 1,436 milk samples. In a study of Canadian human milk samples in 1977, oxychlordane was found in 77% of the samples, *trans*-nonachlor in 68%, each at a mean level of 1 ng/L. Human milk samples of 54 women in Hawaii were collected during 1979 and 1980 (Takei *et al.*, 1983) and oxychlordane was detected in all of the samples. Yasutaka *et al.* (1986) determined chlordane residues in 29 human milk samples and found six compounds in

the milk from each donor. Geometric mean levels in milk (ng/mL) were: *trans*-nonachlor 0.55; oxychlordan 0.39; *cis*-nonachlor 0.14; *cis*-chlordan 0.09; *trans*-chlordan 0.04; heptachlor epoxide 0.66. More recently, Quinsey *et al.* (1996) compared the daily intake of organochlorine pesticide residues in human breast milk in Australia with the acceptable daily intakes (ADI's) set by the World Health Organization. For total chlordan and heptachlor epoxide the mean intakes were 0.46 and 0.25 µg/kg-day. The number of samples exceeding the ADI values were 48 and 100%, respectively. These figures are based on a mean infant weight of 7.08 kg, daily milk intake of 722 mL and milk fat content of 3.73%. In conclusion, the data indicate that breast milk can represent a major source of exposure to chlorinated cyclodienes in the breast-fed infant.

These data suggest widespread chlordan contamination among the general population. The major contributor to total intake of oxychlordan is food; oxychlordan is a known metabolite of chlordan. The relative amounts of chlordan that may be received by adults from air, food and drinking water have been estimated at 86%, 11% and 3%, respectively. These numbers are based on occurrence data (0.0009 µg/kg of food and 0.004 µg/L water) and the estimated data respiratory intake of 0.00003 µg/kg-day (U.S. EPA, 1985). Environmental levels of chlordan have been declining slowly with diminishing uses. Although chlordan is persistent in the environment, it is not expected to leach into ground water since it is almost insoluble in water. Current information indicates that the major route of exposure to chlordan is food.

METABOLISM AND PHARMACOKINETICS

Absorption

A high purity (at least 98%) formulation of the insecticide, designated HCS-3260, contains *cis*- and *trans*-chlordan in a 3:1 ratio. Rats (one of each sex) received a single oral dose of 0.05, 0.2 or 1.0 mg ¹⁴C-HCS-3,260/kg body weight by gavage, or a single oral dose of each ¹⁴C-labeled isomer at 0.2 mg/kg (Barnett and Dorough, 1974). Elimination of radioactivity in the urine over seven days was 6% for females and 2% for males. In other studies, following treatment with *cis*- and *trans*-chlordan-¹⁴C, female rats eliminated in the urine 8.5 and 5% of the administered radioactivity, respectively. These results indicate that at least 2 to 8.5% of the administered dose was absorbed from the rat gastrointestinal tract. Based upon a study in which a male rabbit was administered ¹⁴C-HCS-3260 in a dietary concentration of 25 ppm (25 mg/kg diet) for two days, 33% of the radioactivity was excreted in the urine and 21% in the feces 24 hours after dosing (Barnett and Dorough, 1974). Pulmonary absorption of an unspecified amount of ¹⁴C-chlordan (11,500 dpm/µg) in 20 µL ethanol administered to female Sprague-Dawley rats as an aerosol was measured by Nye and Dorough (1976). No ¹⁴C-chlordan was detected in exhaled air. A peak blood concentration of radioactivity of approximately 4% of the applied dose was reached in less than five minutes.

Distribution

Chlordan and its major metabolite oxychlordan, appear to be distributed preferentially to and stored in adipose tissue. The tissue distribution of *cis*- or *trans*-chlordan-¹⁴C (HCS-3260), and the metabolite, oxychlordan, was compared in male and female rats following treatment with a single oral dose (Barnett and Dorough, 1974). At two days following doses of 0.05 to 2.0 mg/kg of the respective compounds, the concentrations of radioactive equivalents in brain, muscle, liver

and kidney were generally low (0 to 0.08) while the concentrations of radioactivity in fat were somewhat higher (the average for all treatments was approximately 0.47 ppm). Male and female rats treated with 0.1 mg ¹⁴C-HC-3260/kg had higher tissue residue levels of radioactive equivalents in liver (0.05 ppm), kidney (0.26 ppm) and fat (3.71 ppm for females, 2.58 ppm for males). In general, female rats accumulated greater concentrations of radioactivity in fat than male rats after treatment with any preparation. At seven days after dosing with 1.0 mg ¹⁴C-HCS-3260 kg/bw, radioactivity in all tissues declined; radioactivity in fat declined to 2.0 ppm for females and 1.19 ppm for males. Slightly more radioactivity was present in rat tissues following oral doses of *trans*- as compared with *cis*-chlordane-¹⁴C.

When ¹⁴C- HCS-3260 was administered to male rats in the diet at 5 ppm (5 mg/kg diet) for 65 days, the tissue distribution of radioactive equivalents was 0.42, 0.91, 0.55, 0.68 and 14.73 ppm for muscle, brain, kidney, liver and fat, respectively (Barnett and Dorough, 1974). After discontinuing treatment for 28 and 56 days, the concentration of radioactivity in fat declined to 3.67 and 2.49 ppm, respectively. Radioactivity was detected in other tissues 56 days after treatment was terminated. Greater accumulation of radioactivity in all tissues resulting from the absorption of *trans*- rather than from *cis*-chlordane-¹⁴C occurred in female rats treated with 25 ppm (25 mg/kg diet). Analysis of the nature of the radioactivity revealed that approximately 30 to 60% of the radiocarbon was associated with oxychlordane.

Residues of parent isomers and oxychlordane in adipose tissue from male and female rats maintained on diets containing either 50 to 200 ppm, *cis*- or *trans*-chlordane, 100 ppm of fixed ratios of the isomers from 9:1 *trans*: *cis* to 1:9 or 50 ppm technical chlordane for 15 days were determined by Street and Blau (1972). Both male and female rats stored less oxychlordane from *trans*-chlordane, while the reverse trend was observed for storage of the parent compounds. Female rats stored markedly greater concentrations of oxychlordane in comparison to male rats. Female rats fed 50 ppm technical grade chlordane (50 mg/kg diet) stored approximately seven times more oxychlordane than the parent compound in adipose tissue. The results of feeding ratios of *cis*- and *trans*-chlordane indicated that oxychlordane accumulation was additive for each isomer.

Rabbits received *trans*-chlordane-¹⁴C daily *per os* in doses of 14.3 mg/rabbit/day for 10 weeks (Poonawalla and Korte, 1971). Two weeks after treatment was discontinued, low levels of radioactivity were detected in kidney (0.05% of administered dose), liver (0.52%), heart (0.09%), lung (0.04%), spleen (0.03%) and brain (0.04%). Higher levels were found in adipose tissue (2.59% in abdominal fat, 1.53% in subcutaneous fat) and in muscle (5.68%).

Metabolism

Street and Blau (1972) and Brimfield *et al.* (1978) proposed a metabolic pathway for chlordane based on *in vitro* studies with rat liver homogenate. Chlordane is dehydrogenated to 1,2-dichlorochlordene with subsequent epoxidation to oxychlordane. In these experiments, *trans*-chlordane was converted to oxychlordane at a seven-fold greater rate than was the *cis* isomer.

Barnett and Dorough (1974) isolated seven radioactive metabolites (in addition to the unchanged parent compounds) in the feces of rats given *cis*- or *trans*-chlordane-¹⁴C or ¹⁴C-HCS-3260, either as single oral doses (0.2 mg/kg) or by continuous feeding (5 mg/kg diet of ¹⁴C-HCS-3260 for 56 days or 25 mg/kg diet *cis*- or *trans*-chlordane-¹⁴C for 14 days). These metabolites, analyzed by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC), were tentatively

identified as hydroxychlordane, chlordane chlorohydrin, monochloro and dihydroxy derivatives of chlordane, *cis*- and/or *trans*-dihydroxychlordane derivations and a trihydroxylated chlordane metabolite. No oxychlordane or dichlorochlordene was detected in feces. However, oral administration of oxychlordane resulted in fecal excretion of unchanged oxychlordane. The nature of the urinary metabolites was essentially the same as the fecal metabolites in rats administered HCS-3260 at dietary concentrations of 25 mg/kg diet, however, oxychlordane was also present. The 24-hour feces of a rabbit administered ¹⁴C-HCS-3260 at 25 mg/kg in the diet for two days contained the same fecal metabolites found in the rat, although the amounts of unchanged isomers were greater. The urine of the rabbit contained a greater percentage of the conjugated hydroxylated metabolites than did the urine of rats.

In rabbits, Balba and Saha (1978) identified the following urinary metabolites of *cis*-chlordane: 1-hydroxy-2-chlorochlordane, *trans*-chlordane chlorohydrin and 1-hydroxychlordene. Urinary metabolites in rabbits of *trans*-chlordane were 1-hydroxy-2-chlorochlordene, 1,2-dichlorochlordene, *trans*-chlordane chlorohydrin and 3-hydroxychlordane. The metabolism of *cis*- and *trans*-chlordane-¹⁴C was also studied in rats using *in vivo* and *in vitro* techniques by Tashiro and Matsumura (1977). Male rats maintained on diets containing *cis*- or *trans*-chlordane-¹⁴C in a concentration of 100 ppm for four weeks excreted 13 metabolites from *cis*-chlordane and 14 metabolites from *trans*-chlordane in the feces. The metabolites of both isomers, identified by TLC and GLC, included heptachlor, 1,1-dichlorochlordene, oxychlordane, 1-hydroxy-2-chlorochlordene, 1-hydroxy-2-chloro-2,3-epoxychlordene, 1,2-dihydroxychlordene and trihydroxydihydrochlordene. These metabolites were found in different proportions, depending upon the administered isomers. The only urinary metabolite identified from *cis*- and *trans*-chlordane was a glucuronide conjugate of 1-hydroxydihydrochlordene. The *in vitro* incubation of the isomers with rat liver microsomes and cofactors resulted in the same metabolites qualitatively.

The major route of metabolism for both *cis*- and *trans*-chlordane was via dichlorochlordene and oxychlordane (Tashiro and Matsumura, 1977). The results of these studies were in general agreement with the proposal of Street and Blau (1972) and the results of *in vitro* metabolism studies of Brimfield *et al.* (1978).

Excretion

In the experiments of Barnett and Dorough (1974), more than 90% of the administered radioactivity was excreted over seven days by rats that were given single oral doses (0.2 mg/kg diet) of *cis* or *trans*-chlordane-¹⁴C or ¹⁴C-HCS-3260. Females excreted 6% of the radioactivity in the urine, while males excreted only 2%. At higher doses (0.5 or 1.0 mg/kg diet) of HCS-3260, the pattern of elimination was essentially the same. Females excreted slightly, but not significantly, more of the *cis*- than the *trans*-isomer. When ¹⁴C-HCS-3260 was administered in the diet for 56 days, fecal elimination, as measured by radioactivity, was 70% for the 1 mg/kg diet level, 75% for the 5 mg/kg diet level and 80% for the 25 mg/kg diet level, indicating possible saturable absorption; the investigators made no mention of possible biliary excretion. Elimination of *cis*-chlordane (75%) was greater than *trans*-chlordane (65%) following 14 days feeding of the isomers at 25 mg/kg diet.

Tashiro and Matsumura (1977) reported similar results for elimination from rats treated with single oral doses of *cis*-(5.4 mg/kg) and *trans*-chlordane-¹⁴C (9.7 mg/kg) in corn oil. The total seven-day elimination of radioactivity from *cis*- and *trans*-chlordane was 85 and 66% of the

administered dose, respectively. The 24-hour total excretion was 59% for *cis*- and 27% for *trans*-chlordane. Rabbits excreted 18% of an orally administered single dose of 200 mg/kg of chlordane in the urine collected over 16 days when organic chlorine contents were measured (Stohlman *et al.*, 1950). The peak urinary elimination of organic chlorine occurred within two days and amounted to 9% of the dose.

Poonawalla and Korte (1971) observed appreciable urinary excretion of radioactivity by rabbits that received 14.3 mg/rabbit of *trans*-chlordane-¹⁴C daily for 10 weeks. At the end of this period, approximately 70% of the daily dose had been eliminated: 22.7% was excreted in the feces, 30% of which was unchanged *trans*-chlordane and 47% was excreted as urinary metabolites. In agreement with these results, excretion of radioactivity by one rabbit 24-hours after termination of feeding 25 ppm of HCS-3260-¹⁴C for two days was 21% in feces and 33% in urine (Barnett and Dorough, 1974).

Balba and Saha (1978) also observed appreciable urinary excretion of radioactivity of *cis*- or *trans*-chlordane-¹⁴C by rabbits treated with either isomer orally at a dose of 100 mg/rabbit. However, in this study, urinary excretion did not exceed fecal excretion. For the *cis*-isomer, 48.5% and 28.4% of the radioactivity were eliminated in the feces and urine, respectively. For the *trans*-isomer, fecal and urinary excretion were 46.1% and 35.8%, respectively.

Satoh & Kikawa (1992) studied tissue distribution and clearance of *cis*- and *trans*-chlordane in mice. The animals received 20 mg/kg of each isomer by oil gavage. Blood and tissue samples were collected periodically up to 52 weeks following dosing. *Cis*-chlordane disappeared quickly from the tissues with the shortest half-life in the spleen (0.6 day) and the longest in the brain (1.9 days) with muscle, liver, kidney and blood ranging between 1.0 and 1.5 days. For *trans*-chlordane, its shortest half-life was in the spleen (0.5 day) and longest in the liver (two days) with values for the other tissues ranging from 1.0 to 1.5 days. *Cis*- and *trans*-chlordane were quickly metabolized to oxychlordane after dosing and this metabolite had the highest concentration in the liver on day two after dosing and in other tissues on day one. Oxychlordane accumulated in tissues at concentrations several times *cis*- or *trans*-chlordane except in muscle. The tissue elimination curves for oxychlordane were biphasic except adipose tissue. The half-lives were calculated to be 10 days (muscle), 25 days (kidney, blood) before week 8 and 83 days (blood) to 417 days (muscle) thereafter.

Excretion in Humans

In the urine of a four-year-old girl, levels of chlordane declined rapidly during the first three days (1.93 to 0.05 ppm) but rose to 13 ppm by 35 days, presumably due to the release of stored chlordane (Aldrich and Holmes, 1969). Fecal levels also declined rapidly during the first three days and no chlordane was detected in the feces one or two months later. Clearance of ingested chlordane from serum is relatively slow with a biological half-life of 88 days estimated in the case of the four-year-old girl and a serum half-life of 21 days in the case of a 20-month-old boy (Curley and Garrettson, 1969).

A further route of excretion in females is lactation. Several studies on pesticide residues in human breast milk detected chlordane residues. In a survey conducted by Strassman and Kutz (1977) in Arkansas and Mississippi in 1973 to 1974, every analyzed human milk sample contained oxychlordane at trace levels or higher. Breast milk contained mean concentrations of oxychlordane

at 0.005 mg/L, heptachlor epoxide at 0.004 mg/L and *trans*-nonachlor at 0.001 mg/L. Levels of heptachlor epoxide and *trans*-nonachlor were below instrument sensitivity, and could not be confirmed. In another study on 43 samples of breast milk in Northern Mississippi from 1973 to 75, oxychlordanes levels were found at 0.005 mg/L in high-pesticide-usage areas and at 0.002 mg/L in low-usage areas (WHO, 1984). In a survey involving 1,436 lactating mothers in the U.S., the mean levels of oxychlordanes in the milk ranged from 75.4 to 116 µg/L on an adjusted fat basis (WHO, 1984). In a study of Canadian human milk samples in 1974, oxychlordanes was found in 77% of the samples, *trans*-nonachlor in 68%, and heptachlor epoxide in 69%, each at a mean level of 1 mg/L whole milk (Mes and Davies, 1978). Savage *et al.* (1981) found detectable levels of heptachlor epoxide in 67% of 1,436 human milk samples from nursing mothers at 163 hospitals oxychlordanes was reported in 74% of the samples. Human milk samples of 54 women in Hawaii were collected during 1979 and 1980 (Takei *et al.*, 1983). Oxychlordanes was detected in all of the samples, with a mean of 9.068 ppm. The levels of six chlordanes metabolites were determined in human milk of 29 healthy Japanese donors (Yasutaka *et al.*, 1986). All six compounds were detected in the milk from each donor. Geometric mean levels in whole milk (µg/mL) and milk fat (mg/g), respectively, were: *trans*-nonachlor 0.55, 15.7; oxychlordanes 0.39, 11.5; *cis*-nonachlor 0.14, 4.00; *cis*-chlordanes 0.09, 3.08; *trans*-chlordanes 0.04, 1.20 and heptachlor epoxide 0.66, 20.0. Miyazaki *et al.* (1980) obtained mean residue levels of 0.54 to 0.71 ppb for *trans*-nonachlor, 0.48 to 0.52 ppb for oxychlordanes, 0.06 to 0.18 ppb for *cis*-nonachlor, 0.16 ppb for *trans*-chlordanes and 0.13 ppb for *cis*-chlordanes.

TOXICOLOGY

Toxicological Effects in Animals

Acute Toxicity

Acute toxicity of chlordanes to fresh water fish and invertebrate species occurs at concentrations ranging from 3 to 190 µg/L, with most values falling between 15 and 60 µg/L. Acute toxicity of chlordanes to salt water fish and invertebrate species occurs at concentrations ranging from 0.4 to 480 µg/L, with pink shrimp being the most sensitive species (U.S. EPA, 1980).

A number of studies have been conducted to determine chlordanes LD₅₀ values for experimental animals. A review of the literature by the National Institute for Occupational Safety and Health (NIOSH, 1976) indicated a range of chlordanes LD₅₀ values from 100 mg/kg for rabbits from oral administration to 700 mg/kg for rats from dermal administration. Ambrose *et al.* (1953a) reported a chlordanes oral LD₅₀ value of 590 mg/kg for the rat. Gaines (1969) reported technical grade chlordanes oral LD₅₀ values of 335 mg/kg for male and 430 mg/kg for female rats and a dermal LD₅₀ of 530 mg/kg for female rats. Wazeter *et al.* (1968) reported acute oral LD₅₀ values of 392 mg/kg, 327 mg/kg and 371 mg/kg for *cis*-(α) chlordanes, *trans*-(γ) chlordanes and an equal mixture of the two isomers, respectively, in the male rat. Therefore, the data indicate that technical grade chlordanes and the individual purified chlordanes isomers exhibit approximately equal toxicity. Ben-Dyke *et al.* (1970) reported an oral LD₅₀ value of 283 mg technical grade chlordanes/kg body weight for the rat. Oral LD₅₀ values for reference grade technical chlordanes ranged from 137 mg/kg for rats fed a low protein diet, to 311 mg/kg for rats fed a normal protein diet (Boyd &

Taylor, 1969). Truhaut *et al.* (1974) found that hamsters were less sensitive ($LD_{50} = 1,720$ mg/kg) to chlordane than rats.

Chlordane manufactured before 1951 was more toxic than that manufactured during and after 1951. The greater toxicity of the early technical chlordane was partly due to the presence of hexachlorocyclopentadiene in the product (CEC, 1981). Symptoms of acute chlordane intoxication include central nervous system (CNS) stimulation, as evidenced by irritability, tremor and convulsions (U.S. EPA, 1985). Pathological manifestations include hemorrhage in the gastrointestinal tract, kidneys, lung and heart as well as pulmonary congestion and edema, and degenerative changes in the CNS (WHO, 1984). Correlation between respiratory difficulty and electroencephalogram (EEG) patterns suggest that respiratory failure is a contributing factor in chlordane-induced mortality (Hyde & Falkenberg, 1976).

Subchronic and Chronic Toxicity

Khasawinah *et al.* (1989) evaluated the inhalation toxicity of technical chlordane in rats and monkeys. Range-finding (28 days) and subchronic (90 days) inhalation studies with Wistar rats and a 90-day inhalation study with *Cynomolgus* monkeys were conducted. In the range-finding study with 10 animals/sex exposed to 5.8, 28.2, 154 or 413 $\mu\text{g/L}$ chlordane for eight hours/day, five days/week, the threshold for toxicity was about 5.8 $\mu\text{g/L}$. There were no deaths in the control, 5.8 $\mu\text{g/L}$ or 28.2 $\mu\text{g/L}$ groups. Early deaths occurred in the 154 $\mu\text{g/L}$ (11 days) and 413 $\mu\text{g/L}$ (three days) groups. There were no significant treatment-related histopathological changes in tissues of control or 5.8 $\mu\text{g/L}$ chlordane groups. Treatment-related lesions in rats exposed to 28.2 $\mu\text{g/L}$ were centrilobular hepatocyte enlargement of the liver in both sexes and increased height of follicular epithelial cells in the thyroids of male rats. In addition to these effects animals exposed to 154 $\mu\text{g/L}$ chlordane for 12 days showed hepatocyte enlargement with vacuolation or ballooned vacuolated hepatocytes. At 413 $\mu\text{g/L}$ chlordane for four days the livers showed diffuse vacuolation of hepatocytes, foci and areas of hepatic necrosis and centrilobular ballooned vacuolated hepatocytes. In lungs, epithelial degeneration in bronchi and bronchioles and cell debris in bronchi, bronchioles and alveoli were observed.

The 90-day rat inhalation study was conducted with 35 animals/sex/dose group for control, 1.0 $\mu\text{g/L}$ and 9.2 $\mu\text{g/L}$ groups and 47 animals/sex/dose at 0.1 $\mu\text{g/L}$. Twelve animals/sex in the low-dose group were sacrificed during week one of the study for determination of blood chlordane levels. Additional sacrifices were conducted at weeks 9, 13 (final exposure) and 26. Animals were presumably exposed with the same regimen as the range-finding phase but this is not specifically stated. There were no deaths attributable to chlordane exposure. Small but statistically significant differences in body weight gains were not considered toxicologically relevant. There were clinical signs during exposure except that some female rats at 9 $\mu\text{g/L}$ appeared more sensitive to touch from day 18 on and males from day 63 to 67. There were small but statistically significant differences in some red blood cell parameters that were not considered toxicologically relevant. There were also differences noted in females in total white blood cells and lymphocytes at the mid and high-doses ($p < 0.01$), in neutrophil and eosinophil count at the high-dose ($P < 0.05$) and in platelet count at mid and high-doses ($p < 0.01$). At week 27, after the recovery phase, there were no significant hematological differences. The blood chemistry data demonstrated statistically significant increases in calcium at all dose levels in males but only at the high-dose in female rats. Cholesterol levels were 28 to 63% higher in female rats at the high-dose. A dose-dependent increase for male and female rats in liver cytochrome P_{450} concentration and in microsomal protein

was evident after 13 week exposure at the high-dose but declined during recovery showing only a slight elevation at 45 to 46 days post-treatment.

In the 90-day study in monkeys, 48 animals were divided into four groups of six males and six females each and the exposure regimen was the same as for the rats. No treatment-related findings were observed for survival, clinical signs, body weight, food consumption, ophthalmology, urinalysis or macroscopic pathology. A small increase (0.7 °C) in body temperature was observed during exposure to the mid and high-doses. No differences in pulmonary function indicative of adverse effects of chlordane were noted. The blood platelet count was slightly reduced ($p < 0.05$) in the high-dose females at 13 weeks, but all values were within normal limits. There were no statistically-significant differences in organ weights noted, but mean thyroid and liver weights for the high-dose were higher in both males and females. There were no histopathological findings observed in any animals related to chlordane inhalation. Notwithstanding the concern expressed by some authors about the veracity of industry sponsored toxicity studies (Epstein, 1990), this work, within limits, shows a remarkable lack of toxicity in the primate *Cynomolgus*.

Groups of four to seven male and four to seven female dogs were administered 0, 0.3, 3, 15 or 30 mg chlordane/kg in the diet for two years. Abnormalities in clinical liver function tests were seen in the 15 and 30 mg/kg groups. In animals selected for necropsy at the end of the first year, increased relative liver weights and associated hepatocellular changes were found at 30 mg/kg. At the end of two years, dose-dependent increases in relative liver weights were found at 15 and 30 mg/kg, with non-dose-dependent hepatocellular changes. No effects were observed at exposures of 3 mg/kg diet or less (Wazeter, 1967).

In a two-year dietary study in rats, Ingle (1952) demonstrated dose-dependent adverse effects ranging from minor liver damage at 10 mg/kg diet to a high incidence of mortality, eye and nose hemorrhage and severe histopathologic damage to the liver, kidney, heart lung, adrenal, myocardium and spleen at 300 mg/kg diet. No adverse effects were noted at 5 mg/kg diet.

Twenty-four rats, 12 of each gender, were administered dietary levels of 2.5, 25 or 75 mg/kg chlordane for two years (Lehman, 1952b). The two higher levels caused moderate to severe signs of toxicity. The lowest level caused histologic liver changes. Groups, each comprising 20 male and 20 female rats, were administered dietary levels of 0, 5, 15, 25 or 35 mg/kg of *cis*-chlordane, 15, 25, 35 or 75 mg/kg of *trans*-chlordane or 5, 15, 25, 35 or 50 mg/kg of a 1:1 mixture of *cis*- and *trans*-chlordane (Ingle, 1969 cited in WHO, 1984). In the group receiving *cis*-chlordane, growth retardation became apparent in the rats administered 35 mg/kg in the diet after four months in males and after five months in females; with *trans*-chlordane, the 75 mg/kg group of males only displayed growth retardation after eight months. With the mixture, growth retardation was evident in both males and females receiving 50 mg/kg; growth retardation was not evident in any group receiving lower doses of either isomer. Increased mortality rates for both male and female rats became significant in the groups receiving *cis*-chlordane at 35 mg/kg, *trans*-chlordane at 75 mg/kg or the *cis*-/*trans*-mixture at 50 mg/kg. Autopsy did not reveal any gross pathological lesions. Histological examination showed no changes in any organ except the liver.

Groups of six female and six male rats were administered 2.5 mg or 25 mg/kg of a sample of technical chlordane containing 60 to 75% chlordane and 25 to 40% unrelated products in the diet for up to nine months (Ortega *et al.*, 1975). Centrilobular cell hypertrophy, cytoplasmic

margination and cytoplasmic bodies were observed in the liver in one male receiving 2.5 mg/kg and in five males receiving 25 mg/kg. No changes were seen in female rats.

Rats were administered diets containing chlordane for 80 weeks and held until week 109 (NCI, 1977). Decreased body weight gain was noted in males receiving 407 ppm (approximately 20.3 mg/kg) in the diet and in females receiving 242 ppm (approximately 12.1 mg/kg). Tremors were noted in the females at week 44, and the animals that survived to termination were in poor condition (NCI, 1977). B6C3F1 mice were administered diets containing chlordane for 80 weeks and held until weeks 90 to 91. No body weight effects were noted, but tremors were observed in female mice at week 20. Alopecia was noted in male and female mice and a hunched appearance was noted in a few mice. Abdominal distention was observed in all groups but was predominant in the females. Increased mortality incidence was noted for male mice receiving 29.9 ppm (approximately 1.5 mg/kg) ($p \leq 0.02$) and 56.2 ppm (approximately 2.8 mg/kg) ($p \leq 0.01$). Mortality in treated and control groups did not differ significantly in female mice (NCI, 1977).

In a study by Velsicol Chemical Corporation (Khasawinah & Grutsch, 1989a), mice were administered 0, 1, 5 or 12.5 ppm chlordane (approximately 0, 0.15, 0.71 or 1.79 mg/kg) in the diet for two years. In mice receiving 5 or 12.5 ppm, increased levels of serum glutamic-oxaloacetictransaminase (SGOT) and serum glutamic-pyruvictransaminase (SGPT) were noted; hepatocellular swelling and necrosis also occurred with increased liver weight in both male and female mice.

In another study by Velsicol Chemical Corporation (Khasawinah & Grutsch, 1989b), Fischer 344 rats were administered diets containing 0, 1, 5 or 25 ppm chlordane (approximately 0, 0.05, 0.25 or 1.25 mg/kg) for 130 weeks. There were significant dose-dependent changes only in the liver. At 25 ppm chlordane in the diet there were increases in bilirubin levels, liver weight, liver cell volume (centered at the lobules), hepatic necrosis (only in dead or dying animals) and hepatocellular nodules (only after 105 weeks of exposure). Liver weight was significantly increased in males receiving 5 or 25 ppm at week 130, but not at weeks 26 or 52. An increased incidence of hepatocellular swelling was noted in males receiving 1, 5 or 25 ppm. Based on these results, the no-observed-effect-level (NOEL) for female rats was 1 ppm, but a NOEL was not identified for male rats (U. S. EPA, 1986; U. S. EPA, 1987). The original study pathologist identified the NOEL at 5 ppm and the subsequent pathology working group determined that 25 ppm for males and 1 ppm for females were NOELs (see additional discussion on carcinogenicity below).

When male Guinea pigs were exposed to chlordane at 67 mg/kg-day, through dermal painting for 90 days, mild degenerative changes in the skin and testis were evident (WHO, 1984).

Rats (80/sex/dose group) were administered technical chlordane at dietary levels of 0, 1, 5 or 25 ppm for 130 weeks (Yonemura *et al.*, 1983b). Body weight, food consumption and water intake were monitored at regular intervals. Clinical laboratory studies were performed and organ weights were measured on eight animals/sex/group at weeks 26 and 52, and on all survivors at week 130. Gross and microscopic pathology were performed on all tissues. Daily dose levels of 0.045, 0.229 and 1.175 mg/kg for the 1, 5 and 25 ppm treatment groups, respectively, were calculated from food consumption and body weight data. No effects were observed for hematology, clinical chemistry and urinalysis endpoints, and no treatment-related effects were reported for body weight and mortality. Increased liver-to-body weight ratios were reported for male and female mice administered chlordane in the diet for two years at 0.76 ppm (0.09 mg/kg-day), the lowest-dose

administered (Yonemura *et al.*, 1983a). Liver necrosis was observed at 0.43 and 1.1 mg/kg-day for males only.

Developmental and Reproductive Toxicity

Rats, maintained from weaning on a diet containing a chlordane level of 320 mg/kg, exhibited reduced rates of mating and of viable litters and an increased rate of death of progeny prior to weaning. It was concluded that, at this dose level, chlordane interfered with both fertility and litter survival (Ambrose *et al.*, 1953a). Groups of 10 male and 20 female rats, used in a three-generation study, were administered dietary levels of technical chlordane at 0, 0.3, 3, 15, 30 and 60 µg/kg (Ingle, unpublished data, 1967 cited in WHO, 1984). Two litters in each generation were studied. Levels up to and including 30 mg/kg did not have any significant effect on fertility, number of offspring or weight, growth or mortality rate of the young animals up to weaning age. Autopsy of animals after weaning did not reveal any gross or microscopic differences between the groups. At 60 mg/kg, there was a high (10.6%) mortality rate in the second (F₂) generation litters during the latter part of the nursing period; these animals showed gross and microscopic pathological changes, comparable with those characteristic for chlordane intoxication. However, survivors of this generation did not show any tissue changes. A third set of F₃ litters at 60 mg/kg exhibited 17% mortality during the nursing period, with symptomatology and gross and microscopic tissue changes characteristic of chlordane intoxication. Third (F₃) generation litters from dams removed from the 60 mg/kg group and placed on chlordane-free diets for 30 days prior to re-mating showed no significant differences in any respect from control litters. No evidence of teratogenicity was found in this study (WHO, 1984).

Mice administered diets containing 25 to 100 mg/kg chlordane for six generations exhibited decreased viability in the first and second generations at 100 mg/kg; in the third generation at this level no offspring were produced (CEC, 1981). At 50 mg/kg, viability was reduced in the fourth and fifth generations. At 25 mg/kg no statistically significant effects were observed even after six generations.

Chlordane was administered orally to rabbits at levels of 1.0, 5.0 or 15 mg/kg-day on the days 6 to 18 of gestation. A control group and a positive control group were used. No changes were reported in behavior, appearance or body weight. Abortions were reported in three rabbits at the 1.0 mg/kg level and in one rabbit at the 15 mg/kg dose level. No effects on any of the maternal or fetal parameters were noted. No teratogenic effects were noted (WHO, 1984).

Cassidy *et al.* (1994) studied the effects of chlordane exposure during pre- and postnatal periods on sex steroid-mediated behaviors and functions in the rat. Time-pregnant Sprague-Dawley dams (day 4 of gestation through day 21 of lactation) and offspring (day 22 of age through day 80) were administered three dose levels of technical chlordane (100, 500 or 5,000 ng/g) in peanut oil supplemented peanut butter (1 mL of peanut butter) on a daily schedule. The measurements made on three to five animals/sex were: cyclodiene concentrations in rat blood and milk; serum testosterone; body weight; mating behavior; Cincinnati maze; open field activity; auditory startle and [Cl]⁻³⁶ uptake into brain microsacs. The plasma concentrations of heptachlor epoxide and other cyclodienes increased during gestation and then decreased during lactation. In contrast, the plasma levels of cyclodienes in the offspring increased throughout the post-weaning period. Concentrations of the analytes in dam milk on day eight of lactation for the 100 ng/g dose level were: heptachlor, non-detect; heptachlor epoxide, 51 ng/mL; *trans*-chlordane, 2.4 ng/mL;

oxychlordanes, 46 ng/mL. Testosterone levels were significantly reduced in females at 500 and 5,000 ng/g exposure levels. The levels were 20, 60 and 55% below control values for the 100, 500 and 5,000 ng/g doses. Treated males showed very little effect with only a 10% increase at 5,000 ng/g. Dose-dependent increases in body weight were observed in female offspring at all doses but only female offspring at the 500 ng/g level were significantly different from controls ($p < 0.05$).

Of the behavioral tests conducted, only mating behavior, visospatial and auditory-evoked startle tests produced significant effects at the 100 ng/g level. The $[Cl]^{-36}$ uptake in 5,000 ng/g dosed male offspring was significantly reduced. The authors conclude that chlordanes, or more specifically its oxidized metabolites oxychlordanes and heptachlor epoxide, are mimicking sex steroids and/or altering their levels. Only chlordanes-treated females were significantly different in plasma cyclodiene levels, in the Cincinnati maze and in body weight. Treated females exhibited more masculine effects which often mirrored dose-dependent effects in steroid-treated rats. Chlordanes-treated males exhibited increased male-typical mating behaviors and 5,000 ng/g dosed males had decreased $[Cl]^{-36}$ uptake. It is consistent with previous findings that cyclodienes from chlordanes would bind with sex-steroid receptors in the CNS and alter sexually dimorphic behaviors and functions. The study indicates lowest-observed-adverse-effect-level (LOAEL) of 100 ng/g or 0.1 mg/kg-day for sex steroid-related behavioral effects. The authors note that even a lower LOAEL might have been observed with lower doses and U.S. EPA recommended 20 liters/dose.

Genetic Toxicity

Cis-chlordanes, *trans*-chlordanes and mixed chlordanes isomers were tested in the Ames *Salmonella* microsome assay and were not considered mutagenic (WHO, 1984). Chlordanes was not mutagenic when tested using five different strains of *Salmonella typhimurium* in the Ames assay (Ercegovich & Rashid, 1977). Purified chlordanes was not mutagenic in any strain of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98 and TA100) that was tested (IARC, 1979). Chlordanes was the fourth most active of 47 pesticides tested in a modified SOS microplate assay in which the induction of β -galactosidase in *Escherichia coli* PQ37 was used to measure quantitative genotoxic activity (Venkat *et al.*, 1995). A 0.01 mM concentration of chlordanes induced ouabain-resistant mutants in Chinese hamster V79 cells and was considered weakly mutagenic (Ahmed *et al.*, 1977b). Chlordanes induced unscheduled DNA synthesis in SV-40 human cells in culture without activation (Ahmed *et al.*, 1977a).

Chlordanes induced gene conversions in the yeast *Saccharomyces cerevisiae* strain D4 (Chambers & Dutta, 1976). Neither *cis*-chlordanes (42, 58 and 290 mg/kg single intraperitoneal doses or five daily oral doses of 75 mg/kg) nor the *trans*-isomer (five daily oral doses of 50 mg/kg) had a significant effect in a dominant lethal assay on mice (Epstein *et al.*, 1972). Technical chlordanes at dose levels of 50 or 100 mg/kg in a dominant lethal study using mice failed to induce dominant lethal mutations (Arnold *et al.*, 1977). Chlordanes was shown to be clastogenic *in vivo* in mouse bone marrow at a dose of 10 mg/kg (Sarkar *et al.*, 1993).

Studies on animal hepatocytes in culture have shown that chlordanes does not induce unscheduled DNA synthesis (UDS) (Maslansky and Williams, 1981). Telang *et al.* (1982) showed that chlordanes was not genotoxic to adult rat liver cells but inhibited cell-to-cell communication in a rat liver derived 6-thioguanine-resistant cell line. Telang *et al.* (1982) proposed that chlordanes was exhibiting properties exerted by many cancer promoting agents. Schop *et al.* (1990) compared the genotoxicity of topically applied pesticides including chlordanes in CD-1 mice. Chlordanes was

observed to induce a dose-dependent increase in the frequency of hair follicle nuclear aberrations but only induced bone marrow micronuclei at the highest dose tested or about 500 µmol/kg.

Ruch *et al.* (1990) observed that chlordane, endosulfan and heptachlor inhibited gap junction intercellular communication in mouse and rat hepatocytes in a dose-responsive manner. The authors suggest a mechanism involving cyclic AMP (cAMP) since the analog dibutyl-cAMP reversed or partially inhibited the inhibition by these cyclodienes. Bessi *et al.* (1995) demonstrated synergistic effects of chlordane and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in the morphological transformation of SHE cells *in vitro*. Chlordane at 5 to 20 µg/L and 0.1 µg/L TPA highly potentiated each other when applied sequentially. No DNA adducts could be found in SHE cells.

Immunotoxicity

The effects of cyclodiene insecticides on the immune system have been studied in a number of exposure models. Chlordane has been studied in both prenatal and adult exposures. Prenatal exposure to 0.16 to 16 mg/kg chlordane resulted in significantly decreased delayed-type hypersensitivity to oxazolone in the high-dose group and no significant effect in the low-dose group (Barnett *et al.*, 1985 a,b). There were no decreases in T and B cell mitogen-induced blastogenesis (Barnett *et al.*, 1985a) or cytotoxic T lymphocyte responses (Blaylock *et al.*, 1990). In animals immunized with influenza virus, the influenza-specific delayed-type hypersensitivity was also significantly depressed (Barnett *et al.*, 1985b). Prenatal chlordane exposure causes a number of changes to peritoneal macrophages in the offspring. Peritoneal macrophages from chlordane-treated offspring closely resemble inflammatory macrophages from control animals (Theus *et al.*, 1992a). In addition, functions like macrophage-mediated cytolysis of P815 tumor cells show delay when using macrophages from chlordane-treated offspring as compared with normal peritoneal macrophages (Theus *et al.*, 1992b).

Blyler *et al.* (1994) studied the effect of chlordane on the development of myeloid stem and progenitor cells. Female mice were administered either 0 or 8 mg/kg-day chlordane for 18 days during pregnancy. Myeloid hemopoietic activity of bone marrow cells from 16-week old offspring was evaluated for *in vitro* colony-forming units in culture in response to the exogenously added cytokines granulocyte/macrophage-colony stimulating factor (CFU-GM), macrophage-CFU (CFU-M) and interleukin 3 (CFU-IL-3). There was a significant depression of the numbers of bone marrow CFU-GM, CFU-M and CFU-IL-3 only in female offspring. Male offspring consistently demonstrated no difference in the respective CFU's. Prenatal treatment with chlordane did not significantly affect the number of recoverable viable bone marrow cells in either male or female mice. These data demonstrate postnatal defects in myeloid progenitor cell development in female offspring of mice exposed to chlordane. While the mechanism of this effect is unclear it may be related to possible estrogenic effects of chlordane in a manner similar to that postulated for diethylstilbestrol which also shows gender-specific effects in offspring of exposed mothers (Luster *et al.*, 1979).

Johnson *et al.* (1986) observed that chlordane exposure of mice *in utero* may alter immunological competence. Various toxicological and immunological parameters were assessed after exposure of female B6C3F1 mice to 0.1, 1.0, 4.0 or 8.0 mg/kg γ -chlordane for 14 days via oral gavage. Variables evaluated included periodic body weights, terminal organ weights, hematology including leukocyte differentials, antibody response to sheep red blood cells (SRBC), lymphoproliferative

responses to the mitogens phytohemagglutinin (PHA), concanavalin A (Con A) and lipopolysaccharide (LPS) and allogenic cells and the delayed hypersensitivity response to keyhole limpet hemocyanin. When compared to the corn oil (vehicle) controls, chlordane produced a significant, dose-dependent increase in liver weight. Total leukocytes were significantly increased in chlordane-treated groups and seemed to be due to a significant increase in the lymphocyte population. Humoral immunity, as assessed by enumeration of SRBC-specific immunoglobulin M (IgM) antibody forming cells and proliferation of LPS-stimulated spleen cells, was not significantly altered in mice exposed to chlordane. *In vitro* evaluation of cell-mediated immunity, as measured by proliferation of Con A and PHA-stimulated spleen cells from chlordane-treated animals, indicated a significant and dose-dependent increase. The response to allogenic cells was also enhanced. Results from an *in vivo* indicator of cell-mediated immune status, the delayed hypersensitivity response to keyhole limpet hemocyanin, did not support chlordane-enhanced T cell function suggested by mitogen and mixed lymphocyte responses. Therefore, a potent cyclodiene insecticide of environmental concern produced an enhancement of certain indicators of cell-mediated immunity. The expression of a delayed hypersensitivity response *in vivo* and the antibody response to SRBC was unaltered in mice exposed to the chemical. These results suggested that γ -chlordane, at the concentrations utilized, did not produce a biologically significant alteration of the immunocompetence of intact animals.

In a follow-up study, Johnson *et al.* (1987) studied immunosuppression by γ -chlordane by direct addition of chlordane to cultured spleen cells from untreated B6C3F1 mice. The proliferative response of splenocytes was suppressed by 40, 81 and 99% at 1, 10 and 100 μ M chlordane, respectively. No suppression was observed at 0.1 μ M chlordane. In addition chlordane induced a marked suppression of mitogen-induced proliferative responses of both T- and B-lymphocytes at 10 and 100 μ M chlordane. The antibody response to SRBC was suppressed 90% at 10 μ M chlordane. The *in vitro* immunotoxicity of chlordane is associated with cytotoxicity at higher concentrations. However, the effects can be dissociated. Chlordane significantly suppresses (> 50%) both cell-mediated and humoral immunocompetence at concentrations devoid of effects on recovered spleen cell viability. Concentrations of 10 μ M or greater for five days resulted in significant cytotoxicity. No immunosuppressive effects were noted *in vivo*. The failure of chlordane to significantly alter *in vivo* immunity may be related to the close association of chlordane and/or its metabolites with plasma components resulting in reduced distribution to immunocompetent organs.

Neurotoxicity

Moser *et al.* (1995) subjected chlordane and several other toxicants to a battery of neurobehavioral tests. Adult female Fischer 344 rats (number unspecified) received acute exposures of 16, 52, 156 or 291 mg/kg chlordane by corn oil gavage. Doses for repeated exposures were 5, 16, 52 or 156 mg/kg and the chlordane was approximately 40% chlordane and 60% other cyclodiene compounds. No lethality occurred after acute chlordane exposure, but with repeated dosing all rats in the two higher dose groups died with median cumulative doses of 546 to 702 mg/kg. Acute chlordane exposure produced significant effects in the excitability and neuromuscular domains with the most pronounced effects at four hours after dosing but some still evident at 24 hours. Neuromuscular effects included tiptoe gait and increased forelimb grip strength in the highest-dose group. Weight loss was observed in all except the lowest-dose, and piloerection was evident in the two highest-dose groups. Upon repeated exposure chlordane-induced changes in the autonomic and sensorimotor domains were also significant. At four days these included decreased motor activity,

increased removal and handling reactivity and increased responsiveness to touch and auditory stimulation. At nine days, the excitability, sensorimotor and autonomic domains were still affected, handling reactivity and arousal were still increased, and there was an increase in urination and lacrimation. The approach and tail pinch responses were also increased at nine days.

Carcinogenicity

Evaluations of carcinogenicity studies in animals have been published by U.S. EPA (1986) in *Carcinogenicity Assessment of Chlordane and Heptachlor/Heptachlor Epoxide* and summarized in U.S. EPA's IRIS on-line file. Presented below are the data relevant to cancer risk assessment. Chlordane has been studied in four mouse and four rat long-term chronic toxicity carcinogenesis bioassays. In addition, two single dose long-term studies in mice were conducted recently to address mechanistic issues.

Becker and Sell (1979) administered a 90:10 mixture of chlordane:heptachlor to an unspecified number of male C57Bl/6N mice at concentrations of 25 or 50 ppm (approximately 3.57 or 7.14 mg/kg) for 18 months. Specific information as to treatment and observation periods and time of death was not provided. The C57Bl/6N mouse rarely develops spontaneous liver lesions and in a group of 200 control mice no liver tumors or nodular lesions were found over 18 months of observation. In mice receiving chlordane: heptachlor, increased numbers of liver lesions were seen, including both benign proliferative lesions and hepatocellular carcinomas. Of those surviving to the end of the experiment, 27% (16 mice) had primary hepatocellular carcinomas, with the first appearing at 36 weeks. No other information was presented as to early deaths and associated tumor incidences. Data relating tumor incidence to dose were not available. Cells from the carcinomas grew when transplanted, producing tumors that were histologically similar to the primary hepatocellular carcinoma. Cells from the benign lesions did not grow when transplanted. The authors concluded that the benign lesions did not appear to be premalignant lesions. The key finding in this study was that chlordane ingestion induced hepatocellular carcinomas in a strain of mice that does not spontaneously develop hepatocellular carcinomas.

Velsicol/IRDC (1973) administered analytical grade chlordane in the diet at concentrations of 0, 5, 25 or 50 ppm (approximately 0, 0.71, 3.57 or 7.14 mg/kg) for 18 months to groups of 100 male and 100 female CD-1 mice. The mice were six weeks of age when exposure began. A six-month interim sacrifice of 10 mice/sex/group did not reveal compound-related lesions. No effect of chlordane on body weight gain or food consumption was observed. However, the survival was greatly reduced at the high-dose levels and only about one-half of the mice were histologically examined due to tissue autolysis. A dose-dependent increased incidence of hepatocytomegaly was seen in all treated female and male groups. For males, the incidence of hepatocellular carcinoma was: 3/33, 5/55, 41/52 and 32/39 at 0, 5, 25 and 50 ppm, respectively, whereas for females, the incidences were 0/45, 0/61, 32/50 and 26/37, respectively (U.S. EPA, 1985).

The National Cancer Institute (NCI, 1977) conducted a bioassay on groups of 50 male and 50 female B6C3F₁ mice that were fed chlordane consisting of 71.7% *cis*-chlordane, 23.1% *trans*-chlordane, 0.3% heptachlor, 0.6% nonachlor, 1.1% hexachlorocyclopentadiene and 0.25% chlordane isomers in the diet for 80 weeks, at two doses [a predicted maximum tolerated dose (MTD) and 1/2 MTD]. This was followed by a 10-week observation period. As upward or downward adjustments were made in dose levels, the doses were expressed as time-weighted average (TWA) concentrations. The TWA concentrations over the 80-week dosing period for male

mice at the high-dose and low-dose were 29.9 and 56.2 ppm (approximately 4.27 and 8.03 mg/kg), respectively, and 30.1 and 63.8 ppm (approximately 4.3 and 9.11 mg/kg) for female mice. Controls consisted of 20 matched control mice of each gender and 100 and 80 pooled male and female control mice, respectively. The results revealed no differences in body weight gain among groups. Tumors were observed in the high-dose males and females after 20 weeks. A dose-dependent increase in mortality was seen in treated males, but not in females. A significantly higher ($P < 0.001$) incidence of hepatocellular carcinoma was found in both males 2/18, 16/48, 43/49 and females 0/19, 3/47, 34/49 at the respective dose levels.

Velsicol/Research Institute for Animal Science in Biochemistry and Toxicology, Japan (Khasawinah & Grutsch, 1989a) conducted a chronic/carcinogenicity study in mice with technical grade chlordane (Velsicol Lot B-9129A). Chlordane was administered to groups of 80 male and 80 female ICR mice at levels of 0, 1, 5 or 12.5 ppm in the diet (approximately 0, 0.14, 0.71 or 1.79 mg/kg) for a period of 24 months. Each group (sex and dose level) consisted of 80 mice of which eight were sacrificed at 52 weeks. There was no apparent effect of dosing on survival or body weight gain. At terminal sacrifice (104 weeks) the mean weight and organ-to-body weight ratios of the liver were significantly increased in both males and females receiving 12.5 ppm chlordane. In addition, the liver-to-body weight ratios of females receiving 1 or 5 ppm chlordane were statistically significantly greater than in controls ($p < 0.05$). A significant increase in the incidence of hepatocellular adenoma (12/79, 14/79, 14/80, 27/80) ($p < 0.01$) and hemangioma of the liver (4/79, 1/79, 8/80, 14/80) ($p < 0.05$) was found in the 12.5 ppm males dying between 19 and 24 months or at terminal sacrifice (Khasawinah & Grutsch, 1989a). There was no increase in hepatic tumors in female mice. Other than for the liver tumors in male mice, there were no significant differences in tumors at other sites related to chlordane exposure.

Malarkey *et al.* (1995) conducted an analysis of age-specific prevalence for neoplastic liver lesions in B6C3F1 and B6D2F1 mice exposed to 55 ppm chlordane for up to 568 days of age to determine whether neoplasms were dependent on continuous exposure. The overall prevalence of liver tumors at terminal sacrifice was nearly 100% for both mouse strains but the age-specific prevalence increased more rapidly in B6C3F1 mice than in B6D2F1 mice. Also B6C3F1 mice had nearly twice as many tumors per liver than B6D2F1 mice (5.4 vs. 3.3). When chlordane exposure was discontinued for a group of B6C3F1 mice at 491 days of age, overall tumor multiplicity decreased by 30% from an average of 4.4 per tumor-bearing animal at 525 days to 3.1 at terminal sacrifice (568 days). Over the same time period the prevalence of hepatocellular carcinomas significantly decreased from 80 to 54% and adenomas from 100 to 93%. No H- or K-*ras* mutations were detected in the chlordane-induced liver tumors in B6C3F1 or B6D2F1 mice. The authors concluded that mechanisms independent of *ras* oncogene activation are involved in chlordane-induced liver carcinogenesis. Also about 30% of benign and malignant liver tumors in B6C3F1 mice regressed after chlordane exposure was discontinued.

Barrass *et al.* (1993) studied cell proliferation in 100 male C57B1/10J mice administered chlordane at 50 ppm in the diet for up to 24 months. In the second part of the study groups of five male mice were killed on days 2, 3, 4, 5, 8, 15, 29, 99 and 190 after start of dosing. Withdrawal groups were included from days 29 to 99 and 190 to 247 and control groups on days 4, 29, 99 and 190. Replicating cells were labeled by infusing 5-bromo-2-deoxyuridine (BrdU) via mini-osmotic pumps three days before necropsy on days 4, 5, 8, 15, 29, 190 and 247. The body weight data demonstrated a reduction of 12% in chlordane-treated mice after 190 days indicating that 50 ppm is an approximate MTD. No macroscopic abnormalities were noted in the thyroids at necropsy.

At two years, the overall incidence of hepatocellular tumors was about 50% (20/39), with a higher incidence of adenomas (15/39) than carcinomas (5/39). Among the unscheduled deaths, the first tumor was not observed until 21 months of dosing. The incidence of all types of hepatic tumors in 400 untreated male C57B1/10J mice was 2% at two years. While this study confirms the hepatocarcinogenicity of chlordane in a mouse strain with a low background tumor incidence rate, it also indicates that chlordane does not induce thyroid tumors. The data for thyroid follicular cell proliferation demonstrated a peak labeling index (LI) at five days after start of chlordane dosing (6.9% vs. 1.0%). The LI was significantly greater than control values at all time points up to 99 days but there was no significant difference after 190 days. For hepatocytes the peak LI was observed after eight days of dosing (9.0% vs. 0.5%) and unlike the thyroid was significantly higher than control values at all subsequent time points except in the withdrawal groups.

Ingle (1952) administered chlordane to six groups of 20 male and 20 female Osborne-Mendel rats chlordane for up to two years at dietary levels of 5, 10, 30, 150 or 300 ppm (approximately 0.25, 0.5, 1.5, 7.5 or 15.0 mg/kg). Marked toxicity was encountered at 300 ppm in both males and females. This included elevated mortality, reduced growth rates, eye and nose hemorrhaging and histopathologic damage to the liver, kidneys, heart, adrenals, lungs, myocardium and spleen. At 150 ppm, similar but less severe effects were seen. The effects at 30 ppm included tremors and slight liver damage. At 10 ppm, only minor liver damage, such as occasional hepatocytomegaly and mild bile duct hyperplasia, was seen. No symptoms of toxicity, gross or histopathologic changes in the liver, kidneys, lungs, pancreas, testes, ovaries, heart or spleen were noted at 5 ppm. No treatment-related tumor incidence was found.

NCI (1977) conducted a carcinogenicity study in groups of 50 male and 50 female Osborne-Mendel that were administered chlordane (94.8% pure) in the diet for 80 weeks. Two dose levels, predicted MTD and half the predicted MTD, were used. This was followed by a 29-week observation period. The TWA concentrations for male rats at the low dose and high-dose were 203.5 and 407.8 ppm (or approximately 7.49 and 14.97 mg/kg-day for the duration of the study), respectively, for males, and 120.8 and 241.5 ppm (approximately 4.43 and 8.87 mg/kg-day for the duration of the study), respectively, for females. Ten rats of each sex served as matched controls, and 60 rats of each sex served as pooled controls. Complete necropsies and histologic examinations were performed, except in a few cases of spontaneous deaths. The small number of matched control animals severely compromised the statistical power of the study. Two hepatocellular carcinomas were observed, one in a low-dose male and one among the pooled controls. A significant ($p < 0.05$) increase in neoplastic nodules of the liver was seen in the low-dose females (11/47) compared to pooled controls (5/59) but not in the high-dose females (6/39) or in the high- or low-dose males. A dose-dependent trend ($p = 0.01$) was found for neoplastic lesions (adenomas and carcinomas) of the thyroid gland (follicular cell and C-cells) for females when compared with the matched controls (0/10, 4/43, 6/32). NCI (1977) reported that although somewhat suggestive, the data do not appear to be sufficient to indicate a clear carcinogenic effect of chlordane in the thyroid follicular cells of rats.

In a subsequent review of tumors in Osborne-Mendel rats in the NCI studies (over 900 of each sex), Goodman *et al.* (1980) presented data indicating 7.1% incidence of follicular cell tumors in control males and 3.4% in control females. Using these rates the increase in thyroid tumors among treated animals was statistically significant, although U.S. EPA (1986) reported otherwise. A significant dose-dependent increase in the incidence of fibrous histiocytoma (trend, $p = 0.007$) was observed for male rats. This was based on an increase only in the high-dose male group (7/44) as

compared to 1/44, 0/8 and 2/58 for the low-dose, matched control and pooled control groups, respectively. These authors discounted this latter finding as not treatment-related, since similar tumors had occurred spontaneously throughout the bioassay program. NCI noted the instances of increases in neoplasms, yet could not find the evidence of carcinogenicity sufficient in the rat and concluded that "in the judgment of the pathologist, the nature, incidence and severity of neither the thyroid lesions nor histiocytomas were sufficient to indicate clearly a carcinogenic effect of chlordane in rats."

Velsicol/ Research Institute for Animal Science in Biochemistry and Toxicology, Japan (Khasawinah & Grutsch, 1989b) conducted a chronic/carcinogenicity study in rats with "technical grade" chlordane (Velsicol Lot B-9129A). Chlordane was administered to groups of 80 Fischer 344 rats/sex/dose group at levels of 0, 1, 5 or 25 ppm (approximately 0, 0.05, 0.25. or 1.25 mg/kg-day) for a period of 130 weeks. Subsets of eight rats per sex/dose group were sacrificed and examined at 26 and 52 weeks. The dose levels were determined on the basis of a pilot study in which groups of five male and five female Fischer 344 rats were administered diets containing 0, 50, 100, 200, 400 or 800 ppm technical grade chlordane for four weeks. Hepatocellular swelling and fatty degeneration in the liver were found in both male and female rats at 50 ppm. In the 130-week study, there were no dose-dependent effects on mortality, food consumption, water consumption, hematology, clinical chemistry or urinalysis. Virtually all of the toxic effects were restricted to the liver. At 25 ppm chlordane in the diet there were increases in bilirubin levels, liver weight, liver cell volume and hepatic necrosis in animals found dead or killed *in extremis*.

Earlier reviews of this study (CAG, 1986; DHS, 1988) reported a significant increase in adenomas of the liver in males receiving 25 ppm as compared to controls, but no corresponding effect occurred in females. All of these tumors were found after 104 weeks (mean time to tumor death was 121.8 weeks). There was also a significant increase in mammary gland fibroadenomas in females receiving 1 ppm as compared to controls but no significant increase was observed at 5 or 25 ppm. A subsequent peer review by a pathology working group concluded that all the tumors observed in this study were spontaneous and unrelated to chlordane treatment (see citations in Khasawinah & Grutsch, 1989b). The quantal responses for hepatocellular adenomas in male rats were 1/64, 2/64, 3/64 and 9/64 ($p = 0.008$) based on the original RIAST pathology and 2/44, 4/50, 2/49 and 7/52 based on the analysis of animals dying after week 104 at 0, 1, 5 and 25 ppm, respectively. A marginally significant trend was observed with animals dying or sacrificed after week 104 ($p = 0.018$). However, animals sacrificed at week 130 had no treatment related increase in tumors. Thus the pathology peer reviewers concluded that the positive statistical trend is due to extra adenomas found between week 104 and 129 and the apparent dose-response is artifactual.

Nonneoplastic lesions occurred frequently. There was a dose-dependent increase in the incidences of hepatocellular swelling [5/64, 15/64 ($p < 0.05$), 14/64, 42/64 ($p < 0.001$)] and necrosis [3/64, 13/64 ($p < 0.05$), 11/64, 27/64 ($p < 0.001$)] in male rats. When compared to controls, the incidence of hepatocellular swelling was significantly increased in all dosed males and in females receiving 25 ppm. There was also an increase in focal hepatocellular hyperplasia in males receiving 25 ppm, but the increase was not significantly different from controls. Most of these lesions of the liver occurred after 78 weeks of the study. A slight increase in nonneoplastic liver lesions was seen in the 26- and 52-week sacrifice groups (see DHS, 1988 for tabular summaries of data). The Cancer Assessment Group (CAG, 1986) concluded that the NOEL for non-neoplastic lesions in female rats was 1 ppm but that males had no demonstrated NOEL.

Chlordane was considered positive for oncogenicity by the original RIAST authors, since the incidence of hepatic adenomas was significantly increased ($P < 0.001$) in males in the 25 ppm group (9/64 versus 1/64 in controls). The historical incidence of this tumor in F344/CRJ rats for the testing laboratory was 2.5% in males and 2.3% in females. The control incidence in this study was 1.6%. The small increase in benign liver neoplasms was considered as weak evidence for the oncogenicity of chlordane in rats. As noted above the peer reviewers employing National Toxicology Program (NTP) diagnostic criteria (Maronpot *et al.*, 1986) observed slightly different tumor incidences and concluded that the effects were not treatment related. Notwithstanding the reviewer's findings, this assessment concludes that technical chlordane when administered for two years or greater induces a dose-dependent increase of hepatocellular adenomas in Fischer 344 rats. The lack of differential mortality raises the question of whether or not an MTD was achieved during this study. An NOEL and LOEL for chronic toxicity in females based on nonneoplastic changes in the liver are 5 ppm and 25 ppm, respectively; the LOEL in males is 1 ppm.

Toxicological Effects in Humans

Acute Toxicity

Data describing effects in humans range from information collected through clinical case studies and human monitoring data to epidemiological studies. The three effects seen most frequently in clinical case studies are central nervous system toxicity, blood dyscrasias and neuroblastomas (see DHS, 1988 for earlier case studies). Kutz *et al.* (1983) report analytical of chlordane concentrations in various body tissues and fluids following a fatal, accidental ingestion of chlordane in a 59-year-old male with a history of Alzheimer's disease. The decedent drank from a bottle of chlordane thinking it was beer. Shortly thereafter, while seated in a chair, he began jerking and reportedly had a strange smell on his breath. Emergency rescue personnel were unable to resuscitate with cardiopulmonary treatment or atropine and adrenaline and the patient died. A *post-mortem* examination was conducted about two hours after death. Chlordane was detected in all tissues and fluids. The highest concentrations were found in the ingested fluid and stomach contents while the liver and brain showed the highest tissue concentrations. The ingested fluid was 69.8% chlordane. Tissue concentrations of total chlordane ranged from 14.1 ppm ($\mu\text{g/g}$) in kidney to 59.93 ppm in liver. Blood plasma had 4.87 ppm and urine 0.2 ppm. Estimated organ burdens were: liver, 88.0 mg; brain, 23 mg; kidney, 5.0 mg; and spleen, 3.0 mg. The adipose tissue with 22.0 ppm would also represent a significant burden on the order of 150 to 250 mg.

Subchronic Toxicity

Blood dyscrasias have been associated with dermal or inhalation exposure to chlordane at unspecified dose levels. Eight cases of blood dyscrasias have been reported following exposure to chlordane or heptachlor; four aplastic anemias (Infante *et al.*, 1978; Klemmer *et al.*, 1977), one refractory megablastic anemia (Furie and Trubowitz, 1976) and one acute lymphoblastic leukemia (Infante *et al.*, 1978). The American Medical Association's Council on Drugs (Anonymous, 1962) stated that a "specific cause-effect relationship exists" between exposure to chlordane and resulting blood dyscrasias. However, in a case-control study, no association was found between blood dyscrasias and occupational exposure to a number of pesticides including chlordane (WHO, 1984).

Epstein and Ozonoff (1987) reported new cases of blood dyscrasia, including leukemias, production defects and thrombocytopenic purpura, generally following home termite treatment

with the chlorinated hydrocarbon pesticides chlordane and heptachlor (C/H). These more recent cases are consistent with 34 previously published case reporters associating blood dyscrasias with C/H exposure. The leukemias are consistent with epidemiological evidence of excess risk of leukemia and other cancers in C/H-exposed populations and with the carcinogenic action of C/H in animals. Infante *et al.* (1978) described five cases of neuroblastomas in children with a pre- and/or post-natal history of exposure to technical grade chlordane.

Wang and MacMahon (1979 a,b) studied a cohort of workers engaged in the manufacture of chlordane, heptachlor and endrin and another cohort of 16,000 pesticide-spraying personnel, including termite-control workers. Both studies showed a deficit of deaths from all cancers and slight excesses of lung, skin or bladder cancers that were not statistically significant. In 1982, an IARC working group concluded that the above studies were inadequate to evaluate the carcinogenicity of chlordane for human beings (WHO, 1984). In one of these studies there was a statistically significant excess of deaths from cerebrovascular disease (17 observed, 9.3 expected). This excess was not related to duration of exposure or latency and occurred exclusively after termination of employment.

Shindell *et al.* (1981) studied the mortality experience of 783 workers engaged in the manufacture of chlordane and heptachlor. Workers had been employed for a minimum of 3 months, 5, 10, 15 or 20 years. SMRs for cancer were not increased among 124 deaths. However, evidence that chlordane is absorbed from the gastrointestinal tract is derived from reports of systemic toxicity and excretion data following oral exposure to the compound.

Genetic Toxicity

No data were available for review.

Developmental and Reproductive Toxicity

Increased incidences of cardiovascular malformations and hip dislocation were reported following milk contamination by heptachlor on Oahu Island, Hawaii (Le Marchand *et al.*, 1986; IARC, 1991).

Immunotoxicity

McConnachie & Zahalsky (1992) studied lymphocyte phenotype frequencies and *in vitro* functional assays of 27 individuals who had been exposed to technical chlordane in their homes or workplaces. A control group of 118 individuals similar in age and sex distribution was used. A significantly increased frequency of cortical thymocytes in the circulation ($p < 0.001$) and a decreased frequency of the suppressor-inducer phenotype CD45RA/T4 ($p < 0.01$) were noted in the exposed group. Both κ and λ light-chain frequencies were elevated ($p < 0.01$). Proliferative responses to three mitogens tested and to allogenic lymphocytes in the mixed-lymphocyte culture assay were significantly lower than controls ($p < 0.01$). Of 12 individuals tested for evidence of autoimmunity, 11 demonstrated some increased titer of a form of autoantibody. The data indicate significant alterations in the immune system of individuals exposed to technical chlordane, 2 to 10 years previously. The authors propose that metabolic activity in bone marrow fat, a process that slowly releases chlordane/metabolites, could account for the long-term persistence of the phenotypic and functional differences observed.

Neurotoxicity

Kilburn & Thornton (1995) studied 216 adults and children exposed to chlordane when their apartment complex was treated with chlordane in 1987. Indoor concentrations of chlordane were as high as 13.6 $\mu\text{g}/929\text{ cm}^2$ on wipe samples and of 24/294 apartments sampled eight-hour levels were above 0.5 $\mu\text{g}/\text{m}^3$. Eight residents of the treated apartments exhibited elevated levels of chlorinated insecticides in blood or fat: the heptachlor range was 110 to 186 ppb; oxychlordane was 70 to 150 ppm; and *trans*-nonachlor was 76 to 200 ppm. Neurobehavioral functions were measured and questionnaires completed for symptom frequency, mood status, confounding factors and medical, rheumatic and respiratory disorders in 1994. Measurements included simple and choice reaction time, balance, blink reflex latency, color vision, cognitive, perceptual motor, memory and recall functions. The referent group consisted of 174 adults of similar age, weight, gender and educational level. Performance of balance, reaction times, verbal recall, Culture Fair A score, digit symbol score, pegboard dominant score (females only) and trail-making scores were significantly impaired in exposed persons compared to referents. Mood state scores were elevated as were the frequencies of respiratory and rheumatic symptoms. In contrast long-term memory function was apparently unaffected by chlordane exposure.

Carcinogenicity

There were three epidemiological studies of workers exposed to chlordane and/or heptachlor. Wang and MacMahon (1979 a,b) studied a cohort of workers engaged in the manufacture of chlordane, heptachlor and endrin and another cohort of 16,000 pesticide-spraying workers, including termite-control workers. Both studies demonstrated a deficit of deaths from all cancers and slight excesses of lung, skin or bladder cancers that were not statistically significant (DHS, 1988). U.S. EPA's review of these data concluded that there was marginal statistically significant increased mortality from bladder cancer based on three observed cases (IRIS, 1993). In a study of pesticides and other agricultural risk factors for non-Hodgkin's lymphoma, Cantor *et al.* (1992) concluded that elevated risks were found, with odds ratios of 1.5 or greater, for persons handling several pesticide groups and for individual pesticides including chlordane.

