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Carcinogenicity Assessment of Chlordane and Heptachlor/ Heptachlor Epoxide

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CARCINOGENICITY ASSESSMENT OF CHLORDANE AND HEPTACHLOR/HEPTACHLOR EPOXIDE

Carcinogen Assessment Group
Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

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PREFACE

The Carcinogen Assessment Group within the Office of Health and Environmental Assessment has prepared this carcinogenicity assessment at the request of the Hazard Evaluation Division within the Office of Pesticides and Toxic Substances. The assessment contains a qualitative and quantitative evaluation of the carcinogenicity data available as of November 1985.

ABSTRACT

Evidence pertaining to the carcinogenicity of chlordane and heptachlor/heptachlor epoxide is reviewed and evaluated. This report covers studies completed before 1985. Case reports and epidemiologic studies of pesticide applicators and pesticide manufacturing workers are reviewed, but because of methodologic limitations, these studies establish neither a positive nor a negative association between cancer and chlordane or heptachlor/heptachlor epoxide exposure. A number of independent studies of laboratory animals, however, demonstrates that chlordane and heptachlor/heptachlor epoxide cause liver cancer in mice and rats. Based on the accumulated evidence, chlordane and heptachlor/heptachlor epoxide are classified as probable human carcinogens, Group B2 using EPA's Guidelines for Carcinogen Risk Assessment. The carcinogenic potency of chlordane and heptachlor/heptachlor epoxide is estimated by fitting mathematical models to the laboratory animal data. These estimates indicate that chlordane and heptachlor/heptachlor epoxide are rather potent carcinogens, ranking in the second quartile of potential carcinogens evaluated by EPA's Carcinogen Assessment Group. A separate mutagenicity assessment of chlordane and heptachlor/heptachlor epoxide is attached as an appendix to this report. The report also includes an extensive list of references pertinent to the carcinogenicity of chlordane and heptachlor/heptachlor epoxide.

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The Carcinogen Assessment Group (CAG) within EPA's Office of Health and Environmental Assessment is responsible for the preparation of this carcinogenicity assessment. The document was written by Dynamac Corporation, Rockville, Maryland, under EPA contract number 68-02-4131, and was reviewed by the staff of the CAG. The CAG provided overall direction and coordination for document production (Dharm V. Singh, Project Officer).

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1. CONCLUSIONS

Based on the accumulated evidence, chlordane is a probable human carcinogen, classified in Group B2 under the EPA's Guidelines for Carcinogen Risk Assessment. Animal studies provide sufficient evidence for carcinogenicity: chlordane increased the incidence of liver carcinomas in C57Bl/6N, CD-1, and B6C3F1 mice; liver adenomas and hemangiomas in ICR mice; and liver adenomas in Fischer 344 rats. Epidemiologic studies provide inadequate evidence due to methodology and data limitations.

According to the criteria in the guidelines, the above evidence puts chlordane in Group B2. However, the guidelines allow for the possibility of downgrading the classification from Group B2 to Group C when the only tumor response is that of mouse liver tumors in strains with high background rates, or when warranted by a number of other factors. In the case of chlordane these conditions do not apply, since chlordane caused tumors in C57Bl/6N mice--which do not have a high background rate--and caused tumors in rats as well. Other pertinent evidence includes highly significant tumor responses, up to 77 percent increased incidence over controls, increased incidence in both males and females, increased incidence at medium and high doses, a dose-related increase in the proportion of malignant tumors, and induction of tumors by structurally related chemicals. In light of these factors, downgrading is clearly not warranted, and chlordane remains in Group B2.

For chlordane, the carcinogenic potency, averaging estimates from the most sensitive species tested, is 1.3 per mg/kg/day. The potency using the most sensitive sex and strain is 4.7 per mg/kg/day. These are plausible upper bounds for the increased cancer risk from chlordane, meaning that the true risk is not likely to exceed these estimates and may be lower. The molecular potency

index for chlordane is 5.2×10^2 per mmol/kg/day. This places chlordane in the second (upper middle) quartile of suspect carcinogens ranked by the Carcinogen Assessment Group (CAG).

Heptachlor/heptachlor epoxide is a probable human carcinogen, classified in Group B2 under the EPA's Guidelines for Carcinogen Risk Assessment. Animal studies provide sufficient evidence for carcinogenicity: heptachlor/heptachlor epoxide increased the incidence of liver carcinomas in C3H, CD-1, and B6C3F1 mice and in CFN rats. Epidemiologic studies provide inadequate evidence due to methodology and data limitations.

The guidelines consider this evidence sufficient for Group B2, but they allow downgrading from Group B2 to Group C when the only tumor response is that of mouse liver tumors in strains with high background rates, or when warranted by a number of other factors. The evidence, however, shows highly significant tumor responses, increased incidence in both males and females, increased incidence at medium and high doses, and induction of tumors by structurally related chemicals. In light of these factors, downgrading is clearly not warranted, and heptachlor/heptachlor epoxide remains in Group B2.

For heptachlor, the carcinogenic potency, averaging estimates from the most sensitive species tested, is 4.5 per mg/kg/day. The potency using the most sensitive sex and strain is 14.9 per mg/kg/day. These are plausible upper bounds for the increased cancer risk from heptachlor, meaning that the true risk is not likely to exceed these estimates and may be lower. The molecular potency index for heptachlor is 1.7×10^3 per mmol/kg/day. This places heptachlor in the second quartile of suspect carcinogens ranked by the CAG.

For heptachlor epoxide, the carcinogenic potency, averaging estimates from the most sensitive species tested, is 9.1 per mg/kg/day. The potency using the most sensitive sex and strain is 36.2 per mg/kg/day. These are plausible upper

bounds for the increased cancer risk from heptachlor epoxide, meaning that the true risk is not likely to exceed these estimates and may be lower. The molecular potency index for heptachlor epoxide is 3.5×10^3 per mmol/kg/day. This places heptachlor epoxide in the most potent quartile of suspect carcinogens ranked by the CAG.

2. INTRODUCTION

In preparing this assessment, the CAG has considered the Drinking Water Criteria Document for Heptachlor, Heptachlor Epoxide, and Chlordane recently drafted by the Environmental Criteria and Assessment Office (U.S. EPA, 1985a). Much of the prior material presented in the 1977 Risk Assessment of Chlordane and Heptachlor, prepared by the CAG (U.S. EPA, 1977) has also been incorporated into this current assessment. As guidance in preparing this assessment, the EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986) were followed.

3. GENERAL BACKGROUND INFORMATION

Chlordane and heptachlor are structurally related chlorinated hydrocarbon insecticides that have been and/or are being used to control termites and pests on field crops. They are synthesized by the condensation of two cyclopentadiene rings (hexachlorocyclopentadiene and cyclopentadiene) to form chlordane. In the second step (chlorination), the reaction conditions can be adjusted to give either technical chlordane or technical heptachlor.

Technical grade chlordane is a light-yellow-colored liquid consisting of approximately equal parts cis- and trans-isomers, along with chlordene isomers, heptachlor, and their impurities. Technical grade heptachlor is a white crystalline solid containing approximately 73 percent heptachlor and 22 percent transchlordane. Heptachlor epoxide is a degradation product of heptachlor and has an estimated vapor pressure of 3×10^{-4} mmHg at 25°C.

3.1. CHEMICAL AND PHYSICAL PROPERTIES

Those properties most relevant to carcinogenesis and human exposure are listed in Table 1.

3.1.1. Identification

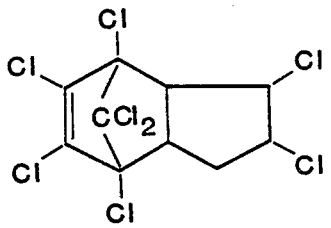
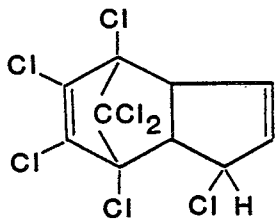
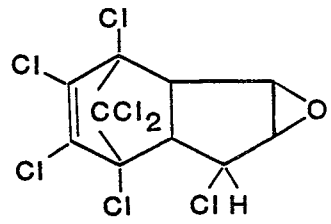
	Chlordane	Heptachlor	Heptachlor Epoxide
			
Molecular Formula:	$C_{10}H_6Cl_8$	$C_{10}H_5Cl_7$	$C_{10}H_5Cl_7O$
Molecular Weight:	409.76	373.32	389.32
CAS No.:	57-74-9	76-44-8	1024-57-3

TABLE 1. CHEMICAL AND PHYSICAL PROPERTIES OF CHLORDANE, HEPTACHLOR, AND HEPTACHLOR EPOXIDE

	Chlordane	Heptachlor	Heptachlor epoxide
Boiling point	175°C at 2 mm	135-145°C at 1-1.5 mmHg	160-161.5°C (99.5% pure)
Melting point	106-107°C (cis-isomer) 104-105°C (trans-isomer)	93°C	--
Specific gravity	1.59-1.63 at 25°C	1.57-1.59 (unknown purity)	--[a]
Vapor pressure	1×10^{-5} mmHg at 25°C	3×10^{-4} mmHg at 25°C	3×10^{-4} mmHg at 25°C
Solubility	9 µg/L at 25°C in water for technical grade and 56 µg/L in water for cis:trans (75:25) chlordane; miscible with aliphatic and aromatic hydrocarbon solvents	0.056 mg/L in water at 25°C; soluble in many organic solvents	0.35 mg/L in water at 25°C
Evaporation half-life ($t_{1/2}$) from water	28 hr (trans-isomer) from 1 m depth; 33 hr (cis-isomer) from 1 m depth	--	--
Log octanol/water partition coefficient	3.32	3.87-5.44	2.65-5.40
Conversion factor	1 mg/L = 59.7 ppm and 1 ppm = 16.76 mg/m^3 at 25°C and 760 mmHg	1 mg/L = 65.1 ppm and 1 ppm = 15.35 mg/m^3 at 25°C and 760 mmHg	1 mg/L = 62.4 ppm and 1 ppm = 15.9 mg/m^3 at 25°C and 760 mmHg

[a]Data not provided.

SOURCE: Adapted from U.S. EPA, 1985a.

3.1.2. Synonyms

Each chemical has a number of synonyms and trade names (U.S. EPA, 1985a).

1) Chlordane

1,2,4,5,6,7,8,8-octachlor2,3,3a,4,7,7a-hexahydro-4,7-methano -1H-indene

1,2,4,5,6,7,8,8-octachloro-4,7-methano-3a,4,7,7a-tetra-hydroindane

1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanindan

Dichlorodene	Chlor Kil
Octachlor	Corodane
Topiclor 20	Ortho-Klor
Velsicol 1068	Synklor
Dowchlor	Belt
Kypchlor	Niran

2) Heptachlor

1,4,5,6,7,8,8-heptachlor-3a,4,7,7a-tetrahydro-4,7-methanoindene

3-chloro-chlordene

3,4,5,6,7,8,8a-heptachlorodicyclopentadiene

Heptagran	Velsicol 104
Rhodiachlor	E3314
Drinox	Heptamul

3) Heptachlor epoxide

2,3,4,5,6,7,7-heptachloro-1a,1b,5,5a,6,6a-hexahydro-2,5-methano-2H-indene(1,2-b)oxirene

1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindan

Epoxyheptachlor

Velsicol 53-CS-17

ENT 25

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3.2. USES

Chlordane and heptachlor, both contact insecticides, have generally had the same uses in the past, primarily in a wide variety of soil applications (U.S. EPA, 1977; U.S. EPA, 1985a, b). The main uses for chlordane have been in the control of cutworms, ants, root weevils, rose beetles, grasshoppers, grubs, and termites. It had been used mainly in agriculture (25 percent), home use such as turf treatment, garden, and household use (25 percent), and soil treatment around buildings (50 percent). Technical grade chlordane is a variable mixture of chemicals that typically consists of 24 percent trans-chlordane, 21.5 percent chlordene isomers, 19 percent cis-chlordane, 10 percent heptachlor, 7 percent nonachlor, and 18.5 percent other miscellaneous impurities.

Heptachlor has some fumigant action and has been used primarily to control termites and insects that attack field crops. Heptachlor has been used in agriculture (about 70 percent) and in termite control (about 28 percent). Technical heptachlor consists of approximately 73 percent heptachlor, 22 percent trans-chlordane, and 5 percent nonachlor. Heptachlor epoxide is not commercially available in the United States, but it is a degradation product of heptachlor (U.S. EPA, 1985a, b).

3.3. ROUTES AND PATTERNS OF EXPOSURE

Both chlordane and heptachlor are chemically stable and persist in the soil, although heptachlor is slightly less persistent than chlordane in soils. Heptachlor is oxidized in soil to heptachlor epoxide, which then is more persistent than chlordane (U.S. EPA, 1977). Residues of chlordane have been detected for up to 10 years after a single application.

Because of their long history of use in a wide variety of applications, residues of chlordane, heptachlor, their oxidation products (oxychlordane and heptachlor epoxide), and impurities (mainly nonachlor) are found in foods,

soils, sediments, wildlife, and in air near application sites.

Heptachlor in soils, water, and plants is metabolized to heptachlor epoxide and to 1-hydroxychlