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TOXICOLOGICAL REVIEW

OF

CHLORDECONE (KEPONE)

(CAS No. 143-50-0)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

January 2008

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LIST OF ABBREVIATIONS AND ACRONYMS

AIC	Akaike's Information Criterion
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMD_{10}	benchmark dose associated with a 10% extra risk
$BMDL_{10}$	benchmark dose lower 95% confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
CHO	Chinese hamster ovary
conA	concanavalin A
CYP	cytochrome
CYP450	cytochrome P450
DEN	diethynitrosamine
ELISA	enzyme-linked immunosorbent assay
FSH	follicle-stimulating hormone
GGT	γ-glutamyl transpeptidase
HDL	high density lipoproteins
HSDB	Hazardous Substances Data Bank
IRIS	Integrated Risk Information System
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
LSPC	Life Science Products Company
NCI	National Cancer Institute
NIOSH	National Institute for Occupational Safety and Health
NK	natural killer
NLM	National Library of Medicine
NOAEL	no-observed-adverse-effect level
PBTK	physiologically based toxicokinetic
PFC	plaque-forming cell
PHA	phytohemagglutinin
POD	point of departure
PVE	persistent vaginal estrus
RfC	reference concentration
RfD	reference dose
SALP	serum alkaline phosphatase
s.c.	subcutaneous
SCK	serum creatine kinase
SER	smooth endoplasmic reticulum
SGOT	serum glutamic oxaloacetic transferase
SGPT	serum glutamic pyruvic transferase
SRBC	sheep red blood cell

STM	*Salmonella typhimurium* mitogen
TD	toxicodynamic
UF	uncertainty factor
U.S. EPA	U.S. Environmental Protection Agency
U.S. DHHS	U.S. Department of Health and Human Services

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to chlordecone. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of chlordecone.

The intent of Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, is to present the major conclusions reached in the derivation of the reference dose, reference concentration, and cancer assessment, where applicable, and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of the data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of chlordecone. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal of entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is an upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is an upper bound on the estimate of risk per ug/m^3 air breathed.

Development of these hazard identification and dose-response assessments for chlordecone has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines and technical reports that may have been used in the development of this assessment include the following: *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplimental Guidance for Assessing Susceptibility from Early-Life*

Exposure to Carcinogens (U.S. EPA, 2005b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a, 2005c), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), *Supplimentary Guidance for Conducting Helath Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000d) and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through May 2007.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Chlordecone is a tan to white crystalline odorless solid (NIOSH, 2004). The structure of chlordecone is shown in Figure 2-1. Synonyms include Kepone, decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]-pentalen-2-one, and GC-1189 (O'Neil, 2001). Selected chemical and physical properties of chlordecone are listed below.

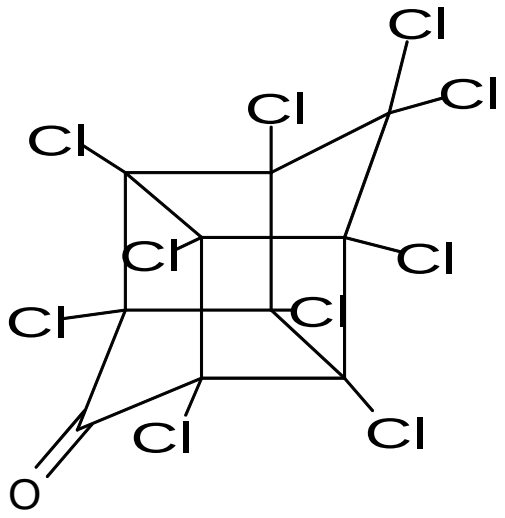


Figure 2-1. The structure of chlordecone.

CAS number:	143-50-0 (Lide, 2000)
Molecular weight:	490.64 (O'Neil, 2001)
Chemical formula:	$C_{10}Cl_{10}O$ (O'Neil, 2001)
Melting point:	Decomposes at 350°C (Lide, 2000)
Vapor pressure:	2.25×10^{-7} mm Hg at 25°C (Kilzer et al., 1979)
Density:	1.61 g/mL at 25°C (Lide, 2000)
Water solubility:	2.70 mg/L at 25°C (Kilzer et al., 1979)
Other solubilities:	Slightly soluble in hydrocarbon solvents; soluble in alcohols, ketones, acetic acid (O'Neil, 2001)
Partition coefficient:	log K_{ow} = 5.41 (Hansch et al., 1995)

Chlordecone production begins with the condensation of hexachlorocyclopentadiene with sulfur trioxide under heat and pressure (NLM, 2004a; ATSDR, 1995). Antimony pentachloride is used as a catalyst. The product of this reaction is hydrolyzed and then neutralized (ATSDR, 1995; IARC, 1979). Chlordecone is obtained by centrifugation or filtration and hot air drying. Chlordecone is also a contaminant in mirex formulations and is a degradation product of mirex (Bus and Leber, 2001).

Chlordecone was first produced in the United States in the early 1950s (IARC, 1979). It was introduced commercially in 1958 (Bus and Leber, 2001). Approximately 3.6 million pounds of chlordecone were produced in the United States between 1951 and 1975 (ATSDR, 1995). Chlordecone production in the United States ended in 1975 after intoxication from severe industrial exposure was observed in employees who worked at the only chlordecone manufacturing plant in the country (Bus and Leber, 2001). Typical signs of chlordecone intoxication include nervousness, headache, and tremor (Cannon et al., 1978). Its registration was cancelled in 1978 (Metcalf, 2002; IARC, 1979).

Chlordecone was primarily used as an insecticide (IARC, 1979). Specific applications have included control of the banana root borer, application on non-fruit-bearing citrus trees to control rust mites, control of wireworms in tobacco fields, control of apple scab and powdery mildew, control of the grass mole cricket, and control of slugs, snails, and fire ants (NLM, 2004a; ATSDR, 1995). The registration of pesticide products containing chlordecone was cancelled by the US EPA in 1976 (US EPA, 1976).

Chlordecone is resistant to degradation in the environment. It is not expected to react with hydroxyl radicals in the atmosphere or to hydrolyze or photolyze (NLM, 2004a). Chlordecone in the air is likely to be removed by deposition of particles (NLM, 2004a). Studies have shown that microorganisms degrade chlordecone slowly (NLM, 2004a). Chlordecone is expected to adsorb to soil and to stick to suspended solids and sediments in water (NLM, 2004a). Small amounts of chlordecone will evaporate from soil or water surfaces (NLM, 2004a). Chlordecone has a high potential for bioaccumulation in fish and other aquatic organisms (ATSDR, 1995).

3. TOXICOKINETICS

The available data for humans and animals indicate that chlordecone is well absorbed following oral exposure. Once absorbed, it is widely distributed and eventually concentrates in the liver. It is metabolized by humans and some animal species to chlordecone alcohol. Glucuronide conjugates of chlordecone and chlordecone alcohol, as well as unconjugated chlordecone, are slowly excreted in the bile and eliminated in the feces. Fecal excretion is limited by enterohepatic recirculation.

3.1. ABSORPTION

Chlordecone absorption in humans has been demonstrated by the measurement of chlordecone concentrations in blood, subcutaneous fat, and other body fluids and tissues following subchronic occupational exposure, presumably through ingestion, inhalation, and dermal contact (Taylor, 1982; Adir et al., 1978; Cannon et al., 1978; Cohn et al., 1978). Workers with neurological symptoms of chlordecone toxicity (i.e., tremors, ataxia) had whole blood concentrations ranging between 0.009 and 11.8 ppm. Chlordecone blood concentrations for workers without neurological symptoms were between 0.003 and 4.1 ppm. Chlordecone was also detected in the blood of Hopewell community residents living near a pesticide plant with concentrations ranging from 0.005 to 0.0325 ppm. Potential exposure routes for community residents included inhalation of chlordecone associated with fine particulate matter and ingestion of contaminated soil and drinking water. Neurological symptoms were reported for some residents living near the plant site. In general, the highest blood chlordecone concentrations were observed in affected workers, and lower concentrations were measured in unaffected workers and community residents (Table 3-1) (Cannon et al., 1978).

No data were available in laboratory animals to evaluate chlordecone absorption following inhalation exposure. Quantitative data on absorption of orally administered chlordecone are limited; however, studies on the distribution and excretion of chlordecone in rats, mice, gerbils, and pigs following oral administration of chlordecone indicate that this chemical is readily absorbed from the gastrointestinal tract in animals (Hewitt et al., 1985; Aldous et al., 1983; Fujimori et al., 1982a; Wang et al., 1981; Kavlock et al., 1980; Egle et al., 1978). One study (Egle et al., 1978) attempted to estimate oral absorption quantitatively. Male Sprague-Dawley rats received a single oral dose of 40 mg/kg-day $C^{[14]}$-labeled chlordecone in corn oil solution. The percentage of radioactivity excreted in the feces was measured over time. Approximately 10% of the dose was detected in the feces on the first day after dosing, suggesting that 90% of the orally administered dose was absorbed from the corn oil vehicle.

Animal studies suggest that chlordecone is absorbed only to a limited extent through the skin (Heatherington et al., 1998; Shah et al., 1987). The in vivo percutaneous absorption of

chlordecone was evaluated in young (33 days old) and adult (82 days old) F344 rats (Shah et al., 1987). Acetone solution that contained $C^{[14]}$-labeled chlordecone was applied to the shaved backs of animals, with the treatment area constituting 2 to 3% of the total body surface area.

Table 3-1. Whole blood chlordecone level by group of exposed persons

Group	No. tested	No. with detectable level	% with detectable level	Range of detectable level, ppm	Mean of detectable level, ppm
Affected LSPC[a] workers	57	57	100	0.009–11.8	2.53
Unaffected LSPC workers	49	48	99	0.003–4.1	0.60
Family members, LSPC workers	32	30	94	0.003–0.39	0.10
Allied[b] chlordecone workers	39	30	77	0.003–0.45	0.06
Neighborhood workers	32	23	72	0.003–0.031	0.011
Sewage treatment plant workers	10	6	60	0.004–0.014	0.006
Cab drivers	5	1	20	0.003	0.003
Truck drivers	2	1	50	0.004	0.004
Hopewell community residents[c]	214	40	19	0.005–0.0325	0.011

[a]LSPC = Life Science Products Company.
[b]Allied Chemical Corporation.
[c]Excludes chlordecone factory workers.

Source: Cannon et al. (1978).

Urine and feces were collected over a 72-hour period, after which animals were sacrificed to determine the recovery of radioactivity and the percutaneous absorption of chlordecone. Three dose levels were used to compare dermal absorption in young and adult rats (three rats per dose group). No age-related differences in dermal absorption of chlordecone were noted in this study. Dermal absorption decreased over the dose range in both young and adult rats. In adults, 9% of the applied dose was absorbed at the lowest dose (0.2587 µmol/cm^2), 6% absorption occurred at the medium dose (0.536 µmol/cm^2), and 1% absorption occurred at the highest dose tested (2.679 µmol/cm^2). In young rats, 10% of the applied dose was absorbed at the lowest dose (0.3357 µmol/cm^2), 7% absorption occurred at the medium dose (0.536 µmol/cm^2), and 2% absorption was seen at the highest dose tested (2.679 µmol/cm^2). The nonlinear relationship between in vivo dermal absorption and dose described by Shah et al. (1987) was confirmed by Heatherington et al. (1998) in young and adult rats. In adults, 8% of the applied dose was absorbed at the lowest dose (0.29 µmol/cm^2), 6% absorption was seen at the medium dose (0.535 µmol/cm^2), and 1% absorption occurred at the highest dose tested (2.68 µmol/cm^2). In young rats, 9% of the applied dose was absorbed at the lowest dose (0.340 µmol/cm^2), 7% absorption

occurred at the medium dose (0.535 µmol/cm^2), and 2% absorption was seen at the highest dose tested (2.68 µmol/cm^2).

The time course of chlordecone dermal absorption was studied in young and adult rats by using a serial sacrifice study design (Heatherington et al., 1998). Young and adult F344 rats were dermally exposed to 0.285 µmol/cm^2 chlordecone by using the procedure described above for the Shah et al. (1987) study. Rats were sacrificed at 6, 24, 48, 72, and 120 hours posttreatment. No significant age-related differences were noted in the time course for dermal penetration of chlordecone. In adult rats, the average cumulative absorption was 0.4, 3, 6, 9, and 14% measured at 6, 24, 48, 72, and 120 hours, respectively. In young rats, the average cumulative absorption was 0.6, 4, 7, 10, and 14% measured at 6, 24, 48, 72, and 120 hours, respectively. In vitro test systems using static and flow through diffusion cells were also employed by Heatherington et al. (1998). Only 1% of the applied chlordecone dose penetrated excised dorsal skin from young and adult rats under in vitro conditions. Based on the in vivo dermal absorption data obtained, a biophysically based percutaneous absorption model was developed to describe the movement of chlordecone through the skin. This model was embedded in a whole animal physiologically based toxicokinetic (PBTK) model that was employed to predict tissue concentrations of chlordecone following dermal exposure (see Section 3.5).

3.2. DISTRIBUTION

In 32 workers exposed to chlordecone for a period that ranged from 3 to 16 months, high concentrations of chlordecone were found in blood, liver, and subcutaneous fat. Modest amounts of chlordecone were detected in muscle, gall bladder, bile, and stool, while only trace amounts were detected in aqueous body fluids such as cerebrospinal fluid, urine, saliva, and gastric juice (Cohn et al., 1978). The ratio of the chlordecone concentration in fat as compared to the chlordecone concentration in the blood was 7:1, which is relatively low for a lipophilic organochlorine pesticide. The liver to blood concentration ratio in exposed workers was reported to be 15:1 (Table 3-2).

Table 3-2. Distribution of chlordecone in man

Tissue	Number of patients	Concentration range (µg/g)	Partition	
			Tissue:blood	Range
Whole blood	32	0.6–32.0	1.0	
Liver	10	13.3–173.0	15.0	4.6–31
Subcutaneous fat	29	1.7–62.1	6.7	3.8–12
Muscle	5	1.2–11.3	2.9	1.8–4.5
Gallbladder bile	6	2.5–30.0	2.5	1.4–4.1

Source: Cohn et al. (1978).

The preferential uptake and slow elimination of chlordecone from the liver was confirmed in laboratory animals (Belfiore et al., 2007; Hewitt et al., 1985; Egle et al., 1978). Chlordecone concentrations in rat plasma, kidney, liver, and adipose tissue were determined at various time points following a single oral dose of 50 mg/kg (Hewitt et al., 1985). Chlordecone concentrations persisted in rat tissues throughout the 32-day study period. The highest tissue concentrations were observed in the liver, and this organ had the slowest elimination rate. Between days 8 and 32, liver concentrations were reduced by 73%, while plasma, kidney, and adipose levels were reduced 90, 88, and 81%, respectively. The distribution of chlordecone was also studied in rats receiving a single oral dose of 40 mg/kg-day $C^{[14]}$-labeled chlordecone in corn oil solution (Egle et al., 1978). Initially, the highest levels of radioactivity were found in the adrenal glands followed by liver, lung, and fat. By 3 days following dosing, the highest concentration was in the liver, and this continued throughout the 182-day study period. Chlordecone is eliminated more slowly from the liver as compared with other tissues. The liver to blood ratio increased from 28:1 on day 1 to 126:1 on day 84. The fat to blood ratio reached a maximum of 31:1 on day 7 and declined thereafter, while other organ to blood ratios remained constant. Belfiore et al. (2007) measured chlordecone concentrations in rat liver, fat, blood, kidney, and muscle at 1, 14, or 30 days following a single oral dose of 40 mg/kg. The highest tissue concentrations were observed in the liver, followed by the kidney. The slowest elimination rate was seen in the liver, with chlordecone concentrations reduced 25% between day 1 and day 30. At day 30, levels were reduced by 65, 69, 73, and 75% in blood, fat, muscle, and kidney, respectively. Liver to blood ratios increased from 71:1 on day 1 to 150:1 on day 30.

The preferential retention of chlordecone by the liver is related to chlordecone binding to plasma proteins and lipoproteins. Serum gel filtration indicated that chlordecone was predominantly bound to albumin and lipoproteins in exposed workers. Electrophoresis of normal human plasma following the addition of $C^{[14]}$-labeled chlordecone demonstrated 80% binding to lipoproteins, with most of this binding associated with high-density lipoproteins (HDLs) (Skalsky et al., 1979). The preferential binding of chlordecone to albumin and HDL was demonstrated in human, rat, and pig plasma (Soine et al., 1982). In human plasma, the in vitro distribution of $C^{[14]}$-labeled chlordecone was 46% protein, 30% HDL, 20% low density lipoprotein, and 6% very low density lipoprotein. Similar distributions were seen for pig plasma and for in vitro and in vivo distribution studies in rat plasma. Albumin was identified as the major component of the protein fraction that binds chlordecone. Experiments in isolated perfused pig liver demonstrated that an increase in HDL can affect the distribution of chlordecone, favoring chlordecone uptake and retention in the liver and decreased chlordecone elimination in the bile (Soine et al., 1984). Chlordecone and cholesterol have been shown to compete for similar intracellular binding and transport proteins, which are inducible by chlordecone pretreatment (Gilroy et al., 1994; Carpenter and Curtis, 1991, 1989).

The brain and plasma levels of chlordecone in mice were measured after daily oral dosing with 10 or 50 mg/kg-day (Wang et al., 1981). At the lower dose, the plasma level of chlordecone increased steadily throughout the 12-day treatment period, while the brain chlordecone level reached a plateau on day 10. Brain and plasma levels decayed biphasically following administration of 50 mg/kg-day chlordecone for 1 or 2 days. Brain and plasma concentrations were correlated with loss of motor control at both administered dose levels. Chlordecone was distributed to discrete areas of the mouse brain following a single gavage dose of 50 mg/kg (Fujimori et al., 1982a). The striatum and the medulla/pons had significantly higher chlordecone levels than the cortex, midbrain, or cerebellum.

The distribution of chlordecone following dermal absorption was studied by Heatherington et al. (1998) in young and adults rats (see Section 3.1 for study design information). Less than 15% of the applied dose was absorbed within 120 hours. Organ concentrations increased slowly over time, with the highest concentrations observed in the liver followed by (in decreasing order) kidney, carcass, skin, and blood. Kinetic differences in liver accumulation of chlordecone were suggested between young and adult rats, but all other organ concentrations were comparable. Tissue levels did not appear to have reached steady-state conditions by 120 hours of dermal exposure to chlordecone.

Kavlock et al. (1980) studied the distribution of chlordecone in fetal and neonatal rats. Pregnant rats were given an oral dose of 5 mg/kg chlordecone on days 15, 18, or 20 of gestation. For the prenatal study, animals were killed at 4, 24, or 48 hours after dosing, and maternal and fetal tissues were obtained for chlordecone analysis. In the postnatal study, the dams were given chlordecone at a dose of either 1 or 10 mg/kg-day on days 2 through 5 of the lactation period. Maternal milk was obtained following an injection of oxytocin on days 5, 9, and 15 of gestation. Pups were sacrificed for chlordecone tissue analysis on days 3, 5, 7, 9, 12, 15, and 17 of lactation. Chlordecone crossed the placenta and was observed in fetal tissues as early as 4 hours after maternal dosing. The maximum concentrations of chlordecone on the placenta were 3.5 and 4.00 ppm. Maternal tissue levels were 4 to 5 times higher than fetal concentrations, indicating that the placental barrier retards the distribution of chlordecone to the fetus. Chlordecone levels in the fetus were highest in the liver, followed by the brain, heart, and kidneys. Chlordecone excretion into milk was an important pathway for elimination in nursing dams. Neonatal organ concentrations of chlordecone increased steadily over the lactation period. Tissue uptake for neonates was highest in the liver, followed by the brain and the eyes. Day 5 liver and brain levels rose from 2 to 23 µg and 16 to 150 µg, respectively, in pups nursed by 10 mg/kg-day dosed dams. Tissue concentrations were correlated with chlordecone levels in milk.

The tissue distribution of chlordecone was investigated in rats following pretreatment with phenobarbital, an inducer of hepatic metabolism (Aldous et al., 1983). Repeat doses of phenobarbital (65 mg/kg) were administered intraperitoneally to adult male Sprague-Dawley rats

6, 12, and 24 hours prior to gavage administration of $C^{[14]}$-labeled chlordecone. Phenobarbital pretreatment resulted in an increase in the specific activity in the liver and uniformly reduced the specific activity in other tissues. In phenobarbital pretreated rats, 87% of the $C^{[14]}$-labeled chlordecone was found in the liver, compared to 55% in control rats not receiving phenobarbital. Fecal and urinary excretion of chlordecone was reduced. A single dose of phenobarbital (12 or 24 hours prior to chlordecone administration) similarly altered the distribution of chlordecone; however, changes were more marked with multiple dose administration.

3.3. METABOLISM

Based on available data, a proposed metabolic scheme for chlordecone is shown in Figure 3-1. Although chlordecone is not extensively metabolized in mammals, chlordecone alcohol is formed in humans and some laboratory animal species by reduction of the hydrated carbonyl group (Fariss et al., 1980; Blanke et al., 1978). A cytosolic aldo-keto reductase enzyme appears to be responsible for the formation of chlordecone alcohol (Molowa et al., 1986). Chlordecone alcohol is excreted in bile primarily as a glucuronide conjugate, while chlordecone is excreted into bile mostly in the unconjugated form (Fariss et al., 1980).

The metabolism of chlordecone to chlordecone alcohol occurs in humans, gerbils, and pigs but not to a significant extent in rats, mice, guinea pigs, or hamsters (Houston et al., 1981; Fariss et al., 1980; Blanke et al., 1978). Species differences were also observed in phase II conjugation reactions, with chlordecone conjugation occurring in humans but not in gerbils or rats (Houston et al., 1981). In humans, a reduced form of chlordecone was first identified in the stool of pesticide workers experiencing symptoms of chlordecone toxicity, including nervousness, headache, and tremor (Blanke et al., 1978). Fariss et al. (1980) utilized human bile samples for further analysis of chlordecone and possible metabolites. Human bile was obtained from exposed workers by either aspirated duodenal contents (six workers) or bile collected directly from a T-tube that was implanted during gallbladder surgery (one worker). The initial analysis of human bile using gas-liquid chromatography revealed significant amounts of free chlordecone and small amounts of free chlordecone alcohol in exposed workers. Subsequent treatment of bile samples with β-glucuronidase prior to the analysis resulted in large amounts of measurable chlordecone alcohol. It was estimated that >90% of the chlordecone alcohol in human bile is present as a glucuronide conjugate, while <10% of the chlordecone parent compound is conjugated prior to biliary excretion. The ratio of chlordecone to chlordecone alcohol following β-glucuronidase, sulfatase, and acid hydrolysis treatments was between 1:2 and 1:4 in human bile. In contrast, rat bile contained only trace amounts of chlordecone alcohol, with a corresponding chlordecone to chlordecone alcohol ratio of 155:1.

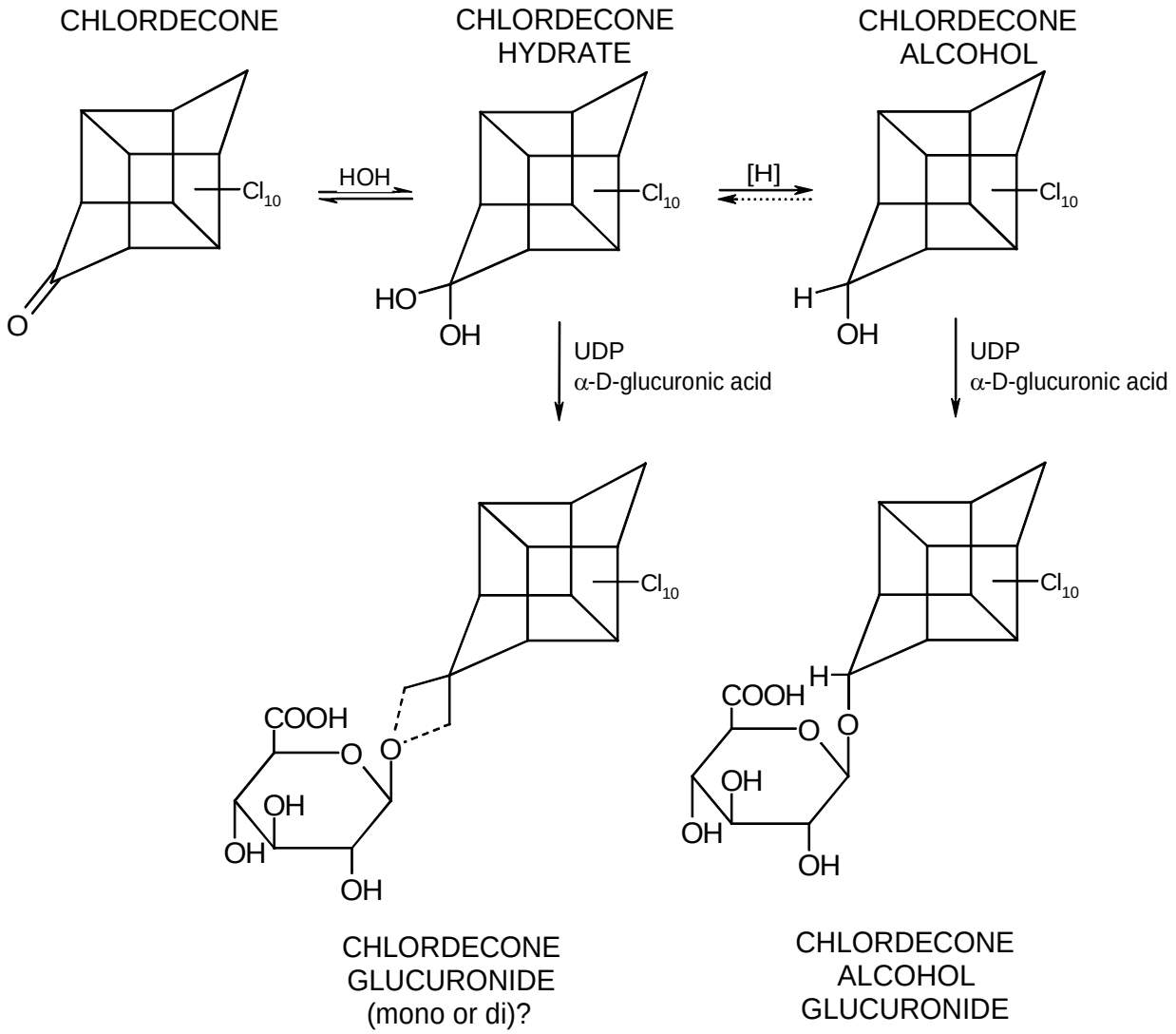


Figure 3-1. A proposed metabolic scheme for chlordecone.

Molowa et al. (1986) characterized a unique cytosolic aldo-keto reductase enzyme responsible for the conversion of chlordecone to chlordecone alcohol. Chlordecone reductase activity was detected in the liver cytosol of rabbits, gerbils, and humans but was absent in rats, mice, hamsters, and guinea pigs. Pretreatment of gerbils with a single oral dose of chlordecone (20 mg/kg) resulted in a 38% increase in the specific activity of chlordecone reductase 7 days later. Soine et al. (1983) also demonstrated the metabolism of chlordecone to chlordecone alcohol in the pig. Pigs were given an intraperitoneal dose of either 40 or 80 mg/kg-day, and chlordecone and chlordecone alcohol concentrations in the blood and gallbladder bile were measured at regular intervals over a 35-day study period. At the end of the study, hepatic bile, liver, and feces were also analyzed for chlordecone and chlordecone alcohol levels. The plasma half-life of chlordecone in the pig was determined to be 12 days at the higher dose and 22 days at the lower dose. Chlordecone metabolites were generally not detected in the plasma; however,

free chlordecone, free chlordecone alcohol, and conjugated chlordecone alcohol were measured in gallbladder bile at both doses. Conjugated chlordecone was only observed in gallbladder bile at the high-dose level. The induction of chlordecone reductase in the pig was suggested by the observed increase in the chlordecone alcohol to chlordecone ratio in the gallbladder bile over the time course of the study. On the last day of the study, 20% of chlordecone was conjugated in the plasma and bile, while only 3% of chlordecone was conjugated in the liver and feces. Chlordecone alcohol was not detected in the plasma or the liver but was 85% conjugated in the bile and 15% conjugated in the feces.

Chlordecone has been shown to induce the cytochrome (CYP) 450 (CYP450) mixed function oxidase enzyme system in male and female rats (Gilroy et al., 1994; Hewitt et al., 1985; Mehendale et al., 1978, 1977). Mehendale et al. (1978, 1977) exposed male and female rats to 0, 50, 100, or 150 ppm chlordecone in the diet for 16 days. A dose-related decrease in body weight gain was observed, while liver weights were unaltered by chlordecone treatment. Enzyme activities that were increased by chlordecone treatment at each dose level include aniline, pentobarbital and hexobarbital hydroxylation, and aminopyrine and ethylmorphine demethylation. CYP450, cytochrome c reductase, and aniline binding were all increased, while cytochrome b5 and NADPH dehydrogenase activity were unaffected by chlordecone treatment. Hewitt et al. (1985) demonstrated increases in microsomal CYP450 and NADPH cytochrome c reductase following a single oral dose of 50 mg/kg (days 2 to 32). Cytochrome b5 was also increased, but not until 24 to 32 days after chlordecone administration. A single oral dose of 15 mg/kg to Sprague-Dawley rats resulted in an increase in CYP450 and ethoxyresorufin-O-deethylase and ethoxycoumarin-O-deethylase enzyme activities (Gilroy et al., 1994). Weanling pups of Sprague-Dawley rat dams exposed to chlordecone from day 2 of gestation to day 21 postpartum (0, 0.1, 1, or 1.5 mg/kg-day) exhibited a dose-related increase in metabolism and excretion of lindane (Chadwick et al., 1979).

Chlordecone was shown to selectively induce CYP2B2 in adult rat hepatocyte cultures (Kocarek et al., 1991). Chlordecone selectively increased the mRNA for CYP2B2, and both chlordecone and chlordecone alcohol induced the immunoreactive protein levels for CYP2B2. Chlordecone did not affect the mRNA or immunoreactive protein levels for CYP2B1 in isolated rat hepatocytes. In addition to its selective induction of CYP2B2, chlordecone also suppressed the induction of CYP2B1 and CYP2B2 when coincubated with phenobarbital in hepatocyte culture. Mechanistic studies suggest that selective induction of CYP2B2 is not due to the estrogenic properties of chlordecone, while the ability to suppress phenobarbital induction may relate to the gem-diol configuration of chlordecone (Kocarek et al., 1994).

3.4. ELIMINATION

Chlordecone and chlordecone alcohol are eliminated from the body primarily through biliary excretion into feces. In humans, chlordecone is eliminated slowly from the blood.

Estimates of the chlordecone serum half-life ($t_{1/2}$) in chemical plant workers ranged from 63 to 128 days (Adir et al., 1978). Analysis of excretory fluids in exposed pesticide workers showed that, while chlordecone was undetectable in sweat and present only in minor quantities in urine, saliva, and gastric juice, concentrations in gallbladder bile were approximately equivalent to chlordecone concentrations in blood (Cohn et al., 1978). The excretion rate of chlordecone into hepatic bile was estimated from either aspirated duodenal contents (six workers) or bile collected directly from a T-tube that was implanted during gallbladder surgery (one worker) (Cohn et al., 1978). The biliary excretion rates varied widely among workers (~1 to 10 mg/day); however, the daily excretion amount expressed as a percent of the total body content was relatively constant (0.29 to 0.85%). For workers who underwent duodenal aspiration, only 5 to 10% of the chlordecone that entered the duodenal lumen via the bile was detected in the feces. Similarly, the rate of chlordecone excreted in bile collected from a surgically implanted T-tube was 19 times greater than the rate of elimination in the stool. These results suggest that enterohepatic recycling plays an important role in the slow excretion of chlordecone. In order to prevent the reabsorption of chlordecone into the gastrointestinal tract, cholestyramine was investigated as a possible treatment for chlordecone intoxication. Cholestyramine is an anion-exchange resin that binds chlordecone but is not absorbed in the gastrointestinal tract. Treatment with cholestyramine reduced the average $t_{1/2}$ in the blood of workers from 165 days to 80 days (Cohn et al., 1978).

Gastrointestinal secretion of chlordecone also appears to play a role in fecal excretion in humans (Boylan et al., 1979). Diversion of the bile stream from the intestine was accomplished in a chlordecone-exposed worker with a surgically implanted T-tube. Chlordecone excretion in stool increased eightfold when bile was diverted from the gut. This nonbiliary mechanism for fecal excretion does not appear to be related to salivary or gastric juice, because chlordecone concentrations in these fluids were minimal in exposed workers. Chlordecone is apparently transferred from the bloodstream to gastrointestinal lumen via a secretory process governed by diffusion (Bungay et al., 1979). High concentrations of chlordecone in the lumen inhibit gastrointestinal secretion. Experimental data in rats confirmed the presence of a nonbiliary pathway for fecal excretion of chlordecone. Bungay et al. (1979) evaluated the transport of chlordecone in and out of the gut and utilized a PBTK model to describe the results (see Section 3.5). The transport of chlordecone into and out of the gut was studied following intravenous administration to the bile duct of cannulated rats and oral administration to intact rats. The primary route of elimination of chlordecone is in the feces.

Animal studies evaluated the elimination of chlordecone following oral exposure. Egle et al. (1978) studied chlordecone excretion in male Sprague-Dawley rats receiving a single oral dose of 40 mg/kg-day $C^{[14]}$-labeled chlordecone in corn oil solution. The percentage of radioactivity excreted in the feces was measured over time. Approximately 30% of the administered chlordecone was excreted within the first 7 days, after which the rate of excretion

steadily declined. After 12 weeks, 65.5% of the dose had been excreted into the feces and after 26 weeks, the cumulative excretion in feces was only 69.8%. A small amount of the administered chlordecone was excreted in the urine. Only 1.6% of the administered dose was found in the urine by 12 weeks, one-third of which was excreted into urine in the first 24 hours. Chlordecone was measured in expired air on days 1 and 9 after dosing, and less than 1% of the administered dose was detected in expired air.

Heatherington et al. (1998) studied the excretion of chlordecone following dermal absorption in young and adult rats (see Section 3.1 for study methods). Higher concentrations of chlordecone were detected in the urine of young rats as compared with adults. Chlordecone elimination was primarily in the feces, with limited urinary excretion. Feces to urine ratios 120 hours following dermal application of chlordecone were 3:1 and 3:8 in young and adult rats, respectively.

Chlordecone treatment has been shown to decrease the biliary excretion of other chemicals (Curtis and Mehendale, 1979). Male Sprague-Dawley rats were fed diets containing 0, 10, 50, or 150 ppm chlordecone for 15 days. Food consumption and body weight data were used to estimate daily dose levels of 0, 0.69, 3.2, and 8.0 mg/kg-day. Clinical signs of chlordecone toxicity were not apparent in the 10 or 50 ppm groups, but hyperexcitability and tremors were observed at 150 ppm. Decreased body weight gain was observed at the two highest dose levels. Biliary function was evaluated in bile-duct-cannulated intact animal preparations. The highest dose of chlordecone reduced the biliary excretion of the polar metabolites of imipramine (31% of control) and phenolphthalein glucuronide (27% of control). These decreases occurred despite an increase in cumulative bile flow at the 150 ppm dose level. Oligomycin-sensitive mitochondrial ATPase activity was inhibited by chlordecone in this study; however, the dose-response data do not suggest a direct correlation between enzyme inhibition and hepatobiliary dysfunction. Chlordecone was also shown to inhibit oligomycin-sensitive Mg^{2+}-ATPase activity in the rat bile canaliculi-enriched fraction of the liver; however, it is not known whether this inhibition represents a causal factor in hepatobiliary dysfunction or simply an indication of membrane perturbation (Curtis, 1988).

Teo and Vore (1991) studied the effect of chlordecone on bile acid secretory function (i.e., bile flow, bile acid concentration, bile acid secretory rate) in the isolated perfused rat liver. Rats were given an oral dose of 18.75 mg/kg-day chlordecone for 3 days prior to measurement of bile secretory parameters. Chlordecone treatment resulted in an increase in bile flow while decreasing bile acid concentration and bile acid secretory rate. These results suggest that chlordecone acts primarily at the bile canalicular membrane to decrease biliary excretion. Rochelle et al. (1990) demonstrated that chlordecone perturbs the membrane and inhibits the active transport of glutamate at the bile canalicular membrane. Hepatobiliary dysfunction does not appear to be related to the concentration of chlordecone associated with the liver plasma membrane (Rochelle and Curtis, 1994); however, inhibition and recovery of 5'-nucleotidase

activity in the liver plasma membrane suggest that biochemical alterations in membrane function may be involved.

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

Physiologically-based toxicokinetic (PBTK) models have been used to describe the hepatic sequestration of chlordecone (Belfiore et al., 2007), movement of chlordecone in and out of the gut (Bungay et al., 1979), percutaneous absorption and disposition of chlordecone (Heatherington et al., 1998), and toxic interactions between chlordecone and carbon tetrachloride (el-Masri et al., 1995) in laboratory animals. PBTK models are not available to describe toxicokinetic processes in humans.

Belfiore et al. (2007) developed a PBTK model to describe sequestration of chlordecone in the liver of rats. Male Sprague-Dawley rats received a one-time treatment of 40 mg/kg-day of chlordecone in corn oil by gavage. Rats were sacrificed at 1, 14, or 30 days following dosing, and liver, fat, kidney, and muscle specimens were removed and assayed for chlordecone concentration. Data from this time course and from distribution studies in the literature (Hewitt et al., 1985 Egle et al., 1978) were used to develop and validate a toxicokinetic model to describe the preferential sequestration of chlordecone in the liver. A model was constructed in which liver, fat, and slowly perfused and rapidly perfused tissues were flow limited. Metabolism was not included due to the low biotransformation rate for chlordecone. The model fit to the experimental data was greatly improved by adding blood and liver binding coefficients derived from data from Soine et al. (1984, 1982). This model provides additional support for the hepatic sequestration of chlordecone in Sprague-Dawley rats; however, several factors limit its use in the derivation of reference values. It is not known how the measured blood, fat, or liver tissue levels would correlate other organ compartments not included in the model. This model also does not provide information on inhalation exposure that would be needed for route-to-route extrapolation and thus cannot be used for the derivation of an RfC. Additionally, the model is not parameterized for humans, so it cannot be used to evaluate interspecies toxicokinetic differences.

Bungay et al. (1979) conducted experiments comparing intravenous administration of chlordecone in bile-duct-cannulated rats and oral administration in intact rats. The data were used in the gut portion of a whole body PBTK model. The gastrointestinal tract was divided into six segments, and the lumens of these segments were connected in series in the model. Flow rates were measured in each segment, and the net secretion or absorption was determined for each compartment. Diffusional processes were assumed to govern chlordecone exchange between blood, gut tissue, and the lumen. In the rat, the PBTK model yields a maximum clearance estimate for gut secretion of 25 mL/hour. Measurement of biliary clearance in bile-duct-cannulated rats was 5 mL/hour, suggesting a total maximum clearance rate of 30 mL/hour. Assuming that the permeability of the gut to chlordecone is similar in rats and humans, a maximum human clearance rate of 1000 mL/hour was calculated by the authors by using a body-

weight scaling factor (body-weight ratio raised to the 2/3 power). The chlordecone clearance rate estimated for pesticide workers not receiving cholestyramine treatment (Cohn et al., 1978) was only 40 mL/hour due to the presence of chlordecone in the lumens and the inhibition of diffusion from the gut.

A PBTK model was developed to describe the percutaneous absorption and disposition of chlordecone in young and adult rats (Heatherington et al., 1998). The experimental data for the dose effect and time course of chlordecone dermal absorption are described in Section 3.1. The distribution and excretion data for this study are reported in Sections 3.2 and 3.3. A biophysically based percutaneous absorption model was developed based on in vivo dermal absorption data. The absorption model consisted of five first-order rate constants describing the movement of chlordecone by diffusion from the site of application to the stratum corneum, where it undergoes partitioning with the viable epidermis, followed by entry into the blood and distribution throughout the body. The rate constants for movement among compartments were based on chlordecone physical and chemical characteristics, skin physiology, and experimental data. The absorption model was significantly limited by its inability to describe the nonlinear dose effect of percutaneous exposure (i.e., decreasing percent absorption with increasing dose). Therefore, the data for only one dose level could be used for PBTK disposition modeling (i.e., time course data for 0.285 μmol/cm^2). The absorption model was embedded in the whole body PBTK model to describe the distribution and excretion of chlordecone in young and adult rats. The distribution of chlordecone from blood to various tissue compartments was described. The PBTK model took into account chlordecone binding to albumin and lipoproteins in the blood, preferential uptake by the liver, and the predominant fecal excretion pathway for chlordecone. Once optimized using the experimental data for chlordecone, the PBTK model was used to predict partition coefficients and excretion rates. Tissue concentrations at varying dose levels were reasonably well estimated if the nonlinear dermal absorption at high doses and the nonlinear uptake of bound chlordecone into the liver were considered.

el-Masri et al. (1995) utilized PBTK and toxicodynamic (TD) modeling to evaluate the toxic interaction between chlordecone and carbon tetrachloride. Chlordecone significantly potentiates the hepatotoxicity and lethality of carbon tetrachloride by interfering with the regeneration process in the liver (see Section 4.4.2). A PBTK model for carbon tetrachloride was adapted and verified using experimental data. The PBTK model was then linked with a TD model based on the mechanistic data for the interaction between chlordecone and carbon tetrachloride in liver cells. The combined model yielded a time course simulation of mitotic, injured, and pyknotic cells following treatment with carbon tetrachloride alone or in combination with chlordecone. The PBTK/TD model was coupled with Monte Carlo simulation techniques to predict the acute lethality of carbon tetrachloride under various exposure conditions. Predictions of lethality were in agreement with experimentally derived values except at very high doses where neurotoxicity led to significant mortality.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

Information regarding the health effects of chlordecone in humans comes from studies of a single group of 133 men exposed occupationally to chlordecone at a facility in Hopewell, Virginia (Taylor, 1985, 1982; Guzelian, 1982a; Guzelian et al., 1980; Sanborn et al., 1979; Cannon et al., 1978; Martinez et al., 1978; Taylor et al., 1978). Of the 133 men, 76 experienced neurological symptoms, especially nervousness, headaches, and tremors, sometimes persisting as long as 9–10 months after cessation of exposure and the start of treatment (Cannon et al., 1978). In addition, some of the men experienced oligospermia.Sperm count and motility had returned to normal by 5 to 7 years following the cessation of chlordecone exposure and treatment with cholestyramine to reduce chlordecone blood levels (Taylor, 1982). Some workers exposed to high levels of chlordecone developed skin rashes, enlarged livers, and joint pain. Liver enlargement developed in 20 out of 32 workers with high blood levels of chlordecone (>0.6 μg/mL) after an average duration period of 5–6 months, although evidence of significant liver toxicity was not found (Guzelian, 1982a; Guzelian et al., 1980; Taylor et al., 1978). Normal results were obtained in all patients for serum bilirubin, albumin, globulin, prothrombin time, cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transpeptidase (GGT), and serum alkaline phosphatase was only minimally elevated in seven patients. Sulfobromophthalein retention, a measure of liver clearance, was normal in a subset of 18 workers tested (Guzelian et al., 1980). Liver biopsy samples taken from 12 workers with hepatomegaly showed histological changes in the liver that were characterized as nonadverse in nature. These included proliferation of the smooth endoplasmic reticulum (SER) and cytoplasmic accumulation of lipofuscin. No evidence of liver neoplasia, fibrosis, cholestasis, or hepatocellular necrosis was found. Neurological symptoms were reported in workers exposed to high doses of chlordecone for a period of months to years (Taylor, 1985, 1982; Guzelian, 1982a; Guzelian et al., 1980; Sanborn et al., 1979; Cannon et al., 1978; Martinez et al., 1978; Taylor et al., 1978). These symptoms included tremor, headache, irritability, poor recent memory, rapid random eye movements, muscle weakness, gait ataxia, incoordination, and slurred speech. The effects persisted for as long as 9–10 months after cessation of exposure and the start of treatment (Cannon et al., 1978). Martinez et al. (1978) reported that nerve conduction velocity tests, electroencephalography, radioisotope brain scans, computerized tomography, and analyses of cerebral spinal fluid content from these workers were all normal. Sural nerve and skeletal muscle biopsies in workers with detectable neurological impairment exhibited a reduction in the number of unmyelinated axons and a disruption in Schwann cell metabolism (Martinez et al., 1978).

The factory did not follow good industrial hygiene practices. Substantial inhalation, dermal, and oral exposures could have occurred to the workers (Guzelian, 1982a; Guzelian et al., 1980; Cannon et al., 1978). Because of uncertainties regarding exposure routes and levels at the facility and concomitant exposure to the precursors used to manufacture chlordecone, no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) could not be established for the adverse effects observed on the nervous systems, livers, and reproductive systems of these men. Liver biopsy samples taken from 12 workers with hepatomegaly resulting from intermediate- or chronic-duration exposures to high levels of chlordecone showed no evidence of significant liver toxicity or cancer (Guzelian et al., 1980); however, conclusions from this study are limited by the very small number of workers sampled, uncertainties concerning exposure dose and route, the relatively brief duration of exposures, and the absence of a sufficient latency period for tumor development. The average exposure of the subjects was 5–6 months, and they were examined immediately after exposure (Cannon et al., 1978). A review of biological and epidemiological evidence of cancer found no population-based studies on cancer in humans related to chlordecone exposure (Ahlborg et al., 1995). These case reports of occupationally exposed workers at the pesticide plant (who were repeatedly exposed to high but unmeasured levels for less-than-lifetime durations) indicate that primary target organs for chlordecone toxicity in humans are the nervous system, reproductive organs, skin, and liver.

4.2. SUBCHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

Animal studies show effects similar to those reported in occupationally exposed humans: neurological effects, oligospermia, hepatomegaly, and skin rashes, as well as kidney lesions that were not reported in humans. Chlordecone is moderately lethal by single exposures; oral median lethal dose (LD_{50}) values range from 71 mg/kg body weight for rabbits to 250 mg/kg body weight for dogs (Larson et al., 1979a). The oral LD_{50} value for rats is 125 mg/kg body weight (Gaines, 1969). In experimental animals, the effects of chlordecone following short-term exposures generally include nervous system effects (tremor and hyperexcitability), liver hypertrophy (with induction of mixed-function oxidases), and structural and ultrastructural changes in the liver, thyroid, adrenal glands, and testes (ATSDR, 1995; U.S. EPA, 1986c; WHO, 1984). In subchronic studies with experimental animals, chlordecone produced tremors and other neurological symptoms, liver hypertrophy with induction of mixed function oxidases, hepatobiliary dysfunction, and centrilobular hepatocellular necrosis. Chlordecone also interferes with reproductive processes in both males (oligospermia) and females (disruption of estrous cyclicity), and it is fetotoxic in experimental animals. Chronic testing of chlordecone in laboratory animals is limited to two studies: NCI (1976a) and Larson et al. (1979a). In a dietary cancer bioassay with chlordecone, NCI (1976a) found a statistically significant increase in the

incidence of and a reduction in the time to detection of hepatic tumors among male (marginal) and female Osborne-Mendel rats and male and female B6C3F1 mice. The Larson et al. (1979a) study also reported hepatic proliferative lesions, but the determination of whether these represented tumors was equivocal. No data are available concerning the toxicity of chlordecone in animals following inhalation exposure. Studies demonstrating adverse effects in experimental animals following oral exposures are presented below. No studies were available for inhalation or dermal routes of exposure.

4.2.1. Subchronic Studies

4.2.1.1. *Oral Exposure Studies*

Huang et al. (1980) administered chlordecone by gavage to adult male ICR mice (15 per dose group) at 0 (corn oil vehicle), 10, 25, or 50 mg/kg-day. Animals were gavaged daily for up to 24 days. Tremor and hyperexcitability were observed in all mice receiving chlordecone; time to onset was dose dependent. Loss of body weight (accompanied by reduced food and water consumption) was also apparent in chlordecone-exposed animals, with the greatest loss of body weight coincident with the onset of tremor. The authors speculated that the reduction in body weight among treated mice was due to paralysis and loss of motor control, which resulted in a decreased ability to eat. Upon termination of chlordecone administration, a diminution of tremor and corresponding recovery of body weight were observed in surviving animals. Chlordecone-treated mice showed a high degree of mortality. Time to onset of first death and slope of the mortality curves (cumulative mortality per day) were dose dependent. Mortality among mice exposed to 50 mg/kg-day began on day 4, and all were dead by day 6. For the 25 mg/kg-day group, mortality began on day 6 and reached 100% by day 11. For the 10 mg/kg-day group, mortality began on day 12 and reached nearly 90% by day 24 of treatment. The control group had no deaths. The cumulative oral LD_{50} was estimated by the authors to be between 180 and 200 mg/kg. Due to the high incidence of mortality among even the lowest dose group, these doses are considered frank effect levels and cannot be used to establish effect levels for quantitative risk assessment. In a follow-up study, Fujimori et al. (1982b) administered chlordecone by gavage to male ICR rats at 0 (corn oil vehicle), 10, 25, or 50 mg/kg-day for 9 consecutive days. The results of this study also demonstrated a dose response in the time to onset of chlordecone impairment of motor coordination (days 2, 4, and 9 for 50, 25, and 10 mg/kg-day dose groups). The authors examined dopamine and serotonin levels and speculated that the dopaminergic pathway may be involved (though not necessarily the sole player) in mediating chlordecone-induced tremor and neurotoxicity.

4.2.1.2. *Inhalation Exposure Studies*

No inhalation exposure studies were found in the literature.

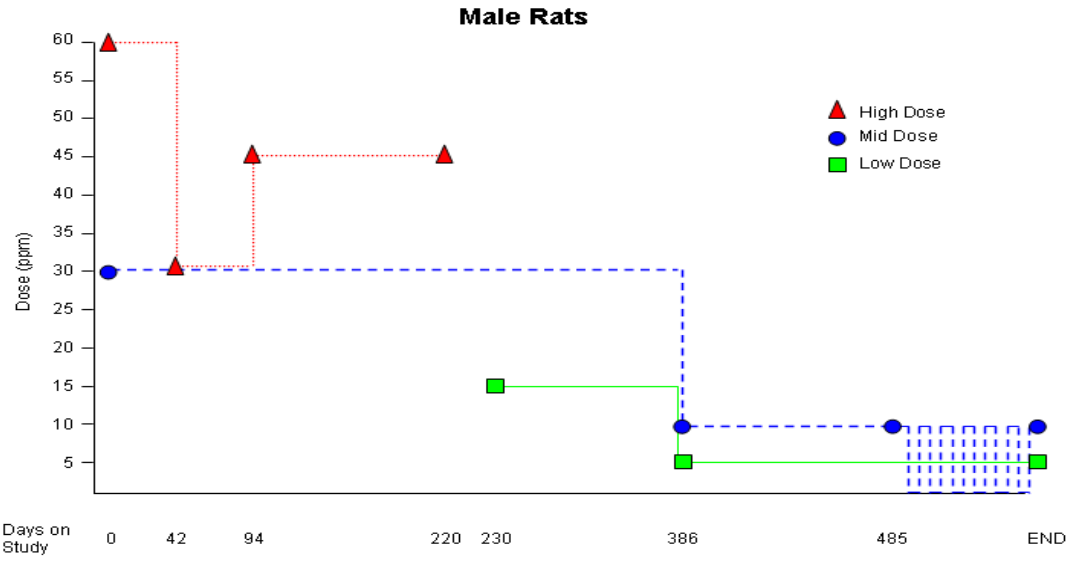
4.2.2. Chronic Studies

4.2.2.1. *Oral Exposure Studies*

Chu et al. (1981a) fed male Sprague-Dawley rats (10 per group) diets containing 0 or 1 ppm of chlordecone (0 or 0.07 mg/kg-day reported by the authors) for 21 months. Corn oil was used to dissolve the chemicals, and control diets contained 4% corn oil. Survival and weight gain were similar in treated and control rats. Hematology and clinical chemistry were also unaffected by treatment. Histopathological findings included apparent increases in the incidence of lesions in the liver (5/6 [83%] vs. 3/7 [43%] in controls) and thyroid (4/6 [67%] vs. 1/7 [14%] in controls). The differences in incidences were not statistically significant (Fisher's exact test conducted for this review), although the power of the statistical test to detect a difference at such small sample sizes is low. The lesion in the liver was described as pericentral cytoplasmic vacuolation with mild anisokaryosis, while the thyroid lesion was described as a mild degenerative and proliferative change in the epithelium. Severity of both lesions was reported to be increased in chlordecone-treated rats in comparison with controls, although the nature and extent of these differences were not described. This study was a follow-up to an earlier short-term (28 day) study with the same exposure protocol (Chu et al., 1980). Twenty-eight days of oral exposure to chlordecone produced no statistically significant alteration in hepatic microsomal or serum enzyme activities. The 28-day study found chlordecone-induced lesions in the liver (3/10 exhibited multiple lymphoid aggregates, perivenous cytoplasmic ballooning, and perinuclear halos in the portal area) and kidney (2/10 showed eosinophilic inclusions in proximal tubules) but not the thyroid. Interpretation of these findings is difficult due to the small number of rats used, use of a single exposure dose, and occurrence of the reported effects among control rats.

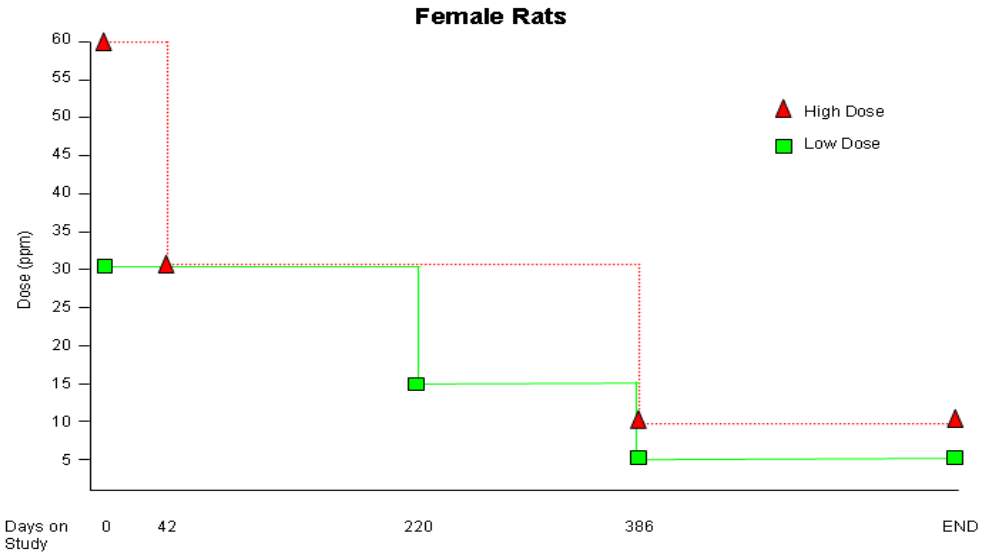
Osborne-Mendel rats and B6C3F1 mice were exposed to technical-grade chlordecone in the diet for 80 weeks (NCI, 1976a,b). The test material was reported to contain no more than 2% impurities other than water. Chlordecone was added to finely ground rat chow in acetone (to aid uniform dispersion of the chemical); the diets were mixed for homogeneity and to allow the acetone to evaporate. Corn oil (2%) was added to the diet as a dust suppressant. Dietary concentrations of chlordecone began at 0, 15, 30, or 60 ppm for male rats and 0, 30, or 60 ppm for female rats. Treatment groups comprised 50 rats per sex; however, only 10 animals per sex were used in matched control groups. Pooled control groups (from the same laboratory with birth dates within 3–4 months of the animals in the matched control and exposed groups) contained 105 male rats and 100 female rats. Overt clinical signs of toxicity observed in the treated animals indicated that the initial doses exceeded the maximum tolerated dose in the high exposure groups; consequently, concentrations of chlordecone in feed were reduced (to one-third to one-sixth of the original concentration) during the experiment (after durations ranging from as

short as 42 days in high-dose female rats to as long as 386 days in high-dose male rats). The specific dosing regimens for male and female rats are illustrated in Figures 4-1 and 4-2.



*Lines represent changes in the dose levels made throughout the study period. The undulating line for the mid dose from day 485 until the end of the study represents a recovery period of a week between doses for the last 75 days of the study. Additionally, the low dose group was added in the middle of the study period.

Figure 4-1. Dosing regimen for male rats in the study by NCI (1976b).



*Lines represent changes in the dose levels made throughout the study period.

Figure 4-2. Dosing regimen for female rats in the study by NCI (1976b).

The initial group of high-dose male rats was discontinued due to excess toxicity; however, nine rats were transferred to the lower dose group in the study. A new dose group of male rats was started 8 months after the beginning of the study. Time-weighted-average dietary concentrations were reported by the authors (and confirmed for this review) to be 0, 8, or 24 ppm for male rats and 0, 18, or 26 ppm for female rats. Doses estimated from U.S. EPA (1988) reference values for body weight and food consumption were calculated[1]: 0, 0.6, or 1.7 mg/kg-day for male rats and 0, 1.4, or 2.0 mg/kg-day for female rats. Following the 80-week exposure, surviving rats were sacrificed at 112 weeks. The following tissues were taken from sacrificed animals, and those dying early, for histological examination: brain, pituitary, lymph nodes, thyroid, parathyroid, salivary glands, lung, heart, diaphragm, stomach, duodenum, jejunum or ileum, large intestine, pancreas, adrenal glands, kidney, liver, skin, gonads, bladder, prostate or uterus, and femur with marrow.

Clinical signs of chlordecone toxicity, including tremor and dermatological changes, were indicated in the NCI (1976a) report, although incidence by dose was not reported (NCI, 1976a,b). Survival was reduced for high-dose male and female rats (NCI, 1976a). Percentages of male rats surviving to study termination (112 weeks) were 63% for pooled controls, 90% for matched controls, 60% for the low-dose group, and 42% for the high-dose group; for female rats, the respective percentages were 61%, 70%, 56%, and 40%. The decreases in survival occurred primarily during the second year of the study, although some early mortality was observed among high-dose male rats (four animals in the first 4 months). Many of the treated rats also showed decreases in food consumption and body weight gain (NCI, 1976b). In male rats, body weight gain at 79 weeks was 82% and 79% of control for the low- and high-dose groups, respectively. Body weight gain in female rats at 79 weeks was 76% and 66% of control for the low- and high-dose groups, respectively. Some high-dose males were observed to have bleeding of the eyes and nose during the first 4 months of the study, and, by week 5 of the study, most high-dose females showed generalized tremors. By week 28, many low-dose females were also experiencing tremors. The incidence of tremors and other clinical signs (rough hair coat, dermatitis, anemia) was low to moderate during the remainder of the first year but gradually increased during the second year of the study. The authors reported that rats surviving to study termination were generally in very poor physical condition, though more specific data regarding occurrence of clinical signs were not reported.

In rats, the incidence of noncancer lesions was reported in summary tables included in the microfiche for the bioassay (NTP, 1976b). These tables showed chronic kidney inflammation in low-dose male rats and high-dose female rats but did not confirm the presence of extensive noncancer liver lesions in male or female rats. Liver tumors described as hepatocellular

[1]Calculation: mg/kg-day = (ppm in feed × kg food/day)/kg body weight. Reference food consumption rates of 0.036 kg/day (males) and 0.030 kg/day (females) and reference body weights of 0.514 kg (males) and 0.389 kg (females) were used (U.S. EPA, 1988).

carcinomas were observed in high-dose female rats at an incidence that was significantly elevated compared with the pooled control incidence (0/100, 0/10, 1/49, and 10/45 in the pooled control, matched control, low-dose, and high-dose groups, respectively). Incidences of male rats with hepatocellular carcinomas were 0/105, 0/10, 1/50, and 3/44, respectively. The incidence of carcinomas in high-dose males was significant ($p = 0.049$) in comparison with pooled controls. The incidence of hepatocellular carcinomas was not statistically significant in comparison with matched controls for rats of either sex. A significant dose-response trend was observed for the incidence of hepatocellular carcinoma in both male and female rats (Cochran-Armitage test conducted for this review). Hepatocellular carcinomas were described as large, poorly circumscribed masses that were well differentiated without vascular invasion or metastases. Liver tumors described as neoplastic nodules were also found but not at elevated incidences in exposed groups compared with control groups. Neoplastic nodule incidences were reported to be 0/10, 2/50, and 0/44 in the matched control, low-dose, and high-dose male groups and 1/10, 0/49, and 2/45 in female rats. The incidence and time to tumor data for hepatocellular carcinoma in rats in the NCI (1976a) report are summarized in Table 4-1.

Table 4-1. Incidence and time to tumor of hepatocellular carcinoma in rats

Osborne-Mendel rats	Exposure group			
	Matched control	Pooled control	Low dose	High dose
Male (0, 0.6, or 1.7 mg/kg-day)[a]	0/10	0/105	1/50	3/44[b,c]
Time to first tumor (weeks)	NA[d]	NA	112 weeks	108 weeks
Female (0, 1.4, or 2.0 mg/kg-day)[a]	0/10	0/100	1/49	10/45[c]
Time to first tumor (weeks)	NA	NA	87 weeks	83 weeks

[a]Doses were calculated for this review using the allometric equation for food consumption by laboratory animals with time-weighted concentrations from NCI (1976a) and reference body weights from U.S. EPA (1988).
[b]Marginal increase ($p = 0.049$) compared with pooled controls.
[c]Statistically significant increase in incidence as compared with pooled controls, using one-tail ($p < 0.05$) Fisher's exact test for 2 × 2 contingency table (NCI, 1976a).
[d]NA = not available.

Source: NCI (1976a).

In addition to the liver, the rats developed tumors in other organs of the endocrine system (NCI, 1976a). Table 4-2 shows the incidence of these tumors by organ and tumor type. The incidence rate for all tumor types combined for each of these systems (endocrine or reproductive) was not statistically increased as compared with controls (Fisher's exact test conducted for this review). Individual tumor types were also not significantly increased, and no dose-response trend was observed (Cochran-Armitage test conducted for this review).

Table 4-2. Summary of endocrine and reproductive system tumor incidence among rats exposed to chlordecone

		Males			Females		
		Control	0.6 mg/kg-day	1.7 mg/kg-day	Control	1.4 mg/kg-day	2.0 mg/kg-day
Number of rats		10	50	44	10	49	45
Number of rats with any type of tumor[a]		3 (30%)	24 (48%)	16 (36%)	7 (70%)	29 (59%)	31 (69%)
Endocrine Organs	Pituitary chromophobe adenoma	2 (20%)	12 (24%)	5 (11%)	3 (30%)	13 (26%)	4 (9%)
	Pituitary adenocarcinoma	–	–	1 (2%)	–	–	–
	Thyroid follicular-cell carcinoma	–	3 (6%)	–	–	–	1 (2%)
	Thyroid follicular-cell adenoma	–	2 (4%)	–	–	1 (2%)	–
	Thyroid C-cell adenoma	–	3 (6%)	–	–	2 (4%)	1 (2%)
	Thyroid C-cell carcinoma	–	1 (2%)	–	–	–	1 (2%)
	Parathyroid adenoma	–	–	1 (2%)	–	–	–
	Pancreatic islet cell adenoma	–	1 (2%)	1 (2%)	–	1 (2%)	–
	Adrenal cortical adenoma	–	1 (2%)	–	–	–	2 (4%)
Reproductive Organs	Mammary gland fibroadenoma	–	1 (2%)	1 (2%)	4 (40%)	4 (8%)	1 (2%)
	Mammary gland adenoma	–	–	1 (2%)	2 (20%)	1 (2%)	–
	Mammary gland fibroma	1 (10%)	–	–	–	–	–
	Mammary gland adenocarcinoma	–	–	–	–	2 (4%)	–
	Mammary gland fibrolipoma	–	–	–	–	–	1 (2%)
Reproductive Organs	Uterus endometrial/ stromal polyp	–	–	–	–	3 (6%)	1 (2%)
	Uterus malignant lymphoma	–	–	–	–	1 (2%)	–
	Uterus squamous cell carcinoma	–	–	–	–	–	–
	Ovary arrhenoblastoma	–	–	–	–	1 (2%)	–
	Ovary granulosa-cell tumor	–	–	–	–	–	1 (2%)
	Cervix uteri squamous cell carcinoma	–	–	–	–	1 (2%)	–

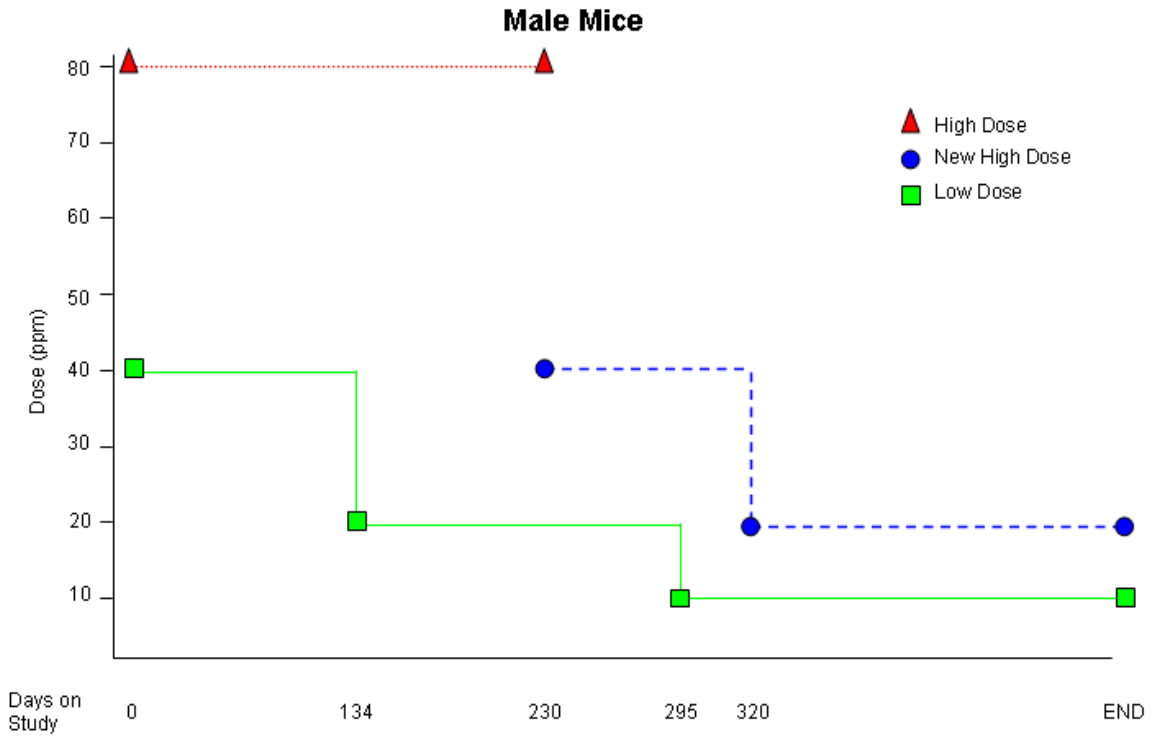
[a]Some animals had multiple tumors.

Source: NCI (1976a).

In mice, dietary concentrations of chlordecone began at 0 or 40 ppm (two groups at this concentration) for males and 0, 40, or 80 ppm for females. Treatment groups comprised 50 mice per sex; however, only 10 female mice and 19 male mice were used as matched controls. Pooled control groups (from the same laboratory with birth dates within 3–4 months of the animals in the matched control and exposed groups) contained 49 male mice and 40 female mice. Overt clinical signs of toxicity observed in the high-dose male and female mice indicated that the maximum tolerated dose was exceeded in those exposure groups; consequently, concentrations of chlordecone in feed for all dose groups were reduced (to one-fourth to one-half of the original concentration) during the experiment. The specific dosing regimen for male and female mice was described in the microfiche for the bioassay (NCI, 1976b) and is illustrated in Figures 4-3 and 4-4. The initial high-dose group of male mice was discontinued due to excess toxicity, and a new group was started 7 months later after the beginning of the study. Time-weighted-average dietary concentrations were reported by the authors (and confirmed for this review) to be 0, 20, or 23 ppm for male mice and 0, 20, or 40 ppm for female mice. Doses estimated from U.S. EPA (1988) reference values for body weight and food consumption were calculated[2]: 0, 3.4, or 3.9 mg/kg-day for male mice and 0, 3.5, or 7.0 mg/kg-day for female mice. Following the 80-week exposure, surviving mice were sacrificed at 90 weeks. Histological examination was similar to that described previously for rats.

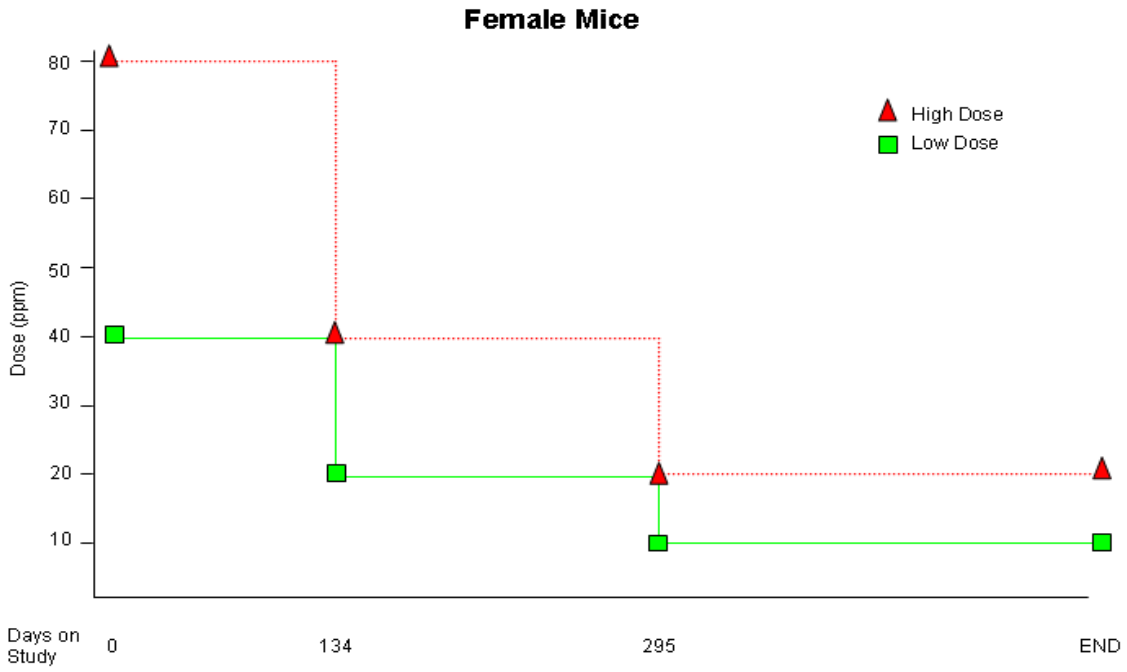
Survival was reduced for male mice at both the high and low dose; though survival rates in female mice at both dose levels were comparable with those of controls (NCI, 1976a). The percentages of male mice surviving to study termination at 90 weeks were 92% for pooled controls, 90% for matched controls, 58% for the low-dose group, and 50% for the high-dose group. The percentages of survival for female mice were 85% for pooled controls, 90% for matched controls, 84% for the low-dose group, and 84% for the high-dose group. The decreases in survival occurred primarily during the second year of the study, although some early mortality was observed. Decreases in food consumption and body weight gain were less pronounced in mice as compared to rats (NCI, 1976b). In male mice, body weight gain at 81 weeks was 93% and 88% of control for the low- and high-dose groups, respectively. Body weight gain in female mice at 81 weeks was 94% and 88% of control for the low- and high-dose groups, respectively. A comparison of survival rates and body weight gain for animals in the NCI study is presented in Table 4-4.

[2]Calculation: mg/kg-day = (ppm in feed × kg food/day)/kg body weight. Reference food consumption rates of 0.0064 kg/day (males) and 0.0061 kg/day (females) and reference body weights of 0.0373 (males) and 0.0353 kg (females) were used (U.S. EPA, 1988)



*Lines represent changes in the dose levels made throughout the study period.

Figure 4-3. Dosing regimen for male mice in the study by NCI (1976a,b).



*Lines represent changes in the dose levels made throughout the study period.

Figure 4-4. Dosing regimen for female mice in the study by NCI (1976a,b).

Clinical signs of chlordecone toxicity were reported in mice; however, the incidence by dose was not reported (NCI, 1976a,b). High-dose female mice developed tremors during the first week of the study that persisted to study termination. Tremors were also observed in some high-dose male mice, and about 20% of high-dose males were highly excitable during the second year of the study. Abdominal distention was first observed in high-dose males at week 45 and high-dose females at week 68, presumably associated with hepatic hypertrophy. Palpable abdominal masses were found in high- and low-dose males during the second year of the study. Alopecia, rough hair coats, and tail sores were seen primarily in males and were thought to be due to fighting. More specific data regarding occurrence of clinical signs were not reported.

In mice, statistically significant elevated incidences of hepatocellular carcinomas were found in both exposed groups compared with matched and pooled control incidences (NCI, 1976a). Incidences for matched control, low-, and high-dose groups were 6/19, 39/48, and 43/49 for male mice and 0/10, 26/50, and 23/49 for female mice. The incidence in control male mice was reported as abnormally high. Two of the pooled control male mice had hepatocellular carcinomas. Combining the matched and pooled control male mouse groups resulted in an overall incidence of 8/49 for control male mice. Hepatocellular carcinomas in mice were described as varying from demarcated nodules to large masses that were well differentiated without vascular invasion or metastases. Extensive liver hyperplasia also was found in both sexes in both low- and high-dose mouse groups. Incidences for liver hyperplasia were not specified, but the report noted that "a few matched controls of each sex also had liver hyperplasia although the incidence was quite low as compared to the treated groups." No tumors of other endocrine organs were reported, aside from one ovary cystadenoma in a single high-dose female (1/49 or 2% incidence rate). No elevated incidences of tumors at other tissue sites were found in exposed mice compared with controls. The incidence and time-to-tumor data for hepatocellular carcinoma in the NCI (1976a) report are summarized in Table 4-3. No exposure-related noncancer lesions were mentioned other than the liver atypia and nodular and diffuse hyperplasia (NCI, 1976a,b). Induction of noncancerous liver lesions (i.e., hyperplasia) was observed at all dose levels for each sex and species. Thus, freestanding LOAELs identified for this study are 0.6, 1.4, 3.4, and 3.5 mg/kg-day for male rats, female rats, male mice, and female mice, respectively.

Table 4-3. Incidence and time to tumor of hepatocellular carcinoma in mice

Mouse/B6C3F1	Exposure group			
	Matched control	Pooled control	Low-dose group	High-dose group
Male (0, 3.4, or 3.9 mg/kg-day)[a]	6/19 (31%)	8/49 (16%)	39/48[b] (81%)	43/49[b] (88%)
Time to first tumor (weeks)	87 weeks	87 weeks	70 weeks	62 weeks
Female (0, 3.5, or 7.0 mg/kg-day)[a]	0/10 (0%)	0/40 (0%)	26/50[b] (52%)	23/49[b] (47%)
Time to first tumor (weeks)	NA[c]	NA	87 weeks	76 weeks

[a]Doses were calculated for this review using the allometric equation for food consumption by laboratory animals with time-weighted concentrations from NCI (1976a) and reference body weights from U.S. EPA (1988).
[b]Statistically significant increase in incidence as compared to matched or pooled controls, using one-tail ($p < 0.05$) Fisher's exact test for 2 × 2 contingency table (NCI, 1976a).
[c]NA = not available.

Source: NCI (1976a).

The NCI (1976a) study provides evidence of carcinogenicity in Osborne-Mendel rats and B6C3F1 mice; however, decreases in survival rates and decreased body weight gain indicate that excessively high doses were utilized in all animal groups except the low- and high-dose female mice (see Table 4-4).

Table 4-4. Percent body weight gain and percent survival of chlordecone-exposed rats and mice

	Time-weighted-average daily dose (mg/kg-day)	Survival (%)	Body weight gain (%)	Liver tumor incidence (%)	Time to 1st tumor (weeks)
Male rats	0 (room controls)	63		0/105	NA[b]
	0 (matched controls)	90		0/10	NA
	0.6	60	82	1/50 (2)	112
	1.7	42	79	3/44 (7)[a]	108
Female rats	0 (room controls)	61		0/100	NA
	0 (matched controls)	70		0/10	NA
	1.4	56	76	1/49 (2)	87
	2.0	40	66	10/45 (22)[a]	83
Male mice	0 (room controls)	92		8/49 (16)	87
	0 (matched controls)	90		6/19 (31)	87
	3.4	58	93	39/48 (81)[a]	70
	3.9	50	88	43/49 (88)[a]	62
Female mice	0 (room controls)	85		0/40	NA
	0 (matched controls)	90		0/10	NA
	3.5	84	94	26/50 (52)[a]	87
	7.0	84	88	23/49 (47)[a]	76

[a]Statistically significant increase in incidence as compared with matched per pooled controls, using one-tail Fisher's exact test ($p < 0.05$).
[b]NA = not available.

Source: NCI (1976a).

In another chronic study, groups of 40 male and 40 female Wistar rats were fed diets containing 0, 5, 10, 25, 50, or 80 ppm of chlordecone for up to 2 years (Larson et al., 1979a). Larson et al. (1979a) added chlordecone to warmed corn oil before combining it with the food. From food consumption and body weight data graphically presented in Larson et al. (1979a) for 5-8 time points measured throughout the study, time-weighted-average food consumption rates were estimated for the 5 through 80 ppm groups as 49, 53, 59, 73, and 80 g food/kg body weight-day for males and 56, 55, 69, 83, and 93 g food/kg body weight-day for females. Using average food consumption rates and averaged body weights (between males and females), doses were estimated to be 0, 0.3, 0.5, 1.6, 3.9, and 7.0 mg/kg-day. In a separate phase of the experiment, groups of 40 males and 40 females were exposed to 0 or 1 ppm for up to 2 years. Because food consumption data were not reported for the 1 ppm group, an estimated dose of 0.06 mg/kg-day was calculated by assuming food consumption equal to the 5 ppm group. Groups of five rats per sex per dose were sacrificed at 3 and 12 months. Another three to five rats per sex per group were sacrificed after 12 months of exposure and a 4-week recovery period. Remaining rats were sacrificed at 24 months. From samples collected at 3-month intervals, hematocrit, hemoglobin, and total and differential white cell counts were measured in blood, and reducing substances and protein were measured in urine. Additional blood studies were performed at 3 months for platelet count, prothrombin clotting time, and serum calcium. Oxygen consumption was measured by spirometry at 9 months. Organ-to-body-weight ratios (liver, kidneys, heart, spleen, and testes) were determined in sacrificed rats. The following tissues were taken from sacrificed rats for histopathological study: brain, spinal cord, heart, lung, liver, kidney, spleen, gut, urinary bladder, bone marrow, skeletal muscle, skin, pancreas, thyroid, adrenal, pituitary, and gonad.

Tremors developed in the 3.9 and 7.0 mg/kg-day groups within a few weeks of the start of the study and became progressively more severe with time (Larson et al., 1979a). Slight tremors were noted in some rats at 1.6 mg/kg-day after 3 months, becoming moderate in severity after 5–6 months, but then regressing. Tremors were not observed at 0.5 mg/kg-day or below. The incidence of tremors was not reported. All rats in the 3.9 and 7.0 mg/kg-day groups died during the first 6 months. Long-term survival was reduced in the 1.6 mg/kg-day females (measured at 1 and 2 years, data not shown). Body weights were depressed after 3 weeks of study in males at $\geq$1.6 mg/kg-day and in females at $\geq$0.3 mg/kg-day. Food consumption (per body weight) tended to increase with concentration of chlordecone in the feed. Metabolic rate (measured by oxygen consumption) increased with dose in both males and females, although statistical significance was achieved only in males at 1.6 mg/kg-day (the highest dose with survivors remaining when tested at 9 months). Hematology analyses revealed no differences related to treatment. Apparent increases in urinary protein concentrations or proteinuria (a clinical indicator of glomerular dysfunction) were reported in both male and female rats exposed to $\geq$0.3 mg/kg-day for 6–24 months, though statistical analysis was not performed on these data because of incomplete data reporting. Proteinuria was not observed in rats exposed to 0.06

mg/kg-day in a separate phase of the experiment (the time of analysis and other details were not reported). Relative liver weight increased with dose at 3, 12, and 24 months in both male and female rats. The difference from controls was statistically significant at ≥1.6 mg/kg-day in males and ≥0.5 mg/kg-day in females. Relative testes weights were significantly decreased in the 3.9 and 7.0 mg/kg-day groups at the 3-month sacrifice. Relative weight changes in the kidneys and other organs were not remarkable. Absolute organ weights were not reported.

Histopathological examination of five rats (randomly selected) from each sex at each feeding level at 13 weeks revealed minimal congestion of the liver at 0.5 mg/kg-day and more degenerative changes in the liver at higher doses (Larson et al., 1979a). There was a trend in dose response for degenerative liver changes. Swollen liver cells were noted in 4/5 males and 5/5 females in the 3.9 mg/kg-day group and 5/5 males and 3/5 females in the 7.0 mg/kg-day group (compared with 0/10 males and 0/10 females in the control groups [both male and female rat studies combined]). The liver-to-body-weight ratios were significantly increased in the 3.9 and 7.0 mg/kg-day groups for both sexes. Histological examination also uncovered a dose-related increase in the incidence and severity of testicular atrophy at 13 weeks, though not at 1–2 years. The study authors did not speculate as to why testicular atrophy was observed after 13 weeks, but not at the chronic time point. Interim (3-month) gross and histopathologic examinations performed on 10 control males and 5 chlordecone-treated males per group revealed statistically significantly increased incidences of chlordecone-induced testicular atrophy (Table 4-5). The atrophy was described as minimal in the control male and generally increased in severity with increasing chlordecone concentration. Also, the testes-to-body-weight ratios in males were significantly decreased in the 3.9 and 7.0 mg/kg-day groups. The study identified a NOAEL of 0.5 mg/kg-day and a LOAEL of 1.6 mg/kg-day for testicular atrophy in male rats exposed to chlordecone in the diet for 13 weeks.

Table 4-5. Testicular atrophy in male rats receiving chlordecone in the diet for 3 months

Dietary level (ppm)	0	5	10	25	50	80
Average dose[a] (mg/kg-day)	0	0.3	0.5	1.6	3.9	7.0
Incidence of testicular atrophy[b]	1/10	0/5	1/5	4/5[c]	4/5[c]	5/5[c]

[a]Average dose to rats, based on graphically depicted food consumption data presented by the authors.
[b]Statistically significant dose-response trend according to the Cochran-Armitage trend test ($p < 0.01$) performed for this review.
[c]Statistically significantly different from controls according to Fisher's exact test ($p < 0.05$) performed for this review.

Source: Larson et al. (1979a).

At the 12-month sacrifice, congestion of the liver was reported for treated groups, but details were not reported. No treatment-related lesions were observed after 12 months of treatment and a 4-week recovery period.

Histopathological examination of rats sacrificed after 2 years and rats that died during the second year showed exposure-related lesions only in the liver and kidney (Larson et al., 1979a). Incidence data for liver and kidney effects are presented in Table 4-6. The principal renal lesion was glomerulosclerosis, or scarring of the system of capillaries that comprise the glomeruli. The increased incidence of glomerulosclerosis was statistically significant (Fisher's exact test performed for this review) in the 0.3, 0.5, and 1.6 mg/kg-day females compared with controls. The background incidence of glomerulosclerosis in male rats was high (56% as compared to 12% in female rats) and, as such, male rat incidence data for glomerulosclerosis did not achieve statistical significance. Incidences of liver lesions (predominately fatty changes and hyperplasia) in male and female rats were also statistically increased by chlordecone administration. The hepatic lesions in three females in the 0.5 mg/kg-day group and one female and two males in the 1.6 mg/kg-day group were described by the authors as being possibly "carcinomatous in nature"; however, the authors reported that an independent review by four pathologists found the evidence for carcinogenic responses in this study to be equivocal. Thus, this study provides equivocal evidence of chlordecone carcinogenicity in Wistar rats.

Table 4-6. Incidence of histopathologic liver lesions (fatty changes and hyperplasia) and renal glomerulosclerosis in male and female Wistar rats following administration of chlordecone in the diet for 1–2 years

Endpoint[a]	Dose (mg/kg-day)				
	0	0.06	0.3	0.5	1.6
Liver lesions[b]					
Male rats	1/22	1/11	2/6	2/9	3/4[c]
Female rats	2/34	1/13	2/17	4/12[c]	1/4
Glomerulosclerosis[b]					
Male rats	12/22	3/11	4/6	6/9	3/4
Female rats	4/34	2/13	8/17[c]	8/12[c]	3/4[c]

[a]The number of animals reported relates to the number of animals analyzed between 1 and 2 years. Due to interim measurements, the approximate number of animals per sex per dose group after 12 months is 25.
[b]The dose-response trend was also statistically significant for each data set according to the Cochran-Armitage trend test performed for this review.
[c]Statistically different from control groups according to Fisher's exact test ($p < 0.05$) performed for this review.

Source: Larson et al. (1979a).

This study identified 5 ppm (0.3 mg/kg-day) as a LOAEL and 1 ppm (0.06 mg/kg-day) as a NOAEL for kidney effects (proteinuria and increased incidence of glomerulosclerosis) in female rats. Also observed were increased incidences of hepatic lesions; these increases were statistically significant (Fisher's exact test performed for this review) starting at 1.6 mg/kg-day in

males and at 0.5 mg/kg-day in females. Higher doses (3.9 and 7.0 mg/kg-day) produced overt clinical signs (tremors) and mortality in the rats.

Larson et al. (1979a) also conducted a long-term study in dogs. Groups of two male and two female purebred beagle dogs were fed diets containing 0, 1, 5, or 25 ppm of chlordecone for up to 128 weeks, beginning at an age of about 6 months. Although not specified, Larson et al. (1979a) likely added chlordecone to warmed corn oil before combining it with the food (the protocol used in the rat study in the same report). Two dogs in the 25 ppm group were sacrificed at the end of week 124; the remaining dogs were sacrificed during week 128. Organ-to-body weights were determined, and 17 tissues were taken for histopathological examination: brain, spinal cord, heart, lung, liver, kidney, spleen, gut, urinary bladder, bone marrow, skeletal muscle, skin, pancreas, thyroid, adrenal, pituitary, and gonad. The same hematological and urine endpoints as those described for the rat studies were determined in samples collected before exposure and at 3-month intervals during exposure. Using reference body weights and food consumption rates of 10.5 kg and 0.2 kg dry food/day, respectively, for beagle dogs (U.S. EPA, 1988), doses were estimated to be 0, 0.02, 0.1, and 0.5 mg/kg-day (the authors did not report food consumption data, body weight data, or estimated dose levels for the dogs). Three dogs died during the study, showing severe dermatitis that did not appear to be related to exposure (one control dog during week 71, one 0.02 mg/kg-day dog during week 48, and one 0.1 mg/kg-day dog during week 50). Body weight gain in the 0.5 mg/kg-day group was reported to be lower than the weight gain in the control dogs during the second year of exposure, but the magnitude of the decrease was not reported and the data were not shown. Decreased food efficiency (kg body weight gain/kg food consumed) was suggested by measurements of food consumption, but again the data were not shown. The only statistically significant changes associated with exposure to chlordecone were a moderate (37%) increase in relative liver weight in dogs from the 0.5 mg/kg-day group (males and females combined) and slight changes (less than about 25%) in relative kidney (increase), heart (increase), and spleen (decrease) weight in the same group. Absolute organ weights were not reported. No exposure-related changes were reported for clinical signs of toxicity; hematological, histopathological, or urinalysis endpoints; sulfobromophthalein retention; or serum cholinesterase. Interpretation of this study is limited by the small number of dogs tested, the deaths of three dogs during the study for reasons not apparently related to treatment, and the reporting of results, including failure of the researchers to present data to support the reported decrease in body weight in dogs from the 0.5 mg/kg-day group during the second year of the study. Nevertheless, the statistically significant changes in organ-to-body-weight ratios support occurrence of an adverse effect on body weight, and the increase in relative liver weight is consistent with other studies demonstrating hepatic toxicity with chlordecone exposure. Therefore, upon review, the results of this study suggest a LOAEL of 25 ppm (0.5 mg/kg-day) and a NOAEL of 5 ppm (0.1 mg/kg-day), based on decreased body

weight and organ-to-body-weight changes (without histological changes) in beagle dogs fed chlordecone in the diet for up to 128 weeks.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

4.3.1. Reproductive Toxicity Studies

Information on reproductive effects in humans is restricted to findings of oligospermia, reduced sperm motility, and decreased libido in a group of men who were occupationally exposed to chlordecone for periods up to 1.5 years (Taylor, 1985, 1982; Guzelian, 1982a; Taylor et al., 1978). Sperm concentration and motility had returned to normal upon follow-up 5 years following cessation of chlordecone exposure. Even though two of seven workers sired children, there is no indication of the true denominator of how many were trying to conceive and/or the fertility rate. In one worker, low sperm count persisted (Taylor, 1985). No information is available concerning chlordecone-induced reproductive effects in women.

Reproductive toxicity has been assessed in some animal studies but not in adequately designed multiple generation studies. Available animal data suggest that chlordecone is a male reproductive toxicant, causing alteration of sperm parameters at low doses and testicular atrophy at higher doses. Persistent vaginal estrus is reported to occur in exposed females and decreased reproductive success has been demonstrated. No animal studies are available to assess the developmental or reproductive toxicity of chlordecone by the inhalation route of exposure.

Huber (1965) performed a series of experiments designed to assess reproductive toxicity in mice exposed to chlordecone in the diet. In a pilot reproduction test (group A), 3-month-old male and female mice of mixed parentage (eight pairs per group) were administered chlordecone (technical-grade chlordecone, 93.6% purity) in the diet at concentrations of 0, 10, 30, or 37.5 ppm for 1 month prior to mating and during the 100 days following individual pairing within each exposure group. Corresponding chlordecone doses of 0, 1.9, 5.6, and 7.0 mg/kg-day were estimated for males and females combined by using reference values for food consumption and body weight from U.S. EPA (1988). The 100-day treatment period allowed sufficient time for mating pairs to produce two litters. Individual males were housed with individual females except during the period of gestation and weaning of offspring. Reproductive parameters assessed included number of pairs producing first and second litters, average number of young per litter, percent survival of offspring, and the average time required to produce the offspring (expressed as pair days per litter [number of pairs × 100 days/number of litters produced] and pair days per offspring [number of pairs × 100 days/number of offspring). Vaginal smears were taken daily for 3–4 weeks for analysis of the estrous cycle following the termination of the reproduction phase. Smears were taken in one group after their reproduction test and in another group prior to mating.

In the chlordecone-treated groups, the number of pairs producing first and second litters, the average number of young per litter, and the percent survival of offspring appeared to be

lower compared with controls. The average time required to produce offspring during the treatment period was greater in chlordecone-treated pairs than controls. However, except for the quantal data presented for pairs producing litters, the data presented for the continuous parameters (average number of offspring, pair days per litter, and percent survival of offspring) did not include a measure of the variance and thus were not adequate for statistical analysis, though visual evaluation of the data appear to indicate a clear reduction in reproductive success at doses greater than 5.6 mg/kg-day. Statistical analysis of the number of pairs producing second litters (Fisher's exact test performed for this review) revealed a significant reduction in the 5.6 and 7.0 mg/kg-day exposure groups relative to controls (Table 4-7).

In another phase (group B) of the study, 4-month-old BALB/cJaxGnMc mice (14 pairs per group) were administered chlordecone in the diet at concentrations of 0 or 40 ppm for 2 months before mating and during a 100-day reproduction period (Huber, 1965). Otherwise, the study design was the same as that used for group A. The corresponding chlordecone dose was 7.6 mg/kg-day (estimated for males and females combined, using reference values for food consumption and body weight from U.S. EPA [1988]). Following the termination of treatment, a second reproduction phase was performed for 100 days and consisted of crossover matings (control males with control females, control females with chlordecone-treated males, and chlordecone-treated females with control males).

Table 4-7. Effects of dietary chlordecone on reproduction in male and female mice (of mixed parentage) treated for 1 month prior to mating and for 100 days following the initiation of mating

Dietary level (ppm)	Average dose[a] (mg/kg-day)	Pairs producing first litter	Pairs producing second litter	Average number offspring per litter	Percent survival of offspring	Pair days per litter	Pair days per offspring
0.0	0.0	7/8	5/8	7.7	89	67	8.7
10.0	1.9	6/8	4/8	7.1	87	80	11.3
30.0	5.6	4/8	0/8[b]	4.7	26	200	42.1
37.5	7.0	3/8	0/8[b]	4.0	42	267	66.7

[a]Average doses to male and female mice (combined), based on reference values for subchronic body weight and food consumption taken from U.S. EPA (1988).
[b]Statistically different from control groups according to Fisher's exact test ($p < 0.05$), performed for this review.

Source: Huber (1965).

The results are summarized in Table 4-8. During the initial reproduction period, each of the control (0 ppm) pairs produced two litters. No offspring were produced by the pairs of mice treated with 7.6 mg/kg-day of chlordecone. The ability to produce offspring was restored during the posttreatment reproduction period. Results of crossover matings indicate that female mice were slightly more affected by chlordecone than males; however, information concerning the statistical significance of the findings was not provided by the author.

Table 4-8. Effects of dietary chlordecone (0 or 40 ppm) on reproduction in BALB/cJaxGnMc mice during 100 days of treatment (preceded by 2 months of pre-mating treatment) and during 100 days of a crossover-mating period following the termination of treatment

	Reproduction period during chlordecone treatment		Crossover reproduction period following termination of chlordecone treatment		
	Controls	Treated pairs	Controls	Control male × treated female	Control female × treated male
Pairs with first litter	14/14	0/14	5/5	8/10	10/10
Pairs with second litter	14/14	0/14	4/5	5/10	6/10
Offspring per litter	7.1	–	7.2	4.5	5.6
Offspring survival (%)	89	–	87.3	76.1	88.3
Pair days per litter	50	–	55.6	76.9	62.5
Pair days per offspring	7	–	7.6	17.3	11.5

Source: Huber (1965).

Huber (1965) also assessed the effect of chlordecone on estrous cyclicity in virgin female mice (20 per group) given either 0 or 40 ppm of chlordecone in the diet for 120 days. After 21 and 120 days of treatment, daily vaginal smears were taken for 3 to 4 weeks. In the 40 ppm females, persistent estrus appeared within 8 weeks of treatment initiation. Seventy-one percent of the smears taken in the 40 ppm females for 4 weeks after termination of chlordecone treatment were in estrus versus only 24% in controls. Huber (1965) also noted persistent estrus in 30 and 37.5 ppm female mice from group A following the reproduction test and 40 ppm female mice from group B prior to mating. The occurrence of persistent estrus is an indication that the treated female mice were under a prolonged stimulation of follicular stimulating hormone (FSH) and estrogen with insufficient luteinizing hormone stimulation. The 30 ppm treatment level represents a LOAEL for this effect.

In summary, the multiple dose reproduction test (Huber, 1965), in which male and female mice were given chlordecone in the diet for 1 month prior to mating and for 100 days following the initiation of mating, resulted in adverse reproductive effects. The 1.9 mg/kg-day dose represents a NOAEL and the 5.6 mg/kg-day dose represents a LOAEL (as determined for this review), based on a statistically significantly reduced number of mouse pairs producing a second litter.

Male and female laboratory mice (7–16 pairs per group) of mixed breeds were administered chlordecone (purity unspecified) in the diet at concentrations of 0, 10, 17.5, 25, 30, or 37.5 ppm for 1 month and then were sex paired within the same exposure grouping and placed on a normal diet throughout mating and production of offspring (Good et al., 1965). Corresponding chlordecone doses of 0, 1.9, 3.3, 4.7, 5.6, or 7.0 mg/kg-day were estimated for males and females combined by using reference values for food consumption and body weight

from U.S. EPA (1988). Reproductive indices (number of litters produced, average number of young per litter, pair days per litter, and pair days per young produced) were assessed for approximately 5 months following the initiation of mating. As shown in Table 4-9, the results suggest a dose-related effect on reproductive success (decreases in number of litters and average number of young per litter, increases in pair days per litter and per young). Though the data presented in the study were not adequate for statistical analysis (no measures of variance were provided for the reproductive parameters), visual evaluation of the data appears to indicate a clear reduction in reproductive success at doses $\geq$5.6 mg/kg-day.

Table 4-9. Effects of dietary chlordecone for 1 month prior to mating on reproductive indices of male and female laboratory mice of mixed breeds

Dietary level (ppm)	Average dose[a] (mg/kg-day)	Number of pairs	Number of litters	Number of offspring per litter	Pair days per litter	Pair days per offspring
0	0.0	9	15	7.93	65.3	8.3
10	1.9	13	26	7.62	54.46	7.15
17.5	3.3	16	25	7.0	72.16	13.09
25	4.7	11	12	6.08	100.42	16.51
30	5.6	7	2	3.0	241.5	80.5
37.5	7.0	10	2	5.0	555.0	111.0

[a]Average doses to male and female mice (combined), based on reference values for subchronic body weight and food consumption taken from U.S. EPA (1988).

Source: Good et al. (1965).

In separate experiments by Good et al. (1965), impaired reproductive success, expressed as significantly ($p < 0.05$) reduced production of a second litter, was observed in mice that were administered chlordecone (purity unspecified) in the diet at a concentration of 5 ppm for 1 month prior to mating and for up to 5 months following initiation of mating (shown in Table 4-10). The corresponding chlordecone dose of 0.94 mg/kg-day was estimated for males and females combined by using reference values for food consumption and body weight from U.S. EPA (1988). The authors reported that continued treatment of offspring of chlordecone-treated mice with either control or 5 ppm chlordecone diets resulted in significantly reduced production of a first litter ($p \leq 0.05$), compared with untreated offspring of untreated parental mice, though reduced production of the second litter did not achieve statistical significance. The results of these studies identified a LOAEL of 0.94 mg/kg-day for impaired reproductive success; a NOAEL was not identified.

Table 4-10. Effects of dietary chlordecone (0 or 5 ppm) 1 month prior to mating and 5 months during mating on reproduction in BALB/c mice

	First generation		Second generation		
	Control	Treated	Control	Offspring of treated mice on control diet	Treated
Number of pairs	24	36	21	23	20
Number of litters	40	52	21	9	10
Number of offspring	275	314	123	42	40
% producing 1st litter	96	81	71	30[a]	25[a]
% producing 2nd litter	78	50[a]	29	9	15
First litter size	6.2	6.2	5.6	4.3	4.4
Second litter size	7.3	5.7	6.5	6.0	5.3
Pair days per litter	70.1	86	120	307	240
Pair days per offspring	10.2	14.2	21	66	60

[a]Reported as significant at $p < 0.05$, using binomial distribution.

Source: Good et al. (1965).

As in the previous data reported by Good et al. (1965), reproductive parameters, including litter size, pair days/litter, and pair days/young produced, were all reported as averages for the treatment or control group without any measure of variance given (i.e., standard deviation). Therefore the degree of variability for the reported reproductive parameters is unclear. Additionally, there appears to be reduced fertility of the BALB/c untreated controls just one generation apart. For instance, 96 and 78% of untreated control animals produced first and second litters, respectively, whereas only 71 and 29% of their untreated progeny produced first and second litters. These inconsistencies limit confidence in this study and the reproducibility of the data.

In a reproductive and neurodevelopmental toxicity study, female F344 rats (10 per group) were fed diets containing 0, 1, or 6 ppm of chlordecone (purity unspecified) for 60 days prior to mating (with nonexposed male rats) through lactation day 12 (Squibb and Tilson, 1982). Corresponding doses of 0, 0.07, and 0.4 mg/kg-day were estimated by using reference values for food consumption from U.S. EPA (1988) and the average of reported body weights of the dams prior to mating and on the day after parturition. Chlordecone treatment did not produce adverse effects on litter size or sex ratio of the offspring. Litters were culled to three male and three female offspring per dam on postpartum day 3. Pup body weights were similar to those of controls at 1, 7, 14, and 30 days of age, but after 100 days body weight was significantly reduced in male pups at 6 ppm (19% decrease relative to controls) and female pups at 1 ppm (27% decrease relative to controls) and 6 ppm (27% decrease relative to controls). Pups were exposed to higher concentrations of chlordecone exposure during the first 2 weeks of life (i.e., during lactation), without any significant effects on body weight. A pharmacokinetic elimination study

in rats (Egle et al., 1978) demonstrated that 65.5% of an orally administered dose of chlordecone had been excreted into the feces by 12 weeks. The chlordecone body burden must therefore be assumed to be much lower at 100 days, when compared with earlier time points. No clear dose-response relationship was demonstrated in this study for decreased pup body weight; thus, the significance of the body weight reductions at 100 days postpartum is uncertain.

One male and one female pup from each litter were chosen at random for behavioral and pharmacological challenge testing (10 males and 10 females from each dose group) (Squibb and Tilson, 1982). The results of behavioral testing, conducted at 30 and 100 days, were primarily negative. Exposed offspring showed no statistically significant changes (compared with controls) in forelimb or hind-limb grip strength, spontaneous motor activity, startle responsiveness (air puff or acoustic stimulus), or tail-flick frequency in response to thermal stimulation. Positive results were found for one test in male offspring exposed to 6 ppm that took significantly longer time to reorient themselves to a vertical position in an assay for negative geotaxis at 100 days of age. The effect was not seen at 30 days in males and was not seen at either time point in female offspring.

The results of pharmacological challenge tests were mixed (Squibb and Tilson, 1982). Motor activity induced by subcutaneously injected 1 mg/kg apomorphine (a dopamine receptor agonist) at 114 days of age was significantly increased in male offspring of the 6 ppm group 30 minutes after dosing and male offspring of the 1 and 6 ppm groups 60 minutes after dosing. This effect was not seen in females. There was no effect on motor activity induced by d-amphetamine (a presynaptic releaser of both dopamine and norepinephrine) at 134 days in either male or female offspring. This study found little evidence of an effect of chlordecone on neurodevelopment in rats. The weight of evidence of behavioral tests was negative, and the one positive finding (increased negative geotaxis latency in males) was not supported by results in females. Similarly, the positive result in the challenge test with apomorphine in males was not supported by the results in females. Further, in the absence of any clear neurological or behavioral response, it is uncertain that a potential alteration in dopaminergic function associated with chlordecone exposure should be considered adverse.

Adult Sherman strain male and female rats (22–25 rats/sex/group) were fed diets containing 0 or 25 ppm commercial grade chlordecone (80.6% purity) for 3 months, during which time they were housed individually and observed for clinical signs of neurotoxicity (Cannon and Kimbrough, 1979). At the end of the treatment period, selected control and chlordecone-treated male and female rats were subjected to gross and histopathologic examinations. The remaining rats 20/sex/group were pair mated, control males with chlordecone-treated females, control females with chlordecone-treated males, and control females with control males, during a breeding period of approximately 2 months. The production of offspring was used as an indicator of reproductive toxicity.

According to the study authors, chlordecone intake ranged from 1.62 to 1.71 mg/kg-day in 25 ppm females and from 1.17 to 1.58 mg/kg-day in 25 ppm males. Body tremors were seen in chlordecone-treated rats after 4 weeks of treatment and appeared to be most marked in treated females. At the end of the exposure period, chlordecone-treated male and female rats exhibited depressed body weight, and gross and microscopic signs of adverse hepatic effects. The adrenals showed hyperplasia of the zona fasciculata and zona reticularis with marked hypertrophy of the cortex. The study authors noted gross and histopathologic signs of adverse adrenal effects in treated females. Twelve of the 20 pairs of control females and chlordecone-treated males produced offspring compared with 13 of 20 pairs in the controls. However, no offspring were produced among the 20 pairs of control males and chlordecone-treated females. Mating of chlordecone-treated females and control males was repeated 9 weeks after exposure cessation. Reproductive function appeared to be partially restored with 9 of 20 pairs producing litters, indicating some reversibility of the observed reproductive deficit in chlordecone-treated females. This study identified a LOAEL of 1.6–1.7 mg/kg-day for impaired reproductive success in female rats.

Groups of sexually mature virgin female CD-1 mice were administered chlordecone by gavage (in sesame oil) at doses of 0, 0.062, 0.125, or 0.25 mg/day (0, 2, 4, or 8 mg/kg-day), 5 days per week for 2, 4, or 6 weeks (Swartz et al., 1988). A positive control group received 17β-estradiol at a dose of 0.1 mg/day. Some mice from each group were assessed for production of oocytes (intraperitoneal administration of pregnant mare's serum gonadotropin followed 48 hours later by human chorionic gonadotropin) during the second, fourth, and sixth week of chlordecone treatment. As shown in Table 4-11, persistent vaginal estrus was noted in most chlordecone-treated mice and positive controls as early as 2 weeks following the initiation of treatment. By week 4, all chlordecone-treated mice exhibited persistent vaginal estrus versus 0/9 vehicle controls.

After 4 and 6 weeks of treatment, ovulation in the highest chlordecone treatment group (8 mg/kg-day) resulted in statistically significantly lower numbers of ovulated oocytes relative to vehicle controls. This study identified a LOAEL of 2 mg/kg-day for persistent vaginal estrus in virgin female CD-1 mice.

Table 4-11. Effects of chlordecone on estrous cyclicity and ovulation in CD-1 mice exposed to chlordecone by gavage 5 days per week for up to 6 weeks

Test	Vehicle controls	Positive controls (17β-estradiol)	Chlordecone dose (mg/kg-day)		
			2	4	8
PVE[a,b]					
Week 2	0/9	7/8	6/8	5/9	8/9
Week 3	0/9	8/8	6/8	7/9	9/9
Week 4	0/9	7/8	8/8	9/9	9/9
Ovulation[c]					
Week 2	19.9 ± 2.4 (15)[d]	30.2 ± 11.8 (6)	26.7 ± 3.2 (10)	19.2 ± 3.2 (10)	17.7 ± 4.5 (15)
Week 4	28.4 ± 2.9 (22)	29.7 ± 2.2 (11)	22.9 ± 4.3 (7)	27.1 ± 5.0 (6)	14.1 ± 2.4 (22)[e]
Week 6	23.7 ± 2.4 (16)	22.1 ± 2.5 (9)	32.4 ± 3.8 (7)	21.0 ± 5.8 (7)	14.5 ± 3.5 (16)[e]

[a]PVE = persistent vaginal estrus, defined as the presence of epithelial cells (without leukocytes) in vaginal smears.
[b]All treatment groups for this endpoint significantly different from vehicle controls ($p < 0.05$), using the Fisher's exact test.
[c]Average number of oocytes in the oviducts at sacrifice.
[d]Number of animals.
[e]Statistically significantly different from vehicle controls ($p < 0.05$) using the Student's *t*-test.

Source: Swartz et al. (1988).

Swartz and Mall (1989) administered chlordecone (98% purity) to groups of female CD-1 mice via gavage (in sesame oil) at doses of 0 or 0.25 mg/day (8 mg/kg-day), 5 days per week for 4 weeks. A positive control group received 17β-estradiol at a dose of 0.1 mg/day. Animals were sacrificed 24 hours following the final treatment, and the ovaries were fixed and sectioned. The abundance of small-, medium-, and large-sized follicles was determined in every tenth section. Significantly fewer small- and medium-sized follicles were found in chlordecone-treated mice relative to vehicle controls (Table 4-12). Based on observations that many of the large-sized follicles in the ovaries of chlordecone-treated mice appeared to be atretic, all histological sections of the ovaries were examined for the presence and condition of large-sized follicles. The number of large-sized follicles in chlordecone-treated mice did not differ significantly from controls; however, a significantly lower abundance of healthy large-sized follicles was noted (Table 4-12). This study identified a LOAEL of 8 mg/kg-day for adverse effects on follicle size and condition.

Table 4-12. Abundance of various-sized follicles and the condition of large-sized follicles in the ovaries of female CD-1 mice exposed to chlordecone by gavage 5 days per week for 4 weeks

Treatment	Number of small-, medium-, and large-sized follicles[a]			Number of healthy and atretic large-sized follicles[b]		
	Small	Medium	Large	Total	Healthy	Atretic
Controls	279.2 ± 39.6	116.2 ± 7.8	21.3 ± 2.5	58.1 ± 7.3	28.4 ± 6.0	29.7 ± 3.4
17β-Estradiol	368.0 ± 47.5	231.9 ± 41.0[c]	28.0 ± 8.3	69.6 ± 6.7	25.4 ± 2.7	44.2 ± 4.3[c]
Chlordecone	190.1 ± 32.8[c]	103.8 ± 11.8	27.5 ± 3.2	58.7 ± 5.8	18.5 ± 1.9[c]	40.1 ± 5.1

[a]Mean ± SEM, based on evaluations of every 10th section.
[b]Mean ± SEM, based on evaluations of all sections.
[c]Statistically significantly different from vehicle controls ($p < 0.05$) using the Student's *t*-test.

Source: Swartz and Mall (1989).

Gellert and Wilson (1979) administered chlordecone (purity unspecified; vehicle: 5% ethanol in sesame oil) to pregnant Sprague-Dawley rats by gavage at doses of 0 or 15 mg/kg-day on gestation days 14–20. Untreated controls were included in the study, as well as groups of dams that were administered other pesticides. The study report did not specify the number of rats in each treatment group. The pregnant rats were allowed to deliver and raise their offspring. At 21 days of age, the offspring were sexed and weaned. At approximately 6 months of age, estrous cyclicity of female offspring was assessed via daily vaginal smears for about 2 weeks. Persistent vaginal estrus (PVE) was defined as 4 or more consecutive days with only cornified or nucleated cells in the vaginal smear. At sacrifice immediately following assessment for estrous cyclicity, the rats were weighed and blood was collected for analysis of serum estradiol. Ovaries, uteri, and adrenals were weighed, and ovaries were histologically examined for the presence of corpora lutea. Animals with visible corpora were considered to be ovulatory. At 6 months of age, the male offspring of the treated dams were subjected to fertility testing by placing them with two experienced female rats for a period of 2 weeks. The resulting offspring of these matings were counted and sexed. At sacrifice, adrenals, testes, and ventral prostates of the F1 generation were individually weighed, and the epididymis was grossly examined for the presence of cysts.

The study authors did not report chlordecone-induced effects in the treated dams. Female offspring of the chlordecone-treated dams exhibited significantly decreased ovarian weight and significantly increased adrenal weight relative to vehicle controls, as well as significantly increased incidences of PVE (Table 4-13). In each of the control groups, all but 1 of the female offspring were ovulatory, whereas none of the 21 of the female offspring of the chlordecone-treated dams were ovulatory (Table 4-13). Serum estradiol levels in control female offspring fluctuated as expected during regular 4- or 5-day estrous cycles, whereas the level in chlordecone-treated female offspring were observed to remain at an intermediate level. The

serum estradiol levels were below 10 pg/mL in 65% of controls and 24% of the chlordecone-treated animals. In 14% of the control animals the estradiol levels were above 47 pg/mL, whereas none of the chlordecone treated animals had the estradiol above this level. Male offspring of the chlordecone-treated dams exhibited no evidence of decreased fertility or altered sex ratios in the resulting F2 generation. This study identified a LOAEL of 15 mg/kg-day for reproductive effects in adult female offspring of rat dams administered chlordecone by gavage during gestation days 14–20.

Table 4-13. Effects of chlordecone on adult female offspring of Sprague-Dawley rat dams administered chlordecone by gavage on gestation days 14–20

Treatment	Number of rats	Body weight (g)	Average weight (mg)			Number of rats with PVE	Number of ovulatory rats
			Ovary	Uterus	Adrenal		
Control							
Untreated	29	372 ± 7[a]	92 ± 3	577 ± 24	64 ± 1	2	1
Sesame oil	25	338 ± 6	96 ± 4	621 ± 26	68 ± 2	1	1
Chlordecone (15 mg/kg)	21	364 ± 13	59 ± 2[a]	686 ± 37	85 ± 3[a]	12[a]	21[a]

[a]Statistically significantly different from sesame-oil-treated controls ($p < 0.001$).

Source: Gellert and Wilson (1979).

Several groups of investigators assessed spermatogenesis in laboratory animals that had been exposed to chlordecone. In a toxicological screen of several chemicals, chlordecone (purity unspecified) was administered to male rats of unspecified strain at dose levels of 0.625, 1.25, 2.5, 5.0, or 10.0 mg/kg once per day for 10 days (U.S. EPA, 1986c). Untreated and vehicle controls were included in the study. Testes and epididymides were removed for assessment of testicular weight; sperm concentration, motility, and morphology; and histopathology. Compared with control values, alteration of sperm concentration was noted in all chlordecone-treated groups. There were no apparent treatment-related effects on sperm motility, testosterone level, or FSH level and no testicular histopathologic findings. A LOAEL of 0.625 was identified for this study.

Linder et al. (1983) exposed male Sprague-Dawley rats (20/group) to dietary concentrations of chlordecone at 0, 5, 15, or 30 ppm for 90-days. The report does not specify how the chlordecone was added to the diet. The corresponding doses were estimated by the researchers as 0, 0.26, 0.83, or 1.67 mg/kg-day, respectively. After 90 days of treatment, half of the animals in each group were sacrificed for weighing and histopathological examination of the reproductive organs and measurement of epididymal sperm characteristics. Each of the remaining males in each group was bred to two untreated females over a 14-day unexposed period immediately following the 90-day exposure period. The mated females were sacrificed

on day 20 of gestation, and fetal weights, fetal viability, and total implants were determined. The mated males were maintained for a 4.5-month recovery period prior to sacrifice and examination of sperm and reproductive organs. Some rats in the 0.83 and 1.67 mg/kg-day groups displayed hyperexcitability and mild tremors during the treatment period. Body weight was significantly lower than that of controls by about 7% in the 1.67 mg/kg-day group at the end of treatment, but the lower dose groups were not affected. The decrease in final body weight was accompanied by significant decreases in absolute prostate and seminal vesicle weight in the 1.67 mg/kg-day group, while testis and epididymis weights were unchanged from controls. Relative weights of all of these tissues were reported to be similar to controls, although the data were not shown. No gross or microscopic pathology related to treatment was found.

Sperm viability, motility, and reserves in the right cauda epididymis were significantly reduced in both the 0.83 and 1.67 mg/kg-day groups but not at 0.26 mg/kg-day (Linder et al., 1983). The findings in the two high-dose groups were similar to each other (no clear increase in severity with increasing dose beyond 0.83 mg/kg-day) (see Table 4-14).

Table 4-14. Sperm parameters in male Sprague-Dawley rats following administration of chlordecone in the diet for 90 days

Endpoint	Dose (mg/kg-day)			
	0	0.26	0.83	1.67
Sperm motility (% motile + SEM)	37.0 ± 3.9	33.2 ± 3.8	19.2 ± 4.4[a]	22.6 ± 5.5[a]
Sperm viability (% alive + SEM)	46.0 ± 4.7	36.2 ± 3.3	25.0 ± 3.3[a]	30.9 ± 4.8[a]
Sperm content of right cauda epididymis (count × 10^6 ± SEM)	308 ± 14	290 ± 10	248 ± 22[a]	249 ± 14[a]

[a]Statistically different from control groups according to ANOVA ($p < 0.05$).

Source: Linder et al. (1983).

Neither sperm morphology nor sperm count in the epididymal fluid was affected at any dose. Reproductive performance (determined by number of pregnant females, number of live litters, average live litter size, number of implants, percentage of resorptions, and fetal weight) was similar in exposed and control groups. No effects of any type were found after the 4.5-month recovery on control diet. In this study, subchronic dietary exposure to 0.83 mg/kg-day or above produced significant reductions in sperm motility, viability, and reserves without affecting sperm morphology or sperm count in the epididymal fluid or without affecting male reproductive performance. Similar effects (oligospermia in the absence of a reduction in reproductive performance) have also been observed in occupationally exposed humans (Guzelian, 1982a,b; Guzelian et al., 1980; Taylor et al., 1978). Doses of 0.83 mg/kg-day and above also produced neurological effects (hyperexcitability and tremors) in the rats, while no effects of any type were

observed at 0.26 mg/kg-day. This study identified a LOAEL of 0.83 mg/kg-day and a NOAEL of 0.26 mg/kg-day, based on the occurrence of neurological and spermotoxic effects.

Additional reproductive studies exist that evaluate the effect of acute injected chlordecone (at doses from 20–80 mg/kg) in experimental animals. Effects observed in these studies were similar to studies of repeat oral administration of chlordecone and generally included changes in estrous cyclicity and fertility (Williams and Uphouse, 1991; Johnson et al., 1990; Pinkston and Uphouse, 1987–1988). These acute injection studies provide information to support reproductive effects at high doses of chlordecone but do not generally contribute additional dose-response information regarding the most sensitive effects of chlordecone exposure.

4.3.2. Developmental Toxicity Studies

The developmental toxicity of chlordecone in humans is not known. Chlordecone produces developmental toxicity in rats and mice at dose levels that also produced maternal toxicity (Seidenberg et al., 1986; Chernoff and Rogers, 1976). Though the database of developmental studies on chlordecone is small, the available animal studies indicate that developmental or fetotoxic effects would not be expected to occur at exposures that are not also associated with maternal toxicity.

Chernoff and Rogers (1976) administered chlordecone (purity unspecified) to groups of pregnant CD rats at gavage doses of 0, 2, 6, or 10 mg/kg-day on gestation days 7–16. Dams were observed for clinical signs and weight gain, and sacrificed on gestation day 21 for assessment of liver/body weight and evaluation of fetuses. Fetal parameters evaluated include number of implants, mortality, weight, and gross developmental abnormalities. Study results are depicted in Table 4-15. Significant maternal toxicity was observed in high-dose dams. All groups of dosed dams exhibited significantly depressed weight gain, and the average liver/body weight ratio was significantly increased in the two highest dose groups (6 and 10 mg/kg-day). Fetotoxicity was observed as significantly depressed fetal body weight and delayed ossification in 6 and 10 mg/kg-day dose groups and significantly increased incidences of litters with fetuses having enlarged renal pelvis, edema, undescended testes, or enlarged cerebral ventricles in the 10 mg/kg-day group relative to controls. The study identified a LOAEL of 2 mg/kg-day for maternal toxicity, based on significantly depressed maternal body weight gain (16% lower than controls). The study identified a NOAEL of 2 mg/kg-day and a LOAEL of 6 mg/kg-day for fetotoxicity. The fetal effects may have been the direct result of maternal toxicity since they occurred at doses that were clearly toxic to the dams.

Table 4-15. Maternal and fetal effects following gavage dosing of pregnant rat dams with chlordecone on gestation days 7–16

	Dose level (mg/kg-day)			
	0	**2**	**6**	**10**
Maternal effects[a]				
Number inseminated	26	31	35	42
Maternal deaths	0	1	0	8[b]
Number pregnant at sacrifice	23	24	33	30
Weight gain (g)	73.5 ± 3.7	62.4 ± 2.9[b]	33.8 ± 2.4[b]	34.0 ± 5.6[b]
Liver/body weight	5.0 ± 0.1	5.1 ± 0.1	5.9 ± 0.1[b]	7.4 ± 0.2[b]
Fetal effects[a]				
Implants/dam	10.2 ± 0.4	10.4 ± 0.6	11.0 ± 0.4	9.1 ± 0.5
Percent mortality	9.5 ± 3.0	8.1 ± 2.7	6.5 ± 1.2	17.7 ± 4.9
Weight at sacrifice (g)	4.1 ± 0.1	4.0 ± 0.1	3.9 ± 0.1[b]	3.7 ± 0.1[b]
Sternal ossification centers	5.6 ± 0.1	5.5 ± 0.1	5.3 ± 0.1	5.3 ± 0.1
Caudal ossification centers	4.7 ± 0.1	4.5 ± 0.1	4.4 ± 0.1[b]	4.0 ± 0.2[b]
Percent supernumerary ribs	24.4 ± 6.3	28.1 ± 5.5	24.5 ± 5.4	17.4 ± 3.9
Enlarged renal pelvis[c]	1	2	5	10[b]
Edema[c]	0	1	0	10[b]
Undescended testis[c]	0	0	1	5[b]
Enlarged cerebral ventricles[c]	0	0	0	5[b]

[a]Mean ± SE.
[b]Statistically significantly different from controls ($p < 0.05$).
[c]Number of litters with one or more fetuses exhibiting the effect.

Source: Chernoff and Rogers (1976).

Chernoff and Rogers (1976) also administered chlordecone (purity unspecified) to groups of pregnant CD-1 mice at gavage doses of 0, 2, 4, 8, or 12 mg/kg-day on gestation days 7–16. Dams were observed for clinical signs and weight gain, and sacrificed on gestation day 18 for assessment of liver and body weight and evaluation of fetuses. Maternal and fetotoxicity were assessed in the same manner as that described for the rats. In the mice, significantly depressed maternal weight gain was noted at 8 and 12 mg/kg-day, and all dose groups exhibited significantly increased maternal liver and body weight (Table 4-16). Signs of fetotoxicity were observed only in the highest dose group and consisted of significantly increased fetal mortality. The study identified a LOAEL of 2 mg/kg-day for maternal toxicity, based on a statistically significant 10% increase in relative liver weight in the 2, 4, and 8 mg/kg-day dose groups. The study identified a NOAEL of 8 mg/kg-day and a LOAEL of 12 mg/kg-day for fetotoxicity. The fetal effects may have been the direct result of maternal toxicity since they occurred at doses that were clearly toxic to the dams.

Table 4-16. Maternal and fetal effects following gavage dosing of pregnant mouse dams with chlordecone on gestation days 7–16

	Dose level (mg/kg-day)				
	0	**2**	**4**	**8**	**12**
Maternal effects[a]					
Number inseminated	26	16	24	25	12
Maternal deaths	0	0	0	0	1
Number pregnant at sacrifice	16	14	16	19	5
Weight gain (g)	4.3 ± 0.5	4.1 ± 0.4	3.3 ± 0.4	0.7 ± 0.9[b]	-2.8 ± 0.9[b]
Liver/body weight	6.8 ± 0.3	7.5 ± 0.2[b]	7.9 ± 0.1[b]	8.6 ± 0.3[b]	7.6 ± 0.6
Fetal effects[a]					
Implants/dam	12.8 ± 0.6	12.0 ± 0.8	12.4 ± 0.7	11.3 ± 0.7	11.8 ± 1.4
Percent mortality	15.6 ± 3.3	12.4 ± 3.5	11.8 ± 2.1	16.9 ± 5.1	53.4 ± 19.4[b]
Weight at sacrifice (g)	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.3 ± 0.1
Sternal ossification centers	5.5 ± 0.1	5.3 ± 0.2	5.6 ± 0.2	5.1 ± 0.3	6.0 ± 0.0
Caudal ossification centers	4.0 ± 0.3	3.5 ± 0.5	4.5 ± 0.4	4.1 ± 0.5	6.4 ± 0.4
Percent supernumerary ribs	33.0 ± 6.8	20.9 ± 9.4	13.8 ± 5.1	26.2 ± 6.4	12.3 ± 4.8

[a]Mean ± SE.
[b]Statistically significantly different from controls ($p < 0.05$).

Source: Chernoff and Rogers (1976).

Additional developmental studies exist on chlordecone administered by injection (from 5–100 mg/kg) during gestation or postnatally. Effects associated with chlordecone exposure generally included alterations in neurological function, as well as impaired learning and behavioral changes, alterations in sexual differentiation, and weak estrogenic effects (Laessig et al., 2007 Sierra and Uphouse, 1986; Cooper et al., 1985; Mactutus and Tilson, 1985; Rosecrans et al., 1985). These acute injection studies help provide information to support developmental effects at high doses of chlordecone but do not generally contribute additional dose-response information regarding the most sensitive effects of chlordecone exposure during development.

4.3.3. Screening Studies

In a neonatal survival screen, chlordecone (purity unspecified) was administered to pregnant F344 rats at a gavage dose level of 0 or 10.0 mg/kg-day during gestation days 7–16 (U.S. EPA, 1986c). Neonatal survival was assessed on days 1 and 3 postpartum. Significantly ($p < 0.5$) reduced survival was noted on day 3 (but not day 1) postpartum (U.S. EPA, 1986c). In a developmental toxicity screen in the ICR/SIM mouse, chlordecone was administered by gavage at a dose of 0 or 24 mg/kg-day during days 8 to 12 of gestation (Seidenberg et al., 1986). Maternal toxicity was observed with decreased body weight gain and mortality in 18% of treated dams. Decreases were also observed in neonatal body weight gain and percent survival (Seidenberg et al., 1986).

4.4. OTHER STUDIES

4.4.1. Acute Toxicity Studies

Oral LD_{50} values for chlordecone range from 71 mg/kg body weight for rabbits to 250 mg/kg body weight for dogs (Larson et al., 1979a). The oral LD_{50} value for rats is 125 mg/kg body weight (Gaines, 1969). In experimental animals, the systemic effects of chlordecone following short-term exposures generally include nervous system effects (tremor and hyperexcitability), reproductive system toxicity (effects on estrous cyclicity and sperm parameters), liver changes (hypertrophy, microsomal enzyme induction, and ultrastructural changes), musculoskeletal effects (resulting from alterations in ATPase activity and calcium homeostasis), and thyroid and adrenal effects (ATSDR, 1995; U.S. EPA, 1986c; WHO, 1984). The adaptive effects observed in the liver are those generally produced by halogenated hydrocarbons; these include increase in liver weight or size and induction of the mixed function oxidase enzyme system (ATSDR, 1995). Chlordecone was also shown to alter lipid storage and metabolism in mice (Carpenter et al., 1996; Chetty et al., 1993a,b), and hepatobiliary excretion of certain chemicals was inhibited by chlordecone following acute exposure (see Section 3.4).

Other systemic effects reported following acute chlordecone exposure include decreases in food intake and body weight gain (ATSDR, 1995; Williams et al., 1992; U.S. EPA, 1986c; Albertson et al., 1985; Chernoff and Kavlock, 1982; Chernoff and Rogers, 1976), altered thermoregulation resulting in a decrease in core temperature that persisted for up to 12 days following ingestion of a single dose of 55 or 75 mg/kg in rats (Swanson and Wooley, 1982), and slight hyperthermia in rats following 12 weeks of exposure at 7.1 mg/kg-day (Pryor et al., 1983). The cardiovascular effects in rats after acute-duration exposure to chlordecone are limited to biochemical changes in cardiac tissue, such as membrane enzyme inhibitions and altered protein phosphorylation (Kodavanti et al., 1990; Desaiah et al., 1980); however, the toxicological implications of these changes are unknown (ATSDR, 1995).

4.4.2. Potentiation of Halomethane Toxicity

Laboratory studies of chlordecone potentiation of halomethane liver toxicity provide insight into potential mechanisms of chlordecone induced liver toxicity, though doses used in these studies are not considered environmentally relevant doses.

Chlordecone potentiates the liver toxicity and lethality of carbon tetrachloride (CCl_4) and other halomethanes (e.g., chloroform, bromotrichloromethane) in rats and mice, and this interaction has been widely studied and reviewed (Mehendale, 1994, 1990; Faroon and Mehendale, 1990; Mehendale et al., 1989; Plaa et al., 1987; Curtis et al., 1981). The exposure of rats to 10 ppm chlordecone in the diet for 15 days greatly increased the liver toxicity of halomethanes, leading to hepatic failure and death (Soni and Mehendale, 1993). Liver toxicity was generally demonstrated by measurement of elevated serum enzyme activities and

histopathological changes, including necrosis, lipid accumulation, and hepatocyte swelling. This effect was specific to chlordecone and was not observed following pretreatment with other organochlorine pesticides (e.g., mirex and photomirex).

Chlordecone enhanced the oxidative metabolism of halomethanes; however, enzyme induction was not correlated with the potentiation of liver toxicity. More efficient enzyme inducers, such as phenobarbital, did not significantly potentiate the toxicity of CCl_4 (Mehendale and Klingensmith, 1988; Curtis et al., 1981). Chlordecone appears to enhance the liver toxicity of halomethanes by suppressing the hepatocellular regeneration that is required to repair liver injury and restore hepatolobular architecture and function (Kodavanti et al., 1992; Faroon and Mehendale, 1990; Mehendale, 1990; Mehendale et al., 1989). Partially hepatectomized rats are protected from chlordecone-CCl_4 toxicity because of an increase in the rate of cell turnover as measured by ^{3}H-thymidine incorporation into hepatocellular DNA and an increase in the percentage of mitotic figures (Kodavanti et al., 1989; Young and Mehendale, 1989). Protection from liver toxicity was also provided by pretreatment with cyanidanol, which stimulated hepatocellular regeneration evidenced by increased ^{3}H-thymidine incorporation (Soni and Mehendale, 1991a,b,c). Polyamine metabolism was inhibited by cotreatment with chlordecone and bromotrichloromethane (Rao et al., 1990). Polyamines are important for the cell growth and proliferation process that results in liver regeneration and repair.

The chlordecone suppression of liver cell regeneration and repair may be related to the compromised energy status of hepatocytes in animals exposed to chlordecone. Treatment of rats with chlordecone and CCl_4 caused a decrease in liver ATP levels and an inhibition of oligomycin-sensitive Mg^{2+}-ATPase (Kodavanti et al., 1990). Chlordecone affects calcium homeostasis in hepatocytes, leading to a decline in glycogen storage and a reduced energy status (Kodavanti et al., 1993, 1990). Chlordecone-CCl_4 administration caused an inhibition in microsomal and mitochondrial calcium uptake and a decrease in the high affinity component of hepatic plasma membrane Ca^{2+}-ATPase. Administration of fructose 1,6-diphosphate to rats resulted in protection from chlordecone-CCl_4 hepatotoxicity due to an increase in the levels of liver cell ATP (Rao and Mehendale, 1989). ATP administration during the early phase of liver injury also helped to restore normal liver function through enhanced regeneration and repair (Soni and Mehendale, 1991a,b,c).

Several studies have indicated an age-related susceptibility to the chlordecone potentiation of CCl_4 hepatotoxicity (Dalu et al., 1995; Cai and Mehendale, 1993). Developing rats have been shown to be resistant to the lethal effects of the chlordecone-CCl_4 combination treatment. Postnatal rats recovered more quickly from CCl_4-induced liver injury than young adult rats, due to the higher level of ongoing cell division and an additional stimulatory response to liver injury (measured by ^{3}H-thymidine incorporation into hepatocellular DNA). The resiliency of postnatal rats was abolished by administration of the antimitotic agent colchicine, highlighting the importance of cell turnover in liver tissue repair (Dalu et al., 1998). Aged rats

(2 years old) were also shown to be resistant to the potentiation of CCl_4 liver toxicity by chlordecone due to the robust and early liver tissue repair in old rats as compared with young adult rats (3 months) (Murali et al., 2002). Gender effects were noted, with female rats being more sensitive to chlordecone-CCl_4 hepatotoxicity than male rats (Blain et al., 1999).

4.4.3. Neurotoxicity Studies

With tremor being the cardinal feature of chlordecone intoxication in humans, research into the mode of action of the neurological changes has been the focus of several studies. A number of studies have associated alterations in neurotransmitter activity (e.g., alpha-noradrenergic, dopaminergic, and serotonergic systems) with chlordecone-induced tremor and exaggerated startle response (Vaccari and Saba, 1995; Brown et al., 1991; Herr et al., 1987; Tilson et al., 1986; Uphouse and Eckols, 1986; Chen et al., 1985; Desaiah, 1985; Gerhart et al., 1985, 1983, 1982; Hong et al., 1984; Fujimori et al., 1982b; Hwang and van Woert, 1979). At the cellular level, changes in ATPase activity and calcium homeostasis in the nervous system have been related to chlordecone exposure across species (ATSDR, 1995). The reported effects of chlordecone exposure on calcium balance in whole animal studies include decreased calcium uptake in rats following a single oral dose of 40 mg/kg (End et al., 1981); decreased total protein-bound, myelin, and synaptosomal calcium following eight consecutive daily oral doses of 25 mg/kg-day in 4- to 6-week-old male ICR mice (Hoskins and Ho, 1982); decreased total protein-bound and mitochondrial calcium content with increased nuclear calcium content in 24-week-old male ICR mice following a single oral dose of 25 mg/kg (Hoskins and Ho, 1982); and decreased brain calmodulin in rats exposed to 2.5 mg/kg-day orally for 10 consecutive days (Desaiah et al., 1985; Desaiah, 1982). In vitro studies have supported that chlordecone may alter calcium regulation of neuronal function (Inoue et al., 1991; Bondy and McKee, 1990; Vig et al., 1989; End et al., 1981, 1979).

4.4.4. Endocrine Disruption Studies

Specific mechanisms of chlordecone-induced reproductive effects are not known, although it is generally believed that an estrogenic mode of action is involved. Observed chlordecone-induced reproductive effects include oligospermia, reduced sperm motility, and decreased libido in occupationally exposed males (Taylor, 1985, 1982; Guzelian, 1982a; Taylor et al., 1978) and decreased offspring production in laboratory animals (Cannon and Kimbrough, 1979; Good et al., 1965; Huber, 1965). Testicular atrophy, altered sperm characteristics, persistent vaginal estrus, and anovulation observed in chlordecone-treated laboratory animals mimic similar effects produced by excessive estrogen (Swartz et al., 1988; U.S. EPA, 1986c; Uphouse, 1985; Linder et al., 1983; Larson et al., 1979a; Huber, 1965). Estrogens appear to function by altering gene transcription in reproductive tissues via nuclear estrogen receptors.

Mechanistic studies, therefore, have been designed to assess the potential of chlordecone to mimic the action of estrogen.

In cell-free preparations containing rat uterine estrogen receptors, 8 µM chlordecone inhibited the binding of [^{3}H]estradiol by nearly 50% (Bulger et al., 1979). It was further demonstrated that chlordecone caused the translocation of estrogen receptors from the cytosolic to the nuclear fraction in both isolated rat uteri and ovariectomized immature rats. These results indicate that chlordecone may act directly on the uterus. In another study, chlordecone-induced uterine effects observed in ovariectomized immature rats were enhanced by coadministration of estradiol, an indication that chlordecone and estradiol act at the same site in uterine tissue (Johnson, 1996). Chlordecone demonstrated a relatively high affinity for recombinant human estrogen receptors; 5.7 µM (Bolger et al., 1998) and 9 µM chlordecone (Scippo et al., 2004) caused 50% inhibition of 17β-estradiol binding. Chlordecone exhibits approximately equal affinity for both subtypes of human estrogen receptors (ERα and ERβ) (Kuiper et al., 1998). In one study, uterine levels of adenosine 3'5'-cyclic monophosphate (cAMP) decreased with increasing uterine weight following repeated exposure to chlordecone in ovariectomized immature rats (Johnson et al., 1995). The levels of cAMP were not decreased in similarly treated rats that were also given the antiestrogen (ICI-182,780), indicating that the chlordecone-induced effect on cAMP is estrogen receptor dependent.

The affinity of chlordecone for estrogen appears to be tissue dependent. Although competition between [^{3}H]estradiol and chlordecone was comparable in magnitude within estrogen receptor preparations from brain or uterine tissues of rats, in vivo binding of chlordecone in the brain of ovariectomized rats was much less than that observed in the uterus (Williams et al., 1989). The basis for this in vivo tissue-specific difference is not clear but may result, at least in part, from a greater time requirement for chlordecone to reach a concentration in the brain that could result in a significant estrogenic effect. Furthermore, although chlordecone may mimic the effect of estrogen in uterine tissue, chlordecone appears to function as an estrogen antagonist in central nervous tissue (Huang and Nelson, 1986; Uphouse et al., 1986).

Chlordecone interacts in vitro and in vivo with the estrogen receptor system in rat uterus. Hammond et al. (1979) found that it competes with estradiol for binding to the cytoplasmic receptor in vitro and also induces nuclear accumulation of estrogen receptor sites in uteri in vitro. Chlordecone translocates estrogen receptor sites to the uterine nucleus, increases uterine weight, and stimulates the synthesis of the progesterone receptor when it is injected into immature female rats (Hammond et al., 1979).

Results of one recent study indicate that chlordecone-induced uterine effects may also be induced via a pathway other than that which includes the estrogen receptor. Chlordecone up-regulated the uterine expression of an estrogen-responsive gene, lactoferrin, in ERα knockout mice, whereas these effects were not elicited by 17β-estradiol (Das et al., 1997). Neither the

estrogen receptor antagonist ICI-182,780 nor 17β-estradiol inhibited the chlordecone-induced uterine expression of lactoferrin in these mice.

Chlordecone has been tested for its potential to bind to other receptors. The chemical exhibited relatively high affinity for recombinant human progesterone receptors (Scippo et al., 2004); 11 μM chlordecone resulted in 50% inhibition of progesterone binding. Chlordecone exhibited characteristics of a partial androgen antagonist, based on 50% reduction of inhibition of 5α-dihydroxytestosterone-mediated activation of luciferase activity by 6.9 μM chlordecone in the human PC-3 prostate carcinoma cell line (Schrader and Cooke, 2000).

4.4.5. Immunological Studies

Several studies have examined the potential for general immunotoxicity associated with chlordecone exposure, and two studies have investigated chlordecone effects on the acceleration of an autoimmune disease. Smialowicz et al. (1985) exposed male F344 rats to technical grade chlordecone (87% pure) in corn oil by gavage for 10 days at doses of 0.625, 1.25, 2.5, 5.0, and 10 mg/kg-day (10 rats/dose). Dose groups also included a vehicle control group (corn oil), an untreated cage-matched control group, and cyclophosphamide (1.5–24 mg/kg-day) exposure groups as positive controls for immunosuppression. Blood samples were taken for total and differential white blood cell counts, and the spleen and thymus weights were recorded. Single cell suspensions were prepared from the spleen, and the lymphoproliferative response of splenocytes to the T-cell mitogens phytohemagglutinin (PHA) and concanavalin A (con A), the T- and B-cell mitogen pokeweed mitogen, and the B-cell mitogen *Salmonella typhimurium* mitogen (STM) were assayed. A single functional immune test, natural killer (NK) cell activity of splenocytes, was also performed. NK activity was measured against W/Fu-G1 rat lymphoma cells and YAC-1 mouse lymphoma cells as the target cell population. The high dose (10 mg/kg-day) of chlordecone caused a 20% reduction in body weight as well as reduced relative spleen and thymus weights (8 and 24% respectively). The high dose was also associated with a 69% reduction in the concentration of circulating neutrophils, but no change was seen in the number of lymphocytes, monocytes, or overall leukocytes. A reduced mitogenic response to PHA was observed in the 2.5 mg/kg-day chlordecone group only. The high dose of chlordecone was associated with a 45% reduced mitogenic response to con A, a 66% increased mitogenic response to STM, and an almost threefold increase in background mitogenic response. In rats exposed to the high dose of chlordecone, NK cell activity was reduced by 62 to 73% against both target cell lines. The authors suggest that the observed effects in the high-dose animals (10 mg/kg-day) were due to overt toxicity. The authors also note that at 10 mg/kg-day rats displayed tremors characteristic of chlordecone intoxication, and therefore the decreased body weight, decreased spleen and thymus weight, altered lymphoproliferative response, and decreased NK cell activity were likely effects secondary to overt toxicity.

The effects of chlordecone exposure on antibody response were examined as part of a study of the consequences of malnutrition on antibody response in male Sprague-Dawley rats (Chetty et al., 1993c). For the purpose of this review, only data from the control and chlordecone-treated rats fed nutritionally sufficient diets are presented. Rats (six per group) were exposed to 0, 10, or 100 ppm (doses calculated as 0, 0.96, or 9.6 mg/kg-day)[3] chlordecone in the diet for 2 or 4 weeks. Rats were immunized by injection of sheep red blood cells (SRBCs) 4 days before the end of chlordecone exposure. In addition to measuring body weight, the authors measured spleen weight and antibody response to SRBCs as determined by the plaque-forming cell (PFC) assay. Chlordecone exposure for either 2 or 4 weeks increased the PFC response. Although the results are only presented graphically, dietary exposure of 10 ppm chlordecone appeared to increase the PFC response about two- to threefold over controls. At this dose, chlordecone treatment significantly reduced body weight by 15% and increased relative spleen weight by 29%. Average body weight and spleen weight were not reported for animals exposed to 100 ppm.

No additional studies of general immunotoxicity of chlordecone were found. As part of an acute neurotoxicity study, however, a single dose of 75 mg/kg chlordecone to Sprague-Dawley rats resulted in significant reductions in thymus weights (Swanson and Woolley, 1982). As with the results from Smialowicz et al. (1985), the dose associated with thymus weight reduction was also associated with severe generalized toxicity.

Several studies from the same laboratory have investigated the potential effects of chlordecone treatment on autoimmune disease (Sobel et al., 2006, 2005; Wang et al., 2007). Sobel et al. (2005) investigated the effect of chlordecone in female (NZB × NZW)F_1 mice, a murine model of systemic lupus erythematosus in which the principal clinical manifestation of lupus is renal disease, specifically immune-mediated glomerulonephritis. In this study, female 8-week-old (NZB × NZW)F1 control, ovariectomized, or sham-operated mice were implanted with 60-day sustained-release pellets containing doses of 0, 0.01, 0.1, 0.5, or 1 mg chlordecone (99.2% pure). Pellets were replaced every 60 days throughout the experiment. For this phase of the experiment, treatment groups consisted of 10 animals per group, whereas the control group consisted of 20 animals. Urine protein, blood urea nitrogen (BUN), and body weight were evaluated monthly for all animals. Mice were euthanized at the conclusion of the experiment if BUN exceeded 50 mg/dL or if proteinuria exceeded 2000 mg/dL. IgG anti-double-strand DNA titers in serum of some treatment groups were determined by indirect enzyme-linked immunosorbent assay (ELISA). Kidneys were removed for histological examination and glomerular damage was scored by light microscopy. Additionally, a subset of treatment groups was examined for IgG-mediated immune complex deposition in glomeruli by using immunohistofluorescence.

[3]Calculation: mg/kg-day = (ppm in feed × kg food/day)/kg body weight. Reference food consumption rates of 0.0179 kg/day (U.S. EPA, 1988) and reported average body weight of 0.188 kg (males) were used.

Mice treated with 1.0 or 0.5 mg chlordecone pellets developed renal disease significantly earlier than did ovariectomized controls ($p < 0.05$). This observation was also correlated with proteinuria and the early appearance of immune complex glomerulonephritis. Additionally, mice treated with chlordecone developed elevated ds-DNA autoantibody titers earlier than ovariectomized controls. Immunohistofluorescence analysis of renal sections from a subset of animals treated for 8 weeks with 1 mg chlordecone showed enhanced deposits of IgG immune complexes as compared with untreated controls. The lowest dose per pellet found to produce a significant decrease in time to onset of renal disease was found to be 0.5 mg. The authors calculated, based on average body weight, that this would result in a dosing rate per unit body weight of 0.2 mg/kg-day. However, blood levels of chlordecone were not examined, and the equivalent oral dose needed to achieve this effect is uncertain.

After the demonstration that chronic chlordecone exposure accelerates the development of autoimmunity in ovariectomized female (NZB × NZW)F_1 mice (Sobel et al., 2005), additional studies were designed to examine the effect of chlordecone on autoimmunity and renal disease in ovary-intact female (NZB × NZW)F_1 mice and female BALB/c mice, a mouse strain that is not predisposed to the development of autoimmune-related renal disease (Sobel et al., 2006). As in the previous study, 8-week-old female mice were implanted with 60-day sustained-release pellets containing 0, 0.001, 0.01, 0.1, 0.5, 1, or 5 mg chlordecone subcutaneously above the shoulders. Blood and urine were collected once per month for the assessment of renal function by BUN analysis and urine protein content. Mice were euthanized at the conclusion of the experiment if BUN exceeded 50 mg/dL or if proteinuria exceeded 2000 mg/dL. Blood was taken for serum analysis and kidneys were removed for later histological analysis by light microscopy. Antigen-specific antibody levels for anti-dsDNA and antichromatin were determined by indirect ELISA.

In the first half of the experiment, involving chlordecone treatment in ovary-intact (NZB × NZW)F_1 mice, Sobel et al. (2006) reported that chlordecone shortened survival, decreased the time to onset of elevated autoantibody titers, and accelerated glomerulonephritis in a dose-dependent manner. Median survival of control groups was 25 weeks, compared with 21 and 18 weeks in mice implanted with the 1 mg and 5 mg chlordecone pellets, respectively. Survival curves for mice treated with chlordecone were significantly different from controls by log rank test for trend ($p = 0.01$). Time to development of renal disease in mice treated with the 5 mg pellets was significantly shorter than in controls ($p < 0.05$). However, histopathology associated with renal disease was similar between the groups. Mice treated with either 1 or 5 mg chlordecone pellets developed anti-dsDNA and antichromatin autoantibody titers significantly earlier than controls ($p \leq 0.005$).

In the second half of the experiment, involving chlordecone treatment of BALB/c mice, Sobel et al. (2006) performed the same assays as for the (NZB × NZW)F_1 mice. No treatment-related effects were seen in mortality, and none of the chlordecone-exposed BALB/c mice developed renal disease. Autoantibody titers (anti-dsDNA and antichromatin) were not different

from controls. Total serum IgG2a and IgG1 were statistically increased in mice treated with the 1 and 5 mg chlordecone pellets ($p < 0.01$). The failure of chlordecone to induce renal disease or autoantibodies in BALB/c mice (a strain not predisposed to the development of autoimmunity or renal disease) emphasizes the importance of genetic background on the effects of chlordecone on autoimmunity.

The mechanism by which chlordecone accelerates autoimmunity in female (NZB × NZW)F_1 mice is unknown. The (NZB × NZW)F_1 mouse is a model of systemic lupus erythematosus, an autoimmune disorder that affects women more frequently than men (Lahita, 1997). Estrogen receptor binding may play a role in some forms of autoimmune disease in rodents and humans (Ahmed et al., 1999), and, in the (NZB × NZW)F_1 mouse model of systemic lupus erythematosus, 17β-estradiol accelerates the development of glomerulonephritis with similar results to the effects observed following chlordecone treatment (Sobel et al., 2005). Sobel et al. (2005) hypothesized that chlordecone's acceleration of autoimmunity may be related to its estrogenic properties and ability of chlordecone to bind the estrogen receptor. However, the poor correlation between autoimmune effects and estrogenic activity of chlordecone as measured by uterine hypertrophy suggests that a non-estrogen-receptor-mediated mechanism may be important (Sobel et al., 2005).

An additional study by the same laboratory was performed to compare the mechanism of chlordecone-accelerated autoimmunity to that of 17β estradiol-accelerated autoimmunity in (NZBxNZW)F_1 mice by examining gene and protein expression of B cells (Wang et al., 2007). As with the earlier experiments, 6-8-week-old ovariectomized female (NZBxNZW)F_1 mice were implanted with 60-day sustained-release pellets. In this experiment, pellets contained 1 mg chlordecone, 5 mg chlordecone, 0.05mg estradiol, or matrix only for controls. Mice were euthanized 5-6 weeks after implantation in order to evaluate the development of autoimmune pathology rather than overt effects. Spleens were removed and splenic tissue and cells were prepared for analysis. Splenocytes were analyzed for proliferation, apoptosis, and mRNA and cDNA expression. The following markers were analyzed for expression: B220, IgM, CD19, CD21, CD24, CD44, CD69, CXCR4, CXCR5, ICAM-1, VCAM-1`, MHC II, B7.2, and GL7. The authors state that germinal center activity and cell surface markers of B cells in the germinal centers were examined because of the importance of the germinal center in negative selection for autoreactive B cells. Both chlordecone exposure and estradiol treatment activated splenic B cells and enhanced germinal center activity as shown by upregulated protein expression of GL7, CXCR5, and CXCR4. Both treatments also resulted in reduced B cell apoptosis and increased patterns of protein and gene expression that may increase survival of autoreactive B cells (i.e., B cell expression of ICAM-1 and VCAM-1 cell adhesion molecules and Bcl-2 and shp-1 gene expression in B cells from the germinal centers). However, major differences were also observed between the effects of chlordecone exposure and that of estradiol, particularly in the lack of an effect of chlordecone on splenic B cell subsets such as $CD138^+B220^-$ populations.

The authors conclude that differences in the effects between chlordecone and estradiol indicate that chlordecone does not accelerate the development to systemic lupus erythematosus by functioning strictly as an estrogen mimic.

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

4.5.1. Genotoxicity

The weight of evidence from in vivo and in vitro studies suggests that chlordecone is not mutagenic. The majority of studies have not shown genotoxic activity in a variety of short-term in vitro assays. There is no evidence that chlordecone is a mutagen in *S. typhimurium* or *Escherichia coli* (Mortelmans et al., 1986; U.S. EPA, 1986c; Probst et al., 1981; Schoeny et al., 1979). Further, chlordecone alcohol, the major metabolite of chlordecone in humans, is not mutagenic in *S. typhimurium* (Mortelmans et al., 1986). Chlordecone also gave negative results when tested for enhancement of unscheduled DNA synthesis in primary cultures of adult rat hepatocytes (Probst et al., 1981; Williams, 1980). The clastogenic activity of chlordecone is unclear. Chlordecone was investigated for potential clastogenic activity in Chinese hamster ovary (CHO) cells (Galloway et al., 1987; Bale, 1983). Bale (1983) reported that chlordecone treatment of CHO (M3-1) cells (2, 4, or 6 µg/mL) produced chromosome breaks, chromatid breaks, dicentric chromosomes, and chromosome interchanges. In a later study employing higher doses, chlordecone did not increase the frequency of CHO cells with abnormal chromosome morphology over a nonactivated concentration range of 10–20 µg/L or over an Aroclor 1254-induced rat liver S9-activated concentration range of 5–15 µg/L (Galloway et al., 1987).

There has been limited testing of chlordecone in whole-animal genotoxicity assays. The available data generally show that chlordecone is not genotoxic in whole-animal tests. Chlordecone was not clastogenic in male Sprague-Dawley rat germinal cells in a dominant lethal assay at doses of 3.6 or 11.4 mg/kg-day orally for 5 consecutive days (Simon et al., 1986, 1978). Although chlordecone clearly increased ornithine decarboxylase activity (indicative of cellular proliferation) in rat livers following oral exposure, it did not induce DNA damage in the target organ (Mitra et al., 1990; Kitchin and Brown, 1989).

4.5.2. Tumor Promotion and Mechanistic Studies

Chlordecone was tested in a two-stage model of liver carcinogenesis in both male and female Sprague-Dawley rats (Sirica et al., 1989). Male rats were subjected to two-thirds hepatectomy and 24 hours later were administered a single gavage dose (20 mg/kg) of the initiator chemical diethynitrosamine (DEN) in water. Ten days following initiation, rats began to receive biweekly subcutaneous (s.c.) injections of chlordecone in corn oil at doses of 0.17, 0.34,

1.7, and 3.4 mg/kg for a total of 44 weeks. Controls for this experiment included rats given DEN after partial hepatectomy without chlordecone administration, rats receiving biweekly administration of chlordecone without DEN initiation, and rats receiving corn oil vehicle only. Chlordecone (30 mg/kg) was also administered by corn oil gavage as an initiating chemical given 24 hours after partial hepatectomy. This treatment was followed 10 days later by administration of the tumor promoter sodium phenobarbital in the drinking water at a daily concentration of 0.05% for 44 weeks. A second experiment was conducted that compared promotion in the two-stage assay in male and female rats. A similar study design was used; however, chlordecone was administered biweekly by s.c. injection at higher doses (3 or 9 mg/kg) and the treatment was continued for only 27 weeks.

At the end of each experiment, rats were killed and their livers were evaluated histologically for the presence of preneoplastic lesions (hyperplastic hepatocellular foci) and tumors (hepatocellular carcinomas). Histological staining for GGT was used to identify preneoplastic foci in nontumorous liver sections. Morphometric measurements of GGT-positive foci were determined, and the total number of foci per cm^3 of liver were quantified. The concentration of chlordecone in the liver was measured by gas-liquid chromatography.

Body weight gain was not altered in male rats receiving chlordecone at doses between 0.17 and 3.4 mg/kg biweekly for 44 weeks (with or without DEN initiation). Higher doses did affect body weight gain (3 and 9 mg/kg in females and 9 mg/kg only in males) when administered biweekly for 27 weeks. The depression in body weight gain was independent of DEN initiation. Doses greater than 3 mg/kg caused increased irritability in male and female rats, but no obvious tremors, dermatologic changes, or liver enlargement were observed. Nonneoplastic liver lesions were observed histologically in both male and female rats given chlordecone doses of 3 and 9 mg/kg biweekly (s.c.) for 27 weeks. The lesions included hypertrophy of Zone 3 hepatocytes, congestion, mild fatty change, focal necrosis, and occasional small nests of proliferated sinusoidal cells. The severity of these lesions appeared to be dose related, although the incidence and severity of noncancer lesions was not quantitatively evaluated.

A dose-related increase in the number of GGT-positive foci/cm^3 of liver was observed in male rats given chlordecone at doses between 0.17 and 3.4 mg/kg biweekly (s.c) for 44 weeks following hepatectomy and initiation with DEN (as compared with control groups that were receiving either initiating or promoting treatment alone). Hyperplastic nodules were also observed in 19% of male rats given the initiation and promotion treatments, while nodular liver lesions were not observed in control rats. Chlordecone (30 mg/kg) was not effective as an initiating chemical following partial hepatectomy and promotion with sodium phenobarbital for 44 weeks. A significant sex difference was noted in the chlordecone promotion response at doses of 3 and 9 mg/kg. Both the median number and the size of the GGT-positive foci were increased in female rats as compared to males following DEN initiation and 27 weeks of

chlordecone promotion. In addition, hepatocellular carcinomas were observed in female rats (11% at 3 mg/kg and 62% at 9 mg/kg) but were not found in male rats given the same initiation-promotion treatment. Male rats exhibited only preneoplastic foci and nodular hyperplasia under the condition of the two-stage assay. Similar concentrations of chlordecone were measured in the livers of male and female rats, suggesting that enhancement of the tumor promotion response is due to increased sensitivity of females and not altered pharmacokinetics.

Chlordecone was demonstrated to be a liver tumor promoter in a two-stage assay of hepatocarcinogenesis (Sirica et al., 1989). The mode of action for liver tumor promotion by chlordecone is unclear; however, liver toxicity and the subsequent repair/regeneration response may play a role at high doses. Liver toxicity (i.e., focal necrosis, hypertrophy, congestion, and fatty change) and decreased body weight gain were evident in male and female rats at doses that induced liver tumor promotion. The mode of action for liver tumor promotion by chlordecone is unclear. Liver toxicity and the subsequent repair/regeneration response may play a role at high doses. However, this study did not evaluate histological evidence of liver toxicity at lower dose levels that were shown to cause an increase in GGT-positive foci in male rats. Therefore, the study did not provide a clear indication of whether liver toxicity precedes liver tumor promotion (Sirica et al., 1989).

Some in vitro evidence suggests that the promotion of liver tumors by chlordecone may be related to suppression of proliferative control through inhibition of gap junctional cell-to-cell communication. The metabolic cooperation between co-cultivated 6-thioguanine-sensitive and resistant Chinese hamster V79 cells was used to evaluate intracellular communication via gap junctions (Tsushimoto et al., 1982). 6-Thioguanine-sensitive cells are wild-type V79 cells that are capable of metabolizing 6-thioguanine to a lethal substrate for nucleic acids that causes cell death. Resistant cells lack the enzyme for 6-thioguanine metabolism; however, cell death can be induced in these cells by a transfer of the lethal 6-thioguanine metabolite across gap junctions from sensitive cells (i.e., metabolic cooperation). Chlordecone was shown to inhibit metabolic cooperation in co-cultivated Chinese hamster V79 cells.

Chlordecone inhibition of cell-to-cell communication was also demonstrated in a dye transfer study in embryonic palatal mesenchymal cells (Caldwell and Loch-Caruso, 1992). Lucifer yellow was scrape-loaded into cell monolayers in the presence or absence of chlordecone. The lucifer yellow dye is too large to cross the plasma membrane but can enter cells through gap junctions. Junctional communication was demonstrated by the movement of lucifer yellow fluorescence away from the scrape line. Chlordecone (20 µg/mL) inhibited dye transfer as demonstrated by the restriction of dye to cells near the scrape line. This effect was reversible with a recovery of dye transfer ability 15 minutes after incubation with control culture medium.

Chlordecone was shown to disrupt adherens junctions in human breast epithelial cells (Starcevic et al., 2001). Human breast epithelial cells cultured on Matrigel (an extracellular

matrix) form lattice-like structures that were disrupted by incubation with 0.1 and 1.0 µM chlordecone (0.01 µM chlordecone had no effect). Chlordecone was also demonstrated to decrease the levels of the transmembrane proteins E-cadherin and β-catenin. These proteins are components of the adherens junctions that mediate cell-to-cell interaction and may play a role in development of neoplastic lesions.

The available data suggest that chlordecone, like many other halogenated hydrocarbons, is not genotoxic, but may act as an epigenetic carcinogen and a tumor promoter. Chlordecone shares similar characteristics with several other well- known tumor promoters. These features include the following: (1) chlordecone induces hepatic enzyme induction (Trosko et al., 1983; Williams, 1980); (2) tumors are found predominantly in rat or mouse livers (NCI, 1976a); (3) chlordecone lacks reactive functional groups and is not genotoxic; (4) there is no evidence of covalent binding to DNA; (5) chlordecone induces ornithine decarboxylase activity (ATSDR, 1995; Mitra et al., 1990; Kitchin and Brown, 1989); and (6) chlordecone inhibits gap-junctional-mediated intercellular communication (Caldwell and Loch-Caruso, 1992; Tsushimoto et al., 1982).

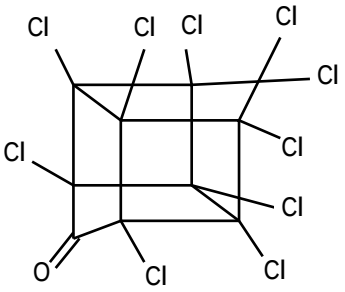
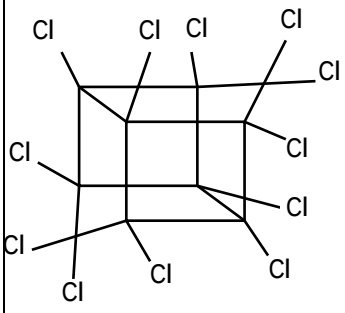
Most of the effects of chlordecone are thought to be produced by the parent compound, primarily by interfering with the function of mitochondrial and cellular membranes. Disruption of cellular homeostasis and energy production within the cell eventually leads to impaired cellular function. In the liver, membrane perturbation and inhibition of transport proteins at the bile canalicular membrane is thought to be related to chlordecone-induced hepatobiliary dysfunction.

4.5.3. Structural Analog Data—Relationship to Mirex

Information on structural analogs can be instructive in predicting biological activity and carcinogenic potential of an agent. Confidence in the conclusions of such a chemical relationship is a function of how similar the analogs are in structure, metabolism, and biological activity. Chlordecone is closely related to the chlorinated pesticide mirex in structure, physiochemical properties, and biological activity.

Mirex is a fully chlorinated molecule, whereas chlordecone has a similar structure with only the substitution of two chlorine atoms for a carbonyl group (a double-bonded oxygen atom). This substitution impartsmore water solubility as compared to mirex. Both compounds have very low vapor pressures and very high melting points and are crystalline solids at standard conditions. A comparison of physiochemical properties of chlordecone and mirex is presented below in Table 4-17.

Table 4-17. Physiochemical properties of chlordecone and mirex

	Chlordecone	Mirex
Structure		
Chemical formula	$C_{10}Cl_{10}O$	$C_{10}Cl_{12}$
Molecular weight	491 g/mol	546 g/mol
Physical state	Crystalline solid	Crystalline solid
Octanol-water partition coefficient	5.41	6.89
Water solubility	2.7 mg/L	0.085 mg/L
Vapor pressure	2×10^{-7} mm Hg	8×10^{-7} mm Hg

Source: NLM (2004b,c).

Mirex and chlordecone are both highly absorbed (75–90%) upon oral exposure and are not substantially metabolized (Egle et al., 1978; Pittman et al., 1976; Wiener et al., 1976). A subset of chlordecone (about 50–75%) is converted into chlordecone alcohol in humans and in some animal species (Fariss et al., 1980; Blanke et al., 1978). No data exist on metabolism of mirex in humans, though animal studies indicate that mirex is not metabolized (Pittman et al., 1976; Wiener et al., 1976; Ivie et al., 1974; Gibson et al., 1972). As a fully chlorinated hydrocarbon, mirex is very hydrophobic and preferentially accumulates in fat (Wiener et al., 1976; Kennedy et al., 1975; Gibson et al., 1972; Mehendale et al., 1972). Chlordecone partitions to fat to a lesser extent. Human data from occupational exposures to chlordecone indicate that chlordecone binds to plasma proteins and lipoproteins and is preferentially sequestered in the liver. The average partitioning of chlordecone among liver, fat, and blood in occupationally exposed workers was found to be 15:7:1 (Cohen et al., 1976).

Chronic exposure studies of chlordecone have indicated that the liver is a target of toxicity. Exposure to chlordecone and mirex in experimental animals results in similar noncancerous liver lesions that may or may not be precursor effects to the development of liver tumors. Liver lesions common to mirex and chlordecone include hypertrophy, hyperplasia, fatty changes, cytoplasmic vacuolation, and anisokaryosis (NTP, 1990; Chu et al., 1981b,c; Larson et al., 1979a,b; NCI, 1976a,b). Though no data exist on liver sensitivity to mirex in humans,

observational studies of workers occupationally exposed to chlordecone found evidence of hepatomegaly in 20 workers. Liver biopsies from 12 of these individuals showed histological changes, including proliferation of the SER and cytoplasmic accumulation of lipofuscin (Guzelian, 1982a; Guzelian et al., 1980; Taylor et al., 1978).

There is inadequate evidence in humans for the carcinogenicity of chlordecone and mirex. Mirex has been shown to induce liver tumors in both sexes of rats and mice in chronic feeding studies at similar dose levels as chlordecone. Incidence of liver tumors in chlordecone-treated male and female rats and mice were found to be significantly elevated at 1.7, 2, 3.4, and 3.5 mg/kg-day. Increased incidence of liver tumors with chronic mirex exposure has been shown in rats and mice at 3.8 and 7 mg/kg-day (NTP, 1990; Innes et al., 1969). In F344/N rats exposed to 0, 0.007, 0.07, 0.7, 1.8, 3.8, and 7.7 mg/kg-day mirex in the diet, statistically significantly increased incidences of combined liver adenomas and carcinomas were found in male and female rats exposed to ≥3.8 mg/kg-day mirex (PWG, 1992; NTP, 1990). Incidences for liver adenomas alone were statistically significantly elevated at concentrations ≥1.8 mg/kg-day in male and ≥3.9 mg/kg-day in female F344/N rats compared with controls. In CD rats exposed chronically in the diet to 0, 4, 7 (males), or 8 (females) mg/kg-day mirex, males showed statistically significantly increased incidences of liver neoplastic nodules and hepatocellular carcinomas at 7 mg/kg-day, whereas females showed increased incidences of neoplastic nodules at 4 and 8 mg/kg-day, with no significant increases in hepatocellular carcinomas at either exposure level (Ulland et al., 1977). In B6C3F1 and B6AKF1 mice exposed for life to 0 or 7 mg/kg-day mirex in the diet, liver tumors reported as hepatomas were found at statistically significantly increased incidence in exposed males and females.

Liver tumors resulting from mirex and chlordecone exposure are generally described as benign, well-differentiated masses without vascular invasion or metastases (PWG, 1992; NTP, 1990; Ulland et al., 1977; NCI, 1976a,b; Innes et al., 1969). The available studies on mirex or chlordecone classified liver tumors as either neoplastic nodules or hepatocellular carcinoma (Ulland et al., 1977; NCI, 1976a,b). However, classification of liver tumor types has changed from the time chlordecone and mirex cancer bioassays were initially published in the 1970s. In early studies, it was common for pathologists to use the term hepatocellular carcinoma for any neoplastic lesion since it was felt that all such lesions had the capacity to become invasive and metastasize. However, current practice is to distinguish between benign (hepatocellular adenoma) and malignant (hepatocellular carcinoma) tumors. Both Ulland et al. (1977) and the NCI study (1976a,b) characterized the observed hepatocellular carcinomas as well-differentiated masses without vascular invasion or metastases. In vivo and in vitro genotoxicity studies for mirex and chlordecone were generally negative. However, the available evidence for chlordecone and mirex is inadequate to establish a mode of action by which these chemicals induce liver tumors in rats and mice.

Mirex and chlordecone have exhibited similarities in reproductive effects. Decreased sperm counts and testicular degeneration have been observed in animals (Larson et al., 1979a; Yarbrough et al., 1981). Additionally, decreased production of litters in animals was observed for both mirex and chlordecone (Cannon and Kimbrough, 1979; Gaines and Kimbrough, 1970).

It should be noted that although chlordecone and mirex have similar biological activity in the liver at comperable dose levels, some of the observed noncancer effects for these structurally related chemicals are dissimilar. For example, chlordecone exposure results in neurological symptoms, most notably tremors, in experimental animals and in occupationally exposed humans (Taylor, 1985, 1982; Linder et al., 1983; Guzelian, 1982a,b; Larson et al., 1979a; Taylor et al., 1978). However, neurological effects have not been observed with mirex exposure (NTP, 1990; Ulland et al., 1977; Innes et al., 1969). In addition, one of the most sensitive effects of mirex exposure is the development of cataracts in offspring exposed in utero and lactationally, whereas the development of cataracts in offspring does not occur as a result of chlordecone exposure. Differences in distribution between chlordecone and mirex may contribute to differences in their low-dose biological effects. For instance, it is known that mirex primarily localizes in adipose tissue, whereas chlordecone preferentially accumulates in the liver (Hewitt et al., 1985; Morgan et al., 1979; Cohn et al., 1978; Egle et al., 1978; Wiener et al., 1976; Kennedy et al., 1975).

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

Table 4-18 presents a summary of the noncancer results for the repeated-dose oral studies of chlordecone toxicity in experimental animals. The primary noncancer health effects of occupational exposure to chlordecone in humans and oral exposure in animals include liver lesions, kidney effects (only in animals), neurotoxicity, and male reproductive toxicity. Other reproductive effects (i.e., persistent vaginal estrus and impaired reproductive sucess) and developmental effects also occur; however, the doses required to elicit these effects were generally higher than those that resulted in other key effects.

Table 4-18. Summary of noncancer results of repeat-dose studies for oral exposure of experimental animals to chlordecone

Species	Sex	Average daily dose (mg/kg-day)	Exposure duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Responses	Comments	Reference
Rat	M	0, 0.07	21 months	ND[a]	ND	Liver and thyroid histopathology	No statistically significant increase in incidence due to small number of animals tested and changes in controls	Chu et al., 1981a
Mouse	M	0, 10, 25, 50	24 days	ND	10 (FEL[b])	Mortality and neurotoxicity	Frank effect levels for hyperexcitability, motor incoordination, and tremor	Huang et al., 1980; Fujimori et al., 1982b
Rat	M F	0, 0.6, 1.7 0, 1.4, 2.0	20 months	ND ND	0.6 1.4	Liver histopathology, neurotoxicity	Hyperplasia and tremors; kidney inflammation observed at higher doses	NCI, 1976a
Mouse	M F	0, 3.4, 3.9 0, 3.5, 7.0	20 months	ND ND	3.4 3.5	Liver histopathology, neurotoxicity	Hyperplasia and tremors	NCI, 1976a
Rat	M/F	0, 0.3, 0.5, 1.6, 3.9, 7.0	13 weeks	0.5	1.6	Reproductive toxicity	Testicular atrophy in a subset of animals from the 2-year study	Larson et al., 1979a
Rat	M/F	0, 0.06, 0.3, 0.5, 1.6, 3.9, 7.0	2 years	0.06	0.3	Kidney histopathology	Glomerulosclerosis; higher doses cause fatty changes, hyperplasia in the liver, and tremors	Larson et al., 1979a
Dog	M/F	0, 0.02, 0.1, 0.5	128 weeks	0.1	0.5	Decreased body weight; organ to body weight changes	Magnitude of body weight reduction not reported; small number of animals detract from reliability of study	Larson et al., 1979a
Mouse	M/F	0, 1.9, 5.6, 7.0	1 month prior to mating, 100 days after pairing	1.9	5.6	Reproductive toxicity	Decrease in the number of pairs producing a second litter; persistent vaginal estrus	Huber, 1965
Mouse	M/F	0, 0.94	1 month prior to mating, 5 months after pairing	ND	0.94	Reproductive toxicity	Decrease in the number of pairs producing a second litter	Good et al., 1965

Table 4-18. Summary of noncancer results of repeat-dose studies for oral exposure of experimental animals to chlordecone

Species	Sex	Average daily dose (mg/kg-day)	Exposure duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Responses	Comments	Reference
Rat	F	0, 0.07, 0.4	60 days prior to mating and throughout gestation and lactation	ND	ND	Developmental toxicity	No neurobehavioral effects; no change in pup body weight at 1, 7, 14, and 30 days of age; decreased pup body weight at 100 days	Squibb and Tilson, 1982
Rat	M F	0, 1.4 0, 1.7	3 months	1.4 ND	ND 1.7	Reproductive toxicity	Impaired reproductive success in females; tremors, liver, and adrenal lesions	Cannon and Kimbrough, 1979
Mouse	F	0, 2, 4, 8	4 weeks	ND	2	Reproductive toxicity	Persistent vaginal estrus; higher doses adversely affect follicle size and condition	Swartz et al., 1988; Swartz and Mall, 1989
Rat	F	0, 15	Days 14–20 of gestation	ND	15	Reproductive and developmental toxicity	Persistent vaginal estrus in offspring, decreased ovary weight, increased adrenal weight	Gellert and Wilson, 1979
Rat	M	0, 0.26, 0.83, 1.67	90 days	0.26	0.83	Sperm parameters, neurotoxicity	Decreased sperm motility and viability, hyperexcitability, and mild tremors	Linder et al., 1983
Rat	M	0, 0.625, 1.25, 2.5, 5, 10	10 days	ND	0.625	Reproductive toxicity	Decreased sperm concentration	U.S. EPA, 1986c
Rat	F	0, 2, 6, and 10	Days 7–16 of gestation	2	6	Developmental toxicity	Fetotoxicity (decreased fetal body weight); maternal toxicity at lower doses	Chernoff and Rogers, 1976
Mouse	F	0, 2, 4, 8, and 12	Days 7–16 of gestation	8	12	Developmental toxicity	Fetotoxicity (fetal mortality); maternal toxicity at lower doses	Chernoff and Rogers, 1976

[a]ND = not determined.
[b]FEL = frank effect level.

4.6.1. Oral Exposure

Liver enlargement developed in 20 out of 32 workers exposed to high levels of chlordecone for an intermediate to chronic exposure duration; however, evidence of significant liver toxicity was not found (Guzelian, 1982a; Guzelian et al., 1980; Taylor et al., 1978). Normal results were obtained for serum biochemistry, and liver biopsy samples showed histological changes in the liver that were characterized as nonadverse in nature (see Section 4.1). Histological changes included proliferation of the SER and cytoplasmic accumulation of lipofuscin. No evidence of fibrosis, cholestasis, or hepatocellular necrosis was found; however, the exposure duration and latency period before examination were relatively short.

Histological changes in the liver have also been demonstrated in laboratory animals. These effects include increased liver size and weight, hepatocellular hypertrophy, proliferation of the SER, increased microsomal protein, CYP450 content, cytochrome c reductase activity, and microsomal enzyme activity (see Section 3.3) (Gilroy et al., 1994; Hewitt et al., 1985; Mehendale et al., 1978, 1977). Histopathological evidence of hepatotoxicity was also demonstrated in animals following chronic exposure to chlordecone. The liver lesions observed in male and female rats given chlordecone doses of 3 and 9 mg/kg biweekly (s.c.) for 27 weeks (average daily doses of 0.86 and 2.6) included hepatocellular hypertrophy, congestion, mild fatty change, focal necrosis, and occasional small nests of proliferated sinusoidal cells (Sirica et al., 1989). Fatty changes and hyperplasia were also observed in rats given doses greater than 0.5 mg/kg-day for up to 2 years (Larson et al., 1979a).

Kidney toxicity was reported in laboratory animals, but was not observed in occupationally exposed pesticide workers (Taylor, 1985, 1982; Guzelian, 1982a,b; Guzelian et al., 1980; Sanborn et al., 1979; Cannon et al., 1978; Martinez et al., 1978; Taylor et al., 1978). It is possible that the clinical signs of glomerulosclerosis (including proteinuria) were not observed in occupationally exposed pesticide workers because of the relatively short exposure duration (average exposure duration was 5–6 months), which may not be a sufficient duration for the development of more obvious renal disease (nephropathy and frank proteinuria). It is unclear whether clinical tests sufficient to detect glomerular damage were performed on the exposed workers. Furthermore, a definitive diagnosis of glomerulosclerosis can only be diagnosed through a kidney biopsy, which was not performed on any occupationally exposed worker. Larson et al. (1979a) identified a chronic LOAEL of 0.3 mg/kg-day for proteinuria and increased incidence of glomerulosclerosis in female Wistar rats with a corresponding NOAEL of 0.06 mg/kg-day. Renal effects were also reported in other studies at higher dose levels. NCI (1976b) included summary tables in which chronic kidney inflammation in male Osborne-Mendel rats (at 0.6 mg/kg-day) and female Osborne-Mendel rats (at 2.0 mg/kg-day) was reported. Chu et al. (1980) reported that 28 days of dietary exposure to chlordecone (at 0.07 mg/kg-day) produced eosinophilic inclusions in proximal tubules in 2/10 male Sprague-Dawley rats.

Neurological symptoms, including tremor, headache, and irritability, were reported in workers exposed to high doses of chlordecone for a period of months to years (see Section 4.1) (ATSDR, 1995; Taylor, 1985, 1982; Guzelian, 1982a; Guzelian et al., 1980; Sanborn et al., 1979; Cannon et al., 1978; Martinez et al., 1978; Taylor et al., 1978). Nearly half (7/16) of the workers reported persistent symptoms (e.g., tremor, nervousness) 5 to 7 years later (Taylor, 1985). In laboratory animals, chlordecone has been shown to cause tremors, decreased motor coordination, hyperexcitability, and an exaggerated startle response (Linder et al., 1983; Huang et al., 1980; Larson et al., 1979a; NCI, 1976a). The hypothesized mode of action for neurotoxicity relates to alteration in membrane transport proteins and disruption of calcium homeostasis (see Section 4.4.3). In the chronic rat study by Larson et al. (1979a), liver lesions were observed at slightly lower doses ($\geq$0.5 mg/kg-day) than those resulting in clinically observable tremors ($\geq$1.6 mg/kg-day); however, hyperexcitability and mild tremors were observed in a subchronic dietary study in rats at doses as low as 0.83 mg/kg-day (Linder et al., 1983).

Chlordecone exposure in humans caused oligospermia, reduced sperm motility, and decreased libido in a group of men who were occupationally exposed to chlordecone for periods up to 1.5 years (see Section 4.1) (Taylor, 1985, 1982; Guzelian, 1982a; Taylor et al., 1978). There was no evidence that the ability of these workers to father children was affected and male reproductive parameters had returned to normal by 5 to 7 years following the cessation of chlordecone exposure and treatment with cholestyramine to reduce chlordecone blood levels (Taylor, 1982). Even though 2 of 7 workers sired children, there is no indication of the true denominator of how many were trying to conceive and/or the fertility rate. Male reproductive toxicity has also been observed in laboratory animal studies (Linder et al., 1983; Larson et al., 1979a). Sperm parameters were altered by chlordecone in a subchronic dietary study (Linder et al., 1983). Sperm viability, motility, and reserves in the right cauda epididymis were significantly reduced at doses of 0.83 and 1.67 mg/kg-day but not at 0.26 mg/kg-day. The sperm parameters evaluated appear to be a precursor effect in this study, because neither sperm morphology nor sperm count in the epididymal fluid was affected at any dose. In addition, reproductive performance (determined by number of pregnant females, number of live litters, average live litter size, number of implants, percentage resorptions and fetal weight) was similar across exposed and control groups. No gross or microscopic pathology of the male reproductive system was found that could be attributed to chlordecone treatment, and recovery from the reported sperm alterations was apparent 4.5 months following cessation of exposure. Decreased sperm concentration was observed in rats exposed to chlordecone doses $\geq$0.625 mg/kg-day for 10 days (U.S. EPA, 1986c). Testicular atrophy was observed in rats at doses $\geq$1.6 mg/kg-day for 13 weeks (Larson et al., 1979a).

No information is available concerning chlordecone-induced reproductive effects in women. Impaired reproductive success was, however, observed in mice and rats exposed to

chlordecone at doses of ≥1 mg/kg-day (see Section 4.3.1) (Cannon and Kimbrough, 1979; Good et al., 1965; Huber, 1965). The mechanism responsible for impaired reproductive success is unknown; however, chlordecone has been demonstrated to affect estrous cyclicity in female mice (Swartz and Mall, 1989; Swartz et al., 1988; Huber, 1965). The doses required to induce persistent vaginal estrus in mice were higher than the doses reported to alter sperm parameters in male rats. Huber (1965) demonstrated that persistent vaginal estrus occurs within 8 weeks of chlordecone treatment at doses of ≥5.6 mg/kg-day. Similar effects on estrous cyclicity were noted by Swartz and Mall (1989) and Swartz et al. (1988) within 2 weeks of chlordecone administration at dose levels of 2, 4, and 8 mg/kg-day. After 4 and 6 weeks of treatment, ovulation was reduced in the highest chlordecone treatment group (8 mg/kg-day), which resulted in statistically significantly lower numbers of ovulated oocytes relative to vehicle controls (Swartz et al., 1988). Persistent vaginal estrus was also observed in offspring of female rats given 15 mg/kg-day chlordecone by gavage on gestational days 14–20 (Gellert and Wilson, 1979). Female offspring also exhibited significantly decreased ovarian weight, significantly increased adrenal weight (relative to vehicle controls), and a decrease in the number of animals ovulating.

No information is available concerning developmental effects of chlordecone exposure in humans. Laboratory animal studies demonstrated developmental toxicity in rats and mice at dose levels that also produced maternal toxicity (Chernoff and Rogers, 1976). Chernoff and Rogers (1976) demonstrated that chlordecone administration via gavage during days 7 to 16 of gestation induced maternal toxicity in mice and rats at doses ≥2 mg/kg-day, while fetotoxicity did not occur until doses of ≥6 mg/kg-day in rats and ≥12 mg/kg-day in mice. Maternal toxicity was evidenced by decreased body weight and increased liver to body weight ratios. Fetotoxicity in rats was observed as significantly depressed fetal body weight and delayed ossification in 6 and 10 mg/kg-day dose groups and significantly increased incidences of fetuses with enlarged renal pelvis, edema, undescended testes, or enlarged cerebral ventricles in the 10 mg/kg-day group relative to controls. Signs of fetotoxicity in mice were observed only in the highest dose group and consisted of significantly increased fetal mortality.

The mode of action of chlordecone-induced toxicity is not completely understood. However, limited evidence suggests that chlordecone may interact with cell membranes and affect the membrane transport proteins (e.g., Mg^{2+}-ATPase, Ca^{2+}-ATPase) that are responsible for cellular homeostasis and energetics. Disruption of cellular homeostasis and energy production within the cell leads to impaired cellular function. In the central nervous system, altered calcium homeostasis leads to changes in neurotransmitter activity (e.g., alpha-noradrenergic, dopaminergic, and serotonergic systems) that may be related to chlordecone-induced tremor and exaggerated startle response (Vaccari and Saba, 1995; Brown et al., 1991; Herr et al., 1987; Tilson et al., 1986; Uphouse and Eckols, 1986; Chen et al., 1985; Desaiah, 1985; Gerhart et al., 1985, 1983, 1982; Hong et al., 1984; Fujimori et al., 1982b; Squibb and

Tilson, 1982; Hwang and Van Woert, 1979). In the liver, membrane perturbation and inhibition of the active transport of glutamate at the bile canalicular membrane is thought to be related to chlordecone-induced hepatobiliary dysfunction (Teo and Vore, 1991). Chlordecone also inhibits oligomycin-sensitive Mg^{2+}-ATPase activity in the rat bile canaliculi-enriched fraction of the liver (Curtis, 1988). Treatment of rats with chlordecone and CCl_4 caused a decrease in liver ATP levels and an inhibition of oligomycin-sensitive Mg^{2+}-ATPase (Kodavanti et al., 1990). Chlordecone alters calcium homeostasis in hepatocytes, leading to a decline in glycogen storage and a reduced energy status (Kodavanti et al., 1993, 1990). Chlordecone-CCl_4 administration caused an inhibition in microsomal and mitochondrial calcium uptake and a decrease in the high affinity component of hepatic plasma membrane Ca^{2+}-ATPase.

An estrogenic mode of action is generally considered to be involved in the reproductive toxicity of chlordecone. Testicular atrophy, altered sperm characteristics, persistent vaginal estrus, and anovulation observed in chlordecone-treated laboratory animals (Swartz et al., 1988; U.S. EPA, 1986c; Linder et al., 1983; Larson et al., 1979a; Huber, 1965) mimic the effects produced by excessive estrogen. Mechanistic studies demonstrate that chlordecone binds to the estrogen receptor, as well as other endocrine receptors (see Section 4.4.4).

4.6.2. Mode-of-Action Information—Glomerular Lesions

The mechanism by which chronic dietary chlordecone exposure in rats results in glomerular lesions is unclear. Larson et al., 1979a observed a significant, dose related increase in the incidence and severity of renal lesions in female Wistar rats in the 0.3, 0.5, and 1.6 mg/kg-day dose groups. An apparent increase in proteinuria, a clinical sign of glomerular damage, was also observed in female rats, starting at 0.3 mg/kg-day (see also Section 4.2.2.1).

The Larson (1979a) study itself does not inform the potential mode of action of the observed glomerular lesions; however, there are some data to suggest that the effect may be mediated through an autoimmune mechanism. Glomerular damage is often, though not exclusively, mediated through immune mechanisms (U.S. DHHS, 2006). Some evidence (Sobel et al., 2006, 2005) suggests that chlordecone may accelerate glomerular lesions in susceptible animals by way of increased deposition of immune complexes in the glomeruli (see Section 4.4.5). In similar treatment protocols Sobel et al. (2006, 2005) implanted female (NZB × NZW)F_1 mice with sustained-release pellets containing 0.001, 0.01, 0.1, 0.5, 1, or 5 mg chlordecone subcutaneously above the shoulders. Ovary intact mice treated with either 1 mg or 5 mg chlordecone pellets developed anti-dsDNA and antichromatin autoantibody titers significantly earlier than controls. Additionally, immunohistofluorescence analysis of renal sections from a subset of animals treated for 8 weeks with 1 mg chlordecone showed enhanced deposits of IgG immune complexes as compared with untreated controls. The histopathology associated with renal disease was similar between chlordecone-treated mice and controls.

An alternate theory holds that chlordecone damages the glomeruli directly. Chlordecone predominantly binds plasma proteins and lipoproteins (especially albumin and HDL); this binding has been demonstrated in exposed workers and in animal models (Soine et al., 1982; Skalsky et al., 1979). The glomeruli are the functional units of the kidney that are predominantly responsible for filtering high molecular weight proteins, including albumin, from the blood (Hart and Kinter, 2005). Therefore, this region of the kidney may be subjected to relatively high concentrations of chlordecone that could potentially result in direct chemical insult. Distribution studies of chlordecone in experimental animals (see Section 3.2) have indicated that chlordecone, by various routes of exposure, predominantly localizes in the liver but is also distributed to the kidneys (Belfiore et al., 2007; Heatherington et al., 1998; Hewitt et al., 1985; Kavlock et al., 1980). A dermal study of chlordecone organ distribution found that kidney concentration was second only to liver concentration (Heatherington et al., 1998).

Uncertainty surrounds the two proposed mechanisms for the observed glomerular damage following chlordecone exposure. It is conceivable that chlordecone may not *cause* glomerular damage per se but may accelerate or increase the severity of the disease in animals with preexisting susceptibility to glomerular damage. For example, though a significant dose-response relationship was seen in the principal study between glomerulosclerosis and increasing doses of chlordecone, the control animals also exhibited a background incidence of glomerular lesions, which was particularly high in the male rats (12% incidence in females and 55% in males). In addition, Sobel et al. (2006, 2005) indicated that chlordecone exposure increased the severity and accelerated the development of renal damage and autoantibodies in a susceptible mouse strain, (NZB × NZW)F_1. However, a follow-up experiment by Sobel et al. (2006) treated BALB/c mice, a strain in which spontaneous development of glomerular damage is rare, and found that treatment of these mice with chlordecone for up to 1 year did not produce elevated autoantibody titers or renal disease. Arguably, the two strains tested by Sobel et al. (2006) represent two extremes in genetic propensity to autoimmunity in rodents and are not representative of the genetic heterogeneity of human populations regarding autoimmune susceptibility.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight of Evidence

Under the U.S. EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), there is suggestive evidence of carcinogenic potential in humans based on data from an oral cancer bioassay in rats and mice demonstrating an increase in the incidence of hepatocellular carcinomas in both sexes of both species (NCI, 1976 a,b). This characterization lies at the high end of the continuum for this weight of evidence descriptor. NCI (1976 a,b) demonstrated a statistically significant increase in hepatocellular carcinomas in both sexes of mice. Male and

female rats exhibited increased incidences of hepatocellular carcinomas at high doses that were statistically significant when compared with pooled controls. The incidence of hepatocellular carcinomas was not statistically significant in comparison with matched controls for rats of either sex. The tumor response was particularly robust in male and female mice at the highest doses (Table 4-1). NCI (1976 a,b) also demonstrated a decrease in the time to tumor in both sexes of both species. There are some limitations associated with the design and conduct of the only cancer bioassay for chlordecone (NCI, 1976 a,b) which create uncertainty in the characterization of the overall weight of the evidence for the carcinogenic potential of chlordecone in humans. The major limitations in the NCI (1976 a,b) study are described below and in Section 4.2.2.1. Issues related to the study design and conduct make it difficult to draw a confident conclusion regarding human carcinogenic potential. It should be noted that mirex, a structurally similar chemical, also induces hepatocellular adenomas or carcinomas in both sexes of rats and mice. This weight of evidence conclusion collectively takes into consideration the NCI (1976a,b) cancer bioassay and its limitations, the available human studies, and other chronic animal bioassays.

There are no studies in humans that adequately assess the carcinogenic potential of chlordecone. NCI (1976a,b) reported a statistically significant increased incidence of liver tumors (hepatocellular carcinomas) in rats and mice, following dietary exposure to chlordecone for 20 months. A strong liver tumor response was seen in both sexes of mice and female rats, with a weak response noted among male rats. No other tumor types were significantly increased in either rats or mice in this study. The NCI (1976a,b) study had several limitations in study design, conduct, and reporting. One major issue included early mortality and toxicity, indicating initial use of excessively high doses. Due to the initial toxicity and mortality, the study authors adjusted dose levels to one-half to one-sixth of the initial dose levels. The adjustment in dosing during the study led to inconsistent exposure concentrations and difficulty in determining time-weighted-average daily dosage. In addition, some of the surviving male rats in the discontinued high-dose group were moved into the next lower dose group at 6 weeks, resulting in a lack of homogeneity in this dose group. Decreased survival was also seen in treated groups when compared with matched controls for all dose groups except female mice (see Table 4.4). The authors did not explain whether decreased survival was secondary to toxic or carcinogenic effects. Survival to study termination for male rats in the low- and high-dose groups was decreased 33 and 53% as compared with matched controls. However, liver tumor incidence was low in these groups (1/50 and 3/44, respectively), indicating that decreased survival in male rats was not due to carcinogenic effects. Further limitations of the NCI (1976a,b) study included incomplete reporting in the published study. For example, about 10% of rats in the high-dose groups of both sexes are unaccounted for by the authors. An additional study limitation includes the use of only two dose groups, making characterization of a dose-response relationship difficult.

Similarities in the tumor profile of chlordecone and mirex, a structurally related chemical, have been observed in animals. A statistically significantly increased incidence of liver tumors in F344/N and CD rats and B6C3F1 and B6AKF1 mice has been observed following chronic oral exposure to mirex at similar dose levels as chlordecone. The liver tumors resulting from exposure to mirex, similar to exposure to chlordecone, are described as predominantly benign, well-differentiated masses without vascular invasion or metastases (PWG, 1992; NTP, 1990; Ulland et al., 1977; NCI, 1976a,b; Innes et al., 1969). Mirex and chlordecone also produce noncancer effects in the liver at similar doses. It should be noted that, though chlordecone and mirex appear to have closely related biological activity and carcinogenicity in the liver at similar dose levels (though the mode of action for each is unknown), several noncancer effects reported following exposure to mirex and chlordecone are dissimilar. For example, the characteristic neurotoxicity observed following exposure to chlordecone has not been described for mirex.

The mode of carcinogenic action of chlordecone in the livers of rats and mice is unknown. Most genotoxicity tests for chlordecone are negative. For the liver tumors in rats and mice, some data suggest that chlordecone may induce cell proliferation and lead to a promotion in the growth of preinitiated cells. However, key precursor events linked to observed cell proliferation have not been identified. The liver tumors observed in the NCI cancer bioassay in rats and mice were considered relevant to the assessment of the carcinogenic potential of chlordecone.

No animal cancer bioassay data following inhalation exposure to chlordecone are available. However, EPA's *Guidelines for Carcinogen Risk Assessment* (2005a) indicate that, for tumors occurring at a site other than the initial point of contact, the carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. Thus, for chlordecone, there is *suggestive* evidence of carcinogenic potential by any route of exposure.

This is the first IRIS assessment for chlordecone. Therefore, no previous characterization of cancer potential or quantitative cancer evaluation exists.

4.7.2. Synthesis of Human, Animal, and Other Supporting Evidence

Few studies are available that directly assess the carcinogenic potential of chlordecone. Limited data on the carcinogenic potential in humans can be garnered from observational studies of a single group of 133 workers occupationally exposed to chlordecone at a chlordecone manufacturing plant in Hopewell, Virginia, in the late 1970s (Taylor, 1985, 1982; Guzelian, 1982a; Guzelian et al., 1980; Sanborn et al., 1979; Cannon et al., 1978; Martinez et al., 1978; Taylor et al., 1978). A subset of 32 of these workers with clinical signs or symptoms of chlordecone toxicity and high chlordecone blood levels (>0.6 μg/mL at the time of diagnosis) were examined specifically for hepatotoxicity (Guzelian et al., 1980). Hepatomegaly was

observed in 20 of 30 of these workers. However, liver biopsy samples taken from 12 of these workers showed no evidence of liver neoplasia (Guzelian, 1982a; Guzelian et al., 1980). The average exposure duration of these subjects was 5–6 months, and they were physically examined for this study within 10 months of exposure cessation. Upon follow-up of the exposed workers 2–3 years after exposure cessation, hepatomegaly had resolved in all workers and biopsies were negative for abnormal histopathological findings (Guzelian et al., 1980). Conclusions regarding cancer from this study are limited by the small number of workers examined, uncertainties concerning exposure dose and route, the relatively brief duration of exposures, and the absence of a sufficient latency period for tumor development.

Occupational exposures to chlordecone also provide evidence for the preferential accumulation of chlordecone in the liver. For example, in 32 workers exposed to chlordecone for a period that ranged from 3 to 16 months, high concentrations of chlordecone were found in blood, liver, and subcutaneous fat (Cohn et al., 1978). The ratio of the chlordecone concentration in fat as compared to the chlordecone concentration in the blood was 7:1, which is relatively low for a lipophilic organochlorine pesticide. However, the liver to blood concentration ratio in exposed workers was reported to be 15:1 (Table 3-2). Chlordecone has also been shown to bind plasma proteins and lipoproteins and preferentially accumulate in the liver, where it is slowly eliminated, in experimental animals and exposed workers (Cohn et al., 1978; Egle et al., 1978). Thus, due to the preferential accumulation of chlordecone in the liver, humans may be susceptible to chlordecone-induced liver toxicity.

The human case reports and clinical observations of occupational chlordecone exposure lack sufficient design, power, and follow-up to determine carcinogenic potential of chlordecone in humans; however, the observations from these studies provide valuable information on human susceptibility to chlordecone. A review of biological and epidemiological evidence of cancer found no population-based studies on cancer in humans related to chlordecone exposure (Ahlborg et al., 1995).

Animal studies provide suggestive evidence for the carcinogenic potential of chlordecone. Chlordecone has been shown to induce liver tumors in Osborne-Mendel rats and B6C3F1 mice in a single study performed by the National Cancer Institute (NCI, 1976a,b). B6C3F1 mice (50/sex/group) and Osborne-Mendel rats (50/sex/group) were exposed to chlordecone in the diet for 20 months. Dietary concentrations of chlordecone began at 0, 15, 30, or 60 ppm for male rats and 0, 30, or 60 ppm for female rats. In mice, dietary concentrations of chlordecone began at 0 or 40 ppm (two groups at this concentration) for males and 0, 40 or 80 ppm for females. During the course of the study, concentrations were reduced at least once in each treatment group due to toxicity (see Figures 4-1 to 4-4). Time-weighted-average dietary concentrations were 0, 8, or 24 ppm (0, 0.6, or 1.7 mg/kg-day) for male rats and 0, 18, or 26 ppm (0, 1.4, or 2.0 mg/kg-day) for female rats. In mice, time-weighted-average dietary concentrations were 0, 20, or 23 ppm (0, 3.4, or 3.9 mg/kg-day) for male mice and 0, 20, or 40

ppm (0, 3.5, or 7.0 mg/kg-day) for female mice. Liver tumors described as hepatocellular carcinomas were observed in high-dose female rats at an incidence that was significantly elevated compared with the pooled control incidence (0/100, 0/10, 1/49, and 10/45 in the pooled control, matched control, and low-dose and high-dose groups, respectively). Incidences of male rats with hepatocellular carcinomas were lower at 0/105, 0/10, 1/50, and 3/44, respectively. The incidence of carcinomas in high-dose males was significant ($p = 0.049$) in comparison with pooled controls. The incidence of hepatocellular carcinomas was not statistically significant in comparison with matched controls for rats of either sex. A significant dose-response trend was observed for the incidence of hepatocellular carcinoma in both male and female rats (Cochran-Armitage test conducted for this review). In mice, statistically significant elevated incidences of hepatocellular carcinomas were found in both exposed groups compared with matched and pooled control incidences (NCI, 1976a). Incidences for matched control, low-, and high-dose groups were 6/19, 39/48, and 43/49 for male mice and 0/10, 26/50, and 23/49 for female mice. No other tumor types in rats or mice were found to be significantly elevated in this study.

Decreases in survival rates and decreased body weight gain were observed in all animal groups except the low- and high-dose female mice (see Table 4.4). A robust liver tumor incidence of 26/50 (52%) was observed in the low-dose group (3.5 mg/kg-day) of female mice, a group that had survival rates and body weight gains that were comparable with controls. While is it is true that high toxicity was observed in the high-dose groups (specifically of male rats and mice), the conclusion that high toxicity is required for tumor induction may not be warranted.

Significant limitations in the study design and outcome with respect to toxicity and mortality exist for the NCI (1976a,b) cancer bioassay. The primary limitation of the NCI (1976a,b) bioassay relates to the dose selection. The initial dietary concentrations in the high-dose groups were excessively high and induced high mortality, tremors, anemia, and dermatitis in both sexes of both species. During the course of the study, concentrations were reduced at least once in each treatment group due to toxicity (see Figures 4-1 to 4-4). In both male rats and mice, the initial high-dose group was discontinued due to excessive toxicity and mortality (animals were sacrificed). Issues related to the dosing regimen of this study make it unsuitable for quantification of cancer risk. Because of changes in chlordecone dietary exposure levels, the dose metric related to the development of liver tumors cannot be determined. The study reports time-weighted-average dietary concentrations for chlordecone in rats and mice; however, the tumorigenic effects observed may not occur following chronic exposure to these lower average concentrations. For example, it is not known whether the high initial dietary concentrations caused significant early liver injury resulting in the subsequent development of the observed liver tumors.

Conclusions from cancer bioassays utilizing potentially excessive doses are regarded with caution for several reasons. Doses of an agent that cause high toxicity to the animals may result in early deaths directly resulting from toxicity, which could decrease the ability of the assay to

detect tumor effects. Animal mortality in the NCI (1976a,b) study was high in comparison to controls; however, this did not prevent the detection of elevated rates of hepatocellular carcinoma in the high-dose groups. Alternately, there is concern that high doses may result in tumor effects that are secondary to toxic effects (e.g., cytotoxicity) or altered toxicokinetics (U.S. EPA, 2005a). It is possible that high doses of chlordecone used in the NCI study resulted in tumor effects that were secondary to liver cytotoxicity and thus would not be likely at low doses. However, there is not sufficient data to support this mode of action. In the absence of data that indicate that direct liver cytotoxicity at high doses precedes tumor development, the increased incidence of liver tumors observed in the NCI cancer bioassay cannot be completely discounted. There are no data to support the concern that elevated levels of hepatocellular carcinoma detected by the NCI study in rats and mice are the direct result of altered toxicokinetics from excessive chlordecone levels. In fact, animal data support the conclusion that the liver is especially sensitive to chlordecone-induced lesions even at very low doses that do not result in overt toxicity to the animal or decreased survival (Chu et al., 1981a; Larson et al., 1979a). Additionally, chlordecone has been demonstrated in humans and animals to preferentially accumulate in the liver (Cohen et al., 1987; Hewitt et al., 1985; Egle et al., 1978). Therefore it is not likely that liver tumors arising after high exposures to chlordecone are due to altered toxicokinetics.

Besides the NCI (1976a,b) cancer bioassay, Larson et al. (1979a) and Chu et al. (1981a) are the only additional chronic dietary studies of chlordecone exposure in animals. Larson et al. (1979a) fed groups of Wistar rats (40/sex/group) diets estimated to result in dose levels of 0, 0.06, 0.3, 0.5, 1.6, 3.9, or 7.0 mg/kg-day for up to 2 years. Increased incidence of liver lesions (characterized as fatty changes and hyperplasia) were seen in females at 0.5 mg/kg-day and in males at 1.6 mg/kg-day. Liver lesions in three females in the 0.5 mg/kg-day group and one female and two males in the 1.6 mg/kg-day group were described by the authors as being possibly carcinomatous in nature, though the authors reported that an independent review by four pathologists was equivocal. Therefore, this study can only be considered to provide a lack of positive evidence for chlordecone carcinogenicity in Wistar rats. However, it should be noted that very few animals were available for pathological examination at the end of the study, limiting the study's power to detect carcinogenic effects.

A chronic dietary exposure study by Chu et al. (1981a) detected an apparent increase in liver lesions in rats in the single chlordecone exposure group (5/6 compared to 3/7) of 0.07 mg/kg-day but did not report tumors. However, the very small number of animals and use of only a single low-dose group severely limit this study's power to assess carcinogenic potential. Additionally, neither toxicity nor changes in body weight gain were observed in the dose tested. Therefore, the dose utilized cannot be considered adequately high to detect carcinogenic potential for chlordecone.

The structurally related chemical mirex has been shown to induce liver tumors in both sexes of rats and mice in chronic feeding studies at similar dose levels as chlordecone. Incidences of liver tumors in chlordecone-treated male and female rats and mice were found to be significantly elevated at 1.7, 2, 3.4, and 3.5 mg/kg-day. Increased incidence of liver tumors (adenomas or carcinomas) with chronic mirex exposure has been shown in rats and mice at 3.8 and 7 mg/kg-day (PWG, 1992; NTP, 1990; Ulland et al., 1977; Innes et al., 1969). Liver tumors resulting from mirex and chlordecone exposure are generally described as benign, well-differentiated masses without vascular invasion or metastases (PWG, 1992; NTP, 1990; Ulland et al., 1977; NCI, 1976a,b; Innes et al., 1969). The available studies on mirex or chlordecone classified liver tumors as either neoplastic nodules or hepatocellular carcinoma (Ulland et al., 1977; NCI, 1976a,b). In vivo and in vitro genotoxicity studies for mirex and chlordecone were generally negative. However, the available evidence for chlordecone and mirex is inadequate to clearly establish a mode of action by which these chemicals induce liver tumors in rats and mice. Chlordecone and mirex exposure in experimental animals results in similar noncancerous liver lesions that may be precursor lesions to the development of liver tumors. Liver lesions common to mirex and chlordecone include hypertrophy, hyperplasia, fatty changes, cytoplasmic vacuolation, and anisokaryosis (NTP, 1990; Chu et al., 1981b,c; Larson et al., 1979a,b; NCI, 1976a,b). However, though chlordecone and mirex appear to have related biological activity and carcinogenicity in the liver, this evidence is limited by the observation of several dissimilar noncancer effects.

In summary, under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is *suggestive* evidence of carcinogenic potential for chlordecone in humans. This is based primarily on a statistically significant increase in liver tumors in both sexes of mice in response to oral chlordecone exposure in a cancer bioassay by the National Cancer Institute (NCI, 1976a,b). Male and female rats exhibited increased incidences of hepatocellular carcinomas at high doses that were statistically significant when compared with pooled controls. The incidence of hepatocellular carcinomas was not statistically significant in comparison with matched controls for rats of either sex. Uncertainty exists due to limitations in the NCI study related to design and conduct (as reviewed above and in Section 4.2.2.1.).

4.7.3. Mode-of-Action Information

The majority of studies on chlordecone were negative for genotoxic activity in a variety of short-term in vitro and in vivo assays (see Section 4.4.5). One hypothosis for the mode of action of chlordecone induced tumorigenicity is sustained proliferation of spontaneously transformed liver cells, resulting in the eventual formation of liver tumors. Proliferative liver lesions (hyperplasia) were found in a chronic dietary study in Wistar rats at doses greater than 0.5 mg/kg-day in females and 1.6 mg/kg-day in males (Larson et al., 1979). Additionally, the NCI (1976a,b) chronic dietary cancer bioassay that reported increased incidences of liver tumors

in both sexes of rats and mice also noted extensive liver hyperplasia in both sexes of both species. Though the incidence of hyperplasia was not noted in the study, the authors reported that the incidence of hyperplasia in the matched control mice was low as compared to the treated groups. In rats, the authors reported that no liver hyperplasia was seen in the matched controls. Chlordecone was demonstrated to be a liver tumor promoter, rather than an initiator or a complete hepatic carcinogen, in a two-stage tumor promotion assay in male and female Sprague-Dawley rats (Sirica et al., 1989). This study also demonstrated a greater tumor response in female rats, suggesting that hormonal involvement may be important in the promotion of chlordecone-induced liver tumors. The NCI (1976a,b) study provides further support for this potential mode of action for chlordecone. Specifically, the authors reported an increased incidence of liver tumors and shorter time to tumor formation in female rats exposed to the high dose compared to male rats exposed to the high dose (NCI, 1976a).

Chlordecone is one of a large number of organochlorine chemicals that produce liver tumors in rodents and do not exhibit genotoxicity in short-term tests. Many of these pesticides (including chlordane, heptachlor, and hexachlorocyclohexane) have been shown to promote liver tumors in rodent livers when administered after an initiating dose of a known carcinogen (Deml and Oesterle, 1987; Williams, 1983; Williams and Numoto, 1984). However, the mode of action by which chlordecone produces liver tumors is unknown.. Precursor events in which chlordecone may promote proliferation of transformed liver cells are uncertain, and data regarding a plausible temporal progression from chlordecone-induced liver lesions to eventual liver tumor formation are not available. Therefore, the available evidence is inadequate to clearly establish a mode of action by which chlordecone induces liver tumors in rats and mice.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

Neurological studies suggest that the immature brain may be sensitive to subtle effects from chlordecone exposure. As reported in Section 4.3, exposure of female rats to chlordecone for 60 days prior to mating through lactation day 12 produced subtle neurological changes in male but not female offspring later in life that suggested an alteration in dopamine sensitivity (Squibb and Tilson, 1982). An abstract by Rosenstein et al. (1977) suggested altered brain activity from gestational and lactational exposure of rats to chlordecone. Brain electrical activity (recorded by EEG) was significantly different in the pups, but not the dams, exposed to chlordecone (maternal dose was 1, 2, or 4 mg/kg-day orally in corn oil) at 24 days postpartum. No additional study details concerning this effect are provided. In a lactation exposure study, Sprague-Dawley rat pups were exposed to chlordecone in milk by treating lactating dams immediately after birth with 0 (corn oil vehicle) or 2.5 mg/kg-day chlordecone by gavage (Jinna et al., 1989). In vitro assays of brain P_2 fractions showed that the exposed pups (through day 20)

exhibited increased activity of Na^+, K^+, and Ca^{++}-ATPase activity. As compared to effective doses in adult rats (8.3 or 10 mg/kg-day orally for 3 days [Kodavanti et al., 1990, and Desaiah et al., 1980, respectively]), the exposure doses expected via lactation are lower, suggesting that the maturing ATPases of neonatal rats may be more sensitive to chlordecone exposure. At the cellular level, Hoskins and Ho (1982) also reported significant differences in calcium content and subcellular distribution in brain in adult (24 weeks old) as compared to young (4–6 weeks old) male ICR mice following acute oral chlordecone exposure (25 mg/kg-day in corn oil).

In summary, some studies have indicated that developing animals may be more susceptible to subtle neurological effects of chlordecone including alterations in dopamine sensitivity, ATP-ase activity, calcium concentration and subcelluar distribution, and EEG activity.

4.8.2. Possible Gender Differences

The extent to which men and women differ in susceptibility to chlordecone toxicity is not known. No human data are available to suggest that there are gender differences in the toxicity or carcinogenicity of chlordecone.

In the NCI (1976a) bioassay of chlordecone carcinogenicity, a strong liver tumor response was seen in female rats, and only a weak response was noted among male rats. Tumors were seen in both genders of mice; however, female mice were more resistant to the lethal effects of chlordecone at high doses. A significant sex difference was noted in the liver tumor promotion response in a two-stage assay of hepatocarcinogenesis (Sirica et al., 1989). Both the median number and the size of the GGT-positive foci were increased in female rats as compared to males following DEN initiation and 27 weeks of chlordecone promotion. In addition, hepatocellular carcinomas were observed in female rats but were not found in male rats given the same initiation-promotion treatment. Similar concentrations of chlordecone were measured in the livers of male and female rats, suggesting that enhancement of the tumor promotion response is due to increased sensitivity of females and not altered pharmacokinetics. It is possible that the estrogenic properties of chlordecone may play a role in the sensitivity of female rats to tumor promotion. Female rats in this study were also more susceptible to decreases in body weight gain, suggesting that enhanced toxicity may play a role in tumor promotion; however, histological examination of noncancerous portions of the liver did not indicate significant gender differences in liver toxicity.

Chlordecone induces reproductive effects in both male and female laboratory animals.However, some evidence exists to suggest that female reproductive toxicity has a larger effect on reproductive success at the same chlordecone dose level. Reproductive toxicity has been demonstrated by altered sperm parameters, testicular atrophy, altered estrous cyclicity, and impaired reproductive success in animals. Although the most sensitive endpoint evaluated appeared to be alterations in sperm parameters induced by subchronic chlordecone exposure in

male rats (Linder et al., 1983), these decreases were observed at doses where reproductive success was unaffected. Effects on the estrous cycle and ovulation are observed at higher doses as compared to sperm effects (Swartz and Mall, 1989; Swartz et al., 1988; Huber, 1965); however, a crossover study in rats that paired control males with treated females and control females with treated males suggests that female reproductive toxicity had a larger effect on reproductive success at the same chlordecone dose level (Cannon and Kimbrough, 1979). In male and female rats fed diets containing 25 ppm chlordecone (1.4 or 1.7mg/kg-day) for 3 months, 12 of 20 pairs of treated males and control females produced offspring, while none of the 20 pairs of treated females and control males produced offspring.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

The only available data concerning health effects of chlordecone in humans are derived from studies of a single group of 133 men exposed occupationally to chlordecone in the late 1970s at a chlordecone manufacturing facility in Hopewell, Virginia (Taylor, 1985, 1982; Guzelian, 1982a; Guzelian et al., 1980; Sanborn et al., 1979; Cannon et al., 1978; Martinez et al., 1978; Taylor et al., 1978). Due to inadequate industrial safety measures at the factory, substantial inhalation, dermal, and oral exposures likely occurred (Cannon et al., 1978). Toxicity observed in the exposed workers included effects on the nervous system, liver, and reproductive system. Of the 133 men, 76 experienced neurological symptoms, especially tremors, nervousness, and headaches, sometimes persisting for as long as 9–10 months after cessation of exposure and the start of treatment (Cannon et al., 1978). In addition, a subset of the men experienced reproductive effects, including oligospermia, reduced sperm motility, and decreased libido (Taylor, 1982). A subset of 32 of the occupationally exposed workers with clinical signs or symptoms of chlordecone toxicity and high chlordecone blood levels (>0.6 μg/mL at the time of diagnosis) were examined specifically for hepatotoxicity (Guzelian et al., 1980). Hepatomegaly was observed in 20 of 32 workers. Minimal elevation (less than two fold) of serum alkaline phosphatase (SALP) was noted in seven patients; however, other liver enzymes were normal including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transpeptidase (GGT) (Guzelian et al., 1980). Sulfobromophthalein retention, a measure of liver clearance, was normal in a subset of 18 workers tested (Guzelian et al., 1980). Upon biopsy of 12 workers with hepatomegaly, histological changes included proliferation of the smooth endoplasmic reticulum (SER) and cytoplasmic accumulation of lipofuscin. These changes in the liver were characterized by the authors as nonadverse in nature and were suggested to be adaptive changes rather than a reflection of hepatotoxicity (Guzelian, 1982a,b; Guzelian et al., 1980; Taylor et al., 1978). Upon follow-up of the exposed workers 2–3 years after exposure cessation, hepatomegaly had resolved in all workers and biopsies were negative for abnormal histopathological findings (Guzelian et al., 1980).

Because of uncertainties regarding exposure routes and exposure levels at the facility, NOAELs or LOAELs could not be established for the observed neurological, liver, and reproductive effects in the occupationally exposed workers. Additionally, workers may have had concomitant exposure to the chemical precursors used to manufacture chlordecone. Because of these major uncertainties, health effects data in these workers are unsuitable for derivation of an RfD.

The toxicity database for oral exposure in laboratory animals includes a few chronic duration studies (Chu et al., 1981a; Larson et al., 1979a, NCI, 1976a) and several subchronic studies (see Section 4.5 and Table 4-18).

Chu et al. (1981a) fed rats (10/group) chlordecone at 0.07 mg/kg-day for 21 months. The authors reported an increase in liver lesions (described as pericentral cytoplasmic vacuolation with mild anisokaryosis) compared to the control group (5/6 compared to 3/7). Chu et al. (1981a) also reported an apparent increase in thyroid lesions (described as mild degenerative and proliferative changes in the epithelium). However, because of small study size and high incidence of effects in the controls, these increases were not statistically significant (Chu et al. 1981a). Thus, due to limited study size, dosing regimen, and high incidence of effects in the control group, this study was not selected as the principal study.

NCI (1976a,b) conducted a 20-month feeding study in B6C3F1 mice and Osborne-Mendel rats. Though treatment groups consisted of 50/sex/group for both rats and mice, only 10 (19 for male mice) matched controls per sex were used. Pooled control groups (from the same laboratory with birth dates within 3–4 months of the treatment groups) contained about 100/sex/group. During the course of the study, toxicity and mortality in the high-dose groups prompted the investigators to reduce dietary chlordecone concentrations to one-half to one-sixth of the previous levels. The resulting time-weighted-average dietary concentrations were 0, 8, or 24 ppm (0, 0.6, or 1.7 mg/kg-day) for male rats and 0, 18, or 26 ppm (0, 1.4, or 2.0 mg/kg-day) for female rats. In mice, time-weighted-average dietary concentrations were 0, 20, or 23 ppm (0, 3.4, or 3.9 mg/kg-day) for male mice and 0, 20, or 40 ppm (0, 3.5, or 7.0 mg/kg-day) for female mice. Noncancer effects reported in response to chlordecone treatment included tremors, dermatologic changes, and liver lesions, though incidences for these effects were not reported. The observed liver lesions were characterized as extensive hyperplasia and atypia in both male and female mice in both dose groups.

This study exhibits several design and conduct issues which limit its interpretation (as reviewed in Section 4.2.2.1). These issues include incomplete reporting (lack of incidence data on observed liver effects in treated animals and controls), inconsistent dose levels (making it difficult to define a dose-response relationship for liver lesions), use of only two dose groups, high early toxicity and mortality in high-dose male animals, decreased 2-year survival in most dose groups, and low numbers of matched controls (10/sex/group). Though the evidence of liver hyperplasia in rats and mice in the NCI (1976a) study provide qualitative information indicating that the liver is a target organ of chlordecone toxicity, for the reasons discussed above, this study is not adequate to support quantitative risk assessment.

Larson et al. (1979a) fed groups of Wistar rats (40/sex/group) diets estimated (based on graphically depicted food consumption and body weight data) to result in dose levels of 0, 0.06, 0.3, 0.5, 1.6, 3.9, or 7.0 mg/kg-day for up to two years. All rats in the highest two dose groups died within the first 6 months. Though the two highest dose groups were uninformative because

of high mortality, four acceptable low-dose exposure groups exist (with adequate numbers of animals). The most sensitive effects observed in this study include kidney lesions in female rats, testicular atrophy in males, and liver lesions in both sexes. The authors reported increased incidence of liver lesions and an increase in relative liver weights in female rats at 0.5 mg/kg-day and male rats at 1.6 mg/kg-day. The liver lesions observed were characterized primarily as fatty changes and hyperplasia.

In addition to liver lesions and testicular effects, Larson et al. (1979a) also observed a significant, dose-related increase in the incidence and severity of renal lesions in female Wistar rats in the 0.3, 0.5, and 1.6 mg/kg-day dose groups. The background incidence of renal lesions in male rats was high (56% as compared to 12% in female rats) and, as such, effects in dosed animals did not achieve statistical significance. An apparent increase in proteinuria, a clinical sign of glomerular damage, was observed in female rats, starting at 0.3 mg/kg-day, though data from individual animals were not reported, precluding statistical analysis for this endpoint. Larson et al. (1979a) identified a LOAEL of 0.3 mg/kg-day for proteinuria and increased incidence of glomerulosclerosis in female Wistar rats with a corresponding NOAEL of 0.06 mg/kg-day.

A supporting study by Sobel et al. (2005) found that chlordecone, at doses estimated to be ≥0.2 mg/kg-day, increased the severity and decreased the latency of glomerular disease in subcutaneously treated mice of a strain known to be susceptible to autoimmunity mediated glomerulonephritis, (NZB × NZW)F_1. Female ovariectomized mice were exposed subcutaneously to sustained-release pellets containing 0.01, 0.1, 0.5, or 1.0 mg chlordecone for up to 30 weeks. Mice treated with 0.5 mg chlordecone pellets (calculated by the authors as an average exposure level of 0.20 mg/kg-day) developed renal impairment (proteinuria and glomerulonephritis) significantly earlier than did ovariectomized controls ($p < 0.05$). Renal sections from the chlordecone-treated mice demonstrated severe proliferative glomerulonephritis with the deposition of immune complexes. A follow-up study by the same group (Sobel et al., 2006), utilizing the same doses and protocol, found that chlordecone treatment of BALB/c mice (a strain not prone to autoimmune disease or glomerular lesions) for up to a year did not produce elevated autoantibody titers or renal disease.

A study by Chetty et al. (1993c) indicates that chlordecone treatment significantly elevates serum indicators of kidney (specifically glomerular) and liver damage in rats treated for 15 days. Male Sprague-Dawley rats (six/group) were treated with 0, 1, 10, 50, or 100 ppm chlordecone in the diet (purity unspecified) for 15 days. Based on the reported average animal weight (175 g) and food intake values (U.S. EPA, 1988) the average doses were calculated as 0.1, 1.0, 4.9, and 9.7 mg/kg-day. After 15 days of chlordecone exposure, serum levels of total protein, urea nitrogen, uric acid, creatinine, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum alkaline phosphatase (SALP), and serum creatine kinase (SCK) were measured. SGPT was elevated at doses starting at 1.0 mg/kg-day,

additionally all other serum enzymes tested were statistically significantly elevated at the highest dose tested (9.7 mg/kg-day). The alterations of serum enzyme levels of SGOT, SGPT, SCK, and SALP suggest chlordecone-induced liver damage. Urea nitrogen was statistically significantly elevated over controls at doses ≥4.9 mg/kg-day. At 9.7 mg/kg-day, urea nitrogen, uric acid and creatinine were statistically significantly elevated. Urea nitrogen is the end product of protein catabolism in most species; >90% is excreted via the kidney, being freely filtered at the glomerulus but also diffusible out of the tubule (Hart and Kinter, 2005). Creatinine is also a compound whose excretion is almost exclusively renal as the consequence of glomerular filtration (Hart and Kinter, 2005). The increased concentrations of these compounds in the serum of chlordecone-treated animals suggest kidney dysfunction. Furthermore, the impaired excretion of creatinine indicates that this dysfunction is most likely glomerular in nature.

Most serum indications of kidney function are insensitive. For example, in order for serum creatinine or blood urea nitrogen concentrations to be detectable, approximately 50% of renal function must be lost (Hart and Kinter, 2005). However, it is unclear whether these elevations in serum proteins and enzymes as seen in Chetty et al. (1993c) were accompanied by pathological lesions, as the scope of the study did not include a histological examination of the liver or kidney.

Renal effects with chlordecone exposure were also reported in other studies. NCI (1976b) reported chronic kidney inflammation in male Osborne-Mendel rats (at 0.6 mg/kg-day) and female Osborne-Mendel rats (at 2.0 mg/kg-day). Chu et al. (1980) reported that 28 days of dietary exposure to chlordecone (at 0.07 mg/kg-day) produced eosinophilic inclusions in proximal tubules in 2/10 male Sprague-Dawley rats. A 32-month oral exposure study in beagles (Larson et al., 1979a) reported increased relative kidney weights in the 0.5 mg/kg-day chlordecone exposure group, though renal histology findings were negative. A 3-month oral study observed increased relative kidney weight in female rats exposed to 1.6–1.7 mg/kg-day, though no histological findings were noted (Cannon and Kimbrough, 1978). Furthermore, Chernoff and Rodgers (1976) reported that gestational exposure to chlordecone (gestation days 7–16) in mice resulted in a statistically significant increase in the incidence of fetuses with enlarged renal pelvis, though this dose was much higher than kidney effects reported in the aforementioned studies (10 mg/kg-day).

Support in the chlordecone database exists for a variety of reproductive effects with chlordecone exposure. Larson et al. (1979a) observed testicular atrophy in male rats treated with chlordecone for 13 weeks at dose levels of ≥1.6 mg/kg-day. The incidence of testicular atrophy at 13 weeks was reported as 1/10, 0/5, 1/5, 4/5, 4/5, and 5/5 at 0, 0.3, 0.5, 1.6, 3.9, and 7.0 mg/kg-day. Other animal studies have also shown male reproductive effects, such as decreased sperm viability, motility, and concentration, following exposure to chlordecone (EPA, 1986c; Linder et al., 1983). However, it is unclear whether these effects on sperm, though statistically significant, can be considered biologically significant. EPA (1986c) reported decreased sperm

concentration in male rats treated orally for 10 days with 0.625 mg/kg-day chlordecone. Linder et al. (1983) saw sperm effects (decreased viability, motility, and concentration) in rats at 0.83 and 1.67 mg/kg-day (90 days of treatment); however, the authors did not see any treatment-related histological lesions or an effect on reproductive performance (number of pregnant females, number of live litters, average live litter size, number of implants, percentage of resorptions, and fetal weight) when treated males were mated to untreated females. This study and a study by Cannon and Kimbrough (1979) indicate that decreased reproductive success in experimental animals may not be solely attributable to male reproductive effects. Cannon and Kimbrough (1979) reported that treated female rats (1.6–1.7 mg/kg-day for 3 months) mated to control rats failed to produce litters, whereas treated males (1.2–1.6 mg/kg-day for 3 months) mated to control females had reproductive success similar to controls. Good et al. (1965) reported in a continuous breeding study that male and female mice treated with 0.94 mg/kg-day for 1 month prior to mating and 5 months during mating had impaired reproductive success; specifically, reduced production of litters was seen in treated mice and the mated offspring of treated mice. However, the general confidence in this study is limited by incomplete reporting of the variance of reproductive parameters and decreased fertility of the control mice one generation apart. Two other reproductive studies (Good et al., 1965; Huber et al., 1965) treated outbred mice in the diet for 1 month prior to mating and 3–5 months during the mating period with doses of chlordecone starting at 1.9 mg/kg-day and did not see a depression of reproductive parameters until doses of 3.3 or 5.6 mg/kg-day (Good et al., 1965, and Huber et al., 1965, respectively). Additional studies have reported reproductive toxicity but at higher doses (Swartz and Mall, 1989; Swartz et al., 1988; Gellert and Wilson, 1979; Huber, 1965). Taken together, the available studies showing reproductive effects following chlordecone exposure suggest that functional reproductive deficits are seen at levels higher than the level reported to cause renal lesions in chronically treated rats (Linder et al., 1983; Cannon and Kimbrough, 1979; Larson et al., 1979a; Good et al., 1965; Huber et al., 1965). Therefore, reproductive effects were not selected as the critical effect of chlordecone exposure. Nevertheless, potential points of departure (PODs) for reproductive endpoints from Larson et al. (1979a) and Good et al. (1965) were considered in the derivation of a RfD (see Section 5.1.2 and Appendix B).

In consideration of the available studies reporting effects of chronic and subchronic chlordecone exposure in humans and animals, Larson et al. (1979a) was chosen as the principal study. This study was adequately designed with several acceptable dose groups and adequate numbers of animals. Results were sufficiently reported for most endpoints. Sensitive endpoints identified in this study include glomerulosclerosis, liver lesions, and testicular atrophy. Though testicular atrophy was observed at 13 weeks, the only lesions observed chronically that were reported to be treatment related were in the liver and kidney. This observation coupled with the lack of support for testicular lesions in other studies in rats of similar dose and duration (Linder et al., 1983; Cannon and Kimbrough, 1978) decreases confidence in this endpoint. Additionally,

the liver lesions observed in the principal study (characterized as fatty changes and hyperplasia) occurred at higher doses as compared with the observed kidney lesions. After consideration of all endpoints, the increased incidence of glomerulosclerosis in female rats was determined to be the most sensitive and biologically significant effect detected in this study. Furthermore, the chlordecone database contains additional support for the specific endpoint of glomerular damage (Sobel et al., 2006, 2005; Chetty et al., 1993c) and general support for the kidney as a target organ as determined by increased kidney weights seen in studies in addition to the principal study (Cannon and Kimbrough, 1978; NCI, 1976a).

Glomerulosclerosis is believed to be an irreversible effect that can result in renal impairment. The mechanism by which chlordecone causes kidney lesions is not known; however, there is no indication that kidney lesions would not occur in humans chronically exposed to chlordecone. Though clinical indications of kidney dysfunction were not detected in workers occupationally exposed to chlordecone, this may be because the relatively short average exposure duration of workers (5–6 months) was not sufficient for the development of detectable kidney impairment. Therefore, for the above reasons, Larson et al. (1979a) was chosen as the principal study and renal lesions as the critical effect.

5.1.2. Methods of Analysis

All available models in the EPA Benchmark Dose Software (BMDS) version 1.3.2 were fit to quantal incidence data for histopathologic renal lesions in female Wistar rats from a 2-year dietary study (Larson et al., 1979a). The data modeled are shown below in Table 5-1.

Table 5-1. Incidence of histopathologic renal lesions (glomerulosclerosis grades 1, 2, or 3 combined) in male or female Wistar rats following administration of chlordecone in the diet for 1–2 years

Gender	Dose (mg/kg-day)				
	0	0.06	0.3	0.5	1.6
Male	12/22 (55%)	3/11 (27%)	4/6 (67%)	6/9 (67%)	3/4 (75%)
Female[a]	4/34 (12%)	2/13 (15%)	8/17 (47%)[b]	8/12 (67%)[b]	3/4 (75%)[b]

[a]Statistically significant trend for increased incidence by Cochran-Armitage test ($p < 0.01$).
[b]Statistically significantly different from controls according to Fisher's exact test ($p < 0.05$) performed for this review.

Source: Larson et al. (1979a).

Biological and statistical considerations were taken into account in the selection of a benchmark response level for this data set. Statistically, a 10% level of response is intended to select a response level near the lower range of detectable observations in typical studies conducted with 50 animals per dose group (U.S. EPA, 2000c). The data set for the critical effect

from Larson et al. (1979a) relies on notably smaller groups of animals (4–22 animals per group), therefore, use of a benchmark response (BMR) below 10% would result in a POD further outside of the observable range and would involve greater uncertainty. Biologically speaking, a BMR of a 10% increase in glomerulosclerosis was selected under an assumption that it represents a minimal biologically significant change. Therefore, for this dataset, a response level of 10% was used. The results of benchmark dose (BMD) modeling of the data are discussed below.

Statistical analysis of the incidence of glomerulosclerosis (grades 1, 2, or 3 combined) in each dose by sex revealed that the incidence of glomerulosclerosis in female rats exhibited a significant dose response trend (according to the Cochran-Armitage test). Therefore, the incidence data for renal lesions in female rats in the 0, 0.06, 0.3, 0.5, and 1.6 mg/kg-day dose groups were used to fit models to derive BMDs. It should be noted that all animals from the two highest dose groups (3.9 and 7.0 mg/kg-day) died within the first 6 months of the study, and thus data from these animals were not available for use in the dose-response assessment.

As shown in Appendix B, most models provided adequate fits to the data for histopathologic renal lesions (glomerulosclerosis) in female rats from the Larson et al. (1979a) study (Table 5-1), as assessed by a chi-square goodness-of-fit test and visual inspection of the respective plots of observed and predicted values from the various models. The log-probit model provided the best fit to the female rat data as assessed by Akaike's Information Criterion (AIC). Additionally, this model exhibits the best fit to the incidence data at low doses (i.e., in the vicinity of the BMR) as evidenced by examining the chi-square scaled residuals and the visual fit of the model to the data in the plot from the BMDS output. Thus, the log-probit model was selected to estimate the BMD for glomerulosclerosis data in female rats from Larson et al. (1979a). The BMD_{10} associated with a 10% extra risk for glomerulosclerosis in female rats was 0.12 mg/kg-day, and its lower 95% confidence limit ($BMDL_{10}$) was 0.08 mg/kg-day.

Reproductive effects observed following oral exposure to chlordecone were also evaluated as potential PODs. Reproductive endpoints, such as testicular atrophy (Larson et al., 1979a), and functional reproductive outcomes, such as decreases in first and second litters (Good et al., 1965; Huber et al., 1965), were investigated. The most sensitive functional reproductive endpoint identified in the chlordecone database of dietary repeat exposure studies is a freestanding LOAEL of 0.94 mg/kg-day identified in Good et al. (1965) for the reduced production of second litters in chlordecone treated BALB/c mice and reduced reproduction in offspring of treated mice (reduced production of first litters). Upon examination of the data set, it was determined that these data were not amenable to BMD modeling; specifically, the continuous endpoints reported (percent of pairs producing first and second litters, pair days per litter) were averages and did not include any measure of the variability, such as standard deviation.

The incidence of testicular atrophy in male Wistar rats, following 3 months of dietary chlordecone exposure (Larson et al., 1979a), was determined to be the only biologically

significant reproductive endpoint with a data set amenable to BMD modeling, though uncertainty surrounds this endpoint since it was not detected in the same study at the chronic time point. Regardless, the BMD modeling results for testicular lesions in rats are included as part of Appendix B. The multistage and quantal linear models provided the best fit for this dataset as assessed by a chi-square goodness-of-fit test, an AIC, and a visual inspection of the respective plots of observed and predicted values from the various models. The BMD_{10} associated with a 10% extra risk for testicular atrophy in rats was 0.206 mg/kg-day, and its lower 95% confidence limit ($BMDL_{10}$) was 0.119 mg/kg-day.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UFs)

Of the endpoints shown in Table 4-18, the increased incidence of histopathological renal lesions (glomerulosclerosis) among female Wistar rats receiving chlordecone in the diet continuously for 2 years (Larson et al., 1979a) is the most sensitive endpoint. BMD modeling revealed that the $BMDL_{10}$ associated with this effect is 0.08 mg/kg-day. The $BMDL_{10}$ provides the POD for the RfD.

A total UF of 300 was applied to the POD of 0.08 mg/kg-day: 10 for interspecies extrapolation from animals to humans (UF_A); 10 for human intraspecies variability (UF_H); and 3 to account for database deficiencies (UF_D).

A 10-fold UF was used to account for uncertainties in extrapolating from laboratory rats to humans. Aside from a difference in metabolism (humans produce chlordecone alcohol, whereas rats do not), the available data do not suggest differential toxicity of these forms, nor do the toxicity data from various animal species provide marked evidence that rats or any other species are more sensitive to chlordecone than humans. Consequently, the default UF of 10 for extrapolating from laboratory animals to humans was applied.

A 10-fold UF was used to account for variation in susceptibility among members of the human population (i.e., interindividual variability). Insufficient information is available to predict potential variability in human susceptibility.

An UF of 3 was applied to account for deficiencies in the chlordecone toxicity database. The database includes limited human data from observational studies of occupationally exposed workers. The database also includes several studies in laboratory animals, including chronic and subchronic dietary exposure studies and several subchronic reproductive and developmental studies, as well as one specifically assessing developmental neurotoxicity. The chlordecone database does not have an appropriately designed multigenerational reproductive study, but includes approximately 10 oral repeat-exposure studies assessing reproductive and developmental toxicity, including several single-generation reproductive toxicity studies and three developmental studies in rats and mice (Linder et al., 1983; Squibb and Tilson, 1982; Cannon and Kimbrough, 1979; Chernoff and Rogers, 1976; Good et al., 1965; Huber et al., 1965). Several of these reproductive studies have indicated decreased reproductive success in

chlordecone-treated animals (Cannon and Kimbrough, 1979; Good et al., 1965; Huber et al., 1965). The database also includes two nonstandard multigenerational studies that evaluate reproductive success of chlordecone-treated animals (Gellert and Wilson, 1979; Good et al., 1965). Due to limited scope and design, these studies are not considered adequate for the assessment of potential multigenerational reproductive toxicity.

In addition, some limited evidence exists to suggest that the critical effect (glomerular lesions) may be mediated through an autoimmune mechanism. Therefore, in consideration of the entire database for chlordecone, a partial database UF of 3 is considered appropriate to account for the lack of an adequately designed two-generational reproductive study and for the lack of immunotoxicity studies where some data indicate chlordecone may exhibit immunological effects. There are no available data to indicate whether these effects would be expected to occur at doses lower than those observed for the critical effect.

Because the POD was selected from a dose associated with an endpoint identified by a chronic dietary study (Larson et al., 1979a), no uncertainty factor is needed for exposure duration (subchronic to chronic). A UF for LOAEL-to-NOAEL extrapolation was not used because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% increase in glomerulosclerosis was selected under an assumption that it represents a minimal biologically significant change.

The oral RfD for chlordecone was calculated as follows:

$$\begin{aligned} \text{RfD} &= \text{BMDL}_{10} \div \text{UF} \\ &= 0.08 \text{ mg/kg-day} \div 300 \\ &= 0.0003 \text{ or } 3\text{E-}4 \text{ mg/kg-day} \end{aligned}$$

5.1.4. RfD Comparison Information

Kidney (glomerular) lesions, liver lesions, testicular atrophy, and decreased fertility are observed low-level effects, following subchronic or chronic oral exposure to chlordecone (Larson et al., 1979; Good et al., 1965). Table 5-2 provides a tabular summary of potential PODs and resulting RfDs for these endpoints. Additionally, Figure 5-1 provides a graphical representation of this information. This figure should be interpreted with caution since the PODs across studies are not necessarily comparable, nor is the confidence the same in the data sets from which the PODs were derived. The PODs presented in this figure are based on either a BMDL_{10} (for kidney, testicular, or liver lesions) or a LOAEL (in the case of Good et al., 1965). Some indication of the confidence associated with the resulting RfD is reflected in the magnitude of the total UF applied to the POD (i.e., the size of the bar); however, the text of Sections 5.1.1 and 5.1.2 should be consulted for a more complete understanding of the issues associated with each dataset and the rationale for the selection of the principal study and the critical effect used

to derive the RfD. As discussed in Section 5.1.1., among the studies considered, the chronic study by Larson et al. (1979a) provided the data set most appropriate for the derivation of the RfD.

Table 5-2. Possible PODs with applied uncertainty factors and resulting RfDs

Effect	POD	Species	Uncertainty factors[a]						RfD
			Total	A	H	L	S	D	
Kidney lesions	0.08[b]	Rat	300	10	10			3	3×10^{-4}
Testicular atrophy	0.12[b]	Rat	3000	10	10		10	3	4×10^{-5}
Liver lesions	0.14[b]	Rat	300	10	10			3	5×10^{-4}
Decreased production of litters	0.94[c]	Mouse	3000	10	10	10		3	3×10^{-4}

[a]Uncertainty factors: A = animal to human (interspecies); H = interindividual (intraspecies); L = LOAEL to NOAEL; S = subchronic-to-chronic duration; D = database deficiency.
[b]POD based on BMDL determined through BMD modeling of a 10% response. Source: Larson et al. (1979a).
[c]POD based on a freestanding LOAEL for a 65% decrease in second-generation animals producing litters. Source: Good et al. (1965).

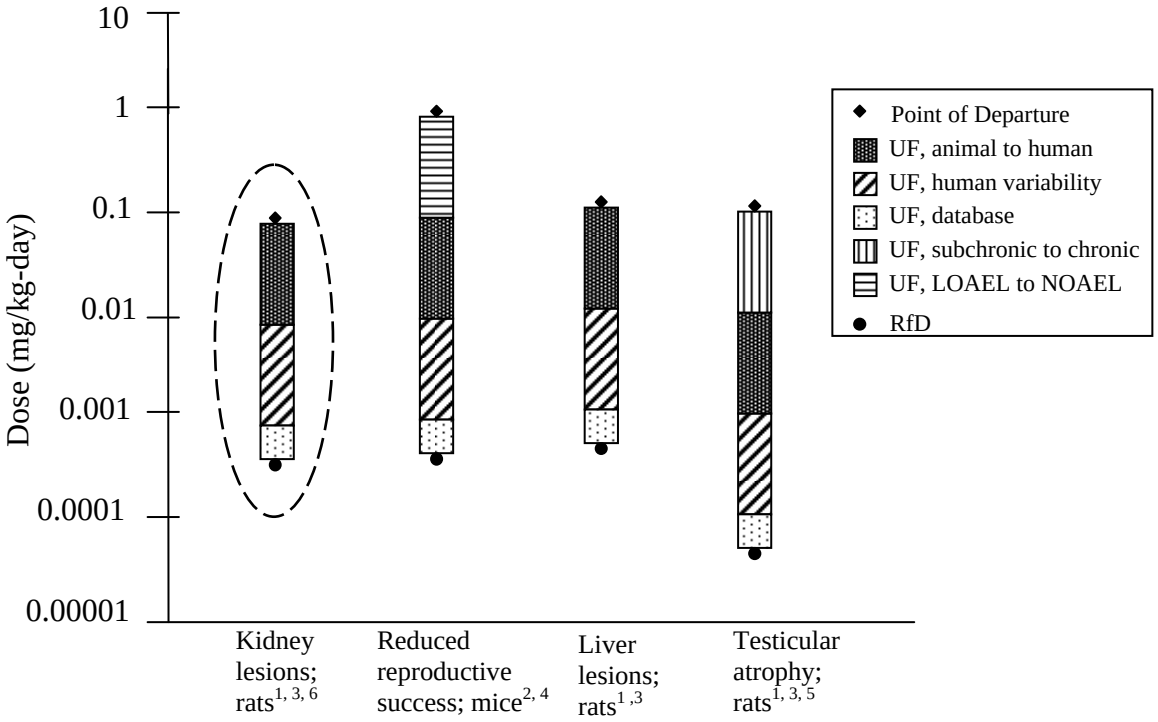


Figure 5-1. RfD comparison array for alternate points of departure.

[1]Larson et al. (1979a).
[2]Good et al. (1965).
[3]$BMDL_{10}$ used as the POD.
[4]POD based on a freestanding LOAEL for a 65% decrease in second-generation animals producing litters.
[5]Subchronic endpoint (13 weeks) not observed at chronic durations (1–2 years).
[6]Selected critical effect for the derivation of the RfD.

The PODs presented for kidney, liver, and testicular lesions were derived through BMD modeling of the dichotomous data by using a 10% response level. BMD modeling outputs for these three endpoints are included in Appendix B. The PODs based on BMD methods have an inherent advantage over the use of a NOAEL or LOAEL by making greater use of all the dose-response data from a given data set. The POD for reduced reproductive success in mice was based on a freestanding LOAEL.

Although the RfD based on kidney lesions has the lowest POD, the RfD based on testicular atrophy results in a lower RfD due to an added magnitude of uncertainty applied for the use of a subchronic endpoint. The POD for testicular atrophy was derived from a 3-month exposure duration (within the chronic study by Larson et al. [1979a]); however, testicular effects were not noted for the longer exposure durations (1–2 years) in the same study, nor were testicular lesions detected in other studies in rats treated with similar doses for the same duration (Linder et al., 1983; Cannon and Kimbrough, 1979). Therefore, because of lower confidence in this endpoint and the evidence in the database for more sensitive effects in the kidney, testicular lesions were not selected as the critical effect for the derivation of the chlordecone RfD.

5.1.5. Previous RfD Assessment

An oral assessment for chlordecone was not previously available on IRIS.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

Although adverse health effects from an occupational exposure incident may have resulted from inhalation exposure (in combination with oral and dermal exposures), the data do not identify doses at which effects occur (Taylor, 1985, 1982; Guzelian, 1982a; Guzelian et al., 1980; Sanborn et al., 1979; Cannon et al., 1978; Martinez et al., 1978; Taylor et al., 1978). Consequently, the human data cannot be used to define a dose-response relationship for inhalation exposure to chlordecone. No studies on the toxicity of chlordecone following inhalation exposure in laboratory animals were located. This lack of data precludes the derivation of an RfC.

Consideration was given to route-to-route extrapolation to derive inhalation doses from existing oral dose-response data for development of an RfC. Route-to-route extrapolation from the oral database, however, is precluded by deficiencies in the database. The available rat PBTK models for chlordecone do not include the inhalation route of exposure (see Section 3.5), and human PBTK models with both oral and inhalation portals of entry have not yet been developed. In the absence of PBTK models that include oral and inhalation routes of exposure, and lacking inhalation absorption efficiency data in humans and rats, a route-to-route extrapolation from oral to inhalation for chlordecone would be highly uncertain. As discussed in Chapter 2, only very

small amounts of chlordecone will evaporate from soil or water surfaces, and any chlordecone in the air is likely to be removed by deposition of particles.

5.3. CANCER ASSESSMENT

Utilizing the EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is *suggestive evidence of carcinogenic potential* for chlordecone. This characterization lies at the high end of the continuum for this weight of evidence descriptor. An animal cancer bioassay (NCI, 1976a,b) provides evidence of carcinogenic potential of chlordecone, following high-dose oral exposure in Osborne-Mendel rats and B6C3F1 mice. In this study the incidence of hepatocellular tumors was statistically significantly increased in both sexes of B6C3F1 mice. Male and female rats exhibited increased incidences of hepatocellular carcinomas at high doses that were statistically significant when compared with pooled controls. The incidence of hepatocellular carcinomas was not statistically significant in comparison with matched controls for rats of either sex. In addition, a decrease in latency for time to tumor appearance was observed in dosed animals compared with controls. Review of the NCI (1976a,b) bioassay for chlordecone raises concerns regarding the study design and conduct (see Sections 4.2.2.1 and 4.7.1). The study was limited in scope (only two dose groups) and utilized high-dose levels designed to elicit a maximal carcinogenic response. Following the observation of marked toxicity in the high-dose groups of both species, dosing levels were lowered to one-half to one-sixth of the initial dose levels. Due to this change in chlordecone exposure levels, the dose metric related to the development of liver tumors cannot be determined. Because of these limitations, the NCI (1976a,b) study is not suitable for low-dose extrapolation for human cancer risk assessment. Therefore, in the absence of adequate cancer bioassay data, no quantitative dose-response cancer assessment can be performed to derive an oral slope factor for chlordecone.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Chlordecone was previously used as an insecticide to control agricultural pests, including slugs, snails, and fire ants. Chlordecone was first produced in the United States in the early 1950s; however, production in the United States ended in 1975 due to intoxication from severe industrial exposure in employees who worked at the only chlordecone manufacturing plant in the country. Its registration was cancelled in 1976. Chlordecone is very resistant to degradation in the environment. It is expected to adsorb to soil and to stick to suspended solids and sediments in water. Very small amounts of chlordecone will evaporate from soil or water surfaces, and any chlordecone in the air is likely to be removed by deposition of particles. Chlordecone has a very high potential for bioaccumulation in fish and other aquatic organisms.

Chlordecone is well absorbed following oral exposure. Once absorbed, it is widely distributed and eventually concentrates in the liver. It is metabolized by humans and some animal species to chlordecone alcohol. Glucuronide conjugates of chlordecone and chlordecone alcohol, as well as unconjugated chlordecone, are slowly excreted in the bile and eliminated in the feces. Fecal excretion is delayed by enterohepatic recirculation.

The primary noncancer health effects of oral exposure to chlordecone in humans and animals include liver effects, kidney lesions (only in animals), neurotoxicity, and male reproductive toxicity. Other reproductive effects (i.e., persistent vaginal estrus, impaired reproductive success) and developmental effects have also been observed in laboratory animals; however, the doses required to elicit these effects were generally higher than those that resulted in liver and kidney effects, neurotoxicity, and/or male reproductive toxicity.

Liver enlargement developed in workers exposed to high levels of chlordecone for an intermediate exposure duration; however, evidence of significant liver toxicity was not found. Histological changes were observed in liver biopsy samples; however, these were characterized as nonadverse in nature. Similar changes in the liver were also demonstrated in laboratory animals, including increased liver size and weight, hepatocellular hypertrophy, proliferation of the SER, increased microsomal protein, CYP450 content, cytochrome c reductase activity, and microsomal enzyme activity. Chronic animal studies also demonstrated evidence of hepatotoxicity, including hepatocellular hypertrophy, hyperplasia, congestion, mild fatty change, focal necrosis, and occasional small nests of proliferated sinusoidal cells.

Neurological symptoms were also reported in workers exposed to high doses of chlordecone, including tremor, headache, irritability, poor recent memory, rapid random eye movements, muscle weakness, gait ataxia, incoordination, and slurred speech. The effects persisted for as long as 9–10 months after cessation of exposure and the start of treatment.

Chlordecone also causes tremors, decreased motor coordination, hyperexcitability, and an exaggerated startle response in laboratory animals.

Chlordecone exposure in humans caused oligospermia, reduced sperm motility, and decreased libido in a group of men who were occupationally exposed to chlordecone for periods up to 1.5 years. There was no evidence that the ability of these workers to father children was affected, and male reproductive parameters had returned to normal by 5 to 7 years following the cessation of chlordecone exposure and treatment with cholestyramine to reduce chlordecone blood levels. Chlordecone also induces reproductive toxicity in male and female laboratory animals, as demonstrated by altered sperm parameters, testicular atrophy, altered estrous cyclicity, and impaired reproductive success. Chlordecone induced developmental toxicity in rats and mice at dose levels that also produced significant maternal toxicity.

Kidney toxicity was reported in laboratory animals but was not observed in occupationally exposed pesticide workers. However, it is unclear if clinical indicators of renal damage were specifically examined in occupationally exposed workers. Several animal studies reported kidney effects from chlordecone exposure. Proteinuria and increased incidence of kidney lesions were observed in female Wistar rats and in (NZB × NZW)F_1 mice. Chronic kidney inflammation was observed in male and female Osborne-Mendel rats. Twenty-eight days of dietary exposure to chlordecone produced eosinophilic inclusions in proximal tubules in male Sprague-Dawley rats. Gestational exposure to chlordecone resulted in a statistically significant increase in the incidences of fetuses with enlarged renal pelvis.

Most of the effects of chlordecone are thought to be produced by the parent compound, primarily by interfering with the function of mitochondrial and cellular membranes. Disruption of cellular homeostasis and energy production within the cell eventually leads to impaired cellular function. In the central nervous system, altered calcium homeostasis leads to changes in neurotransmitter activity. In the liver, membrane perturbation and inhibition of transport proteins at the bile canalicular membrane is thought to be related to chlordecone-induced hepatobiliary dysfunction. The reproductive and developmental effects of chlordecone are most likely related to endocrine disruption. Chlordecone exhibits estrogenic properties that may be related to impaired reproductive success and adverse effects on sperm.

6.2. DOSE RESPONSE

6.2.1. Noncancer

No studies on the toxicity of chlordecone following inhalation exposure in humans or laboratory animals were located. This lack of data precludes the derivation of the RfC.

The database for chlordecone includes limited human data from observational studies of occupationally exposed workers. The database also includes several studies in laboratory animals, including chronic and subchronic dietary exposure studies, and several subchronic studies with a wide variety of tissues and endpoints assessed. The database also includes several

reproductive and developmental studies, including one specifically assessing developmental neurotoxicity. Endpoints associated with oral exposure to chlordecone include lesions in the liver, kidney, and testis; neurological effects (specifically tremors); and reduced fertility. Support for these endpoints exists across a range of diverse studies; nevertheless, data gaps have been identified and uncertainties associated with data are discussed below.

The observation of kidney, liver, and testicular effects in the principal study at similar dose levels creates some uncertainty in the selection of a critical effect that would be most appropriate in a chronic low-dose human exposure paradigm. The most sensitive effect observed from chronic dietary exposure to chlordecone is the increased incidence of kidney lesions in female Wistar rats (Larson et al., 1979a). Furthermore, several additional animal studies, in both rats and mice, support findings of kidney effects with chlordecone exposure (Sobel et al., 2006, 2005; Chetty et al., 1993c; Chu et al., 1981a; Chernoff and Rodgers, 1976; NCI, 1976b). In light of the weight of evidence for kidney, testicular, and liver lesions seen in the chlordecone animal literature (see Section 5.1.1), kidney lesions were deemed to be the most supported, biologically significant effect on which to base the RfD. Some uncertainty exists regarding the lack of observable effects on the kidney in humans. However, it is unknown whether the relatively short average exposure duration of workers (5–6 months) was sufficient for the development of detectable kidney impairment. Additionally, it is unclear from the literature whether clinical tests sensitive to early kidney impairment were administered to exposed workers.

After consideration of all potential PODs, the RfD of 3×10^{-4} mg/kg-day was based on the increased incidence of kidney lesions in female Wistar rats, following chronic dietary administration of chlordecone (Larson et al., 1979a). To derive the RfD, the uncertainty factor approach, following EPA practices (U.S. EPA, 2002), was applied to the POD determined through BMD modeling of the critical effect of kidney lesions in female rats. Factors to account for uncertainties associated with the extrapolation from the POD derived from an animal study to a diverse human population of varying susceptibilities were applied. This extrapolation was accomplished through the application of default UFs due to limitations in the chlordecone database that precluded the derivation of chemical specific adjustment factors.

The choice of BMD model is not expected to introduce a considerable amount of uncertainty in the risk assessment since the chosen response rate of 10% additional risk is within the observable range of the data. Furthermore, the ratio of the BMD to the BMDL for the model that best describes the incidence data for the critical effect is less than a factor of two, indicating a relatively precise BMD estimate.

Additional BMD modeling for other amenable data sets, including liver lesions and testicular atrophy, was also conducted to provide other PODs for comparison purposes (see Appendix B). A graphical representation of these potential PODs and resulting reference values is shown below in Figure 6-1.

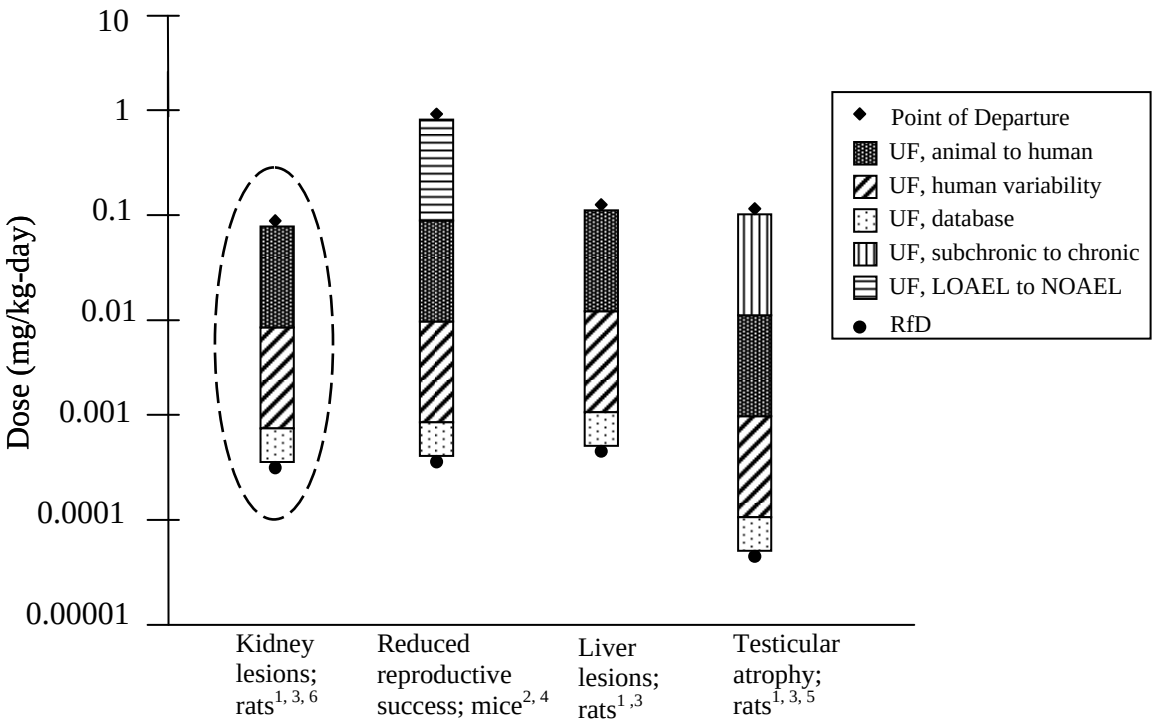


Figure 6-1. RfD comparison array for alternate points of departure.

[1]Larson et al. (1979a)
[2]Good et al. (1965).
[3]$BMDL_{10}$ used as the POD.
[4]POD based on a freestanding LOAEL for a 65% decrease in second-generation animals producing litters.
[5]Subchronic endpoint (13 weeks) not observed at chronic durations (1–2 years).
[6]Selected critical effect for the derivation of the RfD.

The default UF of 10 for the extrapolation from animals and humans is a composite of uncertainty to account for toxicokinetic differences and toxicodynamic differences between the animal species in which the POD was derived and humans. PBTK models can be useful for the evaluation of interspecies toxicokinetics; however, the chlordecone database lacks an adequate model that would inform potential differences. Data from workers occupationally exposed to chlordecone provide some information on the absorption, distribution, metabolism, and elimination of chlordecone in humans and indicate qualitatively that the toxicokinetics of chlordecone are similar between humans and animals. Additionally, biological effects, including neurological, hepatic, and reproductive effects, observed in animals and humans are similar in nature, indicating similar toxicodynamics. However, the magnitude of the similarities or differences in toxicokinetic and toxicodynamic parameters cannot be calculated due to uncertainties regarding routes of exposure and doses for the occupationally exposed workers. Therefore, an UF of 10 to account for interspecies differences was used.

Limited data exist on effects of chlordecone in a small population of occupationally exposed workers. However, since potential variability in responses to chlordecone in the greater human population is unknown, the default uncertainty factor of 10 for intrahuman variability was not reduced. Human variation may be larger or smaller; however, chlordecone-specific data to examine the potential magnitude of human variability of response are unknown.

Uncertainties associated with data gaps in the chlordecone database have been identified. Specifically, data more fully characterizing potential multigenerational reproductive and immunological effects are lacking. Some data suggest that the selected critical effect of kidney lesions may be an immune-mediated effect.However, additional data to evaluate this potential effect is lacking (Sobel et al., 2006, 2005). Additionally, uncertainty exists in the database concerning the dose-response characterization of potential multigenerational reproductive effects. Several one-generational reproductive studies have indicated decreased reproductive success in chlordecone-treated animals (Cannon and Kimbrough, 1979; Good et al., 1965; Huber et al., 1965). In addition, two nonstandard multigenerational studies exist that evaluate reproductive success of chlordecone-treated animals (Gellert and Wilson, 1979; Good et al., 1965). However, due to limited scope and design, these studies are not considered adequate for the assessment of multigenerational reproductive toxicity. Therefore, for the above data gaps in the chlordecone database, an UF of 3 was applied to the POD in the derivation of the RfD.

The overall confidence in the RfD and the principal study (Larson et al., 1979a) is medium. The principal study involves a sufficient number of animals per group, several acceptable dose levels, and a wide range of tissues and endpoints assessed. Confidence in the database is medium. The chlordecone database includes case studies of occupationally exposed workers, chronic and subchronic dietary exposure studies in laboratory animals, and several subchronic reproductive and developmental studies, including one developmental neurotoxicity study. However, the database is lacking a multigenerational reproductive toxicity study. Therefore, reflecting medium confidence in both the database and the principal study, confidence in the RfD is medium.

6.2.2. Cancer

Though uncertainty exists regarding the classification of the carcinogenic potential of chlordecone due to limitations in the design and conduct of the primary cancer bioassay, under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for chlordecone provides *suggestive evidence* of carcinogenic potential. This characterization lies at the high end of the continuum for this weight of evidence descriptor. This determination is primarily based on the NCI (1976a,b) study, which found positive evidence of liver tumors in both sexes of rats and mice after chronic chlordecone dietary exposure. Additionally, data on mirex, a structurally similar chemical also demonstrates an increase in hepatocellular adenomas or carcinomas in both sexes of rats and mice. However, unlike the observed cancer effects, some

but not all of the noncancer effects noted for these two chemicals are similar as described in Section 4.5.3. This weight of evidence conclusion collectively takes into consideration the NCI (1976a,b) cancer bioassay and its limitations, the available human studies, and other chronic animal bioassays. Due to design and conduct issues in the primary study (NCI, 1976a,b), including inconsistent dosing levels and the use of doses that may have been excessively high, the data available are not sufficient for a stronger conclusion and are not suitable for quantification of cancer risk.

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APPENDIX A. SUMMARY OF EXTERNAL PEER REVIEW AND PUBLIC COMMENTS AND DISPOSITION

[Place holder]

APPENDIX B. BENCHMARK DOSE CALCULATIONS FOR THE RfD

Kidney Lesions (Glomerulosclerosis) in Female Rats Exposed to Chlordecone in the Diet for 1–2 years

The Larson et al. (1979a) study did not include statistics for renal lesions as described in Section 4.2.2. Statistical analysis (performed for this review) of the frequency of renal lesions in each dose by sex (Fisher's exact test) revealed that the incidence of glomerulosclerosis (grades 1, 2, or 3 combined) in some of the exposure groups of female rats was statistically different from control. Additionally, a significant dose-response trend was seen by the Cochran-Armitage test. All available models in the EPA Benchmark Dose Software (BMDS) version 1.3.2 were fit to quantal incidence data (Table B-1) for histopathologic glomerulosclerosis in female Wistar rats from a 2-year dietary study (Larson et al., 1979a). To provide potential points of departure for RfD derivation, benchmark response (BMR) levels were selected as 10% extra risk for quantal incidence data. The results of statistical analysis and BMD modeling for each sex are described below.

Table B-1. Incidence of histopathologic renal lesions (glomerulosclerosis grades 1, 2, or 3 combined) in female Wistar rats following administration of chlordecone in the diet for 2 years

Gender	Dose (mg/kg-day)				
	0	**0.06**	**0.3**	**0.5**	**1.6**
Male	12/22	3/11	4/6	6/9	3/4
Female[a]	**4/34**	**2/13**	**8/17[b]**	**8/12[b]**	**3/4[b]**

[a]Statistically significant trend for increased incidence by Cochran-Armitage test ($p < 0.01$).
[b]Statistically significantly different from controls according to Fisher's exact test ($p < 0.05$) performed for this review.

Source: Larson et al. (1979a).

As shown in Table B-1, the frequency of renal lesions (glomerulosclerosis) in female rats was statistically different from the incidence among control rats at doses of 0.3 mg/kg-day and higher. Most dichotomous models provided adequate fit to the female rat incidence data, based on the summary results reported in the BMDS output and a more detailed examination of the graphs and goodness-of-fit statistics (summarized in Table B-2 and Figure B-1).

As shown in Table B-2, the log-probit model had the best fit as indicated by the lowest Akaike's Information Criterion (AIC) and visual inspection (Figure B-1). Additionally, this model also exhibits the best fit to the incidence data at low doses (i.e., in the vicinity of the BMR) as evidenced by examining the chi-square scaled residuals and the visual fit of the model to the data in the plot from the BMDS output. Thus, it was selected to calculate a potential point

of departure for the RfD based on the incidence data for renal lesions (glomerulosclerosis) among female rats. The model-predicted benchmark dose (BMD) associated with a 10% extra risk for glomerulosclerosis was 0.12 mg/kg-day (Table B-2). The lower 95% confidence limit on the benchmark dose ($BMDL_{10}$), a potential point of departure for the RfD, was 0.08 mg/kg-day (Table B-2).

Table B-2. BMD modeling results for the incidence of histopathologic renal lesions (glomerulosclerosis) in female Wistar rats, following administration of chlordecone in the diet for 2 years

Model	BMD_{10}	$BMDL_{10}$	χ^2 *p*-value	AIC
Log-probit[a]	**0.116**	**0.076**	**0.62**	**84.3**
Quantal linear	0.071	0.045	0.56	84.7
Multistage	0.071	0.045	0.56	84.7
Weibull	0.071	0.045	0.56	84.7
Gamma	0.071	0.045	0.56	84.7
Log-logistic	0.067	0.026	0.72	85.7
Quantal quadratic	0.264	0.188	0.0002[b]	93.0

[a]Form of the probit model:
P(response) = background + [1 – background] × CumNorm[intercept + slope × log(dose)]
Where: CumNorm is the cumulative normal distribution function; background = 0.117647; intercept = 0.723913; slope = 1.
[b]Quantal quadratic model provided inadequate fit to the data.

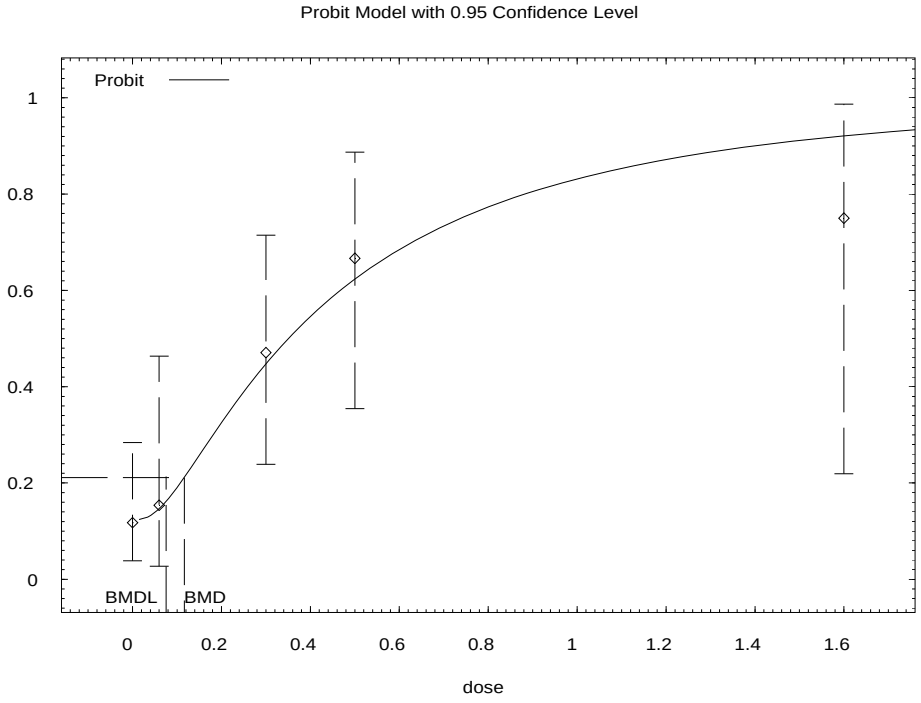

Figure B-1. Observed and predicted incidence of histopathologic renal lesions (glomerulosclerosis grades 1, 2, or 3 combined) in female Wistar rats following administration of chlordecone in the diet for 1–2 years.

Log-Probit Model of U.S. EPA Benchmark Dose Software (Version 1.3.2).

The computer output from the log-Probit model of the glomerulosclerosis data follows:

```
================================================================
        Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
        Input Data File: C:\BMDS\KIDNEY_LESIONS.(d)
        Gnuplot Plotting File:  C:\BMDS\KIDNEY_LESIONS.plt
                                                Wed May 09 15:06:56 2007
================================================================

 BMDS MODEL RUN
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  The form of the probability function is:

  P[response] = Background
        + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

  where CumNorm(.) is the cumulative normal distribution function


  Dependent variable = COLUMN1
  Independent variable = COLUMN3
  Slope parameter is restricted as slope >= 1

  Total number of observations = 5
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008


  User has chosen the log transformed model


            Default Initial (and Specified) Parameter Values
              background =     0.117647
               intercept =     0.723913
                   slope =            1


       Asymptotic Correlation Matrix of Parameter Estimates

       ( *** The model parameter(s)  -slope
            have been estimated at a boundary point, or have been specified by the user,
            and do not appear in the correlation matrix )

         background    intercept

background          1       -0.36

 intercept       -0.36          1
```

Parameter Estimates

Variable	Estimate	Std. Err.
background	0.123642	0.0510126
intercept	0.869701	0.276028
slope	1	NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-39.5379			
Fitted model	-40.1501	1.22434	3	0.7472
Reduced model	-49.6869	20.2979	4	0.0004361

AIC: 84.3002

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.1236	4.204	4	34	-0.1062
0.0600	0.1464	1.903	2	13	0.07598
0.3000	0.4471	7.601	8	17	0.1948
0.5000	0.6232	7.479	8	12	0.3105
1.6000	0.9210	3.684	3	4	-1.268

Chi-square = 1.76 DF = 3 P-value = 0.6241

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.116338

BMDL = 0.0756267

Testicular Atrophy in Male Rats Receiving Chlordecone in the Diet for 3 Months

The Larson et al. (1979a) study did not include statistics for the testicular atrophy observed in male rats (see Section 4.2.2). Statistical analysis (performed for this review) of the frequency of renal lesions in each dose by sex (Fisher's exact test) revealed that the incidence of testicular atrophy in male rats in some of the exposure groups of male rats was statistically different from control. Additionally, a significant dose response trend was seen by the Cochran-Armitage test. All available models in the EPA BMDS version 1.3.2 were fit to quantal incidence data (Table B-3) for testicular atrophy in male Wistar rats, following 3 months of dietary exposure (Larson et al., 1979a). To provide potential points of departure for RfD derivation, benchmark response levels were selected as 10% extra risk for quantal incidence data. The results of statistical analysis and BMD modeling for each sex are described below.

As shown in Table B-3, the frequency of testicular atrophy in male rats was statistically different from the incidence among control rats at doses of 1.6 mg/kg-day and higher. However, the highest dose groups of 3.9 and 7 mg/kg-day were not included in the dose response modeling as animals in these dose groups suffered from overt toxicity, leading to death of all animals in these groups by 6 months into the study. Testicular atrophy in the highest exposed rats may have resulted from frank toxic effects including decreased body weight gain.

Most of the dichotomous models provided adequate fit to the testicular atrophy incidence data based on the summary results reported in the BMDS output and a more detailed examination of the graphs and goodness-of-fit statistics (summarized in Table B-4 and Figure B-2). As shown in Table B-4, the multistage and quantal linear models provided the best fit as indicated by the lowest AIC values and visual inspection (Figure B-2). Both models predicted the BMD associated with a 10% extra risk for testicular atrophy as 0.21 mg/kg-day (Table B-4). The lower 95% confidence limit on the benchmark dose ($BMDL_{10}$), a potential point of departure for the reference dose (RfD), was 0.12 mg/kg-day (Table B-4).

Table B-3. Incidence of testicular atrophy in male rats receiving chlordecone in the diet for 3 months

Dietary level (ppm)	0	5	10	25	50	80
Average dose[a] (mg/kg-day)	0	0.3	0.5	1.6	3.9	7.0
Incidence of testicular atrophy[b]	1/10	0/5	1/5	4/5[c]	4/5[c]	5/5[c]

[a]Average doses to male rats, based on graphically depicted food consumption data presented by the authors.
[b]Statistically significant trend for increased incidence by Cochran-Armitage test ($p < 0.01$).
[c]Statistically significantly different from controls according to Fisher's exact test ($p < 0.05$) performed for this review.

Source: Larson et al. (1979a).

Table B-4. BMD modeling results for the incidence of testicular atrophy in male Wistar rats, following administration of chlordecone in the diet for 3 months

Model	BMD_{10}	$BMDL_{10}$	χ^2 *p*-value	AIC
Gamma	0.393	0.126	0.42	30.97
Logistic	0.560	0.323	0.35	30.52
Log-logistic	0.436	0.125	0.49	30.37
Multistage (1°)[a]	**0.206**	**0.119**	**0.58**	**29.54**
Probit	0.563	0.350	0.36	30.58
Log-probit	0.444	0.203	0.51	30.28
Quantal linear	**0.206**	**0.119**	**0.58**	**29.54**
Quantal quadratic	0.776	0.541	0.26	30.87
Weibull	0.338	0.123	0.41	31.15

[a]Form of the multistage model:
P[response] = background + (1-background) × (1-EXP(-beta × $dose^{-1}$)]
Where: background = 0.0672234; beta(1)= 0.510742.

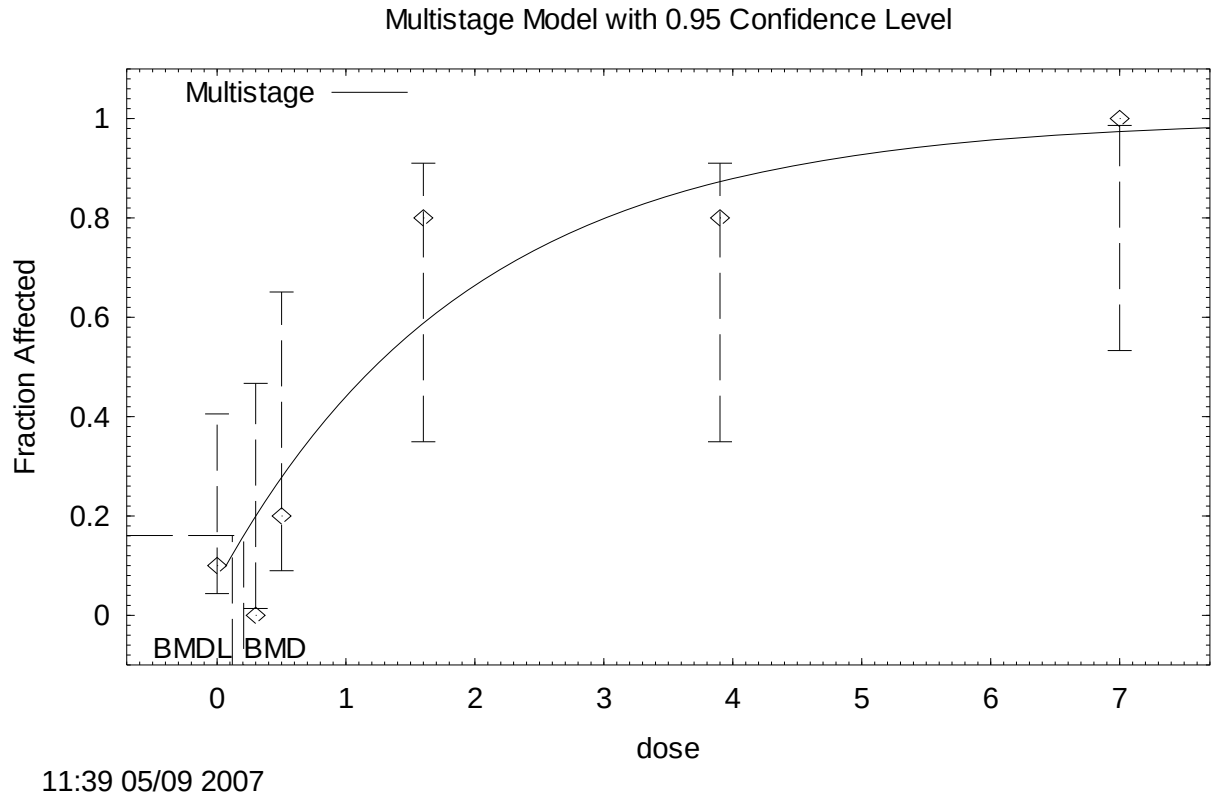

Figure B-2. Observed and predicted incidence of testicular atrophy in male Wistar rats, following administration of chlordecone in the diet for 3 months.

Multistage Model of U.S. EPA Benchmark Dose Software (Version 1.3.2).

The computer output from the Multistage model of the male testicular atrophy follows:

```
================================================================
        Multistage Model. $Revision: 2.1 $ $Date: 2000/08/21 03:38:21 $
        Input Data File: G:\KEPONE DOSE-RESPONSE
MODELING\MALE_RAT_TESTES_LARSON_1979.(d)
        Gnuplot Plotting File:  G:\KEPONE DOSE-RESPONSE
MODELING\MALE_RAT_TESTES_LARSON_1979.plt
                                                  Wed May 09 11:39:01 2007
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Response
   Independent variable = Dose

 Total number of observations = 6
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


            Default Initial Parameter Values
              Background =           0
                Beta(1) = 1.27121e+019


        Asymptotic Correlation Matrix of Parameter Estimates

          Background      Beta(1)
```

Background 1 -0.41

Beta(1) -0.41 1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.0672234	0.22791
Beta(1)	0.510742	0.227823

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-10.7569			
Fitted model	-12.7712	4.02865	4	0.4021
Reduced model	-23.9018	26.2898	5	<.0001

AIC: 29.5424

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.0672	0.672	1	10	0.523
i: 2					
0.3000	0.1997	0.999	0	5	-1.250
i: 3					
0.5000	0.2774	1.387	1	5	-0.386
i: 4					
1.6000	0.5880	2.940	4	5	0.875
i: 5					
3.9000	0.8727	4.364	4	5	-0.655
i: 6					
7.0000	0.9739	4.869	5	5	1.027

Chi-square = 2.87 DF = 4 P-value = 0.5800

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.206289

BMDL = 0.118596

Liver Lesions (Fatty Changes and Hyperplasia) in Male and Female Rats Exposed to Chlordecone in the Diet for 1–2 years

The Larson et al. (1979a) study did not include statistics for liver lesions. Statistical analysis by Syracuse Research Corporation of the frequency of liver lesions in each dose by sex (Fisher's exact test and Cochran-Armitage trend test) revealed that the incidence of liver lesions in some of the exposure groups was statistically different from controls. An examination liver lesion incidence based on sex indicated (by Fisher's exact test) no significant differences; the incidence data for males and females was combined. The incidence data were used to fit various dichotomous models available in the EPA BMDS version 1.3.2. The frequency of liver lesions (fatty changes and hyperplasia) in both sexes combined was statistically different from control at 0.5 and 1.6 mg/kg-day (see Table B-5). In addition, the Cochran-Armitage trend test showed a statistically significant dose-response trend in the frequency of liver lesions (fatty changes and hyperplasia) for both sexes combined.

Table B-5. Incidence of histopathologic liver lesions (fatty changes and hyperplasia) in Wistar rats, following administration of chlordecone in the diet for 1–2 years

Endpoint	Dose (mg/kg-day)				
	0	0.06	0.3	0.5	1.6
Liver lesions[a]					
Male rats	1/22	1/11	2/6	2/9	3/4[b]
Female rats	2/34	1/13	2/17	4/12[b]	1/4
Both	3/56	2/24	4/23	6/21[b]	4/8[b]

[a]Statistically significant trend for increased incidence by Cochran-Armitage test.
[b]Statistically significantly different from controls according to Fisher's exact test performed for this review.

All models for dichotomous variables available in the EPA BMDS version 1.3.2 were fit to the data in Table B-5. All dichotomous models provided adequate fit to the data based on the summary results reported in the BMDS output and a more detailed examination of the graphs and goodness-of-fit statistics (summarized in Table B-6).

The gamma, multistage, quantal linear, and Weibull models had equally good fit as indicated by equally low AIC values for these models. Thus, these were selected to calculate a potential point of departure for the RfD, based on the incidence data for liver lesions (fatty changes and hyperplasia) among rats. The model-predicted BMDs associated with a 10% extra risk for liver lesions (fatty changes and hyperplasia) were all equal to 0.23 mg/kg-day. The lower 95% confidence limit on the BMDL, a potential point of departure for the RfD, was equal to 0.14 mg/kg-day.

Table B-6. BMD modeling results for the increased incidence of liver lesions in rats (both sexes combined), following administration of chlordecone in the diet for 1–2 years

Model	BMD_{10}	$BMDL_{10}$	χ^2 *p*-value	AIC
Gamma	**0.225**	**0.136**	**0.97**	**98.9**
Log-logistic	0.200	0.106	0.95	100.7
Multistage (1°)[a]	**0.225**	**0.136**	**0.97**	**98.9**
Probit	0.327	0.217	0.74	99.9
Quantal linear	**0.225**	**0.136**	**0.97**	**98.9**
Quantal quadratic	0.534	0.380	0.23	102.7
Weibull	**0.225**	**0.136**	**0.97**	**98.9**

[a]Multistage model was run as 3rd degree polynomial with betas > 0.

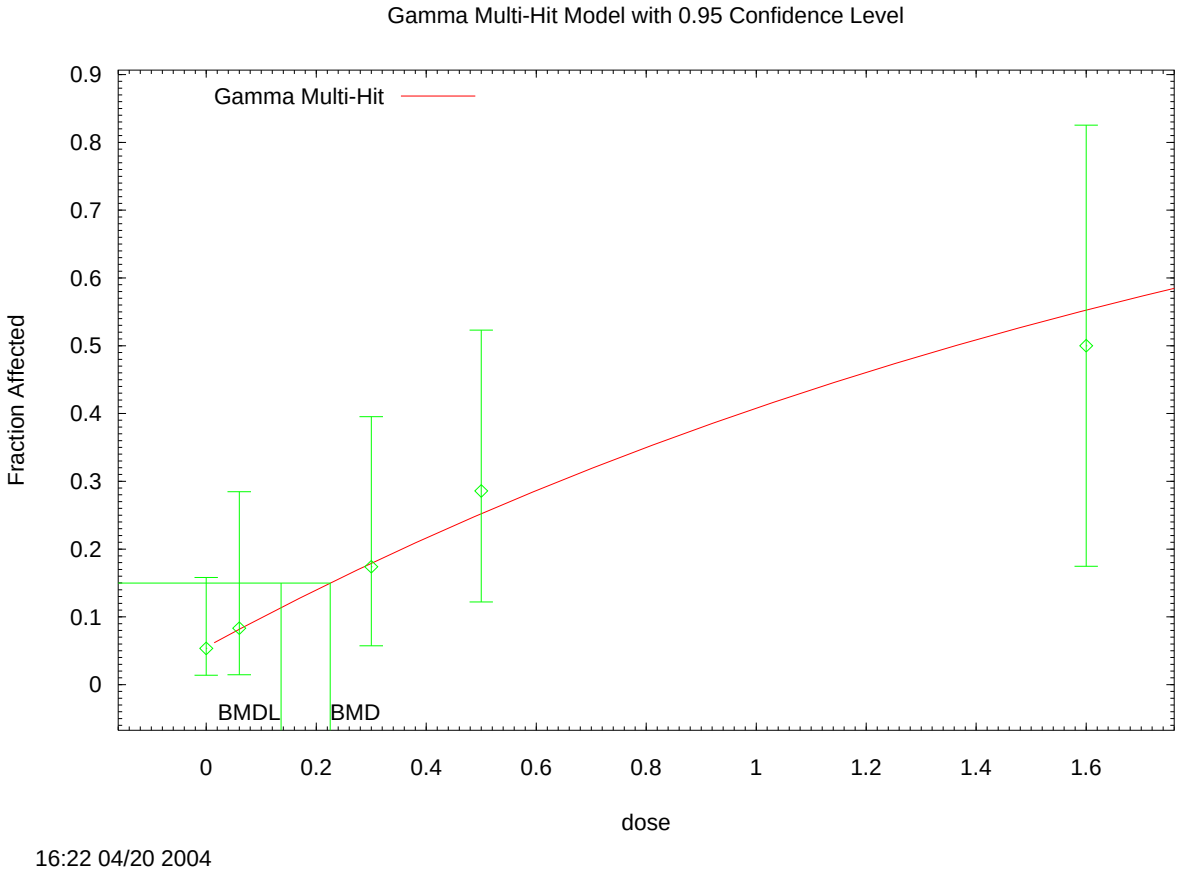

Figure B-3. Observed and predicted incidence of liver lesions in male and female Wistar rats following administration of chlordecone in the diet for 1–2 years.

Source: Larson et al. (1979a).

Gamma Model of U.S. EPA Benchmark Dose Software (Version 1.3.2).

The computer output from the Gamma model of the incidence of liver lesions follows:

```
================================================================
          $Revision: 2.2 $ $Date: 2001/03/14 01:17:00 $
          Input Data File: C:\BMDS\LARSON_BOTHSEXES_DATA.(d)
          Gnuplot Plotting File:  C:\BMDS\LARSON_BOTHSEXES_DATA.plt
                                                Tue Apr 20 16:22:31 2004
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   The form of the probability function is:

   P[response]= background+(1-background)*CumGamma[slope*dose,power],
   where CumGamma(.) is the cummulative Gamma distribution function


   Dependent variable = Frequency
   Independent variable = Dose
   Power parameter is restricted as power >=1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


            Default Initial (and Specified) Parameter Values
              Background =    0.0614035
                 Slope =     0.901339
                 Power =          1.3


        Asymptotic Correlation Matrix of Parameter Estimates

        ( *** The model parameter(s)  -Power
             have been estimated at a boundary point, or have been specified by the user,
             and do not appear in the correlation matrix )

          Background       Slope

Background          1       -0.38

    Slope       -0.38           1
```

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.0554334	0.0274998
Slope	0.467464	0.165121
Power	1	NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-47.3181			
Fitted model	-47.428	0.219805	3	0.9743
Reduced model	-54.3907	14.1451	4	0.006846

AIC: 98.8561

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0554	3.104	3	56	-0.06089
0.0600	0.0816	1.957	2	24	0.03177
0.3000	0.1790	4.118	4	23	-0.06401
0.5000	0.2523	5.298	6	21	0.3525
1.6000	0.5529	4.423	4	8	-0.3009

Chi-square = 0.22 DF = 3 P-value = 0.9737

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.225388

BMDL = 0.136075