DOSE-RESPONSE ASSESSMENT

Noncarcinogenic Effects

The most sensitive noncarcinogenic adverse effects of chlordane exposure appear to be on sex steroid-mediated behavior and functions reported by Cassidy *et al.* (1994). These authors reported an LOAEL of 0.1 mg/kg-day. These data are not readily amenable to a dose-response assessment since no NOAEL was observed for several endpoints. The effects noted in the study are the result of sexual differentiation during critical developmental periods in the rat brain and other tissues containing steroid receptors. Whether humans are similarly susceptible is unknown, although anatomically, the corpus callosum in human females is larger than that in males by 28 weeks of gestation. Notwithstanding the uncertainty of whether these effects in rats are indicative of adverse endocrine effects in developing humans, we have chosen this LOAEL of 0.1 mg/kg-day for the calculation of a PHG for noncarcinogenic effects. This value is two times higher than the NOEL of 0.055 mg/kg-day identified by U.S. EPA for regional liver hypertrophy in female rats.

Carcinogenic Effects

The mechanism of carcinogenic action of chlordane is uncertain. Chlordane does not cause dominant lethal mutations in mice and is non-mutagenic in many other microbial tests. However chlordane does inhibit gap-junction intercellular communication and induce gene mutation in some rodent cells. Chlordane can also damage chromosomes. There is no evidence or valid biological model supporting a threshold or non-linear approach for chlordane or related chlorinated cyclodienes. For the purpose of this risk assessment, a linear approach is adopted.

The most relevant data sets for estimating the carcinogenic potencies or cancer slope factors (CSF's) are the four data sets in CD-1 and B6C3F1 mice which show significant dose-dependent increases in liver tumors. Earlier quantitative estimates using the linearized multistage model (LMS) and (body weight)^{2/3} inter-species scaling are provided in Table 2 for comparison with the present estimates which are based on the LED₁₀ and (body weight)^{3/4} scaling. The LED₁₀ is the 95% lower confidence limit on the dose that gives a 10% excess lifetime individual risk of cancer. Potency values based on the LED₁₀, or the LMS were calculated from the data sets using GLOBAL 86 and TOX_RISK (Version 3.5) software (K. S. Crump Division, Clement International Corp., Ruston, LA). All procedures are for estimates of extra risk. For determination of the LED₁₀ a goodness of fit criterion of $p > 0.05$ was adopted for the Chi-squared test, whereas $p > 0.01$ is usually considered sufficient for the LMS. Using the GLOBAL 86 software the LED₁₀ values ranged from 0.05 to 0.31 mg/kg-day and cancer slope factors (CSFs) ranged from 0.32 to 1.9 (mg/kg-day)⁻¹ (Table 2a). The geometric mean (Gmean1) of the four data sets was 0.69 or rounded off to 0.7 (mg/kg-day)⁻¹. The same geometric mean was obtained for the LMS potency or q₁* value. The potency values in Table 2 differ from those provided by U.S. EPA (IRIS, 1993) primarily because U.S. EPA did not correct these potencies for experiments of shorter duration than two years; the life-span typically assumed for rats and mice. The present estimates and the Office of Environmental Health Hazard Assessment (OEHHA) 1987 values include an intercurrent mortality correction of about 2.4 for a study duration of 78 weeks [(104/78)³]. Most listed U.S. EPA potency values are based on (body weight)^{2/3} scaling and have not yet been recalculated based on the newer (body weight)^{3/4} scaling policy.

The largest CSF estimate by GLOBAL 86 of 1.9 (mg/kg-d)⁻¹ was derived from the data on hepatocellular carcinoma in male CD-1 mice (IRDC, 1973). Estimates of the LED₁₀s by TOX_RISK v.3.5 were expressed as ppb dietary concentrations and were converted to CSFs on a 1.5 kg diet/day/70 kg human basis. The CSFs based on the complete data sets (Table 2b) ranged from 0.62 to 5.1 (mg/kg-day)⁻¹. However only one of the four data sets met the goodness of fit criterion of 0.05. Removing the top dose-responses from the CD-1 data sets, resulted in adequate fits and lower CSFs. The geometric mean of the four best fitting CSF's by TOX_RISK (Gmean3) was 1.3 (mg/kg-day)⁻¹, a value identical to U.S. EPA's current CSF based on the LMS procedure and extra risk. Three of the four data sets were examined by plotting the predicted dose-response from GLOBAL 86 which met the fit criterion and comparing the observed tumor incidences. The female CD-1 data set was problematic with observed quantal responses of 0/45, 0/61, 32/50 and 26/37 which required a sixth-order polynomial to adequately fit. The female B6C3F₁ data set was also problematic involving a cubic term and giving an LED₁₀ which exceeded the MLE₁₀. At lower risk values (≤1%) the LED₁₀ behaved normally. This lack of correct calculation of the LED at higher doses indicated that Global 86 was not using the higher order term. In contrast, while the Tox_Risk program indicated slightly poorer fits to the data by the X² test it apparently did use the higher order terms in calculating the 95% lower bounds in the observed range.

The options to derive the best value CSF include: 1) the highest best fitting value of 1.9 (mg/kg-day)⁻¹, 2) the best fitting geometric mean of the four data sets from GLOBAL 86 of 0.7 (mg/kg-day)⁻¹, 3) the best fitting three data sets (GLOBAL 86) with the problematic female CD-1 set removed of 0.8 (mg/kg-day)⁻¹ or 4) the best fitting geometric mean of the four sets from TOX_RISK of 1.3 (mg/kg-day)⁻¹.

The option currently considered the best is option four, using the geometric mean of the best fitting four data sets by Tox_Risk or 1.3 (mg/kg-day)⁻¹. This option was chosen because of the superior ability of the Tox_Risk program to calculate the lower bounds on effective doses in the observed range. The use of the geometric mean follows U.S.EPA practice in estimating the oral Slope Factor for chlordane from the same data sets albeit using a different extrapolation procedure (U.S.EPA, 1996).

Table 2a. Cancer Potencies for Chlordane Based on Mouse Liver Carcinoma (GLOBAL 86)

Dataset	q ₁ * (mg/kg-day) ⁻¹	X ²	p	k	MLE ₁₀ mg/kg-day	LED ₁₀ mg/kg-day	CSF (mg/kg-day) ⁻¹	U. S. EPA (1993)	OEHHA (1987)
CD-1 (M)	1.9	0.74	0.39	2	0.06	0.05	1.9	4.7	5.9
CD-1 (F)	0.5	0.004	0.95	2		0.21	0.47	2.98	9.4
B6C3F1 (M)	0.87	2.8	0.09	2	0.14	0.13	0.79	0.76	0.80
B6C3F1 (F)	0.27	1.2	0.28	2	0.21	0.31	0.32	0.25	0.28
Gmean1	0.69						0.69	1.28	1.88
Gmean2	0.76						0.78		

Note: The CD-1 mouse data sets are from Velsicol/IRDC (1973) (page 21, text), the B6C3F1 mouse data sets are from NCI (1977) (page 21). The q₁* is the carcinogenic potency determined by the linearized multistage model. X² is the value of the Chi-squared goodness of fit statistic; p is the significance of the Chi-squared value where a criterion of ≥ 0.05 is considered an adequate fit of the polynomial equation to a data set; k is the number of non-zero doses used in the fitting procedure. MLE₁₀ is the maximum likelihood estimate of the dose that corresponds to a 10% tumor response. LED₁₀ is the 95% lower confidence limit on the MLE₁₀ dose. The CSF is the carcinogenic slope factor calculated from the LED₁₀. Gmean1 includes all data sets, k = 2; Gmean2 includes all data sets less CD-1 female, k = 2.

Table 2b. Cancer Potencies for Chlordane Based on Mouse Liver Carcinoma (TOX_RISK v.3)

Data Set	q ₁ * (mg/kg-day) ⁻¹	X ²	p	k	MLE ₁₀ mg/kg-day	LED ₁₀ mg/kg-day	CSF (mg/kg-day) ⁻¹	U.S. EPA (1993)	OEHHA (1987)
CD-1 (M)	5.7	11.84	0.0006	3	0.026	0.0197	5.1	4.74	5.9
CD-1 (F)	2.0 3.6	0.74 15.4	0.39 9 x 10 ⁻⁵	2 3	0.062 0.06	0.0398 0.032	2.5 3.1	2.98	9.4
B6C3F1 (M)	0.64 0.91	2.5 2.8	0.11 0.09	2 2	0.077 0.131	0.064 0.086	1.56 1.16	0.76	0.80
B6C3F1 (F)	0.30	6.3	0.01	2	0.199	0.162	0.62	0.25	0.28
Gmean3	0.77						1.29	1.28	1.88
Gmean4	0.82						1.22		
Cal/EPA (1994)	1.2								

NOTE: MLE's and LED's in TOX_RISK were given as dietary concentration on a 100% food basis. These were converted to mg/kg-day assuming 1.5 kg diet/day and a 70 kg human body weight. For example, from the CD-1 female data above: the LED₁₀ = 3.0 mg/kg diet x 1.5 kg diet/day/70 kg = 0.064 mg/kg-day. CSF = 0.1 Risk/0.064 mg/kg-day = 1.555 or 1.56 (mg/kg-day)⁻¹. Gmean3, all data sets k = 2; Gmean4, all data sets k = 2 less CD-1 female.

CALCULATION OF PHG

The data available for residues of chlordane in human adipose tissue and human milk suggest widespread, albeit declining, chlordane contamination of the environment. Food, air and breast milk are the main sources of exposure of the general population to chlordane. Relative source contribution (RSC) from drinking water, based on exposure data, is low primarily due to the hydrophobic properties of this compound.

Noncarcinogenic Effects

An issue surrounding the chronic low level exposures to chlordane, chlorinated cyclodienes and certain other chlorinated pesticide environmental residues centers on their ability to mimic various hormones and potentially disrupt critical endocrine functions. Such disruptions may be transient or irreversible depending which hormone receptors are affected and the timing of the effect. Based on the LOAEL identified in the Cassidy *et al.* (1994) study of sex steroid-mediated effects in rat, a health-protective drinking water concentration (C, in mg/L) can be calculated according to the general equation for noncarcinogenic endpoints as follows:

$$C = \frac{LOAEL \times BW \times RSC}{UF \times L/day} = \text{mg/L}$$

where,

LOAEL	=	Lowest-observed-adverse-effect-level (use 0.1 mg/kg-day for chlordane)
BW	=	Body weight (a default of 10 kg for children) of an exposed child because developing children may be at special risk from the potential hormone mimicking effects of chlordane and related cyclodienes
RSC	=	Relative source of contribution (use a default of 20% or 0.2 for chlordane, with the major exposures being from food, including breast milk rather than drinking water, probably less than 1 to 5% at the current State MCL of 0.1 ppb)
UF	=	Uncertainty factor [use 10,000 for chlordane, the product of UFs for conversion of LOAEL to NOAEL and severity of endpoint (10), short-term to longer-term study data and better dose-response data (10), inter-species variation (10) and human variability(10)]
L/day	=	Daily water intake by a child (a default of 1 L/day for children).

Therefore,

$$C = \frac{0.1 \text{ mg/kg-day} \times 10 \text{ kg} \times 0.2}{10,000 \times 1.0 \text{ L/day}}$$
$$= 2.0 \times 10^{-5} \text{ mg/L} = 0.02 \text{ ppb} = 20 \text{ ppt.}$$

The value of 10 for LOAEL to NOAEL conversion is appropriate because of the severity of the endpoint, and since a limited number of animals were used in the Cassidy *et al.* (1994) study, the authors commented that a lower LOAEL was likely. Another 10-fold UF is appropriate for the limited length of the study and uncertainties in the dose-response relation for the endpoints measured. Very little is known about inter-species extrapolation of sex-steroid mediated endocrine effects or what degree of inter-individual variation might be exhibited by humans. Also dose timing may be critical for some effects or body burdens developed early in life may be expressed at later stages of development. Ten-fold factors for each of these uncertainties seem suitable in view of the limited knowledge. Since these experimental data are relatively new and implications for human endocrine disruption are uncertain this high number represents a relatively low confidence in the value obtained.

Carcinogenic Effects

The primary issue surrounding the calculation of a PHG for chlordane is the potential for chlordane to induce human cancer. According to the U.S. EPA's draft proposed guidelines for carcinogen risk assessment (U.S. EPA, 1996) a greater emphasis is to be placed on other information besides animal tumor data in reaching conclusions about human carcinogenic potential. This aspect of the risk assessment will be discussed below under risk characterization. The risk characterization will provide the risk manager with more information about the weight-of-evidence supporting the PHG and what degree of flexibility from a toxicologic viewpoint might be available in setting a state MCL.

Based on the NCI (1977) dose-dependent increase in the incidence of hepatocellular carcinoma in male and female B6C3F1 mice and the incidence of hepatocellular carcinoma in male and female

CD-1 mice, U.S. EPA (1987) derived a human cancer potency value (q_1^*) of $1.3 \text{ (mg/kg-day)}^{-1}$ for chlordane. This estimate is slightly higher than the potency of $1.2 \text{ (mg/kg-day)}^{-1}$ derived by DHS in 1988. Our current best estimate of potency or CSF based on the same data sets, but employing the low-dose and inter-species extrapolation procedures recommended in the 1996 U.S. EPA draft proposed guidelines for carcinogen risk assessment, is $1.3 \text{ (mg/kg-day)}^{-1}$.

A health-protective drinking water concentration for chlordane (C, in mg/L) for carcinogenic endpoints can be calculated using the general equation for carcinogenic endpoints:

$$C = \frac{R \times BW}{CSF \times L/\text{day}} = \text{mg/L}$$

where,

- R = The *de minimis* theoretical lifetime excess individual life time cancer risk level (1×10^{-6})
- BW = The default adult male body weight (70 kg)
- CSF = Cancer slope factor for humans [use $1.3 \text{ (mg/kg-day)}^{-1}$ for chlordane]
- L/day = Daily default water intake for adult (2 L/day).

Therefore,

$$C = \frac{1 \times 10^{-6} \times 70 \text{ kg}}{1.3 \text{ (mg/kg-day)}^{-1} \times 2 \text{ L/day}}$$

$$= 2.69 \times 10^{-5} \text{ mg/L} = 0.03 \text{ ppb} = 30 \text{ ppt.}$$

A PHG of $3 \times 10^{-5} \text{ mg/L}$ (30 ppt) based on a carcinogenic endpoint and identical to OEHHA's PHG of 0.03 ppb (30 ppt) based on a carcinogenic endpoint and a *de minimis* theoretical excess individual lifetime cancer risk level of 1×10^{-6} is developed for chlordane in drinking water. This is identical to U.S. EPA's current calculated 1×10^{-6} risk value for drinking water of 0.03 ppb (30 ppt). It seems likely in view of the uncertainties mentioned above that a sufficient margin-of-safety (6700) would be provided by this PHG for potential endocrine effects in exposed human populations. The PHG is also three times lower than the current State MCL for chlordane of 0.1 ppb. The concentration of chlordane in drinking water corresponding to 10^{-5} and 10^{-4} risk level are 300 ppt and 3,000 ppt, respectively.

RISK CHARACTERIZATION

While practically all uses of chlordane have been banned or voluntarily canceled, its persistence in the environment has led to continuing albeit slowly declining exposures largely via the diet, milk, fish and other sources. However, due to its persistence in human body fat, and particularly in breast milk, continuing and possibly critical (temporal) exposures are a cause for concern. The cancer data in rodents are sufficient to consider chlordane probably carcinogenic in humans. However, these studies are limited to liver tumors and at least in mice indicate that continued exposure to chlordane is required to produce the usual yield of tumors or else many of the tumors regress. No specific mutations have been observed in chlordane induced tumors possibly indicating a non-genotoxic or promoting type mechanism. Data in human epidemiological studies are

fragmentary and insufficient for risk assessment. Some case reports and studies suggest links between chlordane exposure and blood dyscrasias, leukemia, non-Hodgkin's lymphoma and cancers of the lung, brain, skin, bladder and stomach. The mechanism of cancer causation is unknown but there is insufficient information to depart from a low-dose linear approach to estimate human cancer risks. The newly proposed U.S. EPA methodology for carcinogen risk assessment used in this document results in a cancer slope factor or potency of $1.3 \text{ (mg/kg-day)}^{-1}$ compared to the U.S. EPA or OEHHA values of 1.3 and $1.2 \text{ (mg/kg-d)}^{-1}$, respectively.

For PHGs, our use of the RSC has, with a few exceptions, followed U.S. EPA drinking water risk assessment methodology. U.S. EPA has treated carcinogens differently from noncarcinogens with respect to the use of RSCs. For noncarcinogens, RfDs (in mg/kg-day), drinking water equivalent levels (DWELs, in mg/L) and MCLGs (in mg/L) are calculated using UFs, body weights and water consumption rates (L/day) and the RSC, respectively. The RSC default range is 20% to 80% (0.2 to 0.8).

U.S. EPA follows a general procedure in promulgating MCLGs:

1. if Group A and B carcinogens (i.e., strong evidence of carcinogenicity) MCLGs are set to zero,
2. if Group C (i.e., limited evidence of carcinogenicity), either a RfD approach is used (as with a noncarcinogen) but an additional UF of 1 to 10 (usually 10) is applied to account for the limited evidence of carcinogenicity, or a quantitative method (potency and low-dose extrapolation) is used and the MCLG is set in the 10^{-5} to 10^{-6} cancer risk range,
3. if Group D (i.e., inadequate or no animal evidence) a RfD approach is used to promulgate the MCLG.

For approaches that use low-dose extrapolation based on quantitative risk assessment, U.S. EPA does not factor in an RSC. The use of low-dose extrapolation is considered by U.S. EPA to be adequately health-protective without the additional source contributions. In developing PHGs, we have adopted the assumption that RSCs should not be factored in for carcinogens grouped in U.S. EPA categories A and B and for C carcinogens for which we have calculated a cancer potency based on low-dose extrapolation. This is an area of uncertainty and scientific debate and it is not clear how this assumption impacts the overall health risk assessment.

An additional concern with respect to chlordane and related persistent pesticide residues in the environment is their potential role as endocrine disrupters. U. S. EPA (1997) is concerned about the possibility of impacts to human health and the environment resulting from exposure to these agents, however, U.S. EPA does not consider endocrine disruption to be an adverse endpoint *per se* but rather as a step that could lead to toxic outcomes such as cancer or adverse reproductive effects. The masculinizing effects of chlordane and possibly other chlorinated cyclodienes in rats exposed to low-doses of technical chlordane raise questions about potential effects in humans who are exposed via diet and adipose tissue body burdens. There are many uncertainties regarding significance of such data for human risk assessment. Additional studies will be needed to better characterize these effects in experimental animals. While at this time the data are not sufficient in themselves to support the proposed PHG, they are of sufficient concern, in the context of the PHG, to be taken into account with the other toxic endpoint of significant potential for adverse human health effects (i.e., cancer). No evidence of synergy with other chemicals in the toxicity of

chlordane was found in the literature. However since the toxicity studies were largely conducted with mixtures of cyclodienes (i.e., technical chlordane) such effects cannot be ruled out.

The PHG of 0.03 ppb is identical to the 10^{-6} risk level of 0.03 ppb cited by U.S. EPA (IRIS, 1993) although the derivation is based on a different procedure. The CSFs in the data sets examined varied by a factor of six and a geometric mean CSF derived from the best fit data sets was considered the best overall value. If the highest CSF was chosen (male CD-1 mice) instead of the geometric mean, the PHG would be 0.019 ppb or 40% of the value chosen. This is not considered a significant difference in view of the uncertainties and limitations in the data sets and mode of action noted above and in the text. For risk management purposes, public health-protective concentrations of 0.3 and 3 ppb chlordane in drinking water can be calculated at theoretical excess individual lifetime cancer risk levels of 10^{-5} and 10^{-4} , respectively.

OTHER REGULATORY STANDARDS AND CRITERIA

The U.S. Environmental Protection Agency (U.S. EPA) has established a Maximum Contaminant Level (MCL) for chlordane of 0.002 mg/L (2 ppb) and a Maximum Contaminant Level Goal (MCLG) of zero. U.S. EPA's current calculated 1×10^{-6} risk value for drinking water is 0.03 ppb. The California Department of Health Services (DHS) has established an MCL for chlordane in drinking water of 0.0001 mg/L (0.1 ppb). U.S. EPA also established a reference dose (RfD) for chlordane of 6×10^{-5} mg/kg-day (U.S. EPA, 1987a). The RfD for chlordane used a 1,000-fold uncertainty factor, because it was based on an NOAEL of 0.055 mg/kg-day observed in a 30-month rat feeding study.

In 1990, U.S. EPA estimated the range of excess cancer risks for lifetime exposure to chlordane from drinking water (IRIS, 1996). This range was 3,000 ng/L, 300 ng/L and 30 ng/L respectively, for risks of 10^{-4} , 10^{-5} and 10^{-6} assuming consumption of two liters of water consumed per day by an adult. The ambient water criteria for human health are 0.46 ng/L for water and fish consumption and 0.48 ng/L for fish only (IRIS, 1996). These recommended criteria were adopted for a *de minimis* theoretical excess individual lifetime cancer risk of 10^{-6} . To protect fresh water aquatic organisms, U.S. EPA estimated a concentration of 4.3×10^{-3} µg/L as 24-hour average, and recommended that the concentration should not exceed 2.4 µg/L at any time. For marine aquatic organisms the respective values were 4.0×10^{-3} µg/L and 9.0×10^{-2} µg/L.

The Food and Agricultural Organization/World Health Organization (FAO/WHO) recommended a maximum acceptable daily intake (ADI) of 0.0005 mg/kg of body weight for chlordane (sum of isomers and oxychlordane) (FAO/WHO, 1978). The World Health Organization (WHO, 1988) recommended a drinking water guideline of 0.3 mg/L for chlordane (total isomers).

The National Academy of Sciences-National Research Council (NRC, 1982) recommended an interim guideline of 5 µg/m³ for airborne chlordane in military housing. The American Conference of Governmental Industrial Hygienists (U.S. EPA, 1985) adopted a time-weighted average value (TWA) of 0.5 mg/m³ for chlordane based on inhalation exposure.

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