2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective of the toxicology of chlordane. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

In the studies discussed in this chapter, exposure of animals may have been to analytical grade chlordane, technical grade chlordane, or to individual *cis*- or *trans*-chlordane. Exposure of humans was mainly to technical chlordane. As discussed in Section 3.1, technical chlordane is a mixture of >140 related chemicals, of which the major other constituents include heptachlor, nonachlor, chlordene, among others. In addition to being constituents or contaminants of technical chlordane, these and other chemicals are also formed during the metabolism of chlordane (see Section 2.3.3), and may influence the toxicity of chlordane.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure - inhalation, oral, and dermal; and then by health effect - death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods - acute (14 days or less), intermediate (15-364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-

observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into “less serious” or “serious” effects. “Serious” effects are hose that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). “Less serious” effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, “less serious” LOAEL, or “serious” LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between “less serious” and “serious” effects. The distinction between “less serious” effects and “serious” effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user’s perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of chlordane are indicated in Table 2-2 and Figure 2-2. Because cancer effects could occur at lower exposure levels, the figures also show a range for the upper bound of estimated excess risks, ranging from a risk of 1 in 10,000 to 1 in 10,000,000 (10^{-4} to 10^{-7}), as developed by EPA.

A User’s Guide has been provided at the end of this profile (see Appendix A). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

2.2.1 Inhalation Exposure

2.2.1.1 Death

Retrospective cohort mortality studies of workers in chlordane and other organochlorine manufacturing plants reported no increase in mortality rate and no increase in any specific cause of death attributed to chlordane exposure (MacMahon et al. 1988; Shindell and Ulrich 1986). Wang and MacMahon (1979b) reported no increase in mortality rate in a prospective study of pesticide applicators. However, in a retrospective mortality study of 1,403 men employed for 23 months at two plants, a significantly increased risk of death from cerebrovascular disease was found, but the authors could not definitively attribute this excess to chlordane exposure (Wang and MacMahon 1979a). In another retrospective mortality study of four cohorts (305-1,155 workers/plant exposed for ≥6 months) from four manufacturing plants, there was a significantly increased risk of death from noncancer respiratory disease, and a slight excess risk of cancer of the esophagus, rectum, liver, and hematopoietic system at plant 3, and a slightly greater risk of stomach cancer at plant 1 (Ditraglia et al. 1981). Chlordane was the only pesticide manufactured at plant 1, while aldrin, dieldrin, endrin, and dichlorodiphenyltrichloroethane were manufactured at plant 3. The statistical power did not allow for a conclusion that no association existed between cause-specific mortality and employment at the plants. However, in a follow-up study of these cohorts, which added 11 years of follow-up, no statistically significant excess risk of death from any cause was found (Brown 1992). All of these studies had serious limitations, including unquantified exposure concentrations and exposure to several pesticides.

An early study by Frings and O'Tousa (1950) reported mortality in mice exposed to chlordane. This study will not be discussed further because it has been established that the mortality and other toxic effects observed were due to the use of "early" production chlordane (Ingle 1953, 1965). Early production chlordane was frequently contaminated with hexachlorocyclopentadiene, a highly volatile and toxic reaction intermediate that Ingle (1953, 1965) concluded was largely responsible for the observed effects. In another inhalation study (Ingle 1953), none of the mice died after 14-25 days of continuous exposure to "later" production (more highly purified) chlordane at estimated concentrations of 25-50% saturation (estimated levels in air not reported). In rats exposed to an unspecified concentration of "refined technical grade" of chlordane for l-8 hours for up to 10 days, three of five died between days 4 and 10 of exposure (Ambrose et al. 1953a).

More recently, rats exposed for 2 days (8 hours/day) to 413 mg technical chlordane/m^3 or similarly for 5 days to 154 mg/m^3 died; rats similarly exposed to 28.2 mg/m^3 for 28 days survived (Khasawinah et al. 1989). The female rats were more sensitive than the males. There was no mortality in rats or monkeys exposed intermittently to technical chlordane at 10 mg/m^3 8 hours/day, 5 days/week for 90 days (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). The LOAELs for death in rats after acute duration exposure are recorded in Table 2-1 and plotted in Figure 2-l.

2.2.1.2 Systemic Effects

The systemic effects in humans and animals after inhalation exposure to chlordane are discussed below. The highest NOAEL values and the LOAEL values for each systemic effect from all reliable studies for each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

Respiratory Effects. Physical examination of library workers acutely exposed to high but unquantified concentrations of chlordane from a spill revealed no indication of respiratory effects (NIOSH 1984a). Chest pains, dyspnea, and shortness of breath were reported in a compilation of cases and personal reports of humans accidentally exposed to chlordane by inhalation (EPA 1980a); exposures frequently involved a mixture of chemicals (such as other related and nonrelated pesticides) and vehicles (including petroleum distillates). Therefore, these effects cannot be attributed to chlordane alone. Results of a questionnaire indicated increases (compared with the National Center for Health Statistics 1979 National Health Interview Survey) in sore throat and respiratory infections in humans shortly after their homes were treated for termites (Menconi et al. 1988). Chronic exposure in pesticide treated homes was associated with bronchitis and sinusitis, which increased in incidence with higher concentrations of pesticides in the air. Because aldrin and heptachlor were included with chlordane in the analysis for pesticides in the indoor air, these effects cannot be attributed unequivocally to chlordane exposure. Other limitations of the Menconi et al. (1988) study include self-selection of respondents. Respiratory effects generally were not found in occupational exposure studies (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951). However, in a retrospective mortality study of four cohorts (305-1,155 workers/plant exposed for ≥6 months) from four manufacturing plants, there was a significantly increased risk of death from noncancer respiratory disease at plant 3 where only aldrin, dieldrin, endrin, and dichlorodiphenyltrichloroethane were manufactured (Ditraglia et al. 1981). However, increased mortality risk from noncancer respiratory disease was not observed in workers at plant 1 where only chlordane was manufactured. The

TABLE 2-1. Levels of Significant Exposure to Chlordane - Inhalation

Key to figure[a]	Species	Exposure duration/ frequency	System	NOAEL (mg/m^3)	LOAEL (effect) Less serious (mg/m^3)	LOAEL (effect) Serious (mg/m^3)	Reference	Form
ACUTE EXPOSURE								
Death								
1	Rat	2-5 d 8 hr/d				413 (death by day 2)	Khasawinah et al. 1989; Velsicol Chem Co 1984	Technical
2	Rat	5 d 8 hr/d				154 (death by day 5)	Khasawinah et al. 1989; Velsicol Chem Co 1984	Technical
Systemic								
3	Rat	3-12 d 5 d/wk 8 hr/d	Resp	154		413 (epithelial degeneration and cellular debris in bronchi, alveoli)	Khasawinah et al. 1989; Velsicol Chem Co. 1984	Technical
			Hemato	154				
			Hepatic		154 (increased blood SGPT, SGOT, SGDH, bile acids, cholesterol, liver enlargement and discoloration, centrilobular hepatocyte enlargement)	413 (same effects, more severe)		
			Other		154 (unspecified body weight loss; increased height of thyroid follicular cells)			
Neurological								
4	Rat	12 d 5 d/wk 8 hr/d				154 (abnormal respiratory movements, salivation, convulsions)	Khasawinah et al. 1989; Velsicol Chem Co. 1984	Technical

TABLE 2-1. Levels of Significant Exposure to Chlordane - Inhalation (*continued*)

Key to figure[a]	Species	Exposure duration/ frequency	System	NOAEL (mg/m^3)	LOAEL (effect) Less serious (mg/m^3)	LOAEL (effect) Serious (mg/m^3)	Reference	Form
INTERMEDIATE EXPOSURE								
Systemic								
5	Rat	28 d 5 d/wk 8 hr/d	Resp Cardio Gastro Hemato Musc/skel Hepatic Renal Derm/oc Other	28.2 28.2 28.2 28.2 28.2 5.8 28.2 28.2 5.8	28.2 (centrilobular hepatocellular hypertrophy; decreased blood glucose, increased blood total protein, albumin, and globulin) 28.2 (increased height of follicular epithelium of thyroid)		Khasawinah et al. 1989: Velsicol Chem Co. 1984	Technical
6	Rat	90 d 5d/wk 8hr/d	Resp Cardio Gastro Hemato Musc/skel Hepatic Renal Derm/oc Other	10 10 10 0.1 10 0.1[b,c] 10 10 1	1 (increased leucocyte count, decreased platelet count in females) 1 (centrilobular hypertrophy, hepatocellular vacuolization, increased P-450, decreased albumin, decreased albumin/ globulin ratio) 10 (increased height of follicular cells of the thyroid in males)		Velsicol Chem Co. 1984; Khasawinah et al. 1989	Technical

TABLE 2-1. Levels of Significant Exposure to Chlordane - Inhalation (*continued*)

Key to figure[a]	Species	Exposure duration/ frequency	System	NOAEL (mg/m^3)	LOAEL (effect) Less serious (mg/m^3)	LOAEL (effect) Serious (mg/m^3)	Reference	Form
7	Monkey	90 d 5d/wk 8hr/d	Resp Cardio Gastro Hemato Musc/skel Hepatic Renal Derm/oc Other (thyroid)	10 10 10 10 10 10 10 10 10			Velsicol Chem. Co. 1984; Khasawinah et al. 1989	Technical
Immunological								
8	Rat	28 d 5 d/wk 8 hr/d		5.8	28.2 (decreased thymus weight in females)		Khasawinah et al. 1989; Velsicol Chem. Co. 1984	Technical
9	Rat	90 d 5 d/wk 8 hr/d		0.1	1 (increased leukocyte count in females)		Khasawinah et al. 1989; Velsicol Chem. Co. 1984	Technical
Neurological								
10	Rat	28 d 5 d/wk 8 hr/d		5.8	28.2 (hypersensitivity to touch in females)		Khasawinah et al. 1989: Velsicol Chem. Co. 1984	Technical

TABLE 2-1. Levels of Significant Exposure to Chlordane - Inhalation (*continued*)

Key to figure[a]	Species	Exposure duration/ frequency	System	NOAEL (mg/m^3)	LOAEL (effect) Less serious (mg/m^3)	LOAEL (effect) Serious (mg/m^3)	Reference	Form
CHRONIC EXPOSURE								
Systemic								
11	Human	1-15 yr 8 hr/d 5 d/wk	Resp Cardio Gastro Hemato Hepatic Renal Other	0.0017 0.0017 0.0017 0.0017 0.0017 0.0017 0.0017			Fishbein et al. 1964	

[a]The number corresponds to entries in Figure 2-1.
[b]Used to derive an intermediate-duration inhalation minimal risk level (MRL) of 0.0002 mg/m^3; concentration adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 to extrapolate from animals to humans and 10 for human variability).
[c]The NOAEL of 0.1 mg/m^3 for hepatic effects identified in the intermediate-duration study by Khasawinah et al. (1989; Velsicol Chemical Co. 1984) was used to derive a chronic-duration inhalation MRL of 0.00002 mg/m^3; the concentration was adjusted for intermittent exposure and divided by an uncertainty factor of 1,000 (10 to extrapolate from animals to humans, 10 to extrapolate from an intermediate duration to a chronic duration, and 10 for human variability).

ATPase = adenoside triphosphatase; Cardio = cardiovascular; d = day(s); Derm/oc = dermal/ocular; Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; SGDH = serum glutamic dehydrogenase; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic-pyruvic transaminase; wk = week(s)

FIGURE 2-1. Levels of Significant Exposure to Chlordane - Inhalation

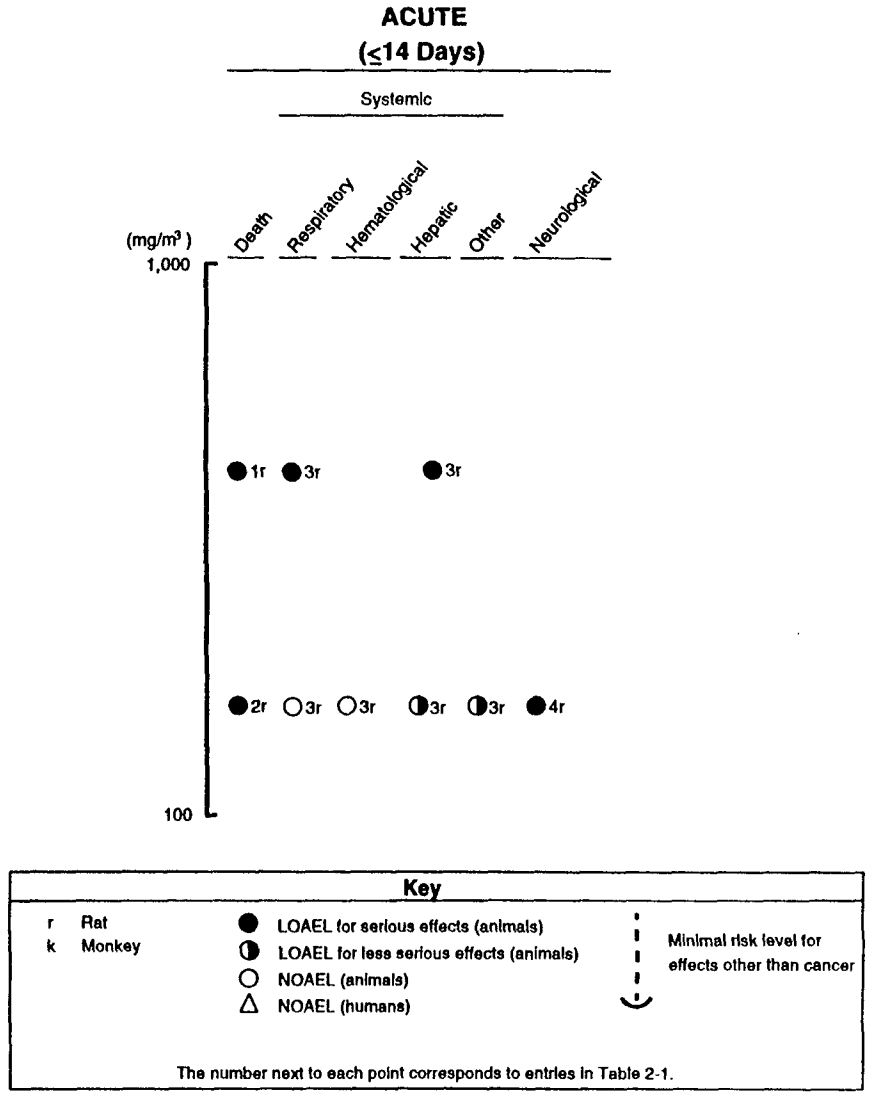
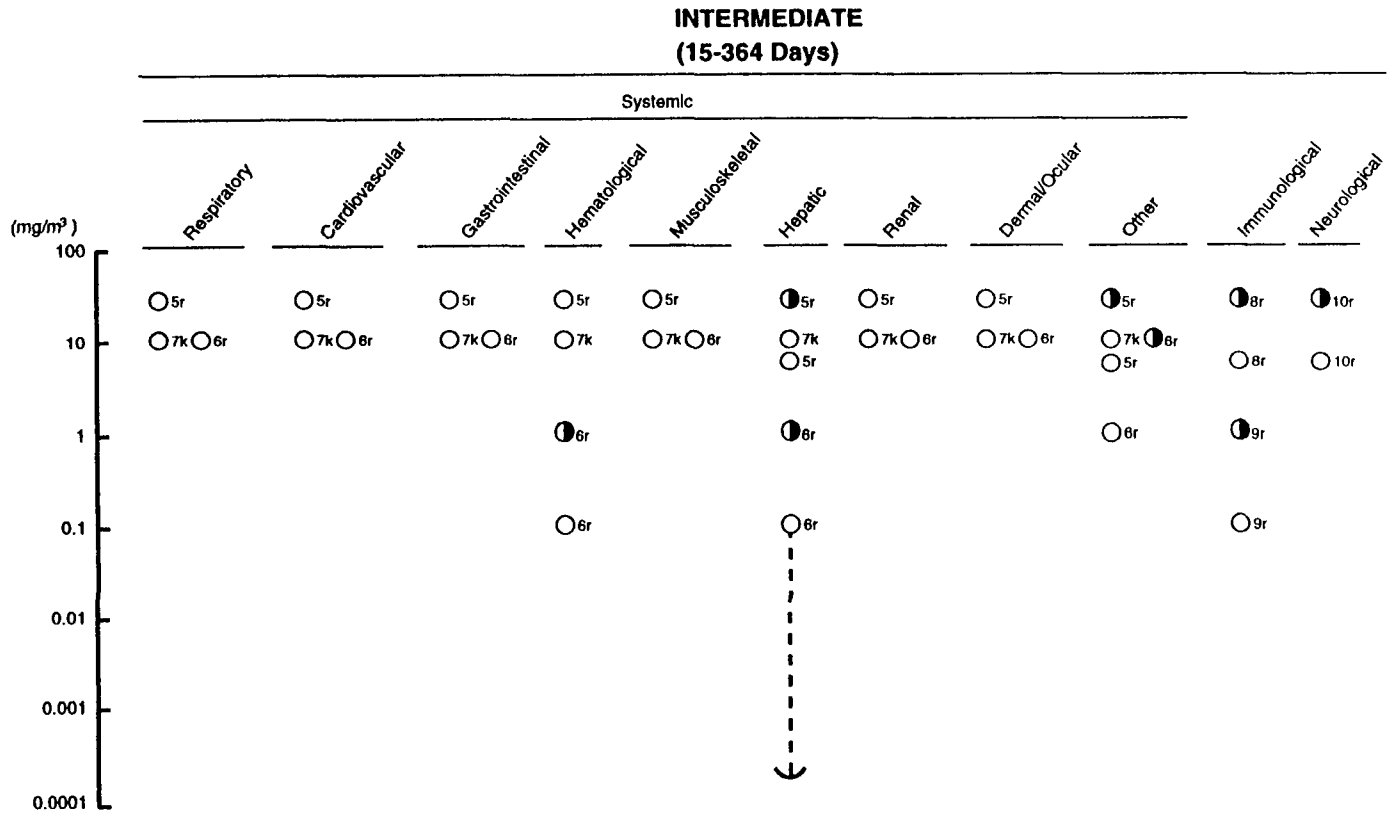


FIGURE 2-1. Levels of Significant Exposure to Chlordane - Inhalation (Continued)



Key

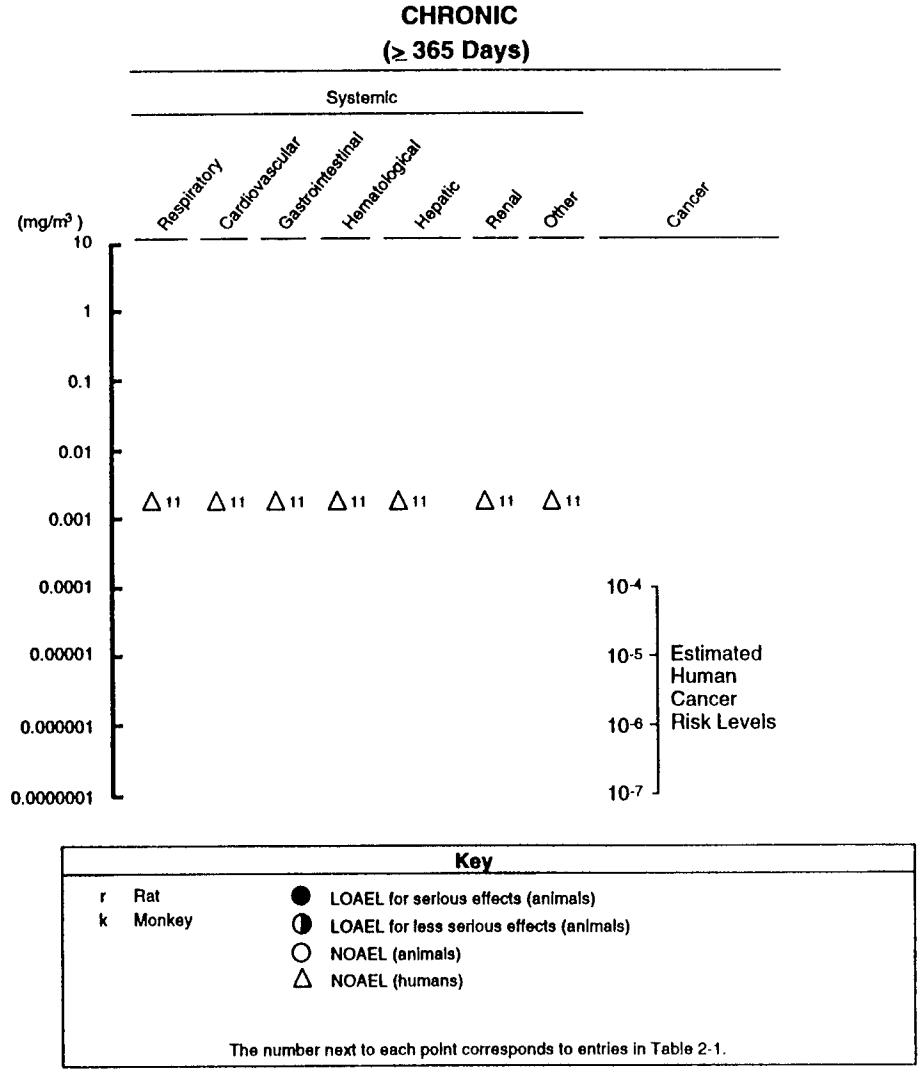
r Rat
k Monkey

● LOAEL for serious effects (animals)
◑ LOAEL for less serious effects (animals)
○ NOAEL (animals)
△ NOAEL (humans)

Minimal risk level for effects other than cancer

The number next to each point corresponds to entries in Table 2-1.

FIGURE 2-1. Levels of Significant Exposure to Chlordane - Inhalation (Continued)



statistical power did not allow for a conclusion that no association existed between cause-specific mortality and employment at the plants. However, in a follow-up study of these cohorts, which added 11 years of follow-up, no statistically significant excess risk of death from any cause was found (Brown 1992).

Mice exposed continuously to an unspecified concentration of chlordane for 14-25 days had slight lung congestion and proliferation of bronchiole lining cells (Ingle 1953). In a series of experiments, rats exposed to 413 mg technical chlordane/m^3 for 3 days had epithelial degeneration and cellular debris in the bronchii and alveoli (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). Respiratory tract lesions were not observed in rats intermittently exposed to technical chlordane at ≤128.2 mg/m^3 8 hours/day, 5 days/week for 28 days or similarly to 10 mg/m^3 for 90 days. Abnormal respiratory movements seen in rats in the 28-day study exposed to ≥154 mg/m^3 were considered signs of neurologic involvement. There were no adverse histopathological changes in the respiratory tract or changes in pulmonary function in monkeys exposed to technical chlordane at 10 mg/m^3 8 hours/day, 5 days/week for 90 days.

Cardiovascular Effects. Tachycardia was among the symptoms attributed to chlordane exposure in a compilation of cases and personal reports of accidental human inhalation exposure to high concentrations of chlordane (EPA 1980b). Cardiovascular effects were not reported in library workers acutely exposed to high but unquantified concentrations of chlordane from a spill (NIOSH 1984a). Cardiovascular effects were not found in occupational exposure studies (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951). Equivocal evidence of increased risk of cerebrovascular disease was reported in workers involved in the manufacture of chlordane (Wang and MacMahon 1 979a) .

Histopathological changes in the heart were not observed in rats exposed to technical chlordane at ≤28.2 mg/m^3 8 hours/day, 5 days/week for 28 days or similarly to 10 mg/m^3 for 90 days (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). No adverse histopathological lesions were observed in the hearts of monkeys exposed to technical chlordane at 10 mg/m^3 8 hours/day, 5 days/week for 90 days (Khasawinah et al. 1989; Velsicol Chemical Co. 1984).

Gastrointestinal Effects. Gastrointestinal effects (cramps, diarrhea, nausea) were a consistent observation in a compilation of cases and personal reports of accidental human inhalation exposure to

high concentrations of chlordane (EPA 1980a). NIOSH (1984a) also reported gastrointestinal symptoms (nausea, diarrhea) in 4 of 13 humans within 4 days of inhalation and/or dermal exposure as a result of 1% chlordane being spilled in a subterranean library room. The greatest prevalence of symptoms occurred in those directly involved in the cleanup, where potential for exposure to higher concentrations was greatest. Concentrations in air, taken ≈4.5 months after the spill, ranged from 0.0001 to 0.0003 mg/m^3. Because air concentration data are not available for the first 4 days of exposure, concentrations associated with the observed effects cannot be estimated. Occupational exposure, however, has not been associated with gastrointestinal effects. Alvarez and Hyman (1953) reported no gastrointestinal effects in a group of 24 workers involved in chlordane manufacture. Both inhalation and dermal exposure occurred. Princi and Spurbeck (1951) reported no effects in workers involved in the manufacture of insecticides (chlordane, aldrin, and dieldrin) when air concentrations of total chlorinated hydrocarbons were ≤10 mg/m^3; exposure was by inhalation and skin contact for 11-36 months. Fishbein et al. (1964) reported no gastrointestinal effects in production workers exposed to chlordane concentrations of 0.0012-0.0017 mg/m^3 over a period of 1-15 years.

No histopathological lesions in the gastrointestinal tract were observed in rats exposed to technical chlordane at ≤28.2 mg/m^3 8 hours/day, 5 days/week for 28 days, or in rats and monkeys exposed to technical chlordane at 0, 0.1, 1.0, or 10 mg/m^3 for 90 days (Khasawinah et al. 1989; Velsicol Chemical Co. 1984).

Hematological Effects. A questionnaire survey revealed that 4% of persons living in homes treated with chlordane to control termites reported anemia as a chronic effect (Menconi et al. 1988). The effect cannot be attributed to chlordane alone, because the quantitative amounts of aldrin and heptachlor were combined with the chlordane measurement in the analysis of indoor air. A number of anecdotal reports of blood dyscrasia associated with organochlorine pesticides (chlordane, lindane, DDT), suggest that there may be an unusually susceptible subpopulation (Ellenhom and Barceloux 1988). Several cases of blood dyscrasia (aplastic anemia, hemolytic anemia, thrombocytopenic purpura, acute disseminated hemorrhages, pernicious anemia, megaloblastic anemia) were observed in persons exposed to chlordane or heptachlor in their home or garden or as a result of their profession as pest control operators (Epstein and Ozonoff 1987; Infante et al. 1978). The usefulness of these reports is limited because exposure to chlordane was unquantified, the individuals were exposed to other chemicals, and there were other confounding factors. Although the existing case reports are not sufficient to indict chlordane as the etiologic agent, they indicate the need for further epidemiologic

study (see also Section 2.2.1.8). No effects on hemoglobin concentrations or sedimentation rates were found in a group of 24 men employed in chiordane manufacture, where exposure was both via inhalation and dermal contact (Alvarez and Hyman 1953). Furthermore, no effects on typical hematological parameters were found in 34 workers exposed to chlordane at unspecified concentrations (Princi and Spurbeck 1951) or in 15 workers exposed to 0.0012-0.0017 mg/m^3 (Fishbein et al. 1964).

No hematological effects were observed in rats exposed to technical chlordane at ≤28.2 mg/m^3 8 hours/day, 5 days/week for 28 days or similarly to 154 mg/m^3 for 11 days, or in monkeys similarly exposed to ≤10 mg/m^3 for 90 days (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). Male and female rats were similarly exposed to 0.1 and 1.0 mg/m^3 for 90 days. Female, but not male rats exposed to 1.0 mg/m^3 had increased leukocyte counts and decreased platelet counts, which suggests that chlordane may have an effect on hematopoiesis and that females may be more sensitive than males.

Musculoskeletal Effects. The only study regarding musculoskeletal effects in humans after exposure to chlordane was a report of increased serum levels of creatine phosphokinase, particularly the isoenzyme associated with skeletal muscle damage, in three of nine pesticide applicators (Ogata and Izushi 1991). This study does not provide convincing evidence of muscular damage, as no other indices were examined.

No histological evidence of musculoskeletal effects were observed in rats exposed to technical chlordane at ≤28.2 mg/m^3 8 hours/day, 5 days/week for 28 days or in rats or monkeys similarly exposed to ≤10 mg/m^3 for 90 days (Khasawinah et al. 1989; Velsicol Chemical Co. 1984).

Hepatic Effects. Hepatic effects were not reported in library workers acutely exposed to high but unquantified concentrations of chlordane from a spill (NIOSH 1984a). Jaundice, reflecting liver effects, was sometimes reported in cases of inhalation exposure to chlordane in a compilation of cases and personal reports of accidental exposure (EPA 1980a). When reported, jaundice was frequently associated with continued exposure such as living in a house in which chlordane had been used to control termites (EPA 1980a). Occupational exposures to chlordane, until recently, have not been associated with liver effects (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951). More recently, however, elevated serum levels of triglycerides, lactic acid dehydrogenase, and gamma-glutamyl transferase were measured in pesticide applicators (Ogata and Izushi 1991).

Biochemical evidence of liver damage (increased blood glutamic oxaloacetic transaminase [GOT], glutamic pyruvic transaminase [GPT], glutamate dehydrogenase [GDH], bile acids, and cholesterol), as well as hepatocellular enlargement and vacuolation, were observed in rats exposed to 154 mg technical chlordane/m^3 8 hours/day, 5 days/week for 11 exposures or to 413 mg/m^3 8 hours/day for 3 exposures (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). Increased liver weight was found in female rats exposed to technical chlordane at 5.8 mg/m^3 8 hours/day, 5 days/week for 28 days. Serum chemistry changes indicative of liver damage occurred in females, and increased liver weight occurred in males exposed to 28.2 mg/m^3 8 hours/day, 5 days/week for 28 days. Both sexes exposed to 28.2 mg/m^3 had centrilobular hepatocyte enlargement. Lesions in the rats exposed to 154 mg/m^3 for 11 exposures included hepatocellular enlargement and vacuolation; frank necrosis occurred in rats exposed to 413 mg/m^3 for 3 exposures. The female rats appeared to be more sensitive than the males.

A 90-day inhalation study in male and female rats exposed intermittently to technical chlordane at 0, 0.1, 1.0, or 10 mg/m^3 reported mild liver lesions (hepatocellular enlargement or vacuolization) and slight changes in serum chemistry at ≤1.0 mg/m^3 and increased liver weight in both sexes at 10 mg/m^3 (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). The lowest concentration, 0.1 mg/m^3, was judged a NOAEL. In monkeys exposed by the same protocol, no effects occurred at 1.0 mg/m^3 but 10 mg/m^3 was associated with increased mean liver weight. The NOAEL of 0.1 mg/m^3 in rats was used to derive an intermediate-duration inhalation MRL of 0.0002 mg/m^3 and a chronic-duration inhalation MRL of 0.00002 mg/m^3 as described in the footnote to Table 2-l and Section 2.4.

Renal Effects. Evidence of altered renal function was not reported in library workers acutely exposed to high but unquantified concentrations of chlordane from a spill (NIOSH 1984a), or in a compilation of cases and personal reports of accidental exposure (EPA 1980a). No kidney effects were found in occupational studies of chlordane manufacture (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951).

Increased kidney weight was noted in male rats exposed 8 hours/day, 5 days/week to 28.2, but not to 5.8 mg/m^3, for 28 days (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). No histopathological lesions of the kidney were observed in either sex. The 28.2 mg/m^3 level is considered a NOAEL. Elevated kidney weights were found in both sexes of rats similarly exposed to technical chlordane at 10 mg/m^3, but not at 1.0 mg/m^3, for 90 days. No histopathological lesions were observed at

1 or 10 mg/m^3. No kidney effects were observed in monkeys in the same study at 10 mg/m^3, which is also considered a NOAEL.

Dermal/Ocular Effects. Dermatitis was found by a questionnaire survey to occur in persons living in homes treated with chlordane, with greater frequency than in a reference population (Menconi et al. 1988). The effects, however, cannot be attributed to chlordane alone, because aldrin and heptachlor were included in the analysis for chlordane in the residents indoor air.

No dermal/ocular effects were found in rats exposed to technical chlordane at ≤28.2 mg/m^3 8 hours/day, 5 days/week for 28 days. No ophthalmoscopic or histopathological changes were observed in the eyes or skin of rats or monkeys similarly exposed to ≤10 mg/m^3 for 90 days (Khasawinah et al. 1989; Velsicol Chemical Co. 1984).

Other Systemic Effects. Body weight loss and increased height of the thyroid follicular cells were observed in rats exposed to 154 mg technical chlordane/m^3, 8 hours/day, 5 days/week for 11 exposures (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). The 28-day inhalation study in rats reported decreased body weight gain in females exposed to 28.2 mg chlordane/m^3 8 hours/day, 5 days/week. Food consumption also decreased, confounding interpretation of this finding. Thyroid weight increased in males exposed to 5.8 or 28.2 mg/m^3 and increased height of thyroid follicular epithelial cells occurred in males exposed to 28.2 mg/m^3 for 28 days. In the 90-day inhalation experiments in rats and monkeys, a slightly increased height in the follicular cells of the thyroid was found in rats intermittently exposed to 10 mg/m^3. No statistically significant histopathological effects on the thyroid were observed at 1.0 mg/m^3. There were no effects on food intake (rats only), body weight, or on the histopathological appearance of other organs or tissues not discussed previously or below.

2.2.1.3 Immunological Effects

Chlordane caused statistically significant immune alterations in humans who had been exposed to chlordane aerosols in the home or in the workplace for periods ranging from 3 days to 15 months (average exposure period, 5.84 months) (McConnachie and Zahalsky 1992). The length of time from exposure to testing ranged from 4 months to 10 years, and the mean interval was 2.4 years. Impaired proliferative responses to all three plant mitogens tested suggested that chlordane exposure was associated with immune deficiency. Eleven of 12 subjects tested for autoimmunity demonstrated an

increased titer of a form of autoantibody. The authors proposed that deposition of chlordane in the bone marrow could account for the long-term persistence of phenotypic and functional differences in the immune system of individuals exposed to chlordane.

Reduced thymus weight was observed in female, but not male rats exposed 8 hours/day, 5 days/week to 28.2 mg chlordane/m^3 for 28 days (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). Altered thymus weight was not observed in females exposed to 5.8 mg/m^3 or in male or female rats exposed 8 hours/day, 5 days/week to 10 mg chlordane/m^3 for 90 days. Female rats exposed 8 hours/day, 5 days/week to 1.0 or 10 mg chlordane/m^3 for 90 days had increased leukocyte counts. No histological lesions in thymus or lymph nodes were observed in monkeys exposed to 10 mg/m^3 8 hours/day, 5 days/week for 90 days, but immune function was not assessed. The NOAEL and LOAEL values for immunological effects in rats exposed for an intermediate duration are recorded in Table 2-l and plotted in Figure 2-l.

2.2.1.4 Neurological Effects

Neurological signs and symptoms including headache, dizziness, vision problems, incoordination, irritability, excitability, weakness, muscle twitching, and convulsions were reported consistently in a compilation of cases and personal reports of humans accidentally exposed by inhalation to unquantified concentrations of chlordane (EPA 1980a). EPA (1986d) reported three cases of optic neuritis that may have been due to chlordane exposure in homes treated for termites. Humans have experienced neurological symptoms (headache, fatigue, sleeping disturbance, blurred vision, weakness, fainting, confusion) shortly after their homes were treated for termites (Menconi et al. 1988). Chronic exposure in the treated homes was associated with migraines and neuritis/neuralgia, which increased in incidence with higher concentrations of pesticide in the air. Because aldrin and heptachlor were included in the analysis for chlordane in indoor air, these effects cannot be attributed unequivocally to chlordane. NIOSH (1984a) reported neurological symptoms (headache, dizziness, blurred vision, irritability, paresthesia, muscle dysfunction) in 4 of 13 humans within 4 days of inhalation and/or dermal exposure as a result of 1% chlordane being spilled in a subterranean library room. The greatest prevalence of symptoms occurred in those directly involved in the cleanup, where rhe potential for exposure to the highest concentrations was greatest. Concentrations in air, taken ≈4.5 months after the spill, ranged from 0.1 to 0.3 μg/m^3. Because air concentration data were not available for the first 4 days of exposure, concentrations associated with the observed effects cannot be estimated. No

neurological effects, however, were found in epidemiological studies of workers in chlordane manufacture (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951). However, in a retrospective mortality study of 1,403 men employed for ≥3 months at two plants, a significantly increased risk of death from cerebrovascular disease was found, but the authors could not definitively attribute this excess to chlordane exposure (Wang and MacMahon 1979a).

Abnormal respiratory movements, excess salivation, and convulsions occurred in rats exposed 8 hours/day, 5 days/week to 154 mg technical chlordane/m^3 for 11 exposures or similarly to 413 mg/m^3 for 3 exposures (Khasawinah et al. 1989; Velsicol Chemical Co. 1984).

Female rats exposed 8 hours/day, 5 days/week to 28.2 mg chlordane/m^3 for 28 days showed hypersensitivity to touch from day 16 of the study (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). This effect did not occur in rats similarly exposed to 5.8 mg/m^3. Intermediate duration inhalation studies in rats and monkeys 8 hours/day, 5 days/week at 10 mg/m^3 for 90 days resulted in no histopathological lesions in the brain, and no overt signs of neurotoxicity were reported. However, tests for more subtle neurological effects were not conducted. The NOAEL and LOAEL values for neurological effects in rats for intermediate-duration exposure are recorded in Table 2-l and plotted in Figure 2-1.

2.2.1.5 Reproductive Effects

Chronic exposure in homes treated with chlordane for termites was associated with an increased incidence of unspecified ovarian and uterine disease, compared with a reference population (Menconi et al. 1988). Because aldrin and heptachlor were included in the analysis for chlordane in indoor air, these effects cannot be unequivocally attributed to chlordane.

Histopathological effects on the reproductive organs were not observed in rats exposed to chlordane in air at 28.2 mg/m^3 8 hours/day, 5 days/week for 28 days, or in rats or monkeys similarly exposed to 10 mg/m^3 for 90 days (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). However, reproductive function was not assessed.

2.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after inhalation exposure to chlordane.

2.2.1.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals after inhalation exposure to chlordane. Genotoxicity studies are discussed in Section 2.4.

2.2.1.8 Cancer

Chronic exposure of humans in homes treated with chlordane for termites was associated with an increased incidence of unspecified skin neoplasms, compared with a reference population (Menconi et al. 1988). Because aldrin and heptachlor were included in the analysis for chlordane in indoor air, these effects cannot be unequivocally attributed to chlordane.

Retrospective mortality studies of workers involved in the manufacture of chlordane (Shindell and Ulrich 1986; Wang and MacMahon 1979a) reported no increased incidence of total deaths due to cancer or to a specific type of cancer. In a prospective study of pesticide applicators, Wang and MacMahon (1979b) reported an increased standardized mortality ratio (SMR) for death due to bladder cancer that was “on the borderline of statistical significance,” but did not attribute this observation to exposure to chlordane because a similar effect was not observed in the manufacturing study (Wang and MacMahon 1979a). A follow-up study on a larger cohort of pesticide applicators found no association of exposure to chlordane with total deaths due to cancer or to a specific type of cancer (MacMahon et al. 1988). In another retrospective mortality study of four cohorts (305-1,155 workers/plant exposed for ≥6 months) from four manufacturing plants, there was a significantly increased risk of death from noncancer respiratory disease, and a slight excess risk of cancer of the esophagus, rectum, liver, and hematopoietic system at plant 3, and a slightly greater risk of stomach cancer at plant 1 (Ditraglia et al. 1981). However, chlordane was the only pesticide manufactured at plant 1, while aldrin, dieldrin, endrin, and dichlorodiphenyltrichloroethane were manufactured at plant 3. The statistical power did not allow for a conclusion that no association existed between cause-specific mortality and employment at the plants. However, in a follow-up to the study by

Ditraglia et al. (1981), the carcinogenic risk among workers exposed to organochlorines was assessed (Brown 1992). This study added 11 years to the previous follow-up study, thus providing 40 years of observation for the cohort. As 23 years was the minimum time elapsed since each cohort member was first employed at the study plants, this allowed more time for diseases with long latency periods to develop. The investigator concluded that the mortality for all causes and all malignant neoplasms was lower than expected. In addition, the study was limited by a lack of exposure data and the potential for exposure to other chemicals. A small but insignificant increase in non-Hodgkin's lymphoma was observed in farmers exposed to chlordane in a case control study (Woods and Polissar 1989). These studies were limited because of unquantified exposure frequency, duration, and concentration, exposure to other compounds, and other confounding factors. Several cases of leukemia and neuroblastoma were reported in persons exposed to chlordane or heptachlor in their home or garden, or as a result of their profession as pest control operators (Epstein and Ozonoff 1987; Infante et al. 1978). Although case reports are not sufficient to indict chlordane as the etiologic agent, they indicate the need for further epidemiological study. A small case-control study found that levels of chlordane residues (heptachlor epoxide, oxychlordane, *trans*-nonachlor) in the breast fat from 20 women with malignant breast disease were not significantly different from 20 women with benign breast disease (largely nonproliferative fibrocystic changes) (Falck et al. 1992).

No studies were located regarding carcinogenic effects in animals after inhalation exposure to chlordane. EPA used the oral q_1* of 1.3 $(mg/kg/day)^{-1}$ (see Section 2.2.2.8) for oral exposure to calculate a unit risk in air of $3.7x10^{-4}$ $(\mu g/m^3)^{-1}$ (IRIS 1992). This corresponds to upper bound individual lifetime cancer risks at 10^{-4} to 10^{-7} of $3x10^{-4}$ to $3x10^{-7}$ mg/m^3. The unit risk should not be used if the concentration in air exceeds $3x10^{-2}$ mg/m^3, above this concentration the slope factor may differ from the slope factor from which the unit risk was derived.

2.2.2 Oral Exposure

2.2.2.1 Death

Most cases of acute human oral exposure to chlordane involved accidental ingestion by children (Aldrich and Holmes 1969; Curley and Garrettson 1969; EPA 1980a). The estimated doses of chlordane ingested are 11.0 mg/kg; complete recovery generally followed medical intervention. In humans, an estimated acute oral lethal dose of chlordane (WHO 1984) was between 25 and 50 mg/kg,

but documentation was not provided and the method of estimation was not discussed. A man who accidentally ingested an unknown quantity of chlordane developed convulsions shortly after the ingestion and subsequently died (Kutz et al. 1983). No studies were located regarding lethality in humans following longer term oral exposure to chlordane.

The dose of chlordane associated with death following acute oral exposure in animals depends on the species and the composition of the chlordane sample administered. Studies published in or prior to 1950 are not included herein because the chlordane produced during this period contained hexachlorocyclopentadiene, which has been shown to be very toxic (WHO 1984; Ingle 1965). In rats, the reported oral LD_{50} value is between 83 and 590 mg/kg (Ambrose et al. 1953a; Ben-Dyke et al. 1970; Boyd and Taylor 1969; Deichmann and Keplinger 1970; Gaines 1960; Lehman 1951; Podowski et al. 1979; Truhaut et al. 1974, 1975). The low value of 83 mg/kg (Podowski et al. 1979) is for the 99.9% pure *cis* isomer, which is less likely than the technical product to be encountered in the environment near waste sites. Oral LD_{50} values for technical grade chlordane in the rat range from 137 to 590 mg/kg. The Gaines (1960) study provided the most information and reported separate LD_{50} values for males (335 mg/kg) and females (430 mg/kg). Oral LD_{50} values for the mouse have been reported at 390 (Gak et al. 1976; Truhaut et al. 1974, 1975) or 145 mg/kg/day (NIOSH 1988). Documentation for the NIOSH (1988) value, however, was unavailable. The hamster appears to be relatively resistent to death following acute oral dosing with chlordane; the reported LD_{50} value in this species was 1,720 mg/kg (Gak et al. 1976; Truhaut et al. 1974, 1975). Truhaut et al. (1975) speculated, on the basis of different activities of liver microsomal enzymes in rats, mice, and hamsters, that species differences in LD_{50} values may reflect differences in the rate of metabolism of the constituents of chlordane. Daily gavage treatment with 50 mg/kg/day caused the death of two of five rats on day 9 and 12 (Ambrose et al. 1953a). No deaths occurred in rats similarly treated with 25 mg/kg/day for 15 days. Technical chlordane at 300 mg/kg/day given by gavage resulted in death of 4 of 10 mice within 7 days, but no mice died at a dose of 100 mg/kg/day for 30 days (Balash et al. 1987). In a developmental study in rats, four of eight rats died after exposure to 80 mg/kg/day technical chlordane in olive oil by gavage on days 7-17 of gestation (Usami et al. 1986). No deaths occurred in 21 rats similarly treated at 40 mg/kg/day. In a developmental study in mice, 3 of 25 mice died after treatment with 50 mg/kg/day technical chlordane in corn oil on days 8-12 of gestation (Chernoff and Kavlock 1982). No other dose levels were included.

In a 6-week dose-range finding study conducted by NCI (1977), mortality was significantly increased in male and female rats fed diets containing analytical grade chlordane (71.7% *cis*- and 23.1% *trans*-chlordane) at doses of 40 mg/kg/day (females) or 80 mg/kg/day (females and males). The deaths occurred within the first 14 days. Mortality did not increase at 20 mg/kg/day. In a 6-week dietary study in mice (NCI 1977), mortality increased significantly at 20.8 mg/kg/day but not at 10.4 mg/kg/day. Doses for rats and mice in the 6-week NCI (1977) study were estimated by applying reference food factors to the average dietary concentrations reported by the investigators. Dietary exposure of rats to technical chlordane at 32 mg/kg/day for 15-163 days resulted in 100% mortality (Ambrose et al. 1953a).

In the chronic (80-week) dietary study by NCI (1977), a dose-related increase in mortality was observed in female rats at doses of 6.0 and 12.1 mg/kg/day. No significant increase in mortality was observed in male rats at doses of 10.2 or 20.4 mg/kg/day in this study. There appears to be a sexrelated difference in mortality in this study. No effect on survival was reported by Velsicol Chemical Co. (1983a) in rats at the highest dose (1.175 mg/kg/day in males, 1.409 mg/kg/day in females) in a 30-month dietary study. In the NCI (1977) study, mortality increased significantly in male mice at doses of 3.9 and 7.3 mg/kg/day for 80 weeks; mortality did not increase in female mice at doses of 3.9 and 8.3 mg/kg/day. Doses in the chronic NCI (1977) study were estimated by applying reference food factors to the time-weighted average dietary concentrations reported by the authors. The relatively high resistance to death among the female mice in this study is not readily explained. Although one may suspect gender-related differences in metabolism, supporting data were not located. Increased mortality occurred in mice exposed to 6.5 mg/kg/day in an l8 month dietary study (IRDC, 1973). In a 24-month dietary study in mice, there was no effect on survival of either sex at 1.21 mg/kg/day, the highest dose tested (Velsicol Chemical Co. 1983b). All reliable LD_{50} and LOAEL values for lethality in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

The systemic effects in humans and animals after oral exposure to chlordane are discussed below. The highest NOAEL values and the LOAEL values for each systemic effect from all reliable studies for each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

TABLE 2-2. Levels of Significant Exposure to Chlordane - Oral

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Form
ACUTE EXPOSURE									
Death									
1	Rat	(F)	2 wk ad lib				40 (4/5 deaths in females) 80 (5/5 deaths in males)	NCI 1977	Analytical
2	Rat	(G)	once				137 (LD_{50})	Boyd and Taylor 1969	Technical
3	Rat	(GO)	11 d Gd 7-17 1x/d				80 (death in 4/8 dams)	Usami et al. 1986	Technical
4	Rat	(GO)	once				335 (LD_{50})	Gaines 1960	Technical
5	Rat	(GO)	once				430 (LD_{50})	Gaines 1960	Technical
6	Rat	(G)	once				283 (LD_{50})	Ben-Dyke et al. 1970	Technical
7	Rat	(GO)	once				420 (LD_{50})	Deichmann and Keplinger 1970	Technical
8	Rat	(GO)	once				590 (LD_{50})	Ambrose et al. 1953a	Technical
9	Rat	(GO)	once				83 (LD_{50})	Podowski et al. 1979	*cis*
10	Rat	(GO)	once				350 (LD_{50})	Truhaut et al. 1974, 1975	Technical
11	Rat	(GO)	3-12 d 1x/d				50 (2/5 died on day 9 and 12)	Ambrose et al. 1953a	Technical
12	Rat	(G)	once				457 (LD_{50})	Lehman 1951	Technical
13	Mouse	(GO)	once				390 (LD_{50})	Truhaut et al. 1975	Technical

TABLE 2-2. Levels of Significant Exposure to Chlordane - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Form
14	Mouse	(GO)	7 d 1x/d				300 (4/10 died)	Balash et al. 1987	Technical
15	Mouse	(GO)	5 d Gd 8-12 1x/d				50 (3/25 died)	Chernoff and Kavlock 1982	Technical
16	Hamster	(GO)	once				1720 (LD_{50})	Truhaut et al. 1974, 1975	Technical
Systemic									
17	Rat	(GO)	once	Resp	200			Truhaut et al. 1975	Technical
				Cardio	200				
				Gastro	200				
				Hepatic		200 (increased SGPT and serum LDH, depressed liver GOT, LDH, ChE, G6PDH, hypertrophy, dilatation of centrilobular sinuses, and congestion)			
				Renal		200 (congestion, tubular dilatation)			
				Other (adrenal)	200				
18	Rat	(GO)	once	Hepatic		200 (increased blood glucose and urea, decreased liver glycogen, increased gluconeogenesis)		Kacew and Singhal 1973	*cis*
				Renal		200 (increased kidney gluconeogenic enzymes, cyclic adenosine monophosphatase and adenyl cyclase activity)			

TABLE 2-2. Levels of Significant Exposure to Chlordane - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Form
19	Rat	(G)	once	Musc/skel		260 (hypertonicity of skeletal muscles)		Santolucito and Whitcomb 1971	Technical
20	Rat	(GO)	4 d 1x/d	Hepatic		100 (increased serum triglyceride, cholesterol, gamma-GTP; reduced blood sugar, increased relative liver weight and lipid content; increased lipid perioxidation; hepatocellular hypertrophy)		Ogata and Izushi 1991	Technical
21	Mouse	(GO)	14 d 1x/d	Hemato	4	8 (increase in total leukocytes)		Johnson et al. 1986	*trans*
				Other (body weight)	8				
22	Mouse	(GO)	once	Resp	200			Truhaut et al. 1975	Technical
				Cardio	200				
				Gastro	200				
				Hepatic		200 (hepatic hypertrophy, congestion, dilatation of centilobular sinuses, elevated SGPT and LDH, and liver GPT and GOT)			
				Renal		200 (congestion and tubular dilatation)			
				Other (adrenal)	200				

TABLE 2-2. Levels of Significant Exposure to Chlordane - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Form
23	Hamster	(GO)	once	Resp	1200			Truhaut et al. 1975	Technical
				Cardio	1200				
				Gastro		1200 (atrophy of gastric mucosa)			
				Hepatic		1200 (decreased liver LDH and increased G6PDH, congestion, dilatation of centrilobular sinuses, and hypertrophy)			
				Renal		1200 (congestion and tubular dilatation)			
				Other (adrenal)	1200				
Immunological									
24	Mouse	(GO)	14 d 1x/d		8			Johnson et al. 1986	*trans*
Neurological									
25	Rat	(GO)	once				200 (convulsions)	Ambrose et al. 1953a	Technical
26	Rat	(GO)	once			100 (hypothermia)	200 (paralylsis, convulsions)	Hrdina et al. 1974	*cis*
27	Rat	(GO)	once			200 (congestion in brain)		Truhaut et al. 1975	Technical
28	Rat	(GO)	9-12 d 7 d/wk		25		50 (convulsions and death in 2 rats on day 9, 12)	Ambrose et al. 1953a	Technical
29	Mouse	(GO)	once			200 (congestion in brain)		Truhaut et al. 1975	Technical

TABLE 2-2. Levels of Significant Exposure to Chlordane - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Form
30	Hamster	(GO)	once			1200 (congestion in brain)		Truhaut et al. 1975	Technical
Developmental									
31	Rat	(GO)	11 d Gd 7-17 1x/d		80			Usami et al. 1986	Technical
32	Mouse	(GO)	7 d (third tri-mester) 1x/d			1[b] (altered conditioned avoidance response, open field test, electroshock seizure threshold)		Al-Hachim and Al-Baker 1973	Technical
33	Mouse	(GO)	5 d Gd 8-12 1x/d		50			Chernoff and Kavlock 1982	Technical
INTERMEDIATE EXPOSURE									
Death									
34	Rat	(F)	15-163 d ad lib				32 (100% mortality)	Ambrose et al. 1953a	Technical
35	Mouse	(F)	6 wk ad lib				20.8 (2/5 deaths in males)	NCI 1977	Analytical
Systemic									
36	Rat	(F)	15-163 d ad lib	Other			32 (unspecified rapid weight loss prior to death)	Ambrose et al. 1953a	Technical
37	Rat	(F)	10-20 wk 2x/wk	Hepatic		0.1 (increased cytochrome P-450 content at 10 weeks; decreased microsomal protein at 20 weeks)		Mahon et al. 1978	*cis* + *trans*

TABLE 2-2. Levels of Significant Exposure to Chlordane - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Form
38	Rat	(F)	2-9 mo ad lib	Hepatic		0.125[c] (centrilobular hypertrophy, cytoplasmic inclusion bodies)		Ortega et al. 1957	Technical
				Renal	1.25				
39	Rat	(GO)	15 d 7 d/wk	Hepatic		6.25 (intracytoplasmic bodies)		Ambrose et al. 1953a	Technical
				Other	25	50 (unpsecified body weight loss)			
Immunological									
40	Mouse	(F)	18 d 7 d/wk		8			Barnett et al. 1990a	Analytical
Neurological									
41	Rat	(F)	12 wk ad lib			1.25 (inhibited brain ATPase)	5 (convulsions)	Drummond et al. 1983	Technical
Developmental									
42	Mouse	(F)	19 d Gd 1-19		0.16		8 (decreased CMI response)	Spyker-Cranmer et al. 1982	Analytical
43	Mouse	(F)	18 d Gd 1-18			8 (decreased liver cell-colony forming capacity)		Barnett et al. 1990b	Analytical
44	Mouse	(F)	18 d Gd 1-18			4 (decreased myeloid cell colony forming capacity)		Barnett et al. 1990a	Analytical
45	Mouse	(GO)	18 d Gd 1-18 1x/d			8.0 (decreased 5'-nucleotidase activity in macrophages; activation of macrophages to inflammatory state in mice exposed prenatally)		Theus et al. 1992	Analytical
46	Mouse	(F)	19 d Gd 1-19				8.0 (death of 55% offspring)	Cranmer et al. 1984	Analytical

TABLE 2-2. Levels of Significant Exposure to Chlordane - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Form
47	Mouse	(F)	18 d Gd 1-18		4	8 (decreased DTH response in offspring)		Barnett et al. 1985a	Technical
48	Mouse	(F)	19 d Gd 1-19			4 (decreased DTH, mixed lymphocyte reactivity)		Barnett et al. 1985b	Technical
Reproductive									
49	Rat	(F)	Weaning through mating, gestation and lactation				16 (decreased fertility and survivability)	Ambrose et al. 1953a	Technical
50	Rat	(F)	90 d ad lib			19.5 (360% increase in androgen receptor content of the ventral prostate gland)		Shain et al. 1977	Technical
51	Mouse	(GO)	30 d 1x/d			100 (reduced size of seminiferous tubules, degeneration in spermatogenic epithelium)		Balash et al. 1987	Technical
Cancer									
52	Mouse	(F)	36 wk ad lib				3.25 (CEL-hepatocellular carcinoma)	Becker and Sell 1979	Technical
CHRONIC EXPOSURE									
Death									
53	Rat	(F)	80 wk ad lib				6.0 (18% decreased survival of females)	NCI 1977	Analytical

TABLE 2-2. Levels of Significant Exposure to Chlordane - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Form
54	Mouse	(F)	18 mo ad lib				6.5 (76-86% mortality)	IRDC 1973	Technical
55	Mouse	(F)	80 wk ad lib				3.9 (40% decreased survival of males)	NCI 1977	Analytical
Systemic									
56	Rat	(F)	30 mo ad lib	Resp	1.409			Velsicol Chemical Co. 1983a; Khasawinah and Grutsch 1989a	Technical
				Cardio	1.409				
				Gastro	1.409				
				Hemato	1.409				
				Musc/skel	1.409				
				Hepatic	0.055[d]	0.273 (hepatocellular hypertrophy in females)			
				Renal	1.409				
				Derm/oc	1.409				
				Other	1.409				
57	Rat	(F)	80 wk ad lib	Resp	20.4			NCI 1977	Analytical
				Cardio	20.4				
				Gastro	20.4				
				Musc/skel	20.4				
				Hepatic	20.4				
				Renal	20.4				
				Derm/oc	20.4				
				Other	10.2	20.4 (unspecified consistent decreased body weight gain in males)			
58	Rat	(F)	80 wk ad lib	Other	6.0	12.1 (unspecified consistent decreased body weight gain in females)		NCI 1977	Analytical

TABLE 2-2. Levels of Significant Exposure to Chlordane - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Form
59	Rat	(F)	407 d ad lib	Hepatic	2	4 (significant increase in liver weight, liver cell inclusion bodies, and liver cell hypertrophy)		Ambrose et al. 1953	Technical
				Other	8	16 (11-18% decrease in body weight)			
60	Mouse	(F)	80 wk ad lib	Resp	8.3			NCI 1977	Analytical
				Cardio	8.3				
				Gastro	8.3				
				Musc/skel	8.3				
				Renal	8.3				
				Hepatic	8.3				
				Derm/oc	8.3				
				Other	8.3				
61	Mouse	(F)	24 mo ad lib	Resp	1.21			Velsicol Chemical Co. 1983b; *Khasawinah and* Grutsch 1989b	Technical
				Cardio	1.21				
				Gastro	1.21				
				Hemato	1.21				
				Musc/skel	1.21				
				Hepatic	0.10	0.47 (hepatocellular hypertrophy, fatty degeneration and necrosis in males hepatocellular hypertrophy in females)			
				Renal	1.21				
				Derm/oc	1.21				
				Other	1.21				
Neurological									
62	Rat	(F)	80 wk ad lib		6.0		12.1 (tremors in females)	NCI 1977	Analytical

TABLE 2-2. Levels of Significant Exposure to Chlordane - Oral (*continued*)

Key to figure[a]	Species	Route	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Form
63	Mouse	(F)	80 wk ad lib		3.9		8.3 (tremors in females)	NCI 1977	Analytical
64	Mouse	(F)	80 wk ad lib		3.9		7.3 (tremors in males)	NCI 1977	Analytical
Cancer									
65	Mouse	(F)	18 mo ad lib				3.25 (CEL-hepatocellular carcinoma)	IRDC 1973; Epstein 1976	
66	Mouse	(F)	80 wk ad lib				8.3 (CEL-hepatocellular carcinoma)	NCI 1977	Analytical
67	Mouse	(F)	80 wk ad lib				3.9 (CEL-hepatocellular carcinoma)	NCI 1977	Analytical
68	Mouse	(F)	24 mo ad lib				1.21 (CEL-hepatocellular carcinoma)	Velsicol Chemical Co. 1983b; Khasawinah and Grutsch 1989b	Technical

[a]The number corresponds to entries in Figure 2-2.
[b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.001 mg/kg/day; dose divided by an uncertainty factor of 1,000 (10 for use of a LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability).
[c]An intermediate-duration oral MRL of 0.0006 mg/kg/day, using the NOAEL of 0.055 mg/kg/day identified in the Khasawinah and Grutsch (1989a; Velsicol Chemical Co. 1983a) chronic-duration study. The Ortega et al. (1957) study was used as a supporting study. The dose was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).
[d]Used to derive a chronic-duration oral MRL of 0.0006 mg/kg/day; dose divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ad lib = ad libitum; ATPase = adenosine triphosphatase; Cardio = cardiovascular; CEL = cancer effect level; CMI = cell-mediated immunity; d = day(s); Derm/oc = dermal/ocular; DTH = delayed type hypersensitivity; (F) = feed; (G) = gavage-not specified; G6PDH = glucose-6-phosphate dehydrogenase; Gastro = gastrointestinal; Gd = gestation day; (GO) = gavage-oil; GOT = glutamic oxalocetic transaminase; GPT = glutamic-pyruvic transaminase; Hemato = hematological; LD_{50} = lethal dose; 50% kill; LDH = lactate dehydrogenase; LOAEL = lowest-observed-adverse-effect level; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; Resp = respiratory; SGPT = serum glutamic-pyruvic transaminase; wk = week(s); wt = weight; x = times

FIGURE 2-2. Levels of Significant Exposure to Chlordane - Oral

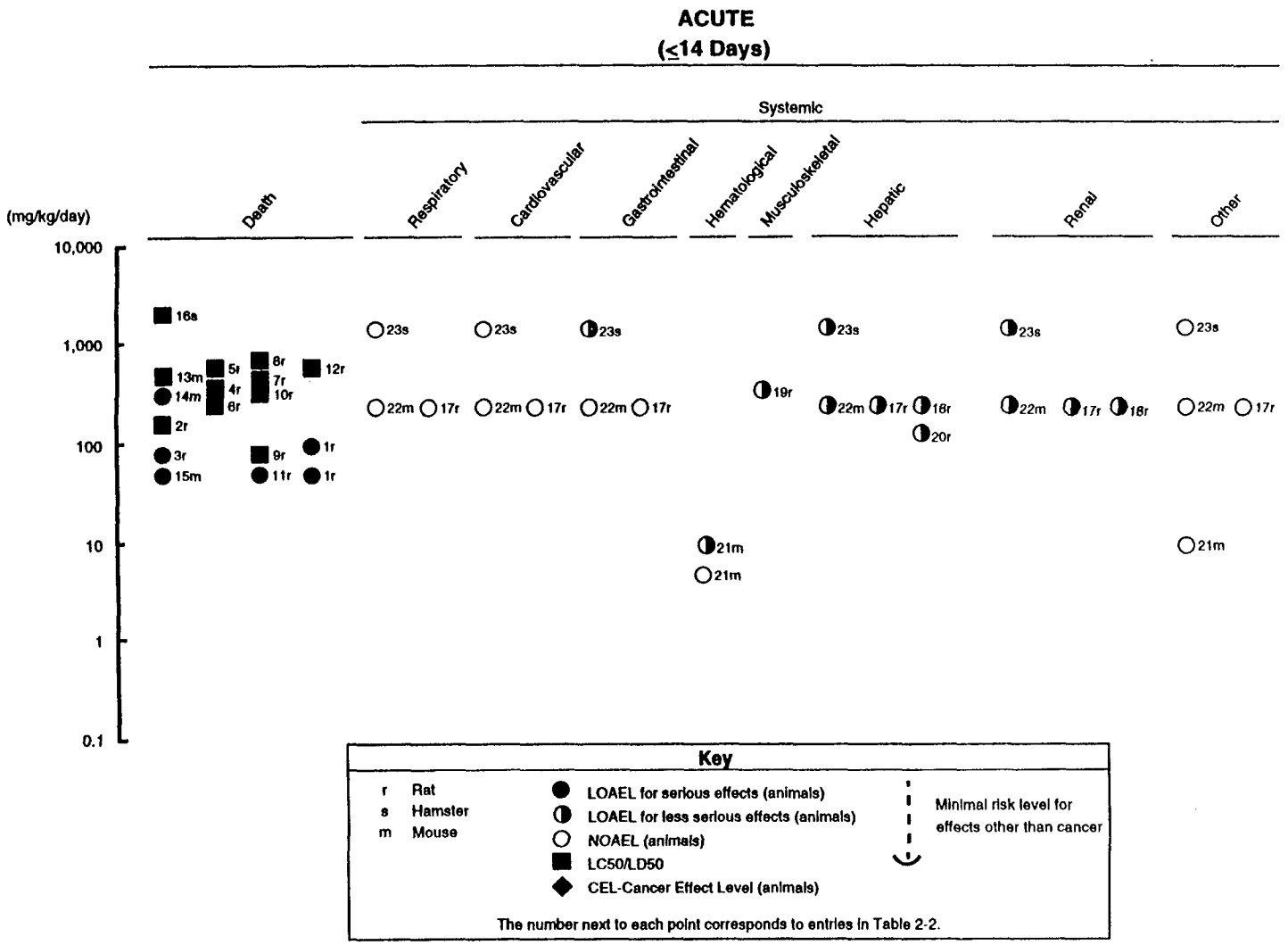


FIGURE 2-2. Levels of Significant Exposure to Chlordane - Oral (Continued)

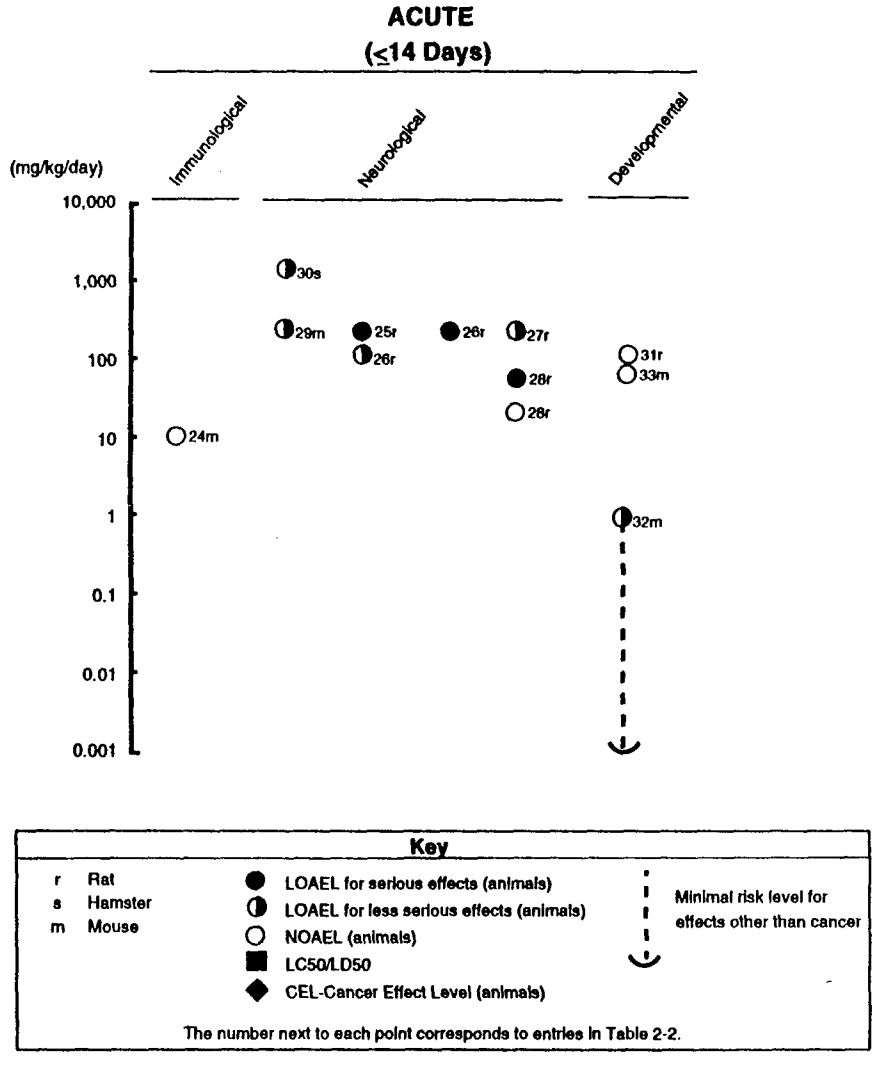


FIGURE 2-2. Levels of Significant Exposure to Chlordane - Oral (Continued)

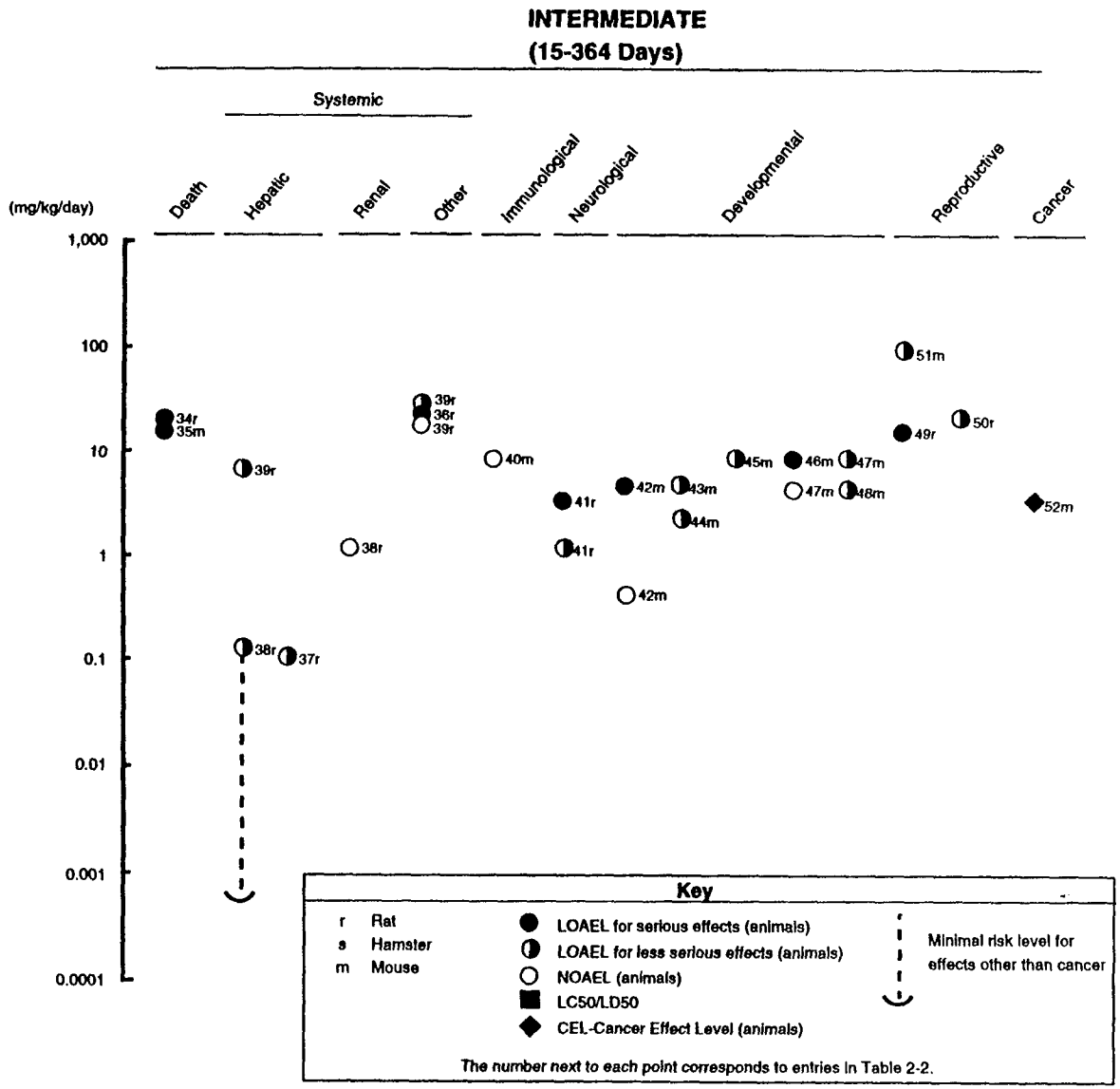
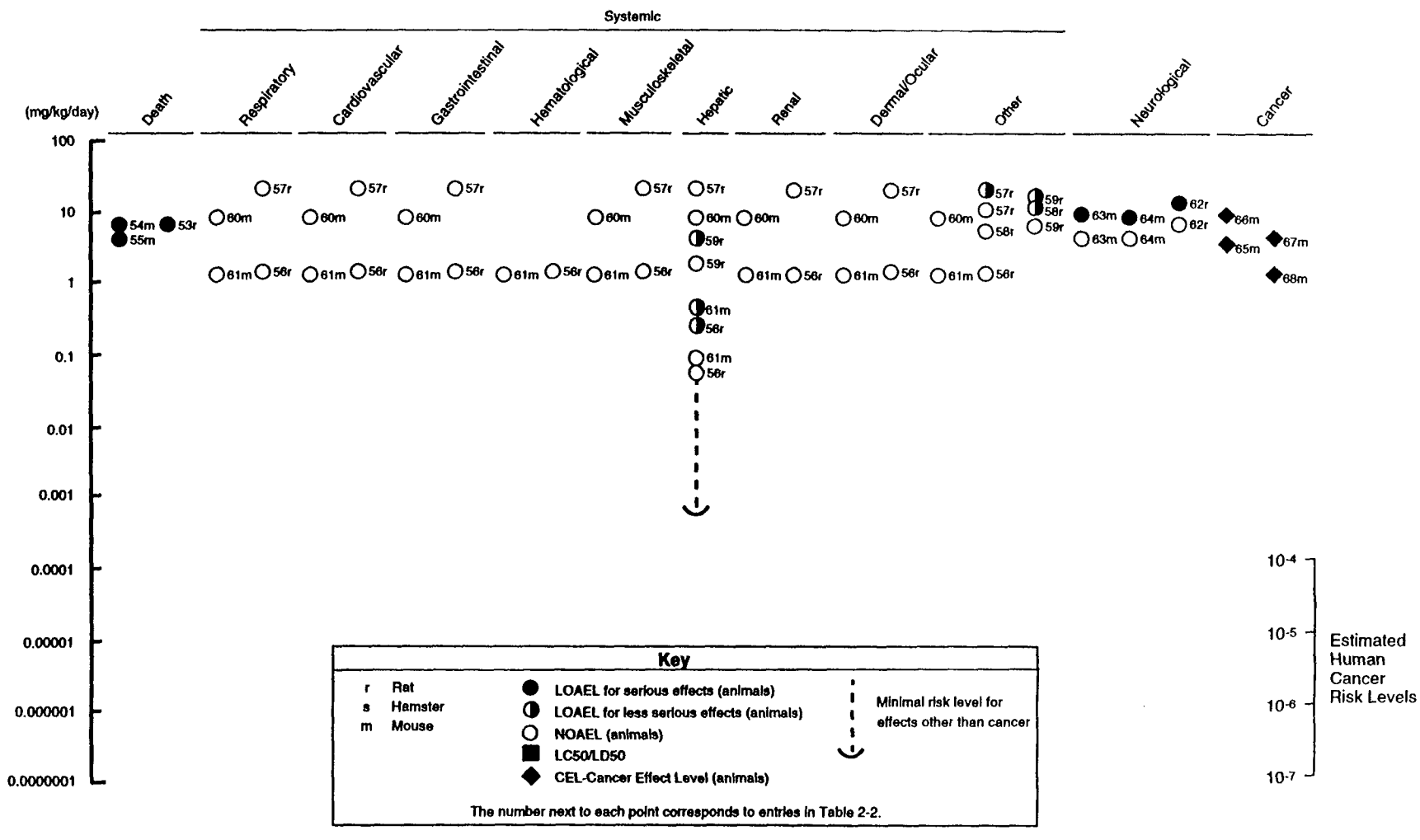


FIGURE 2-2. Levels of Significant Exposure to Chlordane - Oral (Continued)



Respiratory Effects. No respiratory effects were reported in a compilation of cases and personal reports of acute human exposure (EPA (1980a). Reports of other acute oral exposure cases (Curley and Garrettson 1969; Olanoff et al. 1983) did not list pneumonia as a part of the syndrome. Aldrich and Holmes (1969) diagnosed "probable bilateral bronchopneumonia" by radiograph in a 4-year-oId girl with neurological symptoms who had ingested chlordane, but whether this was thought to be due to chlordane exposure was not stated. Approximately 3 hours after a 15-month-old girl ingested 11.1 mg/kg chlordane, respiration rate was irregular and breathing was shallow (Lensky and Evans 1952). The investigators were unable to determine whether respiratory depression was due to chlordane ingestion or to the large amount of barbiturate given to control chlordane-induced seizures.

In animals, acute oral exposure to chlordane does not appear to have adverse respiratory effects. In single-dose experiments, no histopathological lesions of the lungs were found in rats or mice treated by gavage with 200 mg/kg or hamsters treated by gavage with 1,200 mg chlordane/kg (Truhaut et al. 1974, 1975). Comprehensive histopathological examinations performed on rats following dietary exposure for 80 weeks of ≤20.4 mg chlordane/kg/day in males and 12.1 mg chlordane/kg/day in females (NCI 1977) or 30 months of ≤1.175 mg chlordane/kg/day in males and 1.409 mg chlordane/kg/day in females (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a) revealed no evidence of effects in the respiratory tract. Histological examination of the lungs of rats exposed to technical chlordane in the diet at doses ≤16 mg/kg/day for 407 days revealed no lesions; however, only five males and five females per group were used (Ambrose et al. 1953a). Comprehensive histopathological examinations performed on mice following dietary exposure for 80 weeks of ≤7.3 mg chlordane/kg/day in males and 8.3 mg chlordane/kg/day in females (NCI 1977) or 24 months of ≤1.21 mg chlordane/kg/day (Khasawinah and Grutsch 1989b; Velsicol Chemical Co. 1983b) revealed no evidence of effects in the respiratory tract.

Cardiovascular Effects. No cardiovascular effects were reported in a compilation of cases and personal reports of acute human exposure (EPA (1980a) or in the case of a 15 month-old girl who ingested about 11.1 mg/kg chlordane (Lensky and Evans 1952). Elevated pulse rates were also found in other cases of human ingestion of chlordane (Curley and Garrettson 1969; Olanoff et al. 1983).

Acute oral exposure of animals to chlordane does not appear to have adverse effects on the cardiovascular system. In single-dose experiments, no histopathological lesions of the cardiovascular system were found in rats or mice treated with 200 mg chlordane/kg or hamsters treated with

1,200 mg chlordane/kg (Truhaut et al. 1974, 1975). Comprehensive histopathological examinations performed on rats following dietary exposure for 80 weeks of ≤20.4 mg chlordane/kg/day in males and 12.1 mg chlordane/kg/day in females (NCI 1977) or 30 months of ≤l.175 mg chlordane/kg/day in males and 1.409 mg chlordane/kg/day in females (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a) revealed no evidence of effects in the cardiovascular system. Histological examination of the hearts of rats exposed to technical chlordane in the diet at doses≤16 mg/kg/day for 407 days revealed no lesions; however, only five males and five females per group were used (Ambrose et al, 1953a). Comprehensive histopathological examinations performed on mice following dietary exposure for 80 weeks of ≤7.3 mg chlordane/kg/day in males and 8.3 mg chlordane/kg/day in females (NCI 1977) or 24 months of ≤1.21 mg chlordane/kg/day (Khasawinah and Grutsch 1989b; Velsicol Chemical Co. 1983b) revealed no evidence of effects in the cardiovascular system.

Gastrointestinal Effects. Data from a compilation of cases and personal reports reveal that gastrointestinal effects such as upset stomach, nausea, abdominal cramps, and diarrhea are among the earliest signs and symptoms observed in humans who have ingested chlordane (EPA 1980a). A number of other cases of human ingestion of chlordane have reported such effects as gastritis, nausea, vomiting, and diarrhea (Curley and Garrettson 1969; Dadey and Kammer 1953; Olanoff et al. 1983). Gastrointestinal symptoms were reported by 18% of the residents of 42 homes in Chattanooga, Tennessee, in which accidental contamination of part of the public water system with chlordane resulted in concentrations ranging from 0.1 to 92,500 ppb (Harrington et al. 1978). Serum levels of *trans*-nonachlor and oxychlordane, however, were approximately twice as high in asymptomatic as in symptomatic individuals, raising serious questions about the validity of the association between exposure to chlordane and the reported effects. These data are insufficient to estimate an effect level for gastrointestinal effects in humans.

Acute oral exposure of animals to chlordane does not appear to produce adverse effects on the gastrointestinal system. No histopathological lesions were observed in the gastrointestinal tracts of rats or mice treated with 200 mg chlordane/kg or in hamsters treated with 1,200 mg chlordane/kg (Truhaut et al. 1974, 1975). In chronic studies, dietary exposure to chlordane did not produce lesions of the gastrointestinal tracts of rats at doses of 12.1 mg/kg/day (females) or 20.4 mg/kg/day (males) (NCI 1977) or 1.175 mg/kg/day (males) or 1.409 mg/kg/day (females) (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a). Gastrointestinal lesions were not reported in chronic dietary experiments in mice at 7.3 mg/kg/day (males) or 8.3 mg/kg/day (females) (NCI 1977) or at

1.21 mg/kg/day (Khasawinah and Grutsch 1989b; Velsicol Chemical Co. 1983b). Histological examination of the small intestine and stomach of rats exposed to technical chlordane in the diet at doses ≤16 mg/kg/day for 407 days revealed no lesions; however, only five males and five females per group were used (Ambrose et al. 1953a).

Hematological Effects. Limited data were located regarding hematological effects in humans after oral exposure to chlordane. In one study, ingestion of approximately 32 mg/kg chlordane did not adversely affect red blood cell and white blood cell counts up to 5 days after exposure (Dadey and Kammer 1953). However, because the patient vomited after ingestion, the dose of 32 mg/kg does not reflect the dose available for absorption. In another study, a mild hypochromic anemia was diagnosed in a 15-month-old girl following ingestion of 11.1 mg/kg chlordane (Lensky and Evans 1952). Because this hematological effect was observed within a short time period after exposure and no hematological tests were performed prior to exposure, it possible that this hematological effect may have been a preexisting condition in the infant and not due to chlordane exposure. In another case report, white blood cell count decreased from 13,650/mm to 8350/mm 24 hours after a 20-month-old infant had ingested an unknown amount of chlordane (Curley and Garrettson 1969). As with the previous case report, a hypochromic microcytic anemia compatible with a poor iron nutrition was also observed.

Mice treated by gavage with 8 mg *trans*-chlordanelkglday for 14 days developed leukocytosis associated with lymphocytosis (Johnson et al. 1986). A dose of 4 mg/kg/day was without effect. The toxicological significance of this effect to humans is unknown. Chronic dietary exposure of rats (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a) and mice (Khasawinah and Grutsch 1989b; Velsicol Chemical Co. 1983b) produced no hematological effects at doses of 1.175-l .409 mg/kg/day (rats) or 1.21 mg/kg/day (mice). Hematological parameters (erythrocyte counts, leucocyte counts, and hemoglobin concentration), which were monitored frequently in rats exposed to technical chlordane in the diet at doses ≤16 mg/kg/day for 407 days, were not affected; however, only five males and five females per group were used (Ambrose et al. 1953a).

Musculoskeletal Effects. No musculoskeletal effects were reported in a compilation of cases and personal reports of acute human exposure (EPA 1980a).

Single oral doses of 260 mg chlordane/kg caused hypertonicity of skeletal muscle in rats, but this may represent a neurological effect (Santolucito and Whitcomb 1971). There was no effect on the mechanical response of the muscle measured with a strain-gauge transducer *In situ*; therefore, these results are not interpreted as indicating that oral exposure to chlordane was associated with musculoskeletal effects. Furthermore, no significant increase in the serum level of creating phosphokinase was found in rats treated orally with 100 mg/kg/day technical chlordane for 4 days (Ogata and Izushi 1991). Comprehensive histopathological examination performed on rats following dietary exposure for 80 weeks of ≤20.4 mg chlordane/kg/day in males and 12.1 mg chlordane/kg/day in females (NCI 1977) or 30 months of ≤1.175 mg chlordane/kg/day in males and 1.409 mg chlordane/kg/day in females (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a) revealed no evidence of effects in the musculoskeletal system. Comprehensive histopathological examinations performed on mice following dietary exposure for 80 weeks of ≤7.3 mg chlordane/kg/day in males and 8.3 mg chlordane/kg/day in females (NCI 1977) or 24 months of ≤1.21 mg chlordane/kg/day (Khasawinah and Grutsch 1989b; Velsicol Chemical Co. 1983b) revealed no evidence of effects in the musculoskeletal system.

Hepatic Effects. There is little information in the available literature concerning hepatic effects in humans following oral exposure to chlordane. A compilation of cases and personal reports of human exposure (EPA 1980a) did not suggest that liver effects are a predominant part of the clinical picture for acute exposure. Results of various liver function and damage tests were within normal limits in a 20-month-old male who ingested an unknown amount of technical grade (74%) chlordane (Curley and Garrettson 1969). Evaluations were made at 20 hours to 3 days after exposure.

In animals, liver effects from acute oral exposure of animals to chlordane include liver microsomal enzyme induction, alterations in the activities of mitochondrial enzymes, histochemical and histomorphological alterations, and increased liver weight. In a 14-day feeding study in rats, Den Tonkelaar and Van Esch (1974) reported significant liver drug metabolizing enzyme induction (aniline hydroxylase, aminopyrine demethylase, and hexobarbital oxidase) at dietary concentrations equivalent to 0.50-2.5 mg/kg/day, but not at 0.25 mg/kg/day. Liver microsomal enzyme induction is considered an adaptative rather than an adverse effect. It should be noted, however, that in the case of exposure to chlordane, which induces enzymes associated with its own metabolism (see Section 2.3.3), some of the metabolites of chlordane are potentially more toxic than the parent compound. A single oral dose of 200 mg/kg *cis*-chlordane in rats significantly decreased liver glycogen and significantly increased

the activities of hepatic enzymes associated with gluconeogenesis (i.e., pyruvate carboxylase, fructose-1,6-diphosphatase and glucose-6-phosphatase) when measured 1 hour after treatment (Kacew and Singhal 1973; Singhal and Kacew 1976). These investigators also found that the activity of adenyl cyclase increased in the livers of these rats and that levels of hepatic cyclic adenosine monophosphate (AMP) correspondingly increased. Rats treated by gavage with 100 mg/kg/day technical chlordane for 4 days had increased liver weight and liver lipid content, hypertrophy, and increased serum triglyceride, cholesterol, and gamma-glutamyl transferase (Ogata and Izushi 1991). There were no effects on activities of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), creatinine phosphokinase or lactate dehydrogenase (LDH). Liver toxicity characterized by hypertrophy, dilatation of centrilobular sinuses, and congestion by increased SGPT and serum LDH and decreased serum cholinesterase, and by decreased liver GOT, LDH, cholinesterase, and glucose-6-phosphate dehydrogenase occurred in rats given a single oral dose of 200 mg/kg (Truhaut et al. 1974, 1975). In mice given 200 mg/kg, hepatic hypertrophy, congestion, and dilatation of centrilobular sinuses were also seen (Truhaut et al. 1975). In addition, SGPT and serum LDH were increased, as were hepatic GPT and GOT. In hamsters given 1,200 mg/kg, serum cholinesterase was depressed, while hepatic LDH was decreased and hepatic glucose-6-phosphate dehydrogenase was increased (Truhaut et al. 1974, 1975). Hamsters also had congestion, dilatation of centrilobular sinuses, and hypertrophy. In mice treated by gavage for 2 weeks, liver weight increased at 8 mg/kg/day but not at 4 mg/kg/day (Johnson et al. 1986), but histological examination was not performed.

In a 28-day feeding study, a dose-related increase in liver microsomal enzyme activity and relative liver weights was reported in rats at doses of 6.25-25 mg chlordane/kg/day (Casterhne and Williams 1971). Intracytoplasmic bodies were found in the liver cells of rats treated by gavage with ≥6.25 mg/kg/day technical chlordane for 15 days (Ambrose et al. 1953a). In addition, centrilobular hypertrophy and cytoplasmic inclusions were found in rats exposed to technical chlordane in the diet at doses ≥0.125 mg/kg/day for 2-9 months (Ortega et al. 1957). No histopathological liver lesions or increased levels of SGPT or serum alkaline phosphatase were found in rats exposed to chlordane in the diet at 0.1 mg/kg/day for 10-20 weeks (Mahon et al. 1978). However, cytochrome P-450 content was significantly increased at 10 weeks, and microsomal protein content was significantly decreased at 20 weeks, when compared with controls. The 0.125 mg/kg/day dose in the study by Ortega et al. (1957) study is used as a supporting study for an intermediate-duration oral MRL which is based on

the Khasawinah and Grutsch (1989a; Velsicol Chemical Co. 1983a) chronic-duration study (see below).

NCI (1977) reported no compound-related liver lesions in rats of either sex in an 80-week study with pure analytical grade chlordane (72% *cis* and 23% *tram* isomers) at doses of 6.0-20.4 mg/kg/day. In a 30-month study in rats, no liver effects occurred in males at 1.175 mg/kg/day, the highest dose tested, but regional liver hypertrophy occurred in females at 0.273 mg/kg/day (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a). No effects on the liver were found at 0.055 mg/kg/day. The NOAEL of 0.055 mg/kg/day in the study by Khasawinah and Grutsch (1989a) was used to derive an intermediate-duration oral MRL of 0.0006 mg/kg/day and a chronic-duration oral MRL of 0.0006 mg/kg/day as described in the footnote in Table 2-2. Significantly increased liver weight, liver cell inclusion bodies, and hepatocellular hypertrophy were found in rats given technical chlordane in the diet at doses ≥4 mg/kg/day for 407 days (Ambrose et al. 1953a). These lesions were not observed at 2 mg/kg/day.

NCI (1977) reported no compound-related liver lesions in mice of either sex in an SO-week study with pure analytical grade chlordane at doses of 3.9-8.3 mg/kg/day. The 24-month study in mice identified the lowest dose tested, 0.10 mg/kg/day, as a NOAEL for liver effects in both sexes (Khasawinah and Grutsch 1989b; Velsicol Chemical Co. 1983b). The next higher dose, 0.47 mg/kg/day was a LOAEL in both sexes associated with histopathological alterations. Lesions in the females were limited to hepatocellular swelling and degeneration. In males, these lesions had progressed to hepatocellular necrosis. In an unpublished 18-month dietary study in mice (IRDC 1973), which was reviewed by Epstein (1976), significantly increased liver weights and hepatocytomegaly were observed at all dose levels tested (0.65-6.5 mg/kg/day), but this may be related to enzyme induction, which is not considered adverse.

Renal Effects. Few data were located regarding renal effects in humans after oral exposure to chlordane. A compilation of cases and personal reports (EPA 1980a) did not mention kidney effects as a part of the clinical picture of acute human exposure. In one case report, no apparent renal effects were observed in an 18-year-old girl 24-48 hours after an acute exposure to 32 mg/kg of chlordane (Dadey and Kammer 1953). Because the patient vomited after ingestion, the dose of 32 mg/kg does not reflect the dose available for absorption.

In acute gavage studies, Truhaut et al. (1974, 1975) reported congestion of the kidneys in rats and mice treated with 200 mg/kg and hamsters treated with 1,200 mg chlordane/kg, respectively, but kidney weight was unaffected. A single oral dose of 200 mg/kg *cis*-chlordane significantly increased kidney gluconeogenic enzymes, kidney basal and fluoride-stimulated adenyl cyclase, and cyclic AMP (Kacew and Singhal 1973; Singhal and Kacew 1976). No histopathological renal lesions were found in rats exposed to 1.25 mg/kg/day technical chlordane in the diet for 2-9 months (Ortega et al. 1957). No histopathological lesions of the kidney were observed in rats consuming doses of ≤20.4 mg chlordane/kg/day in the males and 12.1 mg chlordane/kg/day in the females, or in mice consuming doses of ≤7.3 mg chlordane/kg/day in the males and 8.3 mg chlordane/kg/day in the females in an 80-week dietary study (NCI 1977). No histopathological lesions of the kidney, no blood chemistry alterations suggesting kidney effects, and no effects on urinalysis were reported in rats consuming ≤1.175 mg chlordane/kg/day in the males and 1.409 mg chlordane/kg/day in the females for 30 months (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a) and in mice consuming ≤1.21 mg chlordane/kg/day for 24 months (Khasawinah and Grutsch 1989b; Velsicol Chemical Co. 1983b). Histological examination of the kidneys of rats exposed to technical chlordane in the diet at doses ≤16 mg/kg/day for 407 days revealed no lesions; however, only five males and five females per group were used (Ambrose et al. 1953a).

Dermal/ocular Effects. Few data were located regarding dermal/ocular effects in humans after oral exposure to chlordane. A compilation of cases and personal reports (EPA 1980a) did not mention dermal/ocular effects as a part of the clinical picture of acute human exposure.

Comprehensive histopathological examination performed on rats following dietary exposure for 80 weeks of ≤20.4 mg chlordane/kg/day in males and ≤12.1 mg chlordane/kg/day in females (NCI 1977) or 30 months of ≤1.175 mg chlordane/kg/day in males and ≤1.409 mg chlordane/kg/day in females (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a) revealed no evidence of derma.l/ocular effects. Comprehensive histopathological examination performed on mice following dietary exposure for 80 weeks to doses ≤7.3 mg chlordane/kg/day in males and 8.3 mg chlordane/kg/day in females (NCI 1977) or 24 months to doses ≤1.21 mg chlordane/kg/day (Khasawinah and Grutsch 1989b; Velsicol Chemical Co. 1983b) revealed no evidence of dermal/ocular effects.

Other Systemic Effects. In single-dose experiments, no histopathological lesions of the adrenal were found in rats or mice treated with 200 mg chlordane/kg or hamsters treated with 1,200 mg chlordane/kg (Truhaut et al. 1974, 1975). Treatment of mice on gestation days 8-12 with 50 mg/kg/day by gavage had no effect on maternal body weight (Chemoff and Kavlock 1982). Gavage treatment of rats with 50 mg/kg/day for 15 days resulted in weight loss (Ambrose et al. 1953a). No effect was observed in rats similarly treated with 25 mg/kg/day. In addition, rapid weight loss occurred prior to death in rats given 32 mg/kg/day technical chlordane for 15-163 days (Ambrose et al. 1953a). Comprehensive histopathological examination performed on rats following dietary exposure for 80 weeks to doses ≤20.4 mg chlordane/kg/day in the males and 12.1 mg chlordane/kg/day in the females (NCI 1977) or 30 months to doses ≤1.2 mg chlordane/kg/day in the males and 1.4 mg chlordane/kg/day in the females (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a) revealed no evidence of other organ effects. Decreased rate of body weight gain and reduced terminal body weights were observed in the NCI (1977) study in rats. Female rats had reduced body weight at 12.1 mg/kg/day, but not at 6 mg/kg/day. Decreased body weight gain occurred in the male rats at 20.4 mg/kg/day, but not at 10.2 mg/kg/day. No effects on body weight were identified in male rats exposed to ≤1.175 mg/kg/day or in female rats exposed to ≤1.409 mg/kg/day for 30 days (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a).

Comprehensive histopathological examination performed on mice following dietary exposure for 80 weeks to doses ≤7.3 mg chlordane/kg/day in males and 8.3 mg chlordane/kg/day in females (NCI 1977) or 24 months to doses ≤1.21 mg chlordane/kg/day (Khasawinah and Grutsch 1989b; Velsicol Chemical Co. 1983b) revealed no evidence of other organ effects. Neither study identified effects on body weight. Body weights were decreased by 1l-l8% in rats exposed to technical chlordane in the diet at 16 mg/kg/day, but not at 18 mg/kg/day, for 407 days (Ambrose et al. 1953a).

2.2.2.3 Immunological Effects

No studies were located regarding immunological effects in humans after oral exposure to chlordane.

In single-dose experiments, no histopathological lesions of the spleen were found in rats or mice treated with 200 mg chlordane/kg or in hamsters treated with 1,200 mg chlordane/kg, but tests of immune function were not performed (Truhaut et al. 1974, 1975). In a 14-day gavage study, no definitive evidence of immune dysfunction was observed in mice treated with 8 mg/kg/day, but

leukocytosis associated with lymphocytosis were observed at this dose (Johnson et al. 1986). Oral treatment of adult mice for 18 days with 8 mg/kg/day had no effect on granulocyte-macrophage and spleen colony forming stem cell populations in the bone marrow (Bamett et al. 1990a). No effects on spleen weight or spleen histology were found in rats exposed to technical chlordane at ≥1.25 mg/kg/day chlordane in the diet for 2-9 months (Ortega et al. 1957) or in rats exposed to technical chlordane in diet at ≤16 mg/kg/day for 407 days (Ambrose et al. 1953a). However, only five of six males and five of six females per group were used, and immune function was not assessed in these studies. The NOAEL values for immunological effects in mice for acute and intermediate durations in studies that assessed immune function are recorded in Table 2-2 and plotted in Figure 2-2. Information on immune effects in prenatally exposed mice is presented in Section 2.2.2.6, Developmental Effects.

2.2.2.4 Neurological Effects

Central nervous system effects including ataxia, headache, dizziness, irritability, excitability, confusion, incoordination, muscle tremors, seizures, convulsions, and coma have been described in a compilation of cases and personal reports as a consistent and predictable part of the clinical picture of acute human oral exposure to insecticidal formulations of chlordane (EPA 1980a). By accident, part of the public water system of Chattanooga, Tennessee, became contaminated, and the tap water of 42 houses had chlordane concentrations ranging from 0.1 to 92,500 ppb (Harrington et al. 1978). When the affected residents were surveyed, 18% reported neurological symptoms consistent with acute chlordane toxicity. Serum levels of trans-nonachlor and oxychlordane, however, were approximately twice as high in asymptomatic as in symptomatic individuals, raising serious questions about the validity of the association between exposure to chlordane and the reported effects. Most of the information on acute human oral exposure comes from cases of accidental or suicidal ingestion; therefore, doses of ingested chlordane are not readily quantifiable. Determination of a dose-effect response is further complicated because vomiting or lavage reduced the amount of ingested chlordane actually available for systemic absorption. In one such case, ingestion of 32 mg/kg of chlordane by a girl resulted initially in diplopia, blurred vision, and twitching of the extremities followed by vomiting and eventually muscle tremors and generalized convulsions (Dadey and Kammer 1953). The investigators also estimated that after vomiting, only about 10 mg/kg was available for absorption. In another case, a man who ingested 3,041 mg/kg chlordane developed seizures and became comatose (Olanoff et al. 1983), but he also vomited, invalidating this dose. Clonic convulsions also developed in a 4-year-old girl who

ingested chlordane (Aldrich and Holmes 1969). A dose of 0.15 mg/kg was estimated after gastric lavage. In a 15 month-old child who ingested 11.1 mg/kg chlordane, tremors and convulsions began about 3 hours after ingestion, which was prior to gastric lavage (Lensky and Evans 1952). These subsided by the second day, followed by moderate ataxia and irritability. Convulsions were also observed in patients who ingested unknown quantities of chlordane (Curley and Garrettson 1969; Kutz et al. 1983).

Central nervous system effects consisting of tremors, convulsions, and paralysis of the hindlimbs occurred in rats following single gavage doses of chlordane ≥200 mg chlordane/kg (Hrdina et al. 1974). Hypothermia occurred at 100 mg/kg. Histological examination of the brains of rats and mice given a single oral dose of 200 mg/kg and hamsters given a single dose of 1,200 mg/kg revealed congestion in the brain (Truhaut et al. 1975). In rats exposed to ≥200 mg/kg/day by gavage once or to 50 mg/kg/day by gavage for 9-12 days, convulsions preceded death (Ambrose et al. 1953a). Neither death nor convulsions occurred in those similarly treated with 25 mg/kg/day for 15 days. No ataxia or change in the level of cerebral amino acids was observed in mice treated by gavage with 25 mg/kg/day for 45 consecutive days (Matin et al. 1977). In a 12-week dietary study, a dose of 5 mg/kg/day caused convulsions in rats (LOAEL for serious effects), and 1.25 mg/kg/day significantly inhibited brain ATPase (LOAEL for less serious effects), which may be involved in the mechanism of chlordane induced neurotoxicity (Drummond et al. 1983). In chronic feeding studies, chlordane induced tremors in female rats at 12.1 mg/kg/day, but not at 6 mg/kg/day and only during week 44 of the 80-week study (NCI 1977). Similar signs were not observed in male rats, which were tested at 10.2 and 20.4 mg/kg/day. No brain lesions were found in rats of either sex. Neither central nervous system signs nor histopathological lesions of the nervous system were found in a 30-month dietary study in which male rats received doses ≤1.175 mg/kg/day and female rats received doses ≤1.409 mg/kg/day (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a). Both sexes of mice in the 80-week chronic study (NCI 1977) had tremors at the high dose (8.3 mg/kg/day in females and 7.3 mg/kg/day in males) but not at the lower dose (3.9 mg/kg/day in both sexes). NCI (1977) reported no histopathological lesions in the brains of treated mice of either sex. Neither central nervous system signs nor histopathological lesions of the nervous system were found in a 24-month dietary study in which mice received doses ≤1.21 mg/kg/day (Khasawinah and Grutsch 1989b; Velsicol Chemical Co. 1983b). No evidence of neurological effects, such as tremors of sensitization to auditory stimuli, were found in rats exposed to technical chlordane in the diet at ≤16 mg/kg/day for 407 days (Ambrose et al. 1953a). However, only five males and five females were used. The highest NOAEL values and all

reliable LOAEL values for neurological effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to chlordane. Histological examination of the testes of rats and mice given a single oral dose of 200 mg/kg or hamsters given a single oral dose of 1,200 mg/kg revealed no lesions (Truhaut et al. 1975), but reproductive function was not assessed. In the only evaluation of fertility with oral exposure, Ambrose et al. (1953a) reported reduced fertility, reflected as a reduction in the number of mated females that delivered litters, when male and female rats were fed a diet that provided chlordane at 16 mg/kg/day. Treatment began at weaning of the parental generation and continued through lactation. None of the litters survived to weaning. Treatment of male mice by gavage with 100 or 300 mg/kg/day for 30 days resulted in reduced size of seminiferous tubules and degeneration of spermatogenic epithelium (Balash et al. 1987). Administration of 19.5 mg chlordane/kg/day to male rats for 90 days increased androgen receptor sites in the ventral prostate (Shain et al. 1977). There were no effects on ventral prostate or testicular weight, or on plasma testosterone level, and the toxicological significance of this observation to humans is unclear. No histopathological lesions were reported in the reproductive tracts of rats consuming doses ≤20.4 mg chlordane/kg/day in the males and 12.1 mg chlordane/kg/day in the females in an 80-week dietary study (NCI 1977) or in rats consuming doses ≤1.175 mg chlordane/kg/day in the males and ≥1.409 mg chlordane/kg/day in the females in a 30-month dietary study (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a). NCI (1977) found no treatment-related histopathological lesions in the reproductive tracts of male (<7.3 mg/kg/day) or female (≤8.3 mg/kg/day) mice in an 80-week dietary study with analytical grade cis and trcllzs isomers. No histopathological lesions in mice were reported in a 24-month dietary study with ≤1.21 mg technical chlordane/kg/day (Khasawinah and Grutsch 1989b; Velsicol Chemical Co. 1983b). In male and female rats given diets providing ≤16 mg/kg/day chlordane for 407 days, no histopathological lesions were found in reproductive organs (Ambrose et al. 1953a). However, only five male and five female rats per groups were used.

The LOAEL values for reproductive effects in rats and mice are recorded in Table 2-2 and plotted in Figure 2-2. However, NOAEL values are not identified in Table 2-2 and Figure 2-2 for studies that

found no histopathological lesions in reproductive organs because these studies did not assess reproductive function.

2.2.2.6 Developmental Effects

No studies were located regarding developmental effects in humans after oral exposure to chlordane.

There was no effect on the incidence of malformations and no evidence of fetal toxicity, including retarded skeletal development, in the fetuses of rats treated by gavage with 0, 20, 40, or 80 mg chlordane/kg/day during gestation, although four of the eight rats treated with 80 mg/kg/day died (Usami et al. 1986). No effects on viability and postnatal growth were observed in the offspring in a developmental toxicity screening test in which mice were treated with an undescribed sample of chlordane at 50 mg/kg/day during gestation days 8-12 (Chemoff and Kavlock 1982). The offspring of mice treated at 1 and 2.5 mg chlordane/kg/day during the third trimester exhibited depressed acquisition of avoidance response, increased seizure threshold, and increased exploratory activity in a study that assessed neurobehavioral effects after *in utero* exposure (Al-Hachim and Al-Baker 1973). The authors concluded that chlordane affected the fetal brain. Exposure could also have occurred via nursing, because the pups were allowed to nurse the treated dams. The LOAEL of 1 mg/kg/day in the study by Al-Hachim and Al-Baker (1973) was used to derive an acute-duration oral MRL of 0.001 mg/kg/day as described in the footnote in Table 2-2.

Cranmer et al. (1984) administered analytical grade chlordane in peanut homogenate at 0, 0.16, or 8.00 mg/kg/day to groups of mice throughout gestation to measure endocrinological performance of adult offspring. Although mice in both treated groups gave birth to approximately equal numbers of viable offspring of “average” body weight that were grossly normal in appearance, 55% of the offspring of the high-dose dams died within the first week of the nursing period. The authors stated only that the cause of death was not apparent from gross necropsy; however, it is possible that exposure to high levels of chlordane and/or metabolites in the dam’s milk may have been responsible for these deaths, based on data reported in Sections 2.3.3 and 2.3.4 on metabolism and excretion of chlordane. Postweaning survival was not affected by treatment. Plasma corticosterone in the offspring measured at 400 days of age was elevated in females at 0.16. but not at 8.0 mg/kg/day, and in males at both dose levels. These effects were not apparent at 800 days of age in either sex, although not enough high-dose males survived for evaluation. The investigators hypothesized that elevated plasma

levels of corticosterone may reflect the diminished ability of the liver to metabolically reduce corticosterone. The effects on plasma corticosterone levels in females did not occur in a dose-related fashion and the toxicological significance of this effect is unclear. Therefore, this effect is not considered in estimating levels of significant exposure.

Studies have been conducted in which pregnant mice were treated with chlordane, and the effects on the immune system of the offspring were assessed (Bamett et al. 1985a, 1985b; Menna et al. 1985; Spyker-Cranmer et al. 1982). These studies suggested to the investigators that *in utero* and/or neonatal exposure to chlordane suppressed cell-mediated immunity, as manifested by depressed delayed-type hypersensitivity reactions in the offspring of treated mice. There was no effect on humoral-mediated immunity. It is likely that the nursing pups continued to be exposed to chlordane because chlordane is excreted in milk (see Section 2.3).

More recent studies by these investigators indicate that prenatal treatment of mice depressed granulocyte-macrophage and spleen-forming stem cells in the bone marrow (Bamett et al. 1990a) and the liver (Bamett et al. 1990b), but had no effect on cytotoxic T-lymphocyte activity (Blaylock *et al.* 1990a). Further mechanistic studies demonstrated that prenatal exposure of mice to chlordane (dams treated with 8 mg/kg/day during gestation days 1-18) alters the macrophage in such a manner that it now has phenotypic characteristics of a cell that has achieved inflammatory status (Theus et al. 1992). The significance of this effect is not well understood. The reliable NOAEL and LOAEL values for developmental effects in each species and duration category for all reliable studies are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.7 Genotoxic Effects

No studies were located regarding genetic effects in humans or animals after oral exposure to chlordane. Genotoxicity studies are discussed in Section 2.4.

2.2.2.8 Cancer

No studies were located regarding cancer in humans after oral exposure to chlordane.

Studies using three different strains of mice (one strain had an historically low incidence of spontaneous liver tumors) have demonstrated that dietary administration of chlordane is associated with the development of hepatocellular carcinomas in this species. An unpublished study by IRDC (1973), which was available only in reviews by EPA (1986c, 1987e), Epstein (1976), IRIS (1992), and Reuber (1978), found significant increases in the incidence of hepatocellular carcinomas in male and female CD-l mice fed analytical grade technical chlordane in the diet at doses of 3.25 and 6.5 mg/kg/day for 18 months. In an NCI (1977) chronic dietary study with a mixture of analytical *cis* (72%) and trans (23%) isomers of chlordane, there was a dose-related increase in the incidence of hepatocellular carcinomas in male and female B6C3Fl mice that was statistically significant in both treated groups of males and in the high-dose females. Becker and Sell (1979) used male C57BL/6N strain mice that are historically resistant to spontaneous liver tumors. Those treated with chlordane in the diet at 3.25 mg/kg/day had a 27% incidence of hepatocellular carcinoma. The incidence of hepatocellular carcinoma in 200 control mice observed over a period of 18 months was zero. This study demonstrated that chlordane was capable of inducing hepatocellular carcinomas in a strain of mouse that does not develop spontaneous liver lesions. An increased incidence of hepatocellular adenomas and hemangiomas developed in male mice, but not female mice, maintained on a diet providing ≈1.21 mg chlordane/kg/day for 2 years (Khasawinah and Grutsch 1989b; Velsicol Chemical Co. 1983b). This report stands in contrast to the NCI (1977) study in which a significantly increased incidence of hepatocellular carcinomas was observed in both sexes of mice. Nonetheless, dose levels in the Khasawinah and Grutsch (1989b) and Velsicol Chemical Co. (1983b) reports were lower than those in the NCI (1977) bioassay, and a different strain of mice was used.

The 80-week NCI (1977) dietary study provided no evidence for the carcinogenicity of chlordane in rats. The IRIS (1992) review of the 30-month dietary study (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a) in rats concluded that an increased incidence of hepatocellular adenomas occurred in male rats at 1.175 mg/kg/day. Subsequent EPA reviews and reevaluations of the original slides (EPA 1985a, 1985b, 1988b) concluded that the increased incidence of hepatocellular adenomas in rats in this study was not statistically significant. Classification of the evidence for carcinogenicity in animals as sufficient is suitable without positive results in this study, and the assignment of chlordane to EPA group B2 is appropriate (IRIS 1992). Using the tumor data from male and female mice in the NCI (1977) and IRDC (1973), studies, EPA (1986c) derived a q_1* for oral exposure of 1.3 $(mg/kg/day)^{-1}$ and a unit risk in drinking water of $3.7x10^{-8}$ mg/L ($3.7x10^{-8}$ ppm) that have been verified (IRIS 1992). This q_1* corresponds to upper bound individual lifetime cancer risks at 10^{-4} to $l0^{-7}$ risk

levels of $8x10^{-5}$ to $8x10^{-8}$ mg/kg/day, which are plotted in Figure 2-2. The unit risk should not be used if the water concentration exceeds $3x10^{-1}$ mg/L ($3x10^{-1}$ ppm), because above this concentration the slope factor may differ from that stated. The Cancer Effect Levels (CELs) are recorded in Table 2-2 and plotted in Figure 2-2.

Chlordane potentiated the development of liver neoplasms in mice fed a diet providing 3.25 mg/kg/day for 25 weeks, following exposure to diethylnitrosamine in the drinking water for 14 weeks (Williams and Numoto 1984). The investigators observed that chlordane enhanced the development of neoplasms in liver cells in which diethylnitrosamine had previously induced subtle biochemical changes. Because chlordane is not strongly genotoxic, the investigators concluded that chlordane acted as a tumor promotor rather than a cocarcinogen.

2.2.3 Dermal Exposure

2.2.3.1 Death

Only one report was found regarding mortality in humans following dermal exposure to chlordane. The usefulness of this report is limited, however, because the individual was exposed to chlordane, DDT, Velsicol AR50, and triton X-100 mixed together in the form of a suspension. After a woman spilled the suspension on the front of her clothes, she became confused, developed convulsions, and died within minutes after exposure (Derbes et al. 1955). At autopsy, the brain, lungs, and kidneys were found to have nonspecific pathological changes. Deaths were not reported in a compilation of cases and personal reports of acute human dermal exposure to chlordane (EPA 1980a).

Dermal toxicity data in animals is largely limited to LD_{50} data. Gaines (1960) reported dermal LD_{50} values of 840 mg/kg in male rats and 690 mg/kg in female rats for technical grade chlordane in xylene. The dermal LD_{50} value of undiluted technical chlordane in female rats (not determined for male rats) was 530 mg/kg. However, Ben-Dyke et al. (1970) reported that the dermal LD_{50} in rats was >1,600 mg/kg (highest dose tested). The vehicle in which chlordane is administered may influence the toxicity. No deaths occurred when 273 mg/kg/day chlordane in alcohol was applied to the depilated skin of rats for 4 days (Ambrose et al. 1953a). However, a single dose of 222 mg/kg in cottonseed oil was fatal to one of five rats, and daily doses of 217 mg/kg/day in cottonseed oil for 2 days was fatal to one of five rats, and for 3-4 days was fatal to all rats. Death occurred from 1 to 12 days after the

ast application. Frings and O'Tousa (1950) reported decreased survival in female mice treated dermally with 7.4 mg chlordane/kg/day for 100 days. This study involved the use of "early" production chlordane containing significant amounts of the reaction intermediate hexachlorocyclopentadiene. Ingle (1965) reported a difference between "early" production and "later" production (i.e., more highly purified) chlordane with respect to dermal toxicity in rabbits; the LD_{50} for "early" production chlordane was <780 mg/kg, and the LD_{50} for "later" production chlordane was 1,100-1,200 mg/kg. The reliable dermal LD_{50} values are presented in Table 2-3.

2.2.3.2 Systemic Effects

No studies were located regarding cardiovascular, hematological, or musculoskeletal effects in humans or animals after dermal exposure to chlordane.

Respiratory Effects. Respiratory effects in humans were not reported in a compilation of cases and personal reports of acute dermal exposure to chlordane (EPA 1980a). Edema and congestion of the lungs were observed in a woman who died within minutes after dermal exposure to a suspension containing chlordane, DDT, and other chemicals (Derbes et al. 1955).

The only information regarding respiratory effects in animals after dermal exposure to chlordane is that hemorrhage of lungs was found in mice treated with 7.4 mg/kg/day 5 days/week for 20 weeks (Frings and O'Tousa 1950). The chlordane used in this study was probably "early production chlordane," which contains hexachlorocyclopentadiene.

Gastrointestinal Effects. Gastrointestinal effects in humans were not reported in a compilation of cases and personal reports of acute human dermal exposure to chlordane (EPA 1980a). Vomiting occurred in an infant who was reported to have "prolonged" derrnal exposure to liquid chlordane (Balistreri et al. 1973). Congestion of the stomach was observed in a woman who died within minutes after dermal exposure to a suspension containing chlordane, DDT, and other chemicals (Derbes et al. 1955).

No studies were located regarding gastrointestinal effects in animals after dermal exposure to chlordane.

TABLE 2-3. Levels of Significant Exposure to Chlordane - Dermal

Species	Exposure duration/ frequency	System	NOAEL (mg/kg/day)	LOAEL (effect) Less serious (mg/kg/day)	LOAEL (effect) Serious (mg/kg/day)	Reference	Form
ACUTE EXPOSURE							
Death							
Rat	once				840 (LD_{50} in males)	Gaines 1960	Technical
Rat	once				530-690 (LD_{50} in females)	Gaines 1960	Technical
Rat	1-4 d 1x/d				217 (1/5 died)	Ambrose et al. 1953a	Technical
Rabbit	once				1150 (LD_{50})	Ingle 1965	Technical
INTERMEDIATE EXPOSURE							
Systemic							
Gn pig	90 d	Derm/oc		168 (hyperkeratosis)		Datta et al. 1977	Technical

d = day(s); Derm/oc = dermal/ocular; Gn pig = guinea pig; LD50 = lethal dose; 50% kill; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; x = times

Hepatic Effects. Liver effects in humans were not reported in a compilation of cases and personal reports of acute dermal exposure to chlordane (EPA 1980a). Balistreri et al. (1973) reported clinical hepatic effects consisting of jaundice, hepatomegaly and elevated serum transaminases in an infant following "prolonged" dermal exposure to liquid chlordane. Liver biopsy revealed centrilobular necrosis, fatty infiltration, and minimal inflammation. Liver function tests were normal in a 47-year-old nurseryman who handled soil sprayed with chlordane and other chemicals for ≈2 years (Barnes 1967); there was no estimate of exposure concentration or dose.

Frings and O'Tousa (1950) reported hepatic centrilobular necrosis in mice treated dermally with "early" production chlordane (7.4 or 29.6 mg/kg/day) 5 days/week for 20 weeks. "Early" production chlordane was frequently contaminated with hexachlorocyclopentadiene, which is very toxic and highly irritating (Ingle 1965). Liver cell inclusion bodies were observed in some rats that received dermal applications of 217-273 mg/kg/day chlordane in alcohol or cottonseed oil for l-4 days (Ambrose et al. 1953a). Additional details were not reported. These data are not considered for estimation of levels of significant exposure.

Renal Effects. Renal effects in humans were not reported in a compilation of cases and personal reports of acute human dermal exposure to chlordane (EPA 1980a). Nonspecific pathological changes were observed in the kidneys of a woman who died within minutes after dermal exposure to a suspension of chlordane, DDT, and other chemicals (Derbes et al. 1955).

White blotches were observed on the kidneys of mice dermally exposed to 7.4 mg chlordane/kg/day, 5 days/week for 20 weeks (Frings and O'Tousa 1950), but the test sample was probably contaminated with hexachlorocyclopentadiene. A large number of yellow droplets in the renal cortex tubular epithelium and many faintly yellow or colorless, rod-shaped, apparently crystalline structures in the cytoplasm of the cortical tubules were found in all rats that received dermal applications of 217-273 mg/kg/day chlordane in alcohol or cottonseed oil for l-4 days (Ambrose et al. 1953a). The toxicological significance of these findings is not clear as other studies by any route did not describe similar effects and no control rats were used.

Dermal/Ocular Effects. In a compilation of many cases and personal reports of accidental dermal exposure to chlordane, frequently reported signs and symptoms included burning sensations, rashes, and pruritus (EPA 1980a). Accidental spraying of chlordane mixtures in the eyes consistently causes

conjunctivitis (EPA 1980a). These reports of dermal and ocular effects are complicated by exposure to mixtures of chemicals including other pesticides and vehicles such as petroleum distillates, known to be irritants. Dermal lesions were not reported in a woman who exhibited generalized convulsions and died within minutes of dermal exposure to a suspension of chlordane, DDT, and other chemicals. The woman's thighs and abdomen were washed after the spill (Derbes et al. 1955).

No local reactions were observed in rats treated dermally with 273 mg/kg/day chlordane in alcohol for 4 days (Ambrose et al. 1953a). Whether skin reactions were observed in the rats treated with chlordane in cottonseed oil was not reported. Datta et al. (1977) reported hyperkeratinization, cellular degeneration such as vacuolization, and a multinucleated condition in cells of the malpighian layer in the skin of dermally treated guinea pigs. No changes were observed in the dermis. Chlordane in acetone was applied to the open, shaved skin at 168 mg/kg once daily for 90 days. This LOAEL for skin effects in guinea pigs is presented in Table 2-3.

2.2.3.3 Immunological Effects

No studies were located regarding immunological effects in humans after dermal exposure to chlordane.

Datta et al. (1977) did not report evidence of sensitization in guinea pigs dermally treated once daily for 90 days with chlordane in acetone (see Dermal/ocular effects). It is not clear, however, that evidence of sensitization would have been detected by the experimental protocol.

2.2.3.4 Neurological Effects

There are several reports regarding central nervous system effects in humans after dermal exposure to chlordane. None of the reports quantified exposure, and in several of the reports, exposure involved chlordane and one or more other pesticides. Derbes et al. (1955) reported the death of a woman dermally exposed to a suspension containing chlordane, DDT, Velsicol AR50, and triton X-100. Mental confusion and convulsions preceded death. Nonspecific pathological changes were found in the brain at autopsy. Balistreri et al. (1973) reported seizures in an infant following "prolonged" dermal exposure to liquid chlordane. Barnes (1967) reported a case involving a 47-year-old nurseryman who handled soil that had been sprayed with chlordane. Exposure took place over a

period of 2 years during which time the nurseryman was also exposed to DDT, plant hormone sprays, and arsenic pesticides. During the exposure period, the nurseryman experienced Jacksonian and grand ma1 convulsions and electroencephalographic evidence of generalized dysrhythmia. Chlordane was considered to be the cause because the patient's condition improved when he ceased using chlordane.

Application of chlordane in alcohol at 273 mg/kg/day or in cottonseed oil at 217 or 222 mg/kg/day for l-4 days to depilated skin of rats did not result in tremors (Ambrose et al. 1953a). "Early production chlordane" induced tremors, convulsions, and seizures in rabbits to which 780 mg/kg was applied to the skin (Ingle 1965). Frings and O'Tousa (1950) also noted tremors, convulsions, and seizures in mice treated topically with "early" production chlordane. These effects were observed at 7.4 and 29.6 mg chlordane/kg/day in mice treated 5 days/week for 20 weeks. Data generated with "early" production chlordane are not used to estimate levels of significant exposure.

2.2.3.5 Reproductive Effects

Only one case report was available that described reproductive effects in humans after dermal exposure to chlordane. In this study, a woman accidentally spilled an unknown quantity of suspension containing chlordane down the front of her clothes and died shortly afterwards (Derbes et al. 1955). An autopsy of the woman revealed endometrial hemorrhage and superficial ulceration of the vaginal mucosa. Because of an inadequate medical history, these lesions may not be treatment-related.

No studies were located regarding reproductive effects in animals after dermal exposure to chlordane.

2.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after dermal exposure to chlordane.

2.2.3.7 Genotoxic Effects

No studies were located regarding genotoxicity in humans after dermal exposure to chlordane.

Chlordane was applied to the plucked skin of male CD-l mice at doses of 0, l/32, l/16, or l/8 of the published dermal LD_{50} (about 0, 51, 102, or 205 mg/kg body weight). The high dose induced a significant incidence of micronuclei formation in the bone marrow cells (Schop et al. 1990). All of the doses induced a significant and dose-related increase in the incidence of nuclei aberrations in the hair follicles. Other genotoxicity studies are discussed in Section 2.4.

2.2.3.8 Cancer

Only one epidemiological study was identified in the literature that investigated cancer in humans after dermal exposure to chlordane. In this population based case-control study, the risk of non-Hodgkins lymphoma (NHL) among farmers was significantly elevated (odds ratio, 1.7) for personal handling, mixing, or application of chlordane as an animal insecticide or as a crop insecticide (Cantor et al. 1992). The odds ratio for NHL was also greater among farmers who first used chlordane before 1965 (15-18 years before diagnosis) or those farmers who did not use protective equipment. The study was, however, limited by incomplete reporting of duration of exposure to chlordane and the amount of chlordane or other pesticides farmers were simultaneously exposed to.

No studies were located regarding cancer in animals after dermal exposure to chlordane.

2.3 TOXICOKINETICS

The fate of chlordane in the body reflects the lipophilicity and metabolism of the chemical. Chlordane appears to be readily absorbed, regardless of the route of exposure, as expected for highly lipophilic substances. Initially, tissue levels are highest in the liver and kidneys, reflecting the high degree of vascularity of these tissues. Subsequently, chlordane and its metabolites are relocated in fat, where they persist for long periods of time. Metabolism results in a number of oxidation products, including oxychlordane, which persist in body fat as the predominant chlordane residues. Free radicals formed by reductive dehalogenation may play an important role in the toxicity of chlordane. Except for the rabbit, chlordane and its metabolites are excreted more readily in the bile than in the urine, due to the general lack of polarity and high lipophilicity of the terminal metabolites. Substantial amounts are also excreted via lactation, even after exposure has ceased, rendering the nursing young particularly susceptible to toxicity.

Effects attributed to chlordane exposure include blood dyscrasia, hepatotoxicity, neurotoxicity, immunotoxicity and cancer. Possible mechanisms of toxicity relevant to all target organs include the ability of chlordane and its metabolites to bind irreversibly to cellular macromolecules, inducing cell death or disrupting normal cell function. In addition, chlordane may increase tissue production of superoxide, which can accelerate lipid peroxidation, disrupting the function of cellular and subcellular membranes. Chlordane induces its own metabolism to toxic intermediates, which may exacerbate its hepatotoxicity. This may involve suppression of hepatic mitochondrial energy metabolism.

The neurotoxicity of chlordane may be related to effects on the levels of endogenous neurotransmitters in various regions of the brain. Immune effects following prenatal exposure may arise from a reduction in the population in the bone marrow of stem cells responsible for differentiation into various types of immunoactive cells. Chlordane is considered an epigenetic carcinogen, causing liver cancer in mice, probably by suppressing gap junction intercellular communication.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Data obtained from humans exposed via inhalation to chlordane in the air of termite-treated homes in Japan (Taguchi and Yakushiji 1988) or as the result of pesticide spraying (Kawano and Tatsukawa 1982; Saito et al. 1986; Takamiya 1987) indicate that blood or tissue levels of chlordane and/or its metabolites increase with exposure duration. Although these data are not sufficient to quantify absorption from the respiratory tract, they confirm qualitatively that absorption occurs.

An intratracheal study suggests that absorption of chlordane by the respiratory system of rats is rapid. A peak blood concentration of radioactivity equivalent to ≈4% of an intratracheal dose of radiolabeled chlordane was reached in <5 minutes (Nye and Dorough 1976). The value of this study was limited by the mode in which the chlordane was delivered, the use of ethanol as a vehicle, and the unknown impact of anesthesia and surgical preparation on uptake from the respiratory tract. In reviewing the data from this study, Nomeir and Hajjar (1987) noted that ≈24% of the dose of radioactivity was present in the lungs 1 hour after treatment and concluded that ≈76% of the dose had been absorbed from the respiratory tract.

2.3.1.2 Oral Exposure

Information on the absorption of chlordane following oral exposure in humans comes largely from case reports involving accidental ingestion. A chlordane level of 2.71 mg/L was measured in the blood of a 20-month-old boy 2.75 hours after he had ingested an unknown amount of technical grade (74%) chlordane (Curley and Garrettson 1969). A chlordane concentration of 3.4 mg/L was measured in a serum sample taken from a 4-year-old girl at an unspecified time following the ingestion of an unknown amount of a 45% chlordane formulation (Aldrich and Holmes 1969). Chlordane was found in the blood plasma (concentration of 4.87 μg/g) and in several tissues (adipose, 22.00 μg; spleen, 19.15 μg/g; brain, 23.27 μg/kg; liver, 59.93 μg/g) of a 59-year-old male ≈2 hours after he ingested an unknown amount of chlordane (Kutz et al. 1983). A whole blood chlordane concentration of 5 mg/L was measured in a 62-year-old male 3.5 hours after he had ingested 300 mL of a 75% chlordane solution (215 g) (Olanoff et al. 1983). While these case reports do not provide information on the rate and extent of absorption in humans after oral exposure to chlordane, they indicate that the compound is absorbed from the gastrointestinal tract.

Excretion of radioactivity in the urine of rats given a single oral dose of radiolabeled chlordane accounted for ≈2-8.5% of the administered dose (Bamett and Dorough 1974). The chlordane doses used in this study were 0.05-1.0 mg/kg of a 3:l mixture of *cis*- and *trans*-chlordane, or 0.2 mg/kg of each isomer separately. Based on these urinary excretion data, EPA (1987e) stated that chlordane absorption from the gastrointestinal tract of rats was at least 28.5% of the administered dose. More recent information (Ewing et al. 1985; Ohno et al. 1986), however, indicates that absorption from the gastrointestinal tract of rats and mice may be substantially higher than previously estimated. Biliary excretion of chlordane (and/or its metabolites) played a significant role in the elimination of this compound in both species, suggesting that a large amount of the radioactivity found in the feces following oral administration of radiolabeled chlordane represents absorbed material. A first-order absorption model suitably described the experimental data in rats (Ohno et al. 1986). The extent of chlordane absorption after oral exposure was estimated by comparing areas under the plasma concentration versus time curves (AUC) of radioactive equivalents of chlordane after oral or intravenous dosing at 0.1-1.0 mg/kg. Absorption was estimated to be ≈80% of the administered dose in rats, and this percentage did not vary significantly over the dose range tested (Ohno et al. 1986).

Chlordane appears to be rapidly absorbed from the gastrointestinal tract of rats; peak blood levels of radioactivity occurred ≈2-4 hours following administration of oral doses ranging from 0.1 to 1.0 mg/kg (Ewing et al. 1985; Ohno et al. 1986). Peak blood levels of radioactivity following a 1.0 mg/kg dose of ^{14}C-chlordane were between ≈81 ng chlordane equivalents/ml for the *cis* isomer (Ewing et al. 1985) and 175 ng chlordane equivalents/ml for the trans isomer (Ohno et al. 1986).

Absorption in the mouse was slower than in the rat after oral exposure to chlordane; a peak blood level of 113 ng equivalents/ml of blood was observed in the mouse 8 hours after administration of a 1 mg/kg dose of ^{14}C-*cis*-chlordane (Ewing et al. 1985). Absorption was not quantified following oral dosing in the mouse, but intraperitoneal studies indicated that a significant degree of biliary excretion also occurs in this species (Ewing et al. 1985). Administration of a mixture (1:1) of *cis* and trans isomers of chlordane (20 mg/kg for each of the isomers) to male mice resulted in peak absorption of *cis*- and *trans*-chlordane within 24 hours (Satoh and Kikawa 1992). The authors did not specify the time of peak absorption for either isomer. Although the distribution data suggest that more *cis*-chlordane was absorbed than *trans*-chlordane, data were insufficient to compare the rate of absorption of *cis*- and *trans*-chlordane. The mouse may be similar to the rat in that a large percentage of an oral dose of chlordane is absorbed in the gastrointestinal tract of both species.

In rabbits, the estimated absorption of chlordane following various repeated oral dosing regimens with radiolabeled compound has been between 30% and 50% of the cumulative ^{14}C dose, based on radioactivity eliminated in the urine (Balba and Saha 1978; Barnett and Dorough 1974; Poonawalla and Korte 1971). The various dosing regimens used in these studies included: radiolabeled *trans*-chlordane given to male rabbits at ≈3.8 mg/kg/day for 10 weeks (Poonawalla and Korte 1971), 25 ppm of a mixture of *cis*- and *trans*-chlordane fed to a male rabbit for 2 days (≈1.22 mg/kg/day) (Barnett and Dorough 1974), and *cis*- or *trans*-chlordane (30 and 67 mg/kg/day, respectively) given in gelatin capsules to male rabbits for 4 days (Balba and Saha 1978). Biliary excretion was not studied in the rabbit, and the estimation of 30-50% absorption (based on urinary excretion) must be considered a minimum estimate.

2.3.1.3 Dermal Exposure

No quantitative data regarding absorption in humans after dermal exposure to chlordane were located. Kazen et al. (1974) reported that chlordane tends to persist on the hands of pest control operators

(exposure duration not reported) for at least 2 years after exposure to the pesticide has been I terminated. This claim is questionable, however, because the subject in the study was not followed closely enough to determine the absence of chlordane exposure during the 2-year period. It is possible, for example, that chlordane may have been present on the subject's clothing or shoes, resulting in continued exposure. Derbes et al. (1955) reported a case of accidental death preceded by neurological signs typical of chlordane toxicity in a woman who was dermally exposed to a mixture of chlordane and other chemicals. These data suggest that chlordane is absorbed through human skin. In a more recent study involving 248 male and 227 female outpatients, the investigators were unable to find a strong correlation between skin chlordane levels and blood chlordane levels in men, although a significant correlation ($r=0.47$; $p<0.01$) was observed in women (Hirai and Tomokuni 1993). In addition, a small correlation ($r=0.37$; $p<0.05$) was observed between skin chlordane levels and blood oxychlordane levels in both sexes.

The dermal route of exposure is relatively significant for chlordane. Ambrose et al. (1953a) indicated that a topical dose of chlordane in rats (50 mg/kg) is more readily absorbed when the compound is administered in an oil vehicle instead of ethyl alcohol. In an *in vitro* study using diffusing cells in which ^{14}C-chlordane (0.0027-0.003 mg/cm^3/day) was applied to human skin from cadavers for 24 hours, the amount of the applied dose of radioactivity recovered from the receptor fluid (human plasma) was 0.04% when the application medium was soil and 0.07% when the application medium was acetone (Wester et al. 1992). Much larger proportions (0.34% from soil and 10.9% from acetone) were retained within the layers of the skin. In an *in vivo* study, ^{14}C-chlordane in soil or acetone was applied to the skin of monkeys for 24 hours (Wester et al. 1992). Absorption accounted for 4.2% of the dose in soil and 6% of the dose in acetone, based on recovery of ^{14}C in the urine following dermal, compared with intravenous, dosing.

2.3.2 Distribution

Several studies report chlordane residues in the blood or fat of pest control operators (spray men), residents in homes treated for termites, or residents with no known mode of exposure other than background. Background exposures include inhalation of the material in ambient air and ingestion through food (Dearth and Hites 1991a; Sasaki et al. 1991a; Wariishi and Nishiyama 1989); dermal exposure also may be possible, although data regarding dermal exposure were not located. In pesticide spray applicators properly attired in protective clothing, the inhalation route is probably most important

(Takamiya 1987). Nevertheless, most humans with any body burden of chlordane residues were probably exposed by multiple routes; therefore, except for those few cases reports in which oral exposure was known, human data are presented here.

Generally, levels of total chlordane residues in blood and milk fat increase as duration of exposure increases (Ogata and Izushi 1991; Saito et al. 1986; Taguchi and Yakushiji 1988; Takamiya 1987). Human milk fat contained total mean chlordane residues of ≤188 ppm, and blood levels were ≤0.015 ppm in exposed individuals (Ogato and Izushi 1991; Taguchi and Yakushiji 1988). Levels in fat and liver exceeded levels in the blood (Mussalo-Rauhamaa 1991).

2.3.2.1 Inhalation Exposure

Data regarding tissue levels of chlordane residues in humans after purely inhalation exposure were not located.

Intratracheal administration of radiolabeled chlordane in rats resulted in the following tissue distributions of radioactivity (expressed as percent of administered dose) 1 hour after administration: 23.9% in lungs, 19.6% in liver, 0.3% in kidney, and 0.1% in the bladder and its contents (Nye and Dorough 1976). Limitations of this study are discussed in Section 2.3.1.1.

Total chlordane residue levels following continual 13-week exposure of rats and monkeys are highest in the fat (69-200 ppm), followed by the liver (3.5-11 ppm) and blood (0.2-0.3 ppm) (Khasawinah 1989). Tissue residues in rats are predominantly oxidation products; residues in monkeys are predominantly unchanged components of technical chlordane, suggesting that monkeys are less efficient metabolizers than rats of chlordane components.

2.3.2.2 Oral Exposure

Information on the distribution of chlordane and/or its metabolites in humans after oral exposure is from case reports involving ingestion of the compound. Approximately 2 hours after a 59-year-old male ingested a fatal dose of chlordane, autopsy revealed concentrations of chlordane in several tissues (Kutz et al. 1983). The tissues analyzed and their respective chlordane concentrations were: adipose tissue 22 μg, spleen 19.15 μg/g, brain 23.27 μglg, kidney 14.10 μg, and liver 59.93 μg/g. The

level of chlordane in the adipose tissue of a 20-month-old boy who drank an unknown amount of technical grade (74%) chlordane was 3.12 μg/g 30 minutes after ingestion (Curley and Garrettson 1969). The concentration in adipose tissue peaked at 30-35 ug/g of fat ≈8 days following the incident. Fifty-eight days after a 62-year-old male ingested 215 g of chlordane, the reported levels of chlordane components and metabolites (oxychlordane, *trans*-nonachlor, and heptachlor epoxide) in the adipose tissue were 5 μg/g of fat (Olanoff et al. 1983). Although this level is considerably below that reported in the 20-month-old boy (30-35 μg/g of fat) on day 8 by Curley and Garrettson (1969), it is still significantly higher than the average concentration of oxychlordane (a metabolite of chlordane) detected in the adipose tissue of members of the general population of the United States. Levels of oxychlordane in human adipose tissue range from 0.03 to 0.49 μg/g of fat, with average concentrations of ≈0.11-0.19 μg/g of fat (Barquet et al. 1981; Biros and Enos 1973; Kutz et al. 1976, 1979).

The pattern of tissue distribution of chlordane and/or its metabolites in animals after oral exposure does not appear to depend on the size of the dose or whether exposure is to single or to multiple doses. The tissue distribution patterns of radioactivity in rats, 1 day after exposure to a single oral dose of a 3:l mixture of radiolabeled *cis*- and *trans*-chlordane, were similar over a dose range of 0.05-1.0 mg/kg (Bamett and Dorough 1974). Levels of tissue radioactivity increased with increasing dose; at all dose levels, the highest concentrations of radioactivity were found in the fat followed, in order, by the liver and kidney. Lower concentrations were found in the brain and muscle. In this same study, oral administration of chlordane over a longer period of time did not change the distribution pattern of radioactivity from that observed following a single oral exposure. Rats fed the same mixture of chlordane (i.e., 3 *cis*-:1 *trans*-) for 56 days at dietary concentrations of 1, 5, or 25 ppm were observed to have high levels of radioactivity in the fat and much lower levels of radioactivity (in decreasing order) in the liver, kidney, brain, and muscle. The concentrations of radioactivity measured in these tissues were dose-dependent.

The accumulation of chlordane and its metabolites in fat appears to depend on exposure duration. Takeda et al. (1984) treated rats by gavage with technical chlordane at 10 μg/kg/day for 7 or 14 days, Chlordane and metabolites measured in the fat reached 30.4 μg/g wet tissue at the end of 7 days of treatment and 77.4 μg/g at the end of 14 days of treatment.

Distribution to the liver and kidneys of rats after a single oral dose of chlordane is more rapid than distribution to adipose tissue. Levels of radioactivity peaked in the liver and kidneys of rats 2-4 hours

after the administration of a single oral dose of radiolabeled γ-chlordane (0.05 or 10 mg/kg) (Ohno et al. 1986). In this same study, the level of radioactivity in the adipose tissue peaked at 16 hours (dose of 10 mg/kg) and 4 days (dose of 0.05 mg/kg) after administration of the compound. The concentrations of γ-chlordane equivalents in adipose tissue 10 days after the administration of either dose (0.05 or 10.0 mg/kg) were ≈10 times higher than levels in the liver and kidney.

More recently, Dearth and Hites (1991b) measured the half-lives of depuration of 14 different chlordane components (e.g., *cis*- and *trans*-chlordane and *cis*- and *trans*-nonachlor) and metabolites (e.g., heptachlor epoxide, oxychlordane) from the fat of rats fed chlordane in the diet for 28 days. Half-lives ranged from 5.92 days (*cis*-chlordane) to 54.1 days (nonachlor III) and were apparently related to the metabolism rate of the various compounds. Structural characteristics associated with slowed depuration included an increasing number of chlorines on ring 1, the chlorine on Cl existing in an endo- (compared with an exo-) configuration, and the presence of two chlorines on C2. In mice treated once or every other day for 29 days, the whole body content of *cis*- and *trans*-chlordane remained at very low levels; the content of *cis*- and *trans*-nonachlor and oxychlordane continued to increase with continued treatment (Hirasawa and Takizawa 1989). The investigators concluded that the chlordane isomers were readily metabolized, but that the nonachlor isomers were not. Oxychlordane, a metabolic intermediate of both chlordane isomers, is very slowly metabolized and tends to persist.

Elimination of radioactivity from the liver and kidney of rats treated with radiolabeled chlordane differs from elimination of radioactivity from peritesticular adipose tissue. Elimination of radioactivity from the kidneys and livers of rats treated with either high (10 mg/kg) or low (0.05 mg/kg) doses of γ-chlordane was biphasic - the initial rapid phase had half-lives in both organs ranging from ≈5.9 to 9.6 hours (Ohno et al. 1986). Half-lives for the slower, terminal phase of elimination in these organs ranged from ≈4.4 to 5.0 days. In contrast, elimination of radioactivity from peritesticular adipose tissue was monophasic and relatively slow (elimination half-lives of 9.1 days in the low-dose group and 8.4 days in the high-dose group). Skin retained radioactivity longer than any other tissue (elimination half-lives of 15.2 and 10.4 days for the low- and high-dose groups, respectively). Ewing et al. (1985) confirmed that peak concentrations of radioactivity were found in the livers of rats and mice 2-4 hours after administration of a single oral dose of radiolabeled *cis*-chlordane (1.0 mg/kg). The investigators observed that radioactivity was eliminated much more slowly from the livers of mice than from the livers of rats. They speculated that this may explain the susceptibility of mice to the

development of hepatocellular carcinomas; whereas, rats appear to be relatively insensitive to the formation of this tumor following chlordane administration (NCI 1977).

Several of these oral studies with radiolabeled chlordane (Ambrose et al. 1953b; Barnett and Dorough 1974; Street and Blau 1972) reported that female rats had higher levels of radioactivity in the fat than did the males. Levels of radioactivity in perirenal adipose tissue from female rats were as much as twice the levels observed in males (Ambrose et al. 1953b), and females tended to store a much larger proportion of this radioactivity in abdominal fat in the form of oxychlordane (Street and Blau 1972). Another recurrent observation in these distribution studies is an isomer effect on the amount of radioactivity that is distributed to the various tissues. Studies by Barnett and Dorough (1974) and *Street* and Blau (1972) indicated that significantly higher concentrations of radioactivity are stored in the tissues of rats following oral administration of the *trans* isomer, compared to the concentrations observed following administration of the cis isomer. This observation also holds true for rabbits. Balba and Saha (1978) administered four doses of either *cis*-chlordane (67 mg/kg body weight/dose) or *trans*-chlordane (30 mg/kg body weight/dose) to rabbits for 4 days. Although the administered dose of the cis isomer was more than twice the dose of the *trans* isomer, tissue levels of radioactivity were higher in animals given the trans isomer. When a 1:l mixture of *cis*- and *trans*-chlordane (total dose: 40 mg/kg) was administeredeto mice, higher concentrations of *cis*-chlordane were found in muscle tissues than of *trans*-chlordane (Satoh and Kikawa 1992). At day one, *cis*-chlordane concentrations in muscle, liver, kidney, brain, and spleen were 1,260, 377, 136, 56, and 34 ppb, respectively, while *tram-chlordane* concentrations in these tissues were 766, 103, 82, 37, and 22 ppb, respectively. The concentrations of *cis*- and *trans*-chlordane were similar in blood (29 and 22 ppb, respectively). Oxychlordane concentrations peaked in the liver (1,918 ppb) at day 2, and peaked at day 1 in the following tissues: muscle (569 ppb), kidney (326 ppb), brain (226 ppb), spleen (126 ppb), and blood (103 ppb). The level of oxychlordane in adipose tissue was 2,890 ppb on week 8, but by week 52, it had decreased to 648 ppb. In addition, oxychlordane levels were higher in adipose tissue than any other tissue at 52 weeks.

2.3.2.3 Dermal Exposure

No studies were located regarding tissue distribution in humans or animals after dermal exposure to chlordane.

2.3.3 Metabolism

Information on the metabolism of chlordane in humans is limited. Tashiro and Matsumura (1978) identified the metabolites of *cis*- and *trans*-chlordane following incubation of these compounds with human liver microsomal preparations. The following rnetabolites (in order of decreasing concentration) were identified: chlordene chlorohydrin, monohydroxylated dihydrochlordene, oxychlordane, and relatively smaller but similar amounts of 1,2-dichlorochlordene, l-hydroxy-2-chlorochlordene, 1-hydroxy-2-chloro-2,3-epoxychlordene, 1,2-hydroxychlordene, trihydroxydihydrochlordene, and β-glucuronide-l-hydroxydihydrochlordene. Patterns of metabolites were similar whether the starting material was the *cis* or *trans* isomer. Kutz et al. (1976, 1979) reported the presence of oxychlordane in most adipose tissue samples taken at surgery or necropsy from humans. Data were not located regarding the levels of chlordane metabolites in the urine of exposed humans.

Tashiro and Matsumura (1978) reported that experiments with liver microsomal preparations from rats yielded results nearly identical to those for human preparations. These investigators noted, however, that rat microsomal preparations efficiently metabolized *trans*-nonachlor (a predominant component of technical chlordane) to *trans*-chlordane, but that human microsomal preparations did not. These data suggest that the metabolism of pure isomers of chlordane by humans and rats is similar, but that metabolism of components other than the pure isomers present in the technical product may differ.

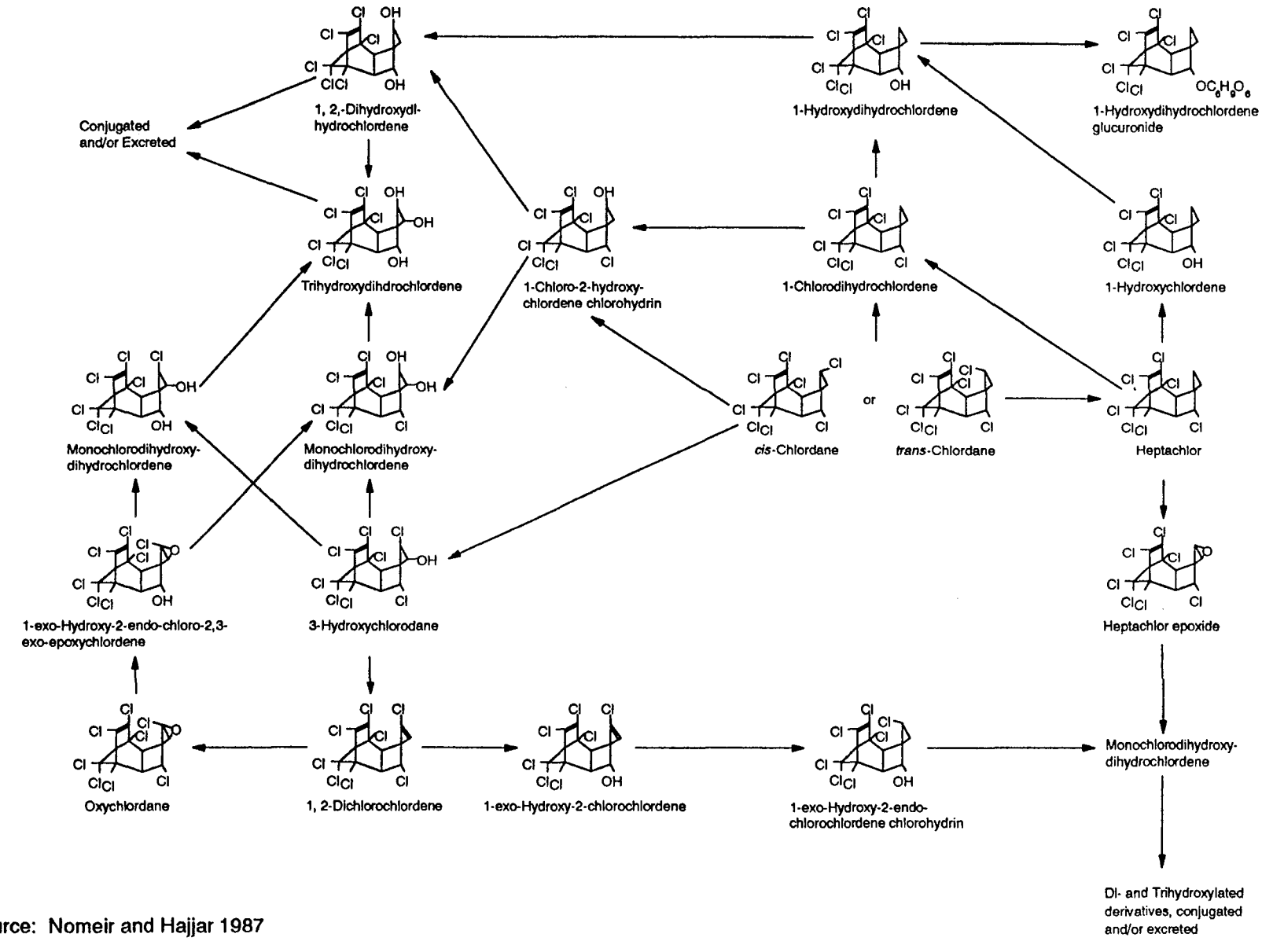
Data regarding the nature of tissue residues in rats and monkeys following continual inhalation exposure for 90 days (see Section 2.3.2.1) indicate that monkeys are less efficient metabolizers of chlordane than are rats (Kbasawinah 1989). Oxychlordane is the predominant metabolite of *trans*-chlordane in rats and monkeys (Khasawinah 1989; Sasaki et al. 1992).

Chlordane has been known to undergo biotransformation in animals since the mid 196Os, when it was demonstrated by Poonawalla and Korte (1964) that 10-80s of the radioactivity found in the tissues and excreta of rats given an intravenous dose of radiolabeled *cis*-chlordane was in the form of water soluble metabolites. Subsequently, several metabolic schemes have been proposed based on information obtained from *in vivo* and *in vitro* studies in rats (Bamett and Dorough 1974; Brimfield et al. 1978; Tashiro and Matsumura 1978) and rabbits (Balba and Saha 1978; Poonawalla and Korte 1971). These proposed schemes lack consistency, and controversial issues include: differences in the

identity of metabolites observed in *in vivo* versus *in vitro* experiments, possible isomer differences (i.e., *cis* versus trans) in the routes of metabolism followed, and whether metabolites thought to be terminal by some investigators (e.g., oxychlordane) are capable of undergoing further biotransformation by mammals. The metabolic scheme for chlordane in animals presented in Figure 2-3 was proposed recently as a synthesis of the available information by Nomeir and Hajjar (1987). This scheme involves four routes of metabolism for the chlordane molecule. No distinction is made between *cis*- and [runs-chlordane in the qualitative nature of the metabolites formed. The first proposed metabolic route starts with hydroxylation at position three of the molecule to form 3-hydroxychlordane. This reaction is thought to be mediated by the microsomal mixed-function oxidase (MFO) system. Dehydration of 3-hydroxychlordane leads to 1,2-dichlorochlordene and eventually to other metabolites such as oxychlordane and l-hydroxy-2-chlorochlordene. Alternatively, 3-hydroxychlordane may undergo replacement of chlorines by hydroxyl groups to form monochlorodihydroxylated and -trihydroxylated derivatives. The second pathway starts with dehydrochlorination to form heptachlor. The mechanism of this reaction is not completely understood but is thought to be mediated by the cytochrome P-450 system and/or by glutathione-S-transferase type enzymes. Further metabolism of heptachlor leads to 1-hydroxychlordene, heptachlor epoxide, or eventually to 1-chloro-2,3-dihydroxydihydrochlordene. The third pathway starts with dehalogenation of chlordane to form l-chlorodihydrochlordene, probably mediated by microsomal MFO systems. Further reactions probably involve hydrolysis and conjugation with glucuronic acid. The fourth metabolic pathway, and probably the least understood, involves hydrolytic removal of a chlorine atom and its replacement by a hydroxyl group to form l-chloro-2-hydroxychlordene chlorohydrin. This product may undergo further metabolism to form monochlorodihydroxy- and trihydroxy- derivatives of dihydrochlordene. Studies with rat hepatic microsomes suggest that cytochrome P-450 may be the most important enzyme to catalyze degradation of *trans*-chlordane (Kawano et al. 1989). Epoxide hydrolase is probably the predominant enzyme to catalyze degradation of oxychlordane. Reductive dehalogenation, with the production of free radicals, may also be important in the toxicity of chlordane (Brimfield and Street 1981; Kawano et al. 1989).

The metabolic rate of various chlordane components appears to depend on three structural features (Dearth and Hites 1991b). First, compounds with two chlorines on ring 1 are metabolized 3 times as rapidly as those with three chlorines. Second, compounds with the chlorine on C2 in an exo-configuration are metabolized 20-25% more quickly than compounds with an endo-configuration. Third, compounds with one chlorine on C2 are metabolized 3 times as rapidly as those with two

FIGURE 2-3. Proposed Metabolic Pathways for Chlordane



Source: Nomeir and Hajjar 1987

chlorines. Dechlorination of compounds with three chlorines on ring one appears to be the first and rate-limiting step of metabolism of these compounds.

In mice treated orally every other day for 28 days with technical chlordane, *cis*- and *trans*-chlordane reached peak levels in the whole body on the first day and declined to lower levels in spite of repeated dosing; *cis*- and *trans*-nonachlor and oxychlordane increased during the entire study period (Hirasawa and Takizawa 1989). The ratio of *cis*- to *trans*-chlordane and *cis*- to *trans*-nonachlor in the test sample (6:7 and 1:4, respectively) and in the mouse body at termination of the experiment (5:3 and 1:7, respectively) suggests that *trans*-chlordane is metabolized more readily than *cis*-chlordane and that *cis*-nonachlor is metabolized more readily than *trans*-nonachlor. The decreasing content of the chlordane isomers and the increasing content of oxychlordane with repeated dosing suggests that chlordane induces its own metabolism.

2.3.4 Excretion

Lactation is probably the route of excretion of most interest, because of concern that nursing mothers may pass chlordane residues to their infants in this manner. Documentation of toxicity in infants induced in this manner, however, was not located in the available literature. While *cis*- and *trans*-chlordane have not been identified in human milk, chlordane metabolites and related chemicals present in commercial products (e.g., oxychlordane, *trans*-nonachlor and heptachlor epoxide) have been identified in human milk. Oxychlordane residues were detected in 46% of 57 human milk samples in Arkansas/Mississippi (Strassman and Kutz 1977), in 68% of 6 samples in low pesticide usage areas of Mississippi (Bamett et al. 1979), and in 100% of 50 samples in Hawaii (Jensen 1983). On a whole milk basis, mean concentrations of oxychlordane ranged from 0.002 to 0.005 mg/L (Bamett et al. 1979; Strassman and Kutz 1977). All three routes of exposure (inhalation, oral, dermal) may have been involved in the accumulation of chlordane residues in the mothers.

2.3.4.1 Inhalation Exposure

No studies were located regarding excretion in humans after inhalation exposure to chlordane.

Six days after the endotracheal administration of radiolabeled chlordane to rats, 52% of the administered dose of radioactivity was recovered from the feces and 12% was recovered from the urine (Nye and Dorough 1976). The limitations of this study are discussed in Section 2.3.1.1.

2.3.4.2 Oral Exposure

Information regarding the excretion of chlordane and/or its metabolites from the human body after oral exposure is from case reports of accidental ingestion. These reports conclude that elimination from the plasma was biphasic in nature (Aldrich and Holmes 1969; Curley and Garrettson 1969; Olanoff et al. 1983). Marked differences existed, however, in the reported half-life of the terminal (slow) phase. Values reported for the terminal phase were 88 days (Aldrich and Holmes 1969), 21 days (Curley and Garrettson 1969), and 34 days (Olanoff et al. 1983). Small amounts of chlordane have been excreted in the urine of humans after oral ingestion of the compound. Aldrich and Holmes (1969) reported that the urinary concentration of chlordane decreased from 1.93 to 0.05 mg/L over the first 3 days following the ingestion of an unknown amount of chlordane by a 4-year-old girl. Curley and Garrettson (1969) reported a chlordane concentration in the urine of 0.309 mg/L 24 hours after the ingestion of an unknown amount of chlordane by a 20-month-old boy. Fecal chlordane concentrations of 719 and 105 ppm have been reported on days 2 and 3, respectively, following chlordane ingestion by a 4-year-old girl (Aldrich and Holmes 1969).

The excretion of chlordane and/or its metabolites has been studied in animals following oral administration. In rats, ≈70-90% of the radioactivity administered (depending on the isomer) was eliminated within 7 days following a single oral dose of the radiolabeled pesticide (Bamett and Dorough 1974; Ewing et al. 1985; Tashiro and Matsumura 1977). In these studies, the cis isomer was eliminated more quickly than the *trans* isomer. From 70% to 90% of the radioactivity orally administered to rats was excreted in the feces; excretion of radioactivity in the urine ranged from 2% to 8% of the administered dose (Bamett and Dorough 1974). In another study, *cis*- and *trans*-chlordane were cleared from the blood within 7 days after male mice received a total dose of 40 mg/kg chlordane mixture (1:1) of both isomers (Satoh and Kikawa 1992). Although the cis isomer tended to accumulate more than the *trans* isomer, no difference in the half-lives in tissues (0.6-2 days) was observed between the two isomers. Both isomers were rapidly metabolized to oxychlordane. Oxychlordane was eliminated very slowly compared to the isomers and its half-life in blood was determined to be 25 days.

Recent studies by Ohno et al. (1986) and Ewing et al. (1985) indicate that biliary excretion of chlordane and/or its metabolites is significant in both rats and mice and is the source of fecal excretion by these species. Ewing et al. (1985) administered a 1.0 mg/kg dose of radiolabeled *cis*-chlordane to rats and mice by intraperitoneal injection and recovered 47% (rats) and 67% (mice) of the dose in the feces within 7 days. By using bile duct-cannulated rats, Ohno et al. (1986) showed that bilk-y excretion occurred more rapidly after oral administration than after intravenous administration, probably as a result of the first pass of blood from the digestive tract through the liver via the hepatic portal circulation. The relative proportions of fecal and urinary excretion of radioactivity after oral administration of radiolabeled chlordane in rats do not appear to change significantly with dose over ranges of 0.05-10.0 mg/kg (Bamett and Dorough 1974; Ohno et al. 1986). In addition, longer-term administration of chlordane in the diet (1, 5, or 25 ppm for 56 days) did not change the excretion pattern significantly in rats from that observed following single oral doses (Bamett and Dorough 1974).

In contrast to the excretion pattern of radioactivity observed in rats following oral exposure to radiolabeled chlordane, rabbits tend to excrete larger percentages of the administered dose in the urine. The percentage of the administered radioactivity excreted in the urine of rabbits following multiple oral doses ranged from ≈28% to 47% (Balba and Saha 1978; Poonawalla and Korte 1971). In these same studies, fecal excretion in the rabbit ranged from ≈22% to 48% of the administered dose. The greater urinary excretion of radioactivity in rabbits compared with rats may be due to the greater ability of rabbits to form water soluble conjugates of chlordane metabolites. Biliary excretion was not studied in these experiments.

2.3.4.3 Dermal Exposure

No studies were located regarding excretion in humans or animals after dermal exposure to chlordane.

2.3.5 Mechanisms of Action

Although the data suggest that chlordane is readily absorbed from the respiratory (Nye and Dorough 1976) and gastrointestinal tracts (Ohno et al. 1986), and that dermal absorption is sufficient to cause toxicity in humans and animals (Derbes et al. 1955; Gaines 1960), data regarding the mechanisms of absorption were not located. Generally, highly lipophilic organic compounds cross membranes largely

by passive diffusion. Since chlordane is highly lipophilic (see Chapter 3), it is expected that absorption of chlordane by all routes of exposure would involve primarily passive diffusion. This is consistent with the observation by Ohno et al. (1986) that little difference in the extent of gastrointestinal absorption occurred over a 10-fold difference in dose.

The metabolism of the components of chlordane and the lipophilicity of the components and metabolites influence their distribution. Initial distribution to the liver and kidneys is more rapid than to fat (Ohno et al. 1986), probably reflecting differences in vascularity of these sites. Subsequently, redistribution results in higher levels in the fat than other tissues. Low levels of *cis*- and *trans*-chlordane in fat and relatively higher levels of oxychlordane (a metabolite) and *trans*-nonachlor (a component) reflect the relative lability of the chlordane isomers and stability of the latter two compounds (Hirasawa and Takizawa 1989; Sasaki et al. 1991a, 1992).

Metabolism of the *cis* and trans isomers of chlordane by humans and laboratory animals appears to be qualitatively similar (Kutz et al. 1976, 1979), although monkeys may be less efficient than rats (Khasawinah 1989), and rats may metabolize *trans*-nonachlor more efficiently than humans do (Tashiro and Matsumura 1978). Metabolism appears to be largely oxidative, involving hepatic microsomal cytochrome P-450 (Kawano et al. 1989). Epoxide hydrolase is probably the predominant enzyme involved in further degradation of oxychlordane, but the process appears to be slow in animals and humans. In addition, reductive dehalogenation, probably resulting in the formation of reactive free radical intermediates, may be important in the toxicity of chlordane (Brimfield and Street 1981; Kawano et al. 1989).

The strong lipophilicity and relatively weak hydrophilicity of chlordane and its metabolites suggest that excretion would be largely by passive diffusion. This is supported by the observation that fecal (biliary) excretion exceeds urinary excretion in humans and rats (Aldrich and Holmes 1969; Ohno et al. 1986), indicating that renal tubular excretion is probably not a major factor in excretion. Passive tubular resorption probably accounts for the lesser role that renal excretion plays in the fate of chlordane, compared with most organic chemicals, for which biotransformation results in the formation of more polar (hydrophilic) products.

The principal effects of exposure to chlordane include liver effects, neurological effects, immunological effects, and liver cancer. Although mechanisms of toxicity specific for each of these is

discussed below, general mechanisms that may apply to many organ systems are discussed here. The first is the ability of *cis*- and *trans*-chlordane and their metabolites to bind irreversibly with cellular macromolecules such as protein, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA) (Brimfield and Street 1981). Binding to these macromolecules may lead to cell death or altered cellular function. In addition, *cis*- and *trans*-chlordane, heptachlor, and heptachlor epoxide increase the generation of superoxide in cultures of guinea pig polymorphonuclear leukocytes (Suzaki et al. 1988). This was probably an indirect effect of activation of phospholipase C, or of increasing the intracellular concentration of free ionized calcium, rather than a direct effect on protein kinase C.

Many animal studies identify the liver as an important target organ for chlordane. Rats appear to be more sensitive than mice (Khasawinah and Grutsch 1989a, 1989b; Khasawinah et al. 1989; Velsicol Chemical Co. 1983a, 1983b, 1984). The primary effect, induction of hepatic cytochrome P-450 and other microsomal protein, is accompanied by a large increase in the volume of the smooth endoplasmic reticulum, which results in hepatocellular enlargement and hypertrophy (Khasawinah et al. 1989). These effects appear to be reversible. Reversible enzyme induction accompanied by hyperplasia is considered to be an adaptive response. In mice treated repeatedly over several weeks, the body burden of the chlordane isomers decreased, and the body burden of oxychlordane increased with time (Hirasawa and Takizawa 1989). This suggests that chlordane induces its own metabolism, probably to intermediates that bind to and disrupt the function of vital cellular macromolecules (Brimfield and Street 1981). A possible mechanism may be that the components and metabolites of chlordane exert their effects by altering the permeability of the mitochondrial membrane, inhibiting mitochondrial oxidative phosphorylation (Ogata et al. 1989). Also, chlordane may induce production of superoxide (Suzaki et al. 1988), which may result in lipoperoxidation, a known mechanism of toxicity to the liver.

Neurotoxicity is a consistent effect of acute exposure to chlordane in humans and animals. Little is known, however, regarding the mechanisms of neurotoxicity. In rats, a single oral 200-300 mg/kg dose induced tremor, paralysis, and tonic-clonic convulsions, and a 100 mg/kg dose induced hypothermia (Hrdina et al. 1974). The convulsive signs were accompanied by a decrease in cortical and striatal acetylcholine and an increase in acetylcholinesterase activity. Although the decrease in availability of the neurotransmitter acetylcholine may alter neuronal calcium ion levels at the synaptic plate, there is no evidence that this accounts for the convulsions induced by chlordane (Grutsch and Khasawinah 1991). In mice, a single 1,000 mg/kg intraperitoneal injection of chlordane induced

convulsions accompanied by reduced ν-aminobutyric acid (GABA) levels in five regions of the brain (Fishman and Gianutsos 1985). Grutsch and Khasawinah (1991) speculate that chlordane may competitively inhibit GABA, reducing the ability of GABA to inhibit post-synaptic neuronal excitability. The hypothermia was accompanied by a decrease in brain stem levels of norepinephrine. The investigators speculated that the reduced levels of norepinephrine arose from release and use of this transmitter amine to activate heat-loss mechanisms, resulting in hypothermia. In more recent investigations, Inoue et al. (1989) reported that chlordane inhibits nicotine-induced neurocellular uptake of Ca^{++}, which *is* required for release of norepinephrine. Therefore, chlordane may inhibit neural transmission by altering membrane permeability to Ca^{++} restricting the release of norepinephrine.

Reduced fertility, observed in mice and rats treated with high doses of chlordane (Ambrose et al. 1953a; Welch et al. 1971), may arise from altered metabolism and circulating levels of steroid hormones (Cranmer et al. 1984; Welch et al. 1971) or from reduced binding of progesterone to its cytosolic receptor site in the endometrium (Lundholm 1988).

There is little evidence that altered immunological function in adults is a prominent effect of chlordane exposure, since exposure induced no clear effects on cellular or humoral immune response or on granulocyte-, macrophage-, or splenocyte-forming stem cell populations in the bone marrow of adult mice. (Bamett et al. 1990a; Johnson et al. 1986). *In vitro* experiments, however, showed that *trans*-chlordane and its metabolites, including oxychlordane, suppressed both cell-mediated and humoral immune responses, possibly by depressing immune cell proliferation early in the chain of events involved in an immune response (Johnson et al. 1987). The addition of small amounts of mouse or human serum blocked chlordane-induced immunosuppression in the *in vitro* studies, suggesting that factors in the serum may have blocked immunosuppression in the *in vivo* studies.

Studies in mice suggest that prenatal and early postnatal exposure may have lasting effects on the immune system, including a reduction in the population of primitive granulocyte-, macrophage-, and splenocyte-forming stem cells in the bone marrow (Barnett et al. 1990a) and liver (Barnett et al. 1990b), without reducing the total cellularity of these tissues. Reasons for the apparent greater sensitivity of the developing immune system are not clear. A weak association of chlordane exposure with human blood dyscrasia, including leukemia, has been suggested (Epstein and Ozonoff 1987;

Infante et al. 1978). Although no mechanism has been suggested, it is not unreasonable to suspect that disruption of hematopoietic stem cell populations could be involved.

Although chlordane clearly induces liver cancer in mice (IRDC 1973; NCI 1977; Khasawinah and Grutsch 1989b; Velsicol Chemical Co. 1983b), epidemiological data provide no convincing evidence that it induces cancer in humans (Ditraglia et al. 1981; MacMahon et al. 1988; Shindell and Ulrich 1986; Wang and MacMahon 1979a, 1979b). Most genotoxicity tests with chlordane yielded negative results (see Section 2.4), suggesting an epigenetic mechanism of carcinogenicity. Chlordane inhibited gap junction intercellular communication in the rat hepatocyte/liver epithelial system metabolic cooperation assay (Tong and Williams 1988) and in the Lucifer yellow CH dye-coupling test in rat and mouse hepatocytes (Ruth et al. 1990). These results suggest that chlordane acts as a tumor promoter, depressing intercellular communication that checks uncontrolled proliferation of transformed or neoplastic cells (Tong and Williams 1988). Ruth et al. (1990) suggested that inhibition of intercellular communication may involve alteration of CAMP-dependent protein kinase phosphorylation of hepatocellular gap junction proteins, which would increase permeability at the gap junctions. Moser and Smart (1989), who noted that chlordane stimulated protein kinase C activity in several tissues of mice *in vitro*, provide support for this theory. Nonetheless, Suzaki et al. (1988) did not observe a chlordane-induced increase in protein kinase C activity *in vitro* in the rat .brain.

2.4 RELEVANCE TO PUBLIC HEALTH

Chlordane, an insecticide formerly used to treat field crops and as a soil treatment to kill termites, is of considerable concern to human health. Reasons for this concern include its potential for inducing adverse health effects, its presence in breast milk, its persistence in adipose tissue in the human body, and its persistence in all environmental media.

Chlordane's persistence in soil may lead to dermal exposure and also to oral exposure from eating field or garden crops grown on the treated soil. Soil ingestion by children is another possible exposure pathway. It is not expected to leach readily into groundwater, except at hazardous waste sites where the presence of organic solvents may dissolve it and facilitate its movement through soil. However, erosion of treated soil has contaminated the sediment found in many bodies of surface water. Human exposure has occurred from ingestion of contaminated drinking water or fish taken from contaminated

waters, or from bathing with contaminated water. Levels that induce subchronic or chronic disease in humans are not known.

Inhalation exposure for the general population arises chiefly from living in homes treated with chlordane, because chlordane volatilizes from the treated soil and accumulates in indoor air. Although levels at which adverse effects occur are not definitely known, neurological symptoms and jaundice have been associated with chlordane in treated homes. Levels in treated homes have been measured at 0.61 mg/m^3. Adverse effects have not been reported for industrial exposure; levels at which adverse effects would occur in occupationally exposed workers are not known. Presumably, exposure to $\leq$0.5 mg/m^3, the current OSHA standard, would not lead to adverse health effects in most healthy persons. No data are available regarding levels of chlordane in the air near hazardous waste sites, so it is not known if adverse effects would be expected to occur in humans in these areas.

Chlordane residues stored in fat in the human body are probably innocuous. It is possible that toxicity may occur when stores of body fat are mobilized in response to stress or dieting, or in the case of nursing mothers, who mobilize substantial amounts of body fat to maintain lactation, although examples were not located in the available literature.

The effects observed in humans or animals exposed to chlordane do not appear to be route-dependent, probably because absorption occurs readily by any route of exposure. It seems reasonable, therefore, that the effects of human exposure would be similar in people exposed at hazardous waste sites or in their homes.

Acute exposure of humans to high levels is characterized by gastrointestinal upset and neurological signs, including tremors and convulsions. Death may ensue, often preceded by convulsions. Neurological signs have been consistently observed in animal poisoning as well, firmly establishing chlordane as a neurotoxicant. Longer term exposure of humans to lower levels also caused neurological signs, including grand-mal seizures and altered EEG, but levels of exposure were not quantified. The occurrence of jaundice in persons living in chlordane treated homes and the alteration of serum enzyme levels in persons working as pesticide applicators suggest that the liver is an important target organ in humans.

Data were not located regarding reproductive or developmental effects in humans. In animals, however, increased mortality was observed in the offspring of treated dams, probably due to transmission of substantial amounts of chlordane residues from the body fat of the dams to the suckling offspring through the milk. Prenatally exposed mice appear to be more sensitive than adults to the immunological and neurological effects of chlordane. Given the generally greater sensitivity to toxins of incompletely developed tissues, it seems possible that prenatal exposure of humans to chlordane could result in compromised immunocompetence and subtle neurological effects.

Only one genotoxicity study in humans was located. Human HeLa cells were negative for evidence of DNA damage. Most genotoxicity studies in microorganisms or other mammalian systems were also negative.

Minimal Risk Levels for Chlordane

Inhalation MRLs

- An MRL of 0.0002 mg/m^3 has been derived for intermediate-duration (15-364 days) inhalation exposure to chlordane.

This derivation was based on the NOAEL of 0.1 mg/m^3 for liver effects in rats exposed intermittently for 90 days (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). Similar exposure to 1.0 mg/m^3 was associated with hepatocellular hypertrophy, and exposure to 10 mg/m^3 was associated with increased liver weight. Hepatocellular hypertrophy was the most sensitive end point in animals exposed by inhalation.

- An MRL of 0.00002 mg/m^3 has been derived for chronic-duration (≥365 days) inhalation exposure to chlordane.

This derivation was based on the study and end point used to derive the intermediate-duration MRL (Khasawinah et al. 1989; Velsicol Chemical Co. 1984), as described above. An additional uncertainty factor of 10 was applied to account for extrapolation from an intermediate-duration to chronic-duration exposure.

An MRL has not been derived for acute-duration inhalation exposure to chlordane. Only one acute inhalation study (Khasawinah et al. 1989; Velsicol Chemical Co. 1984) was located, and this study did not sufficiently identify target organs, and serious effects (death, convulsions) occurred at the lowest concentration tested.

Oral MRLS

- An MRL of 0.001 mg/kg/day has been derived for acute-duration (≤14 days) oral exposure to chlordane.

This derivation is based on a LOAEL of 1 mg/kg/day for developmental (depressed conditioned avoidance response acquisition, increased exploratory activity in open field test, and increased seizure threshold) effects in the offspring of mice exposed to chlordane during the third trimester (Al-Hachim and Al-Baker 1973). Developmental effects, as well as neurological effects, have been observed in a number of other acute-duration oral studies.

- An MRL of 0.0006 mg/kg/day has been derived for intermediate-duration (15-364 days) oral exposure to chlordane.

This derivation was based on a NOAEL of 0.055 mg/kg/day for hepatic effects in female rats given chlordane in the diet for 30 months (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a). The hepatic effects (hepatocellular hypertrophy) were observed at 0.273 mg/kg/day. The Ortega et al. (1957) study which found centrilobular hepatocellular hypertrophy and cytoplasmic inclusions in rats exposed to 0.125 mg/kg/day chlordane in the diet for 2-9 months supports the selection of the Khasawinah and Grutsch (1989a; Velsicol Chemical Co. 1983a) as the critical study. The selection of Khasawinah and Grutsch (1989a; Velsicol Chemical Co. 1983a) as the critical study [as opposed to Ortega et al. (1957)] was based on a higher degree of confidence in the quality of the study. A LOAEL of 0.1 mg/kg/day for increased hepatic cytochrome P-450 content at 10 weeks and decreased microsomal protein at 20 weeks in rats given chlordane in the diet (Mahon et al. 1979) supports the NOAEL of 0.055 mg/kg/day in the Khasawinah and Grutsch (1989a; Velsicol Chemical 1983a) study and the LOAEL of 0.125 mg/kg/day in the study by Ortega et al. (1957).

- An MRL of 0.0006 mg/kg/day has been derived for chronic-duration (≥365 days) oral exposure to chlordane.

This derivation is based on a NOAEL of 0.055 mg/kg/day for hepatic effects in rats given chlordane in the diet for 30 months (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a). The LOAEL was 0.273 mg/kg/day, at which an increased incidence of hepatocellular hypertrophy was observed in female rats.

Death. Absorption following ingestion of or skin contact with chlordane can be fatal to humans. WHO (1984) reported that an acute oral dose ≤25 mg/kg can result in death, although documentation was not available for this estimation. This estimated dose is ≈10 times lower than oral LD_{50} values reported for animals. Mortality increased among mice chronically ingesting 3.9 mg/kg/day through feed (NCI 1977), suggesting that mortality could occur in humans chronically exposed to doses much lower than 25 mg/kg/day. The dermal dose that causes death of humans is not known, but dermal LD_{50} values in animals are relatively low. A dose of 690 mg/kg of technical chlordane was a dermal LD_{50} in rats (Gaines 1960) and 1,100-1,200 mg/kg of "later" production chlordane (not contaminated with hexachlorocyclopentadiene) was a dermal LD_{50} in rabbits (Ingle 1965). Therefore, caution should be exercised when handling chlordane because of the possibility of dermal absorption. Epidemiological studies (Brown 1992; Ditraglia et al. 1981; MacMahon et al. 1988; Shindell and Ulrich 1986; Wang and MacMahon 1979a, 1979b) and case reports of acute exposure (EPA 1980a) do not suggest that inhalation exposure is likely to result in human deaths. Also, animal studies do not suggest that mortality would be expected in humans exposed by inhalation to low levels of chlordane, although exposure to high levels may cause death. Velsicol Chemical Co. (1984) and Khasawinah et al. (1989) reported no mortality in rats intermittently exposed to 10 mg/m^3 for 90 days, but mortality occurred in rats exposed to 154 or 413 mg/m^3 for 3 days. Although accidental or intentional ingestion of high doses of chlordane can lead to death, levels of chlordane found in drinking water, air, or soil near waste sites are not likely to result in noncancer human fatality.

Systemic Effects

Respiratory Effects. Most of the data from human or animal studies do not indicate that respiratory effects are likely in humans exposed to chlordane. Several cases and personal reports involving acute inhalation, oral, or dermal exposure (Curley and Garrettson 1969; EPA 1980a; Harrington et al. 1978;

NIOSH 1984a; Olanoff et al. 1983) and epidemiological studies of chlordane manufacture (Alvarez and Hyman 1953; Brown 1992; Fishbein et al. 1964; Princi and Spurbeck 1951) do not report respiratory effects in exposed humans. One study, however, reported sore throat and respiratory infections were in humans shortly after their homes were treated for termites (Menconi et al. 1988). In addition, a case report of acute ingestion of chlordane by a human described bronchopneumonia as a possible effect (Aldrich and Holmes 1969). Slight lung congestion and proliferation of bronchiole lining cells were also observed in mice exposed continuously to an unspecified concentration of chlordane in air for 14-25 days (Ingle 1953). Repeated exposure to chlordane vapor or aerosols does not appear to cause respiratory effects in rats (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). It is unlikely that respiratory effects would be important in humans exposed to chlordane in food, drinking water, air, or soil near waste sites.

Cardiovascular Effects. Although equivocal evidence of increased risk of cerebrovascular disease was reported in workers involved in the manufacture of chlordane (Wang and MacMahon 1979a), cardiovascular effects associated with exposure to chlordane were not reported in other studies of occupational exposure during manufacture (Alvarez and Hyman 1953; Brown 1992; Fishbein et al. 1964; Princi and Spurbeck 195l), following inhalation and dermal exposure resulting from a chemical spill in a library room (NIOSH 1984a), or in the majority of cases and personal reports of acute oral, inhalation, or dermal exposure compiled by EPA (1980a). A few of the cases compiled by EPA (1980a) reported tachycardia, chest pains, and shortness of breath, but exposure often involved a mixture of pesticides and vehicles such as petroleum distillates that may have caused these effects. Several case reports of accidental or intentional ingestion of chlordane by humans reported tachycardia or elevated pulse rates (Curley and Garrettson 1969; Olanoff et al. 1983). No histopathological heart lesions were found in rats, mice, or hamsters treated acutely with oral doses of chlordane (Truhaut et al. 1974, 1975) or in rats or mice in chronic dietary (Khasawinah and Grutsch 1989a, 1989b; NCI 1977; Velsicol Chemical Co. 1983a, 1983b) or inhalation (Ingle 1953; Khasawinah et al. 1989; Velsicol Chemical Co. 1984) studies. Thus, it is unlikely that exposure to chlordane per se would cause cardiovascular effects in humans exposed to chlordane in food, drinking water, or soil near hazardous waste sites.

Gastrointestinal Effects. Gastrointestinal symptoms are an early and consistent observation in a number of cases and personal reports of acute human oral and inhalation exposure (Curley and Garrettson 1969; Dadey and Kammer 1953; EPA 1980a; Olanoff et al. 1983). These symptoms

include gastritis, nausea, vomiting, intestinal cramps, abdominal pain, and diarrhea. NIOSH (1984a) reported gastrointestinal symptoms in library workers following inhalation and dermal exposure to high (unquantified) levels of chlordane resulting from a spill. Humans accidently exposed to chlordane in their drinking water at levels ranging from 0.1 to 92,500 ppb reported gastrointestinal symptoms (Harrington et al. 1978). Gastrointestinal symptoms were not reported in a number of epidemiology studies of chlordane manufacture (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951), nor in a compilation of cases of acute dermal exposure (EPA 1980a). However, vomiting (Balistreri et al. 1973) has been reported in case reports of humans exposed dermally to chlordane.

No histopathological lesions in the gastrointestinal tract were found in rats, mice, or hamsters treated acutely with oral doses of chlordane (Truhaut et al. 1974), or in rats or mice in chronic feeding studies (Ambrose et al. 1953a; Khasawinah and Grutsch 1989a, 1989b; NCI 1977; Velsicol Chemical Co. 1983a, 1983b), or in rats exposed by inhalation (Ingle 1953; Khasawinah et al. 1989; Velsicol Chemical Co. 1984).

Case reports (EPA 1980a) mention oral irritation after acute accidental ingestion. These observations suggest that chlordane, especially in large doses, is irritating to the gastrointestinal tract when ingested. The gastrointestinal symptoms (vomiting, abdominal pain, and diarrhea) reported in humans following inhalation exposure and ingestion of relatively smaller doses may reflect a primary effect on other organs or systems, such as the central nervous system. These data suggest that gastrointestinal symptoms would be among the earliest complaints of those exposed to chlordane at low levels in the air or drinking water. The threshold concentrations for these effects in environmental media are unknown, and the likelihood of their occurrence near waste sites cannot be predicted.

Hematological Effects. Hematological effects specifically related to exposure to chlordane were not reported in a compilation of cases and personal reports of acute oral, inhalation, or dermal exposures (EPA 1980a). Limited hematological examinations revealed no effects on workers involved in chlordane manufacture (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951). There were several reports of cases of blood dyscrasia in humans exposed to chlordane (Epstein and Ozonoff 1987; Infante et al. 1978), but most were exposed to other chemicals as well, and the association of blood dyscrasia with chlordane is very weak. However, the existence of a number of anecdotal reports of blood dyscrasia associated with several organochlorine pesticides (chlordane, lindane, DDT), suggests that there may be a an unusually susceptible subpopulation (Curley and

Garrettson 1969; Ellenhorn and Barceloux 1988; Lensky and Evans 1952). Leukocytosis associated with lymphocytosis was reported in mice treated by gavage with 8 mg/kg/day for 14 days (Johnson et al. 1986), but the toxicological significance of an elevated leukocyte count is unclear. Hematological examination revealed no effects in rats exposed for 90 days by inhalation (Khasawinah et al. 1989; Velsicol Chemical Co. 1984) or in rats or mice chronically exposed to chlordane in the diet (Ambrose et al. 1953a; Khasawinah and Grutsch 1989a, 1989b; Velsicol Chemical Co. 1983a, 1983b). The available data suggest that hematological effects might occur in unusually sensitive humans exposed to low levels of chlordane in environmental media near waste sites.

Musculoskeletal Effects. Musculoskeletal effects in humans were not reported in a compilation of cases and personal reports of acute oral, inhalation, or dermal exposure to chlordane (EPA 1980a), in library workers exposed to high levels resulting from a spill (NIOSH 1984a), or in workers involved in chlordane manufacture (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951). Three of nine pesticide applicators were reported to have elevated serum levels of creatine phosphokinase (Ogata and Izushi 1991), but this does not constitute convincing evidence of muscle damage.

Musculoskeletal lesions were not found in rats or mice exposed to chlordane chronically in the diet (Khasawinah and Grutsch 1989a, 1989b; NCI 1977; Velsicol Chemical Co. 1983a, 1983b) or in rats or monkeys exposed intermittently by inhalation for 90 days (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). Rats treated orally with chlordane did not have increased levels of creatine phosphokinase in serum, but rats injected intraperitoneally did (Ogata and Igushi 1991). It is not likely that humans would develop musculoskeletal lesions after prolonged exposure to low doses of chlordane.

Hepatic Effects. Hepatic effects were reported in a small proportion of a compilation of cases and personal reports of acute oral, inhalation, or dermal exposure to chlordane (EPA 1980a), but not in library workers acutely exposed to high (unquantified) levels resulting from a spill (NIOSH 1984a). Liver function tests were normal in a 20-month-old male child between 20 hours to 3 days after ingesting chlordane (Curley and Garrettson 1969), in a nurseryman who developed neurological effects after handling soil that contained chlordane and other chemicals (Barnes 1967), and in workers involved in the manufacture of chlordane (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951). Subtle serum chemistry changes indicative of altered liver function (increased triglycerides and lactate dehydrogenase activity), however, were observed in pesticide applicators in

Japan exposed to chlordane (Ogata and Izushi 1991). Jaundice has been reported in humans living in homes treated with chlordane for termite control (EPA 1980a). Acute, prolonged dermal exposure of an infant to liquid chlordane was associated with jaundice, liver necrosis, fatty infiltration, and inflammation diagnosed from a biopsy sample (Balistreri et al. 1973).

Reliable acute oral and parenteral studies in animals exposed to chlordane indicate that enzyme induction, minor histochemical and histomorphological changes, and liver hypertrophy occur within hours of treatment and at very low doses (Casterline and Williams 1971; Cram et al. 1956; Den Tonkelaar and Van Esch 1974: Hart et al. 1963; Johnson et al. 1986; Truhaut et al. 1974, 1975). Enzyme induction in the absence of evidence of liver damage or impaired liver function is considered an adaptative rather than an adverse effect. In the case of chlordane, however, it should be noted that metabolites of chlordane, such as oxychlordane, heptachlor, and heptachlor epoxide, are more toxic than the parent compound. Moreover, enzyme induction can accelerate metabolism of therapeutic drugs and hormones (Welch and Harrison 1966; Welch et al. 1971). Liver cell inclusion bodies were found in some rats,exposed dermally to chlordane for a few days (Ambrose et al. 1953a). In prolonged inhalation (Ambrose et al. 1953a; Khasawinah et al. 1989; Velsicol Chemical Co. 1984) and oral exposure studies (Ambrose et al. 1953a; IRDC 1973; Khasawinah and Grutsch 1989a, 1989b; Ortega et al. 1957; Velsicol Chemical Co. 1983a, 1983b), mild histopathological changes in the liver occurred in mice and rats.

Although reports of serious liver disease in humans exposed to chlordane are uncommon, animal studies suggest that more subtle effects, including altered enzyme activities and mild morphological changes, may be expected from any route of exposure to low doses. The more subtle effects are expected to occur at lower doses and shorter durations of exposure than jaundice or other indications of severe liver dysfunction or histopathological effects. Histopathological effects are not likely to be diagnosed routinely, however, because invasive procedures such as liver biopsy would be required. Physicians prescribing therapeutic drugs or hormones should be aware that doses may require adjustment in patients exposed to chlordane.

Renal Effects. Renal effects were not reported in a compilation of cases and personal reports of acute human oral, inhalation, or dermal exposure to chlordane (Dadey and Kammer 1953; EPA 1980a), in library workers exposed to high levels resulting from a spill (NIOSH 1984a), or in workers involved in chlordane manufacture (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951).

Derbes et al. (1955) reported pathological changes in the kidneys of a woman who died within minutes after an accidental spill resulted in dermal exposure to chlordane and other pesticides.

Kidney congestion was observed in rats, mice, and hamsters given large acute oral doses of 200, 200, and 1,200 mg/kg, respectively (Truhaut et al. 1974, 1975). Kidney lesions were not found in rats exposed to chlordane in the diet at ≤20.4 mg/kg/day or in mice similarly exposed to ≤8.3 mg/kg/day (Ambrose et al. 1953a; Khasawinah and Grutsch 1989a, 1989b; NCI 1977; Ortega et al. 1957; Velsicol Chemical Co. 1983a, 1983b) or in rats or monkeys exposed intermittently by inhalation for 90 days to 10 mg chlordane/m^3 (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). Rats in the inhalation study, however, exhibited elevated kidney weights. It is not known whether humans could develop kidney lesions after prolonged exposure to low doses of chlordane.

Dermal/OcularEffects. Dermal effects were not reported in a compilation of cases and personal reports of acute oral and inhalation exposure to chlordane (EPA 1980a), in a group of library workers following inhalation and dermal exposure resulting from a spill (NIOSH 1984a), in workers involved in chlordane manufacture (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951), or in a woman who died within minutes of spilling a mixture of chemicals including chlordane on the front of her clothing (Derbes et al. 1955). EPA (1980a), however, reported burning sensations of the skin, rashes and pruritus in cases of accidental acute dermal exposure to chlordane. Accidental spraying of chlordane in the eyes consistently resulted in conjunctivitis (EPA 1980a). These reports of dermal and ocular effects are complicated by exposure to mixtures of chemicals including other pesticides and vehicles, such as petroleum distillates, that are known to be irritants.
Oral exposure of rats and mice (NCI 1977; Khasawinah and Grutsch 1989a, 1989b; Velsicol Chemical Co. 1983a, 1983b) and inhalation exposure of rats and monkeys (Khasawinah and Grutsch 1989a, 1989b; Velsicol Chemical Co. 1984) produced no skin or ocular lesions. Datta et al. (1975) reported changes in the malpighian cells of guinea pigs dermally exposed once daily for 90 days to a high dose of chlordane (168 mg/kg). No changes were observed in the dermis. However, no local dermal reactions were found in rats treated dermally with 273 mg/kg/day for 4 days (Ambrose et al. 1953a).

These data suggest that both ocular and dermal exposure to liquid chlordane may cause adverse effects, but these effects are unlikely at concentrations expected in environmental media near waste sites.

Other Systemic Effects. Other effects observed in animals exposed to chlordane include effects on body weight and the thyroid. In intermediate-duration inhalation studies, increased height of the thyroid follicular cells were observed in rats and monkeys (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). Rats also lost body weight. Body weight loss was also observed in rats treated orally with chlordane for intermediate and chronic durations (Ambrose et al. 1953a; NCI 1977). Whether exposure of humans to chlordane at hazardous waste sites would result in body weight or thyroid effects is not known.

Immunological Effects. Chlordane appears to have caused impaired proliferative responses to plant mitogens in humans following inhalation exposure for periods ranging from 3 days to 15 months (McConnachie and Zahalsky 1992). Further testing of similarly exposed individuals demonstrated an increased autoantibody titer; suggesting chlordane-induced autoimmunity.

In intermediate-duration inhalation studies, female rats, but not male rats, exposed to 28.2 mg/m^3 chlordane for 28 days had reduced thymus weight (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). However, thymus weight was not altered in either sex of rats exposed to 10 mglm^3 for 90 days, but female rats had increased leukocyte counts at 1 or 10 mg/m'. No histopathological lesions were found in the thymus or lymph nodes of monkeys similarly exposed to chlordane. Sensitization was not reported in guinea pigs treated once daily for 90 days with dermal applications of chlordane in acetone (Datta et al. 1975). It is not clear, however, that the protocol would have reliably identified dermal sensitization. Oral studies conducted in mice suggest that chlordane causes leukocytosis associated with lymphocytosis (Johnson et al. 1986), depresses cell-mediated immunity, as manifested by a depression in delayed-type hypersensitivity reactions and depressed mixed lymphocyte reactivity (Barnett et al. 1985a, 1985b; Menna et al. 1985; Spyker-Cranmer et al. 1982). There were no effects on humoral-mediated immunity. In some of these studies, the mice tested were the offspring of dams treated during gestation and allowed to nurse their young. Therefore, the mice had been exposed to chlordane and its metabolites during gestation and lactation. The effect on delayedtype hypersensitivity occurred at very low levels in mice. Data from an *in vitro* study suggested that chlordane may interfere with cell-mediated immune proliferative response in rhesus monkeys (Chuang et al. 1992). In this study, cultures of rhesus monkey peripheral blood mononuclear cells (PBMC) were prepared and 10 or 80 μM of chlordane along with conventional mitogens were added at the onset of the culture. At 10 μM concentration, chlordane modulated T cell mitogenic response or acted as a mitogen for T lymphocytes in the absence of conventional mitogens. The study also

demonstrated that at 80 μM concentration, chlordane completely impaired the functions of monkey lymphocytes. It is possible that effects on immune function could occur in humans exposed to chlordane at hazardous waste sites.

Neurological Effects. Chlordane clearly causes neurological effects in humans following acute or prolonged oral, inhalation, or dermal exposure (Aldrich and Holmes 1969; Balistreri et al. 1973; Barnes 1967; Curley and Garrettson 1969; Dadey and Kammer 1953; Derbes et al. 1955; EPA 1980a; Harrington et al. 1978; Kutz et al. 1983; Lensky and Evans 1952; Menconi et al. 1988; NIOSH 1984a; Olanoff et al. 1983). Neurological effects, including headache, dizziness, irritability, muscle tremors, confusion, convulsions, and coma are frequently the earliest reported in cases of human intoxication with chlordane. It is possible that gastrointestinal symptoms such as nausea, abdominal pain, and diarrhea may result from effects on the nervous system. Oral exposure has been associated with central nervous system effects in children (Aldrich and Holmes 1969). The threshold concentration in air for neurological effects in humans has not been identified, but cases and personal reports (EPA 1980a; NIOSH 1984a) suggest that these effects may occur at concentrations encountered *in* recently treated homes. Neurological symptoms, however, have not been reported in studies of occupational exposure (Alvarez and Hyman 1953; Fishbein et al. 1964; Princi and Spurbeck 1951), although equivocal evidence of increased risk of cerebrovascular disease was reported in workers involved in the manufacture of chlordane (Wang and MacMahon 1979a). Abnormal respiratory movement, excessive salivation, and convulsions occurred in rats exposed by inhalation to chlordane for acute durations, and hypersensitivity to touch was observed in female rats exposed by inhalation for intermediate durations (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). Acute (Ambrose et al. 1953a; Hrdina et al. 1974) and prolonged (Drummond et al. 1983; NCI 1977) oral exposure in animals has been associated with frank neurological signs such as tremor and convulsions. Neither human nor animal studies, however, have investigated subtle neurological or behavioral effects, which may occur at doses lower than the frank effects that have been associated with chlordane exposure. Therefore, it is not possible to predict the likelihood of subtle neurological effects in humans exposed to the low levels of chlordane expected in environmental media located near waste sites.

Reproductive Effects. Compared with the control population from the National Center for Health Statistics 1979 National Health Interview Survey (NHIS), the incidence rates of unspecified ovarian and uterine disease were significantly ($p<0.05$) elevated in women who were exposed to chlordane

vapors in their homes (Menconi et al. 1988). Because the case report and the epidemiological study have several limitations, it is doubtful that the reported lesions and diseases are treatment-related.

Male mice treated by gavage with 100 or 300 mg/kg/day for 30 days had reduced size of seminiferous tubules and degeneration of spermatogenic epithelium (Balash et al. 1987). Oral administration of chlordane to male rats (19.5 mg/kg/day for 90 days) increased androgen receptor sites in the ventral prostate (Shain et al. 1977). However, histological examination of testes of rats and mice given a single oral dose of 200 mg/kg or hamsters given a single oral dose of 1,200 mg/kg revealed no treatment-related lesions (Truhaut et al. 1975). Lesions in male or female reproductive organs were not reported in rats or mice in chronic dietary studies (Ambrose et al. 1953a; Khasawinah and Grutsch 1989a, 1989b; NCI 1977; Velsicol Chemical Co. 1983a, 1983b) or in rats or monkeys in a 90-day inhalation study (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). Datta et al. (1975) reported mild degenerative changes in the testes of guinea pigs treated with dermal applications of chlordane in acetone at 168 mg/kg once daily for 90 days. Ambrose et al. (1953a) reported reduced fertiiity, reflected as a reduction in the number of mated females that delivered litters, when male and female rats were fed a diet that provided chlordane at 16 mg/kg/day. Treatment began at weaning and continued through lactation. None of the delivered litters survived to weaning. Reduced fertility, reflected as a reduction in the number of females that became pregnant, was observed in mice given intraperitoneal injections of chlordane (25 mg/kg) once weekly for 3 weeks before being mated to untreated males of proven fertility (Welch et al. 1971). Data indicate that exposure to chlordane can affect metabolism and circulating levels of steroid hormones (Cranmer et aI. 1984; Welch et al. 1971) and can reduce the binding of progesterone to its cytosolic receptor in the endometrium (Lundholm 1988).

Data regarding effects on the fertility of rats and mice are limited by study design. Dose levels were not chosen to detect thresholds for effects on fertility, and the studies were not designed to investigate the mechanisms by which chlordane may interfere with fertility. The data in animals are not sufficient to indicate whether chlordane can cause reproductive effects in humans, or whether such effects would be expected in humans exposed to chlordane near waste sites.

Developmental Effects. No data were located regarding developmental effects in humans after exposure to chlordane.

No developmental effects were observed in rats treated orally during gestation with doses sufficient to cause death of 50% of the dams (Usami et al. 1986). In a screening study, no effects on viability or postnatal growth were observed in the offspring of mice treated orally during gestation (Chemoff and Kavlock 1982). Pregnant mice were treated with chlordane in order to study the immunological, behavioral, and endocrine effects on the offspring. The offspring were allowed to nurse their dams; therefore, they were exposed to chlordane and metabolites during gestation and nursing. The results of immunological tests suggest that chlordane suppressed cell-mediated immunity (Barnett et al. 1985a, 1985b, 1990a, 1990b; Menna et al. 1985; Spyker-Cranmer et al. 1982; Theus et al. 1992). Results of behavioral tests indicate that chlordane depressed the acquisition of avoidance behavior, raised seizure thresholds, and increased exploratory activity (Al-Hachim and Al-Baker 1973). Increased plasma levels of corticosterone were observed in the offspring of mice treated during gestation; the change implies an effect on neuroendocrinological feedback, possibly resulting from the liver's reduced ability to metabolize corticosterone (Cranmer et al. 1984). The relevance of these findings to humans is not known, and the likelihood of effects on human development resulting from exposure to chlordane near waste sites is unpredictable.

Genotoxic Effects. Chlordane has been tested for mutagenicity in several systems. As seen from Table 2-4, mostly negative results have been obtained for reverse mutations, DNA repair, and dominant and recessive lethal assays. Chlordane induced mitotic gene conversion in *Saccharomyces cerevisiae* in the presence, but not the absence, of metabolic activation; and in prophage in *Escherichia coli,* regardless of metabolic activation. Chlordane induced forward mutation in mouse lymphoma L5178Y cells without metabolic activation, and also induced sister chromatid exchange in human lymphoid cells. Dermal application of chlordane to mice induced micronuclei formation in the bone marrow cells and nuclei aberrations in the hair follicles (Schop et al. 1990). The generally negative results for mutagenicity of chlordane are consistent with an epigenetic mechanism of carcinogenicity (see below).

Cancer. Retrospective mortality studies provide no convincing evidence of a link between exposure to chlordane, during its manufacture or during its application as a pesticide, and increased risk of death due to cancer (Brown 1992; Cantor et al. 1992; Ditraglia et al. 1981; MacMahon et al. 1988; Shindell and Ulrich 1986; Wang and MacMahon 1979a, 1979b). Weak evidence suggests a link between chronic residence in chlordane-treated homes or gardens and increased risk of unspecified skin neoplasms (Menconi et al. 1988) or leukemia (Epstein and Ozonoff 1987; Infante et al. 1978) and result

TABLE 2-4. Genotoxicity of Chlordane

Species (test system)	End point	Activation	Response	Reference
Salmonella typhimurium	Reverse mutation	+	—	Probst and Hill 1981; Gentile et al. 1982;
S. typhimurium	Reverse mutation	—	—	Ercegovich and Rashid 1977; Mortelmans et al. 1986; Simmon et al. 1977; Ashby and Tennant 1988
Escherichia coli	Reverse mutation	+	—	Probst and Hill 1981
E. coli	Reverse mutation	—	—	Probst and Hill 1981
Saccharomyces cerevisiae	Mitotic gene conversion	+	+	Gentile et al. 1982
		—	—	
S. cerevisiae	Mitotic gene conversion	Not applicable	+	Chambers and Dutta 1976
E. coli	Prophage induction	+	+	Houk and DeMarini 1987
		—	+	
S. typhimurium	DNA repair and synthesis	—	—	Rashid and Mumma 1986
E. coli	DNA repair and synthesis	—	—	Rashid and Mumma 1986
Rat hepatocytes	DNA repair and synthesis	Not applicable	—	Probst and Hill 1981
Rat hepatocytes	DNA repair and synthesis	Not applicable	—	Maslansky and Williams 1981
Mouse hepatocytes	DNA repair and synthesis	Not applicable	—	Maslansky and Williams 1981
Hamster hepatocytes	DNA repair and synthesis	Not applicable	—	Maslansky and Williams 1981
Human SV—40 fibroblasts	DNA repair and synthesis	+	—	Ahmed et al. 1977a
		—	+	
HeLa cells	DNA repair and synthesis	No data	—	Griffin and Hill 1978; Brandt et al. 1972;
(human uterine cells)			—	Blevins and Sholes 1978
			+	
Human lymphoid cells	Sister chromatid exchange	No data	+	Sobti et al. 1983
Mouse	Dominant lethal	Not applicable	—	Arnold et al. 1977
Mouse	Dominant lethal	Not applicable	—	Epstein et al. 1972

TABLE 2-4. Genotoxicity of Chlordane *(continued)*

Species (test system)	End point	Activation	Response	Reference
Drosophila melanogaster	Recessive lethal	Not applicable	—	Vogel 1980
Chinese hamster ovary	Mutagenic activity	No data	—	Tsushimoto et al. 1983
Chinese hamster ovary	Mutagenic activity	No data	+	Ahmed et al. 1977b
Rat hepatocytes	HGPRT[a]	Not applicable	—	Telang et al. 1981; Tong et al. 1981
Mouse lymphoma L5178Y cells	Forward mutation	—	+	McGregor et al. 1988

[a]Hypoxanthine-guanine phosphoribosyl transferase mutagenesis assay

DNA = deoxyribonucleic acid; — = negative result; + = positive result

between the use of chlordane in farming operations and increased risk of non-Hodgkins lymphoma (Woods and Pollissar 1989). Chronic oral treatment with chlordane at ≥1.21 mg/kg/day significantly increased hepatocellular carcinomas in mice (EPA 1985a, 1986c, 1987e; Epstein 1976; IRDC 1973; IRIS 1992; Khasawinah and Grutsch 1989b; NCI 1977; Reuber 1978; Velsicol Chemical Co. 1983b). Becker and Sell (1979) demonstrated that chlordane could induce hepatocellular carcinomas in a strain of mice that historically is not predisposed to liver tumors. No statistically significant increase in tumor incidence was observed in dietary studies in rats in the NCI (1977) study or in EPA (1988b) reevaluations of the Khasawinah and Grutsch (1989a) and Velsicol Chemical Co. (1983a) study. Negative responses in a number of mutagenicity tests suggest an epigenetic mechanism of carcinogenicity, perhaps involving a promoting effect on predisposed cells (Ashby and Tennant 1988; Maslansky and Williams 1981). Ashby and Tennant (1988) also noted that a positive carcinogenic response in the mouse liver in NCI/NTP studies may reflect an epigenetic as well as a genotoxic mechanism. Williams and Numoto (1984) presented evidence that chlordane acts as a promoter of liver tumor formation in cells initiated by diethylnitrosamine. Telang et al. (1982) observed a marked inhibition of intercellular communication in cultured rat liver cells treated with chlordane. The investigators reported that this effect is common to other organochlorine pesticides that appear to act as tumor promoting agents. EPA (IRIS 1992) considered the data in laboratory animals sufficient to classify chlordane in Group B2, that is, as a probable human carcinogen.

2.5 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the

properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to chlordane are discussed in Section 2.5.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by chlordane are discussed in Section 2.5.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.7, "Populations That Are Unusually Susceptible."

2.5.1 Biomarkers Used to Identify or Quantify Exposure to Chlordane

It is possible to measure chlordane and/or a number of its metabolites in a variety of human tissues and fluids (i.e., blood, adipose tissue, brain, liver, kidney, milk, sebum (or skin lipids), urine, and feces). Generally, total chlordane residue levels are higher in fat and liver than in the blood (Mussalo-Rauhamaa 1991). There is no information in the literature, however, correlating the levels found in these tissues and fluids with the environmental chlordane concentrations to which the individual was exposed. Furthermore, the data do not reveal how long after exposure residues may be detected in the various body tissues and fluids.

Kawano and Tatsukawa (1982) measured "total" chlordane *(cis-* and *trans-*chlordane, heptachlor epoxide, oxychlordane and *trans-*nonachlor) residues in the blood of pest control operators and

nonexposed workers in Japan. Not all of these residues (e.g., heptachlor epoxide) are specific for exposure to chlordane. Levels in the blood of four of five unexposed workers were below 0.10 ng/g, the limit of detection. A level of 0.13 ng/g was reported for the fifth unexposed worker, who had lived for 2 years in a termite-treated home. Levels in the blood of 21 pest control operators ranged from 0.57 to 83 ng/g, with an average of 12 ng/g, approximately two orders of magnitude greater than levels in unexposed workers. There was no mention or indication of signs of chlordane toxicity in either the unexposed workers or pest control operators. Katz (1983) reported mean levels in human serum for each of four components or metabolites of chlordane at <l ppb. It was not possible, however, to estimate the mean level of "total" chlordane from these data. Kutz et al. (1976, 1979) reported mean levels of heptachlor epoxide of ≈0.1 ppm in adipose tissue samples collected from the U.S. population. Levels of oxychlordane in these samples also averaged ≈0.1 ppm. There appeared to be no significant change in the concentration of either chlordane metabolite over a 5-year period from 1970 to 1975. The individuals sampled by Kutz (1983) and Kutz et al. (1976, 1979) are assumed to be asymptomatic, so the reported levels were not associated with effects of chlordane toxicity. Oxychlordane has been measured at concentrations ranging from 0.002 to 0.005 mg/L in human breast milk (whole milk basis) in samples taken at random from subjects with infants assumed to be asymptomatic (Bamett et al. 1979; Strassman and Kutz 1977). In Finnish human milk samples, total chlordane residues in positive samples averaged 0.41 mg/kg of milk fat (Mussalo-Rauhamaa et al. 1988).

Several components of chlordane (*trans*- and *cis*-chlordane, *trans*- and *cis*-nonachlor, heptachlor, gamma-chlordene) were detected in the skin lipids of humans (Sasaki et al. 1991b). The samples were taken by swabbing the face with cotton soaked with 70% ethanol 3-4 hours after the face was washed with soap. Because all samples from inhabitants of an area known to be contaminated with chlordane contained chlordane residues, and because the profile of chlordane components in skin lipids closely resembled those in technical chlordane, the authors suggested that skin lipid analysis is a satisfactory indicator of dermal exposure to airborne chlordane, such as occurs in homes treated for termites. Oxychlordane in the skin lipids was positively correlated (correlation coefficient = 0.68, $p<0.01$) with concentrations in internal adipose tissue. The authors concluded that the concentration of oxychlordane in skin lipids was a satisfactory indicator of body accumulation of chlordane.

A later study in monkeys confirmed that chlordane residues in skin lipids correlate closely with residues in blood (Sasaki et al. 1992). In this study, monkeys were given 5 consecutive, weekly,

subcutaneous doses of 1 or 10 mg *trans*-chlordane/kg, and blood, adipose tissue and skin lipids were sampled up to 28 weeks after the last treatment for analysis for *trans*-chlordane and oxychlordane. *Trans*-chlordane concentrations in adipose tissue declined rapidly after the last dose; oxychlordane concentrations increased for about 5 weeks and leveled off. The correlation coefficients for *trans*-chlordane in the adipose tissue and blood, and in the adipose tissue and skin lipids were 0.93 and 0.72, respectively. The correlation coefficients for oxychlordane in the adipose tissue and blood, and in the adipose tissue and skin lipids were 0.94 and 0.83, respectively. These data suggest that *trans*-chlordane concentrations in skin lipids are a satisfactory biomarker of recent exposure, and that oxychlordane concentrations in skin lipids are a satisfactory marker of previous exposure and of the body burden (in adipose tissue) of oxychlordane.

Data are available that correlate exposure to chlordane with levels of the pesticide and/or its metabolites in biological samples taken from humans. Total chlordane residues in the blood were ≈3-16 times higher in pesticide applicators, and ≈1.5-10 times higher in residents of a heavily contaminated area, where many of the houses were treated for termites with chlordane, than in residents of a relatively noncontaminated area (Wariishi and Nishiyama 1989). Recent information indicates a relatively strong correlation between the length of exposure to atmospheric chlordane in a termite-treated home and the concentration of chlordane in human milk fat (Taguchi and Yakushiji 1988); however, actual atmospheric chlordane concentrations were not reported. A relatively strong correlation between blood chlordane concentration and the number of days that a pest control operator has sprayed has been reported by Saito et al. (1986). Takamiya (1987) demonstrated a strong correlation between total chlordane residues (i.e., *trans*-nonachlor plus oxychlordane) in the blood of pest control operators and their duration of exposure. The atmospheric concentrations of chlordane to which these pest control operators had been exposed were not reported (Saito et al. 1986; Takamiya 1987). Kawano and Tatsukawa (1982) showed that the levels of heptachlor epoxide, oxychlordane, and *trans*-nonachlor in the blood of pest control operators in Japan increased with increased duration of exposure (years of employment).

More recently, elevated serum levels of creatinine phosphokinase (CPK) were measured in Japanese pest control operators exposed to chlordane (Ogata and Izushi 1991). Levels of SGOT and SGPT were not elevated, and the investigators concluded that elevated CPK was somewhat specific for exposure to chlordane, compared with other organochlorine compounds.

2.5.2 Biomarkers Used to Characterize Effects Caused by Chlordane

The most sensitive indicators of acute chlordane toxicity in humans are central nervous system effects including headache, confusion, behavioral aberrations, and tremors (EPA 1980a; Harrington et al. 1978). At high levels of exposure, central nervous system effects include convulsions, coma, respiratory failure, and eventually death. Effects on the liver appear to be the only manifestations in humans of chronic exposure to chlordane (EPA 1980a; Ogata and Izushi 1991).

The most sensitive target organ in animals appears to be the liver, and the most sensitive effect appears to be induction of hepatic microsomal xenobiotic-metabolizing enzymes at ≥0.50 mg/kg/day (Casterline and Williams 1971; Den Tonkelaar and Van Esch 1974). Determination of enzyme induction would be an inappropriate assay for most cases of suspected human intoxication, because enzyme induction is not specific for exposure to chlordane and because invasive techniques would be required to obtain liver tissue. More recently, Ogata and Izushi (1991) observed subtle evidence of liver effects, elevated serum triglycerides, CPK, and LDH in pesticide workers, compared with normal values. The elevated CPK was considered to be somewhat specific for chlordane exposure. Discussions of other biomarkers for liver damage and neurological effects in humans are found in CDC/ATSDR (1990) and OTA (1990). A more detailed discussion of the health effects caused by chlordane can be found in Section 2.2 of Chapter 2.

2.6 INTERACTIONS WITH OTHER SUBSTANCES

Chlordane, like other organochlorine insecticides, functions as a potent inducer of hepatic microsomal enzymes. Induction of these enzymes following chlordane administration is associated with an increased rate of metabolism of many endogenous and xenobiotic compounds, including therapeutic drugs and hormones (Welch and Harrison 1966; Welch et al. 1971). The literature regarding enzyme induction by chlordane is voluminous. It is beyond the intended scope of this document to review all this literature. Instead the reader is referred to several review articles (Albrecht and Manchon 1974; Azamoff 1977; Campbell et al. 1983; Fabacher et al. 1980; Hodgson et al. 1980; Smith 1991). Health practitioners should be aware that doses of therapeutic drugs and hormones may require adjustment in patients exposed to chlordane, although data regardin, 0 enzyme induction in humans exposed to chlordane were not located.

Health practitioners should also be aware that exposure to other pesticides or chemicals that induce hepatic microsomal enzymes may increase the toxicity of chlordane, probably by enhancing the transformation of chlordane to more highly toxic metabolites. For example, previous exposure to aldrin, dieldrin, or DDT increased the acute toxicity of chlordane to rats by ≈2.3-4.6 times (Deichmann and Keplinger 1970). When given simultaneously to rats or mice, the acute lethal effects of chlordane in combination with most pesticides appeared to be roughly additive, except that aldrin or endrin and chlordane, and methoxychlor and chlordane were more than additive in mice (Keplinger and Deichmann 1967).

The acute toxicity of chlordane in rats increased when rats were fed protein deficient diets (Boyd and Taylor 1969). Chlordane treatment has also been demonstrated to enhance the hepatotoxic effects produced by carbon tetrachloride in rats, as indicated by its effect on SGPT levels, presumably by inducing the metabolism of carbon tetrachloride to its toxic metabolite (Mahon et al. 1978; Stenger et al. 1975). On the other hand, chlordane provided some protection against carbon tetrachloride-induced liver necrosis in rats, possibly by inducing a type of cytochrome P-450 with diminished ability to metabolize carbon tetrachloride to its toxic metabolite (Mahon et al. 1978). Pretreatment of rats with chlordane accelerated the metabolism of lindane, presumably by the same mechanism (Chadwick et al. 1977).

2.7 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to chlordane than will most persons exposed to the same level of chlordane in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the pre-existing compromised function of target organs (including effects or clearance rates and any resulting end-product metabolites). For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.6, “Populations With Potentially High Exposure.”

Humans with chronic liver disease or impaired liver function may be unusually susceptible to chlordane toxicity. Infante *et al.* (1978) speculated on the existence of an unusually susceptible population prone to the development of blood dyscrasia (i.e., aplastic anemia and leukemias) following exposure to chlordane. Such a population was thought to have some sort of idiosyncratic response to chlordane exposure, but identification of this population was not thought to be possible. Although data are not available, humans exposed to other chemicals that induce hepatic microsomal enzymes may be unusually susceptible to chlordane, because the induced enzymes may enhance the transformation of chlordane to more highly toxic metabolites. Studies with rats show that males generally respond more than females to xenobiotic-induced enzyme induction (Kinoshita et al. 1966), but information regarding sex differences in enzyme induction in humans was not located.

Evidence in mice indicates that the fetus may be particularly susceptible to compromised immunocompetence due to altered stem cell populations of key immunoactive cells (Bamett et al. 1990a, 1990b). Infants may be unusually susceptible to a chronic seizure disorder following exposure to chlordane, particularly if they have a hereditary predisposition, such as a positive familial history of febrile convulsions (Bernad 1989).

2.8 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to chlordane. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to chlordane. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice.

2.8.1 Reducing Peak Absorption Following Exposure

Methods for reducing absorption of chlordane include those general route-specific measures that would be considered for any poisoning (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990; Rumack and Lovejoy 1991). Generally, little can be done to reduce absorption following inhalation exposure. Recommendations include keeping the victim quiet and removing them to fresh air as quickly as possible. In the case of dermal/ocular exposure, medical reference texts recommend removal of contaminated clothing as quickly as possible and washing contaminated skin with soap and

water. The use of oils could facilitate dermal absorption and their use has not been recommended. If the material enters the eyes, thorough rinsing with sterile physiological saline has been suggested.

If chlordane has been ingested , gastric lavage is not usually considered beneficial unless performed very soon after ingestion of an extremely large dose (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990; Rumack and Lovejoy 1991). Emesis may or may not be induced, depending on the situation. Emesis may be contraindicated if the victim is comatose, convulsing, has lost the gag reflex, or if the chlordane was suspended in petroleum distillates, so that there is danger that emesis would result in aspiration. Emesis may be followed by giving activated charcoal and saline cathartics. Oilbased cathartics are considered to be contraindicated because they may hasten the absorption of the ingested chlordane. Cholestyramine or other agents that may bind to chlordane may be useful in limiting absorption.

2.8.2 Reducing Body Burden

Following absorption, chlordane rapidly leaves the blood for initial distribution to the liver and kidneys, followed by redistribution to adipose tissue (Ewing et al. 1985; Ohno et al. 1986). Once chlordane has been absorbed, little can be done to reduce the body burden (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990; Rumack and Lovejoy 1991). Diuresis is not likely to be effective because of the high lipophilicity of chlordane. Dialysis and hemoperfusion are not expected to be practical because of the rapidity with which chlordane leaves the blood and locates in peripheral compartments, suggesting that chlordane has a large apparent volume of distribution. However, continued dosing with charcoal and cholestyramine may be useful to prevent reabsorption following biliary excretion. The barbiturates, which have been used to control poison-induced convulsions, may hasten metabolism and elimination of chlordane (Smith 1991). The barbiturates may also induce epoxide hydrolase (Sipes and Gandolfi 1991), hastening the elimination of oxychlordane, a toxic metabolite of chlordane.

2.8.3 Interfering with the Mechanism of Action for Toxic Effects

The initial effects of acute toxicity of chlordane are gastrointestinal, which probably result from neurological involvement, and other definite neurological symptoms of central nervous system stimulation, such as tremor, paralysis, and convulsions (Ellenhom and Barceloux 1988). The rapid

onset of these effects suggests that they are due to the parent compound(s) rather than to metabolite(s). The mechanism(s) of action of the neurological effects is (are) not certain, but may involve changes in the levels of neurotransmitters (norepinephrine, acetylcholine) and GABA at synapses (Fishman and Gianutsos 1985; Grutsch and Khasawinah 1991; Hrdina et al. 1974; Inoue et al. 1989). The benzodiazepine sedatives (e.g., diazepam) have been used to control convulsions (Ellenhorn and Barceloux 1988; Rumack and Lovejoy 1991). Epinephrine is usually avoided, unless needed to reverse cardiopulmonary arrest, because of the possibility that chlordane (as an organochlorine compound) may sensitize the heart to epinephrine-induced arrhythmias (Haddad and Winchester 1990). After the mechanisms of toxicity are better understood, it may be possible to develop more specific methods to interfere with the toxic effects of chlordane (i.e., more specific ways of countering GABA inhibition) (Grutsch and Khasawinah 1991). There is no known antidote for chlordane toxicity (Ellenhorn and Barceloux 1988).

Intermediate and chronic duration exposure of humans to chlordane has been associated with blood dyscrasia (Ellenhorn and Barceloux 1988; Epstein and Ozonoff 1987; Infante et al. 1978), liver effects (EPA 1980a; Ogata and Izushi 1991) and neurological symptoms (EPA 1980a; Menconi et al. 1988). Although human data are lacking, animal data suggest that intermediate or chronic duration exposure may induce immunological effects, particularly if exposure is prenatal. It seems likely that the effects of intermediate or chronic duration exposure may result from metabolites of chlordane, rather than from chlordane itself, because the metabolites have been shown to be more toxic than the parent compound (Brimfield and Street 1981). The reductive formation of free radicals may be important in inducing toxicity. Increased dietary vitamins C or E, or increased dietary selenium may be protective by reducing the superoxide formation induced by highly reactive free radicals.

2.9 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of chlordane is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of chlordane.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

2.9.1 Existing Information on Health Effects of Chlordane

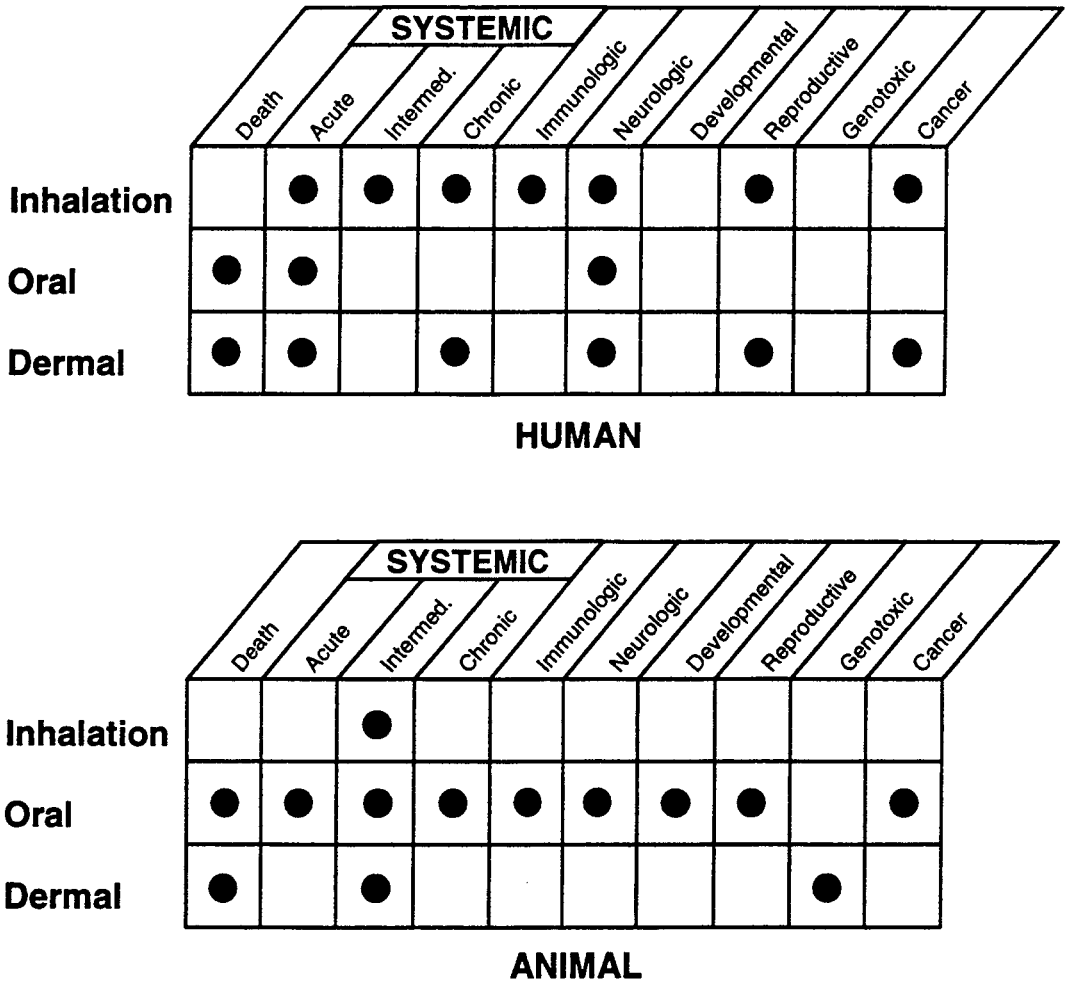
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to chlordane are summarized in Figure 2-4. The purpose of this figure is to illustrate the existing information concerning the health effects of chlordane. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not imply anything about the quality of the study or studies. Gaps in this figure should not be interpreted as "data needs." A data need, as defined in ATSDR's Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (ATSDR 1989a), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

As seen from Figure 2-4, data regarding systemic and neurological effects in humans are available for acute inhalation, oral, and dermal exposure. These data come from case reports of accidental or intentional exposure. Oral and dermal exposure may lead to death, but data were not located regarding death from inhalation exposure. Human data for chronic systemic effects and carcinogenicity come from epidemiological studies involving chlordane manufacture or application of chlordane as a pesticide, or studies of humans living in homes treated with chlordane as a termiticide. One case report of chronic dermal exposure was also located. Notably missing are data regarding developmental, reproductive, and genotoxic effects in humans.

Inhalation data in animals consist of a comprehensive study of intermediate duration in rats and monkeys. The oral toxicity of chlordane in animals has been studied extensively, and data are available for all important effects except genotoxicity. Dermal data in animals consist of acute lethality data, a go-day study in guinea pigs, and a genotoxicity study in mice.

Dermal data in animals are restricted to investigations of lethality and one 90-day study in guinea pigs.

FIGURE 2-4. Existing Information on Health Effects of Chlordane



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2.9.2 Identification of Data Needs

Acute-Duration Exposure. Acute inhalation, oral, and dermal exposure data in humans have identified neurological, gastrointestinal, hematological, and respiratory effects, and jaundice as the presenting symptoms in persons exposed to high doses (Balistreri et al. 1973; Curley and Garrettson 1969; Dadey and Kammer 1953; EPA 1980a; Harrington et al. 1978; Lensky and Evans 1952; Menconi et al. 1988; NIOSH 1984a; Olanoff et al. 1983). Oral exposure to high levels may be lethal (Aldrich and Holmes 1969; Curley and Garrettson 1969; EPA 1980a; Lensky and Evans 1952). The data suggested that the central nervous system and liver are the target organs for acute exposure in humans. Specific levels associated with effects in these organs in acutely exposed humans are not known.

Acute inhalation data in rats have identified a level associated with mortality (Khasawinah et al. 1989). Acute oral and dermal exposure data in animals have identified LD_{50} values and levels associated with mortality in rats, hamsters, and mice (Ben-Dyke et al. 1970; Deichmann and Keplinger 1970; Gaines 1960; Gak et al. 1976; Ingle 1965; Podowski et al. 1979; Truhaut et al. 1975; Usami et al. 1986). Acute oral data also have identified the liver and the central nervous system as target organs in rats (Den Tonkelaar and Van Esch 1974; Hrdina et al. 1974; Kacew and Singhal 1973; Ogata and Izushi 1991; Singhal and Kacew 1976; Truhaut et al. 1974, 1975). Generally, the animal data confirmed the target organs in humans, and, based on similar target organs and metabolic pathways (Tashiro and Matsumara 1978), the rat appears to be an appropriate model for toxicity in humans. The data were insufficient to derive an acute inhalation MRL for chlordane because neurological effects have not been sufficiently studied to aid in the identification of the most sensitive end point. An acute-duration oral MRL of 0.001 mg/kg/day was derived from a LOAEL of 1 mg/kg/day for developmental effects in the offspring of mice exposed to chlordane in the diet during the third timester of pregnancy (Al-Hachim and Al-Baker 1973). The pharmacokinetic data in animals, which indicate that absorption occurs following exposure by any route (Ambrose et al. 1953a; Ewing et al. 1985; Nye and Dorough 1976), and the human effects data indicate that the central nervous system and liver would be the target organs of dermal exposure. More animal studies by all three routes of exposure may be helpful to identify thresholds for the more subtle and less studied manifestations of hepatic and neurological toxicity. These data are important because humans may be exposed by all routes to low or moderate levels of chlordane near hazardous waste sites or in homes and gardens previously treated with chlordane.

Intermediate-Duration Exposure. Intermediate-duration oral or dermal human exposure data were not located.

Intermediate-duration inhalation exposure data in rats, mice, and monkeys have identified the lungs, hematological system, liver, central nervous system, thyroid, and possibly the thymus in female rats, as the target organs (Ingle 1953; Khasawinah et al. 1989; Velsicol Chemical Co. 1984). Intermediate-duration oral studies have identified levels in rats, mice, and rabbits associated with death, and identified the liver, central nervous system, and developing immune system as target organs (Bamett et al. 1985a, 1985b, 1990a, 1990b; Blaylock et al. 1990a; Casterline and Williams 1971; Cranmer et al. 1984; Drummond et al. 1983; Mahon et al. 1978; Matin et al. 1977; Menna et al. 1985; NCI 1977; Ortega et al. 1957; Spyker-Cranmer et al. 1982; Stohlman et al. 1950). Intermediate-duration dermal exposure induces convulsions and liver necrosis in mice and hyperkeratosis of the skin of guinea pigs(Datta et al. 1975; Frings and O'Tousa 1950). An intermediate-duration inhalation MRL of 0.0002 mg/m^3 was derived based on a NOAEL for hepatic effects in rats exposed to chlordane intermittently for 90 days (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). An intermediateduration oral MRL of 0.0006 mg/kg/day was derived based on a NOAEL of 0.055 mg/kg/day for hepatic effects in rats given chlordane in the diet for 30 months (Khasawinah and Grutch 1989a; Velsicol Chemical Co. 1983a). The 2- to 9-month rat dietary study conducted by Ortega et al. (1975) which identified a minimal LOAEL of 0.125 mg/kg/day for hepatic effects supports the Khasawinah and Grutsch (1989a; Velsicol Chemical Co. 1983a) study. The pharmacokinetic data in animals, which indicate that absorption occurs following exposure by any route (Ambrose et al. 1953a; Ewing et al. 1985; Nye and Dorough 1976), and effects data in animals indicate that the liver and central nervous system are the principal target organs by all routes of exposure. Additional animal studies by all three routes of exposure may be helpful for identifying thresholds for the more subtle and less studied manifestations of hepatic, immunological, and neurological toxicity. These data might be important because humans may be exposed by all routes to low or moderate levels of chlordane near hazardous waste sites or in homes and gardens previously treated with chlordane.

Chronic-Duration Exposure and Cancer. Chronic-duration inhalation data include two case report studies of blood dyscrasia, and several studies of humans living in chlordane-treated homes or exposed to chlordane during its manufacture or during its use as a pesticide (Brown 1992; Ditraglia et al. 1981; Epstein and Ozonoff 1987; Fishbein et al. 1964; Infante et al. 1978; Kawano and Tatsukawa 1982; Menconi et al. 1988; Ogata and Izushi 1991; Wang and MacMahon 1979a, 1979b). Dermatitis,

migraine headaches, unspecified skin neoplasms, and unspecified ovarian and uterine disease have been reported by these authors to be associated with chronic inhalation exposure to chlordane. A chronic-duration inhalation MRL of 0.00002 mg/m^3 was derived from the NOAEL for hepatic effects in rats exposed to chlordane intermittently for 90 days (Khasawinah et al. 1989; Velsicol Chemical Co. 1984). Elevated serum levels of hepatic enzymes associated with liver damage were found in pesticide applicators (Ogata and Izushi 1991). No studies of chronic oral exposure to chlordane were located for humans. Chronic-duration dermal data in humans are limited to a report of seizures in a nursery owner who handled soil containing chlordane.

Chronic-duration animal exposure data, located only for oral exposure, have identified levels in rats and mice associated with reduced survival, and identify the liver and central nervous system as target organs (Epstein 1976; IRDC 1973; Khasawinah and Grutsch 1989a, 1989b; NCI 1977; Velsicol Chemical Co. 1983a, 1983b). A chronic-duration oral MRL of 0.0006 mg/kg/day was derived based on the NOAEL for hepatic effects in rats given chlordane in the diet for 30 months (Khasawinah and Grutsch 1989a; Velsicol Chemical Co. 1983a). Chlordane induced liver tumors in mice, and chlordane has not been sufficiently studied for reproductive effects. The pharmacokinetic data in animals, which indicate that absorption occurs following exposure by any route (Ambrose et al. 1953a; Ewing et al. 1985; Nye and Dorough 1976), and the effects data in animals, particularly for acute- and intermediate-duration exposure, indicate that the liver and central nervous system would be principal target organs by all routes of chronic exposure. No chronic inhalation studies of chlordane in animals were located. Additional animal studies by all three routes of exposure may be helpful for identifying thresholds for the more subtle and less studied manifestations of hepatic, immunological, and neurological toxicity. These data are important because humans may be exposed by all routes to low or moderate levels of chlordane near hazardous waste sites or in homes and gardens previously treated with chlordane.

The epidemiology studies and case reports discussed above revealed no convincing evidence of carcinogenicity in humans, except for a weak association with leukemia and neuroblastoma (Epstein and Ozonoff 1987; Infante et al. 1978). One epidemiological study also investigated the risk of non-Hodgkins lymphoma in farmers who were dermally exposed to chlordane for prolonged periods (Cantor et al. 1992). Although odds ratio data were derived, the study was limited by the lack of exposure data. Oral studies in animals have confirmed that chlordane induces liver tumors in mice, but not rats, exposed to high levels (Becker and Sell 1979; Epstein 1976; IRDC 1973; Khasawinah and

Grutsch 1989a, 1989b; NCI 1977; Velsicol Chemical Co. 1983a, 1983b; Williams and Numoto 1984). Chronic-duration inhalation and dermal studies were not located, but it seems likely that the carcinogenicity of chlordane in mice is not route-dependent, because the pharmacokinetic data in animals indicate that absorption occurs following any route of exposure, and because the liver is a target organ for non-cancer effects regardless of route of exposure. Most genotoxicity tests with chlordane yielded negative results (Arnold et al. 1977; Ashley and Tenant 1988; Blevins and Sholes 1978; Brandt et al. 1972; Maslansky and Williams 1981) (see Section 2.4), suggesting an epigenetic mechanism of carcinogenicity. In support of this theory, chlordane inhibited gap junction intercellular communication (Ruth et al. 1990; Tong and Williams 1988). These results suggest that chlordane acts as a tumor promoter, depressing intercellular communication that checks uncontrolled proliferation of transformed or neoplastic cells. Further mechanistic studies may provide useful information regarding the potential carcinogenicity to humans chronically exposed to low levels. These studies are important because humans may be chronically exposed by living in previously treated homes or near hazardous waste sites.

Genotoxicity. Studies of genotoxic effects in humans are limited to an *in vitro* study of chlordaneinduced sister chromatid exchange in lymphoid cells and a positive response was obtained (Sobti et al. 1983). *In vivo* mouse studies provided mixed results; chlordane did not induce dominant lethal mutations in mice (Arnold et al. 1977; Epstein et al. 1972), but did induce micronucleus formation in the bone marrow cells and nuclei aberrations in the hair follicles (Schop et al. 1990). The most prevalent metabolite of the chlordane isomers, oxychlordane, although an epoxide, appears to be relatively inert (Khasawinah 1989; Sasaki et al. 1992), and probably does not bind strongly to tissue macromolecules. Free radicals formed as a result of reductive dehalogenation, however, may bind to DNA and other macromolecules (Brimfield and Street 1981; Kawano et al. 1989), inducing genetic defects or interfering with DNA repair. Additional *in vivo* mutation and chromosomal aberration tests in animals may clarify the ability of chlordane to induce genotoxicity in humans.

Reproductive Toxicity. Data from one human case report involving a woman who was exposed to a lethal dermal dose of chlordane (Derbes et al. 1955) and a cross-sectional epidemiological investigation involving women exposed to chlordane vapors (Menconi et al. 1988) did not provide conclusive evidence that the reproductive system is a potential target organ in humans exposed to chlordane. Inhalation and oral acute-, intermediate-, or chronic-duration exposure studies in animals, in which the reproductive organs were examined histopathologically, did not identify lesions in the

reproductive organs (Khasawinah and Grutsch 1989a, 1989b; Khasawinah et al. 1989; NCI 1977; Truhaut et al. 1975; Velsicol Chemical Co. 1983a, 1983b, 1984). However, treatment of male mice by gavage with chlordane for 30 days resulted in reduced size of seminiferous tubules and degeneration of spermatogenic epithelium (Balash et al. 1987). No studies were located regarding reproductive effects in animals after dermal exposure to chlordane. Of more concern is the observation that oral exposure to a large dose decreased fertility in rats and resulted in the death of all offspring before weaning (Ambrose et al. 1953a). The death of the offspring may have resulted from chlordane residues in milk transferred from the dam. The pharmacokinetic data in animals indicated that absorption occurs following any route of exposure, and that chlordane residues tend to accumulate in body fat; therefore, impaired reproductive performance in humans may occur following any route of exposure. Because of the tendency for chlordane residues to accumulate in body fat, multi-generation reproduction studies in animals by the inhalation and oral routes are recommended. Epidemiological investigations of reproductive effects in humans living in homes previously treated with chlordane, or those exposed during its manufacture or use as a pesticide, would also be useful, if an adequate cohort can be identified.

Developmental Toxicity. Data were not located regarding the developmental effects of chlordane in humans. Effects data from animals, available only for oral exposure, suggest that subtle behavioral and immunological effects occur in developing mice (Al-Hachim and Al-Baker 1973; Bamett et al. 1985a, 1985b, 1990a, 1990b; Chemoff and Kavlock 1982; Cranmer et al. 1984; Menna et al. 1985; Spyker-Cranmer et al. 1982; Theus et al. 1992; Usami et al. 1986). Pharmacokinetic data in animals indicate that absorption occurs following any route of exposure; therefore, developmental effects may occur following any route of exposure. Additional developmental studies in other animal species may clarify the developmental effects that could be anticipated in humans via inhalation, oral, and dermal routes of exposure. Particularly useful would be studies designed to locate thresholds for subtle immunological and neurological effects following both pre- and postnatal exposure. Epidemiological investigation of developmental effects in humans living in homes treated with chlordane, or those exposed during its manufacture or use as a pesticide, would also be useful.

Immunotoxicity. Data from one human study suggest that chlordane may cause autoimmunity as well as impaired proliferative responses to plant mitogens following inhalation exposure to chlordane (McConnachie and Zahalsky 1992). *In vitro* studies with rhesus monkey peripheral blood mononuclear cells suggest that chlordane may impair cell-mediated immunity in rhesus monkeys

(Chuang et al. 1992). Mice exposed to chlordane developed leukocytosis and decreased thymus weight (Johnson et al. 1986; Khasawinah et al. 1989). Decreased myeloid cell colony forming capacity and depressed delayed type hypersensitivity occurred in the offspring of mice exposed orally to chlordane during gestation (Bamett et al. 1985a, 1985b, 1990a, 1990b; Menna et al. 1985; Spyker-Cranmer et al. 1982). Because chlordane is absorbed following any route of exposure, immunological effects may be expected in humans exposed by any route. Further testing of immune function in mice and other animals may provide useful information regarding the immunotoxicity in humans. In addition, epidemiology studies, perhaps comparing persons with high and low levels of chlordane residues in the blood, fat or breast milk, may provide useful information. Parameters evaluated may include the frequency of allergic and autoimmune disorders, susceptibility to opportunistic infections (e.g., colds or flu), and alterations in absolute and differential leukocyte counts.

Neurotoxicity. Neurotoxicity is a consistent and predictable finding in humans (Aldrich and Holmes 1969; Balistreri et al. 1973; Barnes 1967; Curley and Garrettson 1969; Dadey and Kammer 1953; EPA 1980a, 1986d; Harrington et al. 1978; Lensky and Evans 1952; Menconi et al. 1988; NIOSH 1984a; Olanoff et al. 1983) and animals (Drummond et al. 1983; Frings and O'Tousa 1950; Hrdina et al. 1974; Ingle 1952; Khasawinah et al. 1989; NCI 1977; Stohlman et al. 1950) exposed to chlordane by any route. In the human studies, clinical signs and symptoms included migraines, convulsion, and seizures following inhalation, oral, or dermal routes of exposure. In the animal studies, convulsions and seizures were consistent findings after inhalation, oral, and dermal routes of exposure to chlordane (Ambrose et al. 1953a; Hrdina et al. 1974; Ingle 1953; Khasawinah et al. 1989; NCI 1977; Velsicol Chemical Co. 1984). Further testing should be designed to investigate subtle effects on neurobehavior and central nervous system function. Epidemiological investigation of subtle neurological and behavioral effects in humans living in homes treated with chlordane, or those exposed during its manufacture or use as a pesticide, would also be useful.

Epidemiological and Human Dosimetry Studies. Several epidemiological studies have investigated the cancer and noncancer effects of chlordane in humans exposed in their homes or occupationally in the manufacture of chlordane or in its application as a pesticide (Alvarez and Hyman 1953; Brown 1992; Cantor et al. 1992; Ditraglia et al. 1981; Kawano and Tatsukawa 1982; MacMahon et al. 1988; Menconi et al. 1988; Ogata and Izushi 1991; Wang and MacMahon 1979a, 1979b; Woods and Polissar 1989). Limitations of these studies include unquantified exposure levels of chlordane, exposure to a mixture of chemicals, and failure to investigate subtle neurological, behavioral, and

hepatic effects in the exposed persons. Additional multi-end point epidemiological studies should be designed to study these subtle effects, as well as hematological effects such as blood dyscrasia and leukemia, in the exposed populations mentioned above. Further, case-control designed epidemiological studies would help to establish a cause/effect relationship among a better defined population.

Biomarkers of Exposure and Effect

Exposure. Biomarkers of exposure include various components of commercial chlordane (principally *cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor) and its metabolites (principally oxychlordane). These substances are specific for exposure to chlordane. Detection of heptachlor and its metabolite, heptachlor epoxide, could reflect exposure to chlordane, because heptachlor is a component of commercial chlordane, or exposure to heptachlor, which is an insecticide in its own right. Data were not located that permit differentiation of the route, magnitude, or duration of exposure based on biomarkers of exposure.

Detectable levels in urine would probably reflect recent or ongoing exposure, because urinary excretion is not prominent for chlordane (Aldrich and Holmes 1969; Bamett and Dorough 1974; Ewing et al. 1985; Ohno et al. 1986; Tashiro and Matsumura 1977). Higher levels of chlordane residues occur in the feces of acutely poisoned humans (Aldrich and Holmes 1969). Generally, levels of chlordane residues in blood are below those in liver and fat (Mussalo-Rauhamaa 1991); blood (serum) levels may be reasonable indicators of recent or ongoing exposure (Ogam and Izushi 1991).

Recent studies evaluated the use of levels of chlordane residues in skin surface lipids as a biomarker of exposure, to avoid the invasive techniques necessary to obtain blood or body fat (Sasaki et al. 1991b; Wariishi and Nishiyama 1989). In monkeys, levels of *trans*-chlordane and oxychlordane in surface skin lipids correlated fairly well with levels in subcutaneous fat. Further refinement of this technique could increase the utility of chlordane residues in skin surface lipids as biomarkers of exposure.

Effect. Known biomarkers of effect are limited to slight alterations in serum chemistry. Elevated serum triglycerides, CPK and LDH, evidence of subtle liver effects, were observed in pesticide workers (Ogata and Izushi 1991). The elevated CPK was considered somewhat specific for chlordane exposure. Carefully performed epidemiological studies might provide data that clarify the relation

between exposure to chlordane and optic neuritis or other disease states. Such studies may also identify alterations in blood chemistry indices or other clinicopathological end points that are useful for identifying the presence or pathogenesis of disease states associated with chlordane exposure.

Absorption, Distribution, Metabolism, and Excretion. There are no quantitative absorption data regarding human exposure by any route. However, that chlordane is absorbed by humans is indicated from measurement of blood and tissue levels of chlordane in persons exposed via inhalation from homes treated for termite control (Kawano and Tatsukawa 1982; Saito et al. 1986; Taguchi and Yakushiji 1988; Takamiya 1987), in case reports of accidental ingestion (Aldrich and Holmes 1969; Curley and Garrettson 1969; Kutz et al. 1983; Olanoff et al. 1983), and from systemic toxicity after dermal exposure (Barnes 1967). One human study also attempted to correlate skin chlordane levels with blood chlordane levels in 248 male and 227 female outpatients (Hirai and Tomokuni 1993). Although chlordane was potentially absorbed by the dermal route, the data from this study did not demonstrate any strong correlations between skin chlordane levels and blood chlordane levels. The rat data for inhalation absorption are limited to an intratracheal study, which is inadequate for estimating absorption via the respiratory tract (Nye and Dorough 1976). Oral data in rats and mice indicate that gastrointestinal absorption occurs readily (Ewing et al. 1985; Ohno et al. 1986). Quantitative dermal absorption data are lacking, but absorption is indicated by lethality in rats and rabbits exposed dermally (Gaines 1960; Ingle 1965). Quantitative inhalation and dermal absorption data would be useful, because these routes are toxicologically significant to humans.

Distribution data available for humans are limited to levels of chlordane metabolites in several tissues after acute poisoning, and in blood, liver, fat, and breast milk after chronic-duration exposure following the oral route (Aldrich and Holmes 1969; Curley and Garrettson 1969; Dearth and Hites 1991a; Hirai and Tomokuni 1991a, 1991b; Kutz 1983; Kutz et al. 1976, 1983; Mussalo-Rauhamaa 1991; Ogata and Izushi 1991; Olanoff et al. 1983; Sasaki et al. 1991a; Wariishi and Nishiyama 1989). Rat studies regarding inhalation, oral, and parenteral exposure demonstrate that initial distribution is to the liver and kidney, followed by redistribution to body fat (Ambrose et al. 1953b; Balba and Saha 1978; Bamett and Dorough 1974; Dearth and Hites 1991b; Ewing et al. 1985; Khasawinah 1989; Nye and Dorough 1976; Ohno et al. 1986; Poonawalla and Korte 1971; Sasaki et al. 1992; Street and Blau 1972; Takeda et al. 1984). One oral study in mice demonstrated that chlordane is initially distributed to the muscles and that higher levels of the *cis* isomer accumulate in tissues than the *trans* isomer (Satoh and Kikawa 1992). Additional dermal exposure studies would be useful for elucidating patterns

of distribution by this route. Of particular interest would be studies of distribution to the central nervous system, since neurological effects are a consistent part of the clinical picture in humans exposed by any route. The ability of chlordane to cross the placenta and its presence in milk should also be investigated, because data show that prenatally exposed mice are more sensitive than adults to the immunological effects of chlordane (Barnett et al. 1985a, 1985b; Menna et al. 1985; Spyker-Cranmer et al. 1982). Determination of apparent volume of distribution and the extent of binding to tissue proteins may provide data that would be useful in the management of clinical cases of poisoning.

Human metabolism data are limited to *in vitro* studies (Kutz et al. 1976, 1979; Tashiro and Matsumura 1978). *In vivo* and *in vitro* animal studies, however, are sufficient to propose probable metabolic pathways (Balba and Saha 1978; Bamett and Dorough 1974; Brimfield et al. 1978; Nomeir and Hajjar 1987; Poonawalla and Korte 1964; Sasaki et al. 1992; Tashiro and Matsumura 1978). Further studies could be designed to estimate metabolic rate constants and to determine the levels at which saturation of specific pathways occurs with different routes of exposure.

Data from environmentally exposed humans suggest that substantial excretion occurs via lactation (Barnett et al. 1979; Strassman and Kutz 1977; Taguchi and Yakushiji 1988; WHO 1984). Data regarding acute poisoning in humans after acute oral exposure to chlordane indicate that most chlordane-derived material is excreted in the feces (Aldrich and Holmes 1969; Curley and Garrettson 1969; Olanoff et al. 1983). Studies involving intratracheal dosing of rats and acute, oral dosing of rats, mice, and rabbits confirm that fecal, probably biliary, excretion is more important than renal excretion (Barnett and Dorough 1974; Ewing et al. 1985; Tashiro and Matsumura 1977; Nye and Dorough 1976; Ohno et al. 1986). Additional animal studies might elucidate the relative importance of various routes of excretion following inhalation and dermal exposure, and might provide useful information regarding the rate and extent of excretion via lactation.

Comparative Toxicokinetics. Data in rats, mice, and rabbits following oral exposure to chlordane indicate that there are some species differences in absorption, distribution, and excretion (Balba and Saha 1978; Barnett and Dorough 1974; Ewing et al. 1985; Ohno et al. 1986; Poonawalla and Korte 1971; Satoh and Kikawa 1992). The available data on metabolites in human tissues and *in vitro* studies both indicate qualitatively that metabolism of chlordane in rats and humans is similar (Tashiro and Matsumura 1978), and that rats and mice are satisfactory animal models for the toxicity of

chlordane. Data regarding urinary metabolites of chlordane in humans were not available. Analysis of the urine of humans with known exposure to chlordane (e.g., those living in previously treated houses) could provide a means of further studying the differences and similarities between animal species and humans and of monitoring humans for exposure.

Methods for Reducing Toxic Effects. Although it is suspected that absorption occurs by passive diffusion, this has not been established, and data on the mechanism of chlordane absorption is not known. Distribution appears to reflect the lipophilicity of chlordane, but additional studies could determine the extent of binding to serum proteins and the apparent volume of distribution. This information may be useful in the clinical management of cases of poisoning, although such cases would be rare because the use of chlordane in the United States has been canceled. Methods for reducing the toxic effects of chlordane are limited to general methods for reducing absorption and body burden in cases of acute poisoning, and to the use of sedatives to control convulsions. There are no methods to reduce body burden from long-term exposure. Additional information on the mechanisms of toxicity, specifically on methods to counter chlordane-induced reduction of GABA activity, may be helpful in managing clinical cases of poisoning. Further studies in animals may be helpful for determining the usefulness of cholestyramine or other binding agents to sequester chlordane in the gut, thereby reducing body burden, and to locate other agents that would control convulsions without danger of respiratory depression.

2.9.3 On-going Studies

Several on-going investigations of the toxicity of chlordane were located. Kocoshis, sponsored by the National Institutes of Health, is studying liver and psychomotor function in children exposed to chlordane (CRISP 1992); and Zahm et al. (1988) is performing a case-control study of the risk of non-Hodgkin's lymphoma among farmers exposed to herbicides and insecticides. An odds ratio of 2.1 was estimated for farmers exposed to chlordane. Adeshina (1989) developed a method for estimating daily dose in humans based on levels of oxychlordane in adipose tissue. Omiecinski of the University of Washington, sponsored by NIEHS, is testing the hypothesis that animal and human exposure to a various class of chemicals can be monitored through composite measure of expression patterns for key P-450 gene products in peripheral cells (FEDRIP 1993). P-450 gene expression patterns in cultured cells will be evaluated subsequent to exposure to chlordane.

Spyker et al. (1989) observed an increase in neurological effects, associated with increased body burden of chlordane, in persons living in homes treated with chlordane. These patients also had a high incidence of abnormal electromyogram, electronystagmogram, and findings on neuropsychiatric testing. With further information and validation, abnormal electromyogram and electronystagmogram may become reliable biomarkers of effects in humans. Sponsored by the National Institutes of Health, Spyker et al. (1989) is studying the decontamination of humans poisoned by chlordane (CRISP 1992).

Neurotoxicity is a consistent effect in humans intoxicated with chlordane, and several on-going studies in animals are designed to clarify the effects of exposure and elucidate the mechanisms of these effects. Woolley, sponsored by the U.S. Department of Agriculture, is studying the neurochemical and neurobehavioral effects of acute and chronic exposure in rats (FEDRIP 1992). Woolley has further proposed to determine if adrenal steroids and cholecystokinin antagonists can ameliorate or exacerbate toxicity induced by heavy metals and insecticides in rats or mice (CRISUSDA 1993; FEDRIP 1993). Abstracts of studies yet to be published indicate that myoclonic seizures in rats induced with chlordane appeared to be related to increased limbic excitability (Dai et al. 1989; Hasan et al. 1989). That chlordane inhibits nicotine-stimulated neuronal release of norepinephrine, probably by interfering with opening of voltage-dependent calcium channels is suggested in an abstract of a study yet to be published (Inoue et al. 1989). In studies sponsored by the National Institute of Environmental Health Sciences (NIEHS), Matsumura is investigating the effects of chlorinated pesticides on sodium and calcium regulation in the nervous system (CRISP 1992). Uphouse is studying the neuroreproductive effects in rats of chlorinated pesticides (CRISP 1992). These studies are sponsored by the National Institute of General Health Sciences. In an abstract of a yet to be published report, the effects of preand postnatal chlordane exposure on neurobehavioral indices in the rat are being investigated (Cassidy et al. 1992). In this study, time pregnant dams were exposed daily from day 4 of pregnancy and through lactation. The resulting offspring received three levels of technical chlordane from day 22 of age through day 80. At low dose exposure, heptachlor epoxide plasma levels in the dam and in the offspring (day 80) was representative of those found in the United States populace at the 99th percentile (3 μg/L). The rats were tested in the Cincinnati maze between days 74 and 78 of age for alteration of behavior on days 76 and 77. Changes in open field activity, auditory startle, and shuttle box escape were evaluated on day 81 of age.

Researchers at the University of Arkansas (Barnett et al. 1990a, 1990b; Blaylock et al. 1990a; Menna et al. 1985) reported that, in mice, the fetal immune system appears to be markedly more susceptible

to chlordane than the immune system of the adult. Additional studies planned by Barnett, sponsored by NIEHS, will investigate further the effect of prenatal treatment of mice on immune function (CRISP 1992; FEDRIP 1993).

Chlordane is clearly carcinogenic in mice, inducing increased incidence of hepatocellular carcinomas (NCI 1977). Chlordane probably acts as a tumor promoter, rather than a genotoxic agent (Tong and Williams 1988). Smart is conducting further studies on the mechanism of tumor promotion, sponsored by NIEHS (CRISP 1992) and the Department of Agriculture (FEDRIP 1992). Reynolds, in studies sponsored by NIEHS, is investigating the role of oncogene activation in liver tumor induction in mice (CRISP 1992). Goodman is planning similar studies, also sponsored by NIEHS (CRISP 1992; FEDRIP 1993). Goodman and Robens of the University of Michigan, sponsored by the U.S. Department of Agriculture, further propose to determine whether alterations in the methylation status of proto-oncogenes is the mechanism for the promotion stage of carcinogenesis (CRISUSDA 1993; FEDRIP 1993).

The use of the hepatocyte DNA repair assay in combination with the replicative DNA synthesis (RDS) assay in the detection of mouse carcinogens has been reported in an abstract of a study yet to be published (Millner et al. 1988). Mice treated orally with chlordane had increased RDS, but no increase in DNA repair. The investigators suggest that the combination test may be useful in elucidating the mechanism of hepatocarcinogenesis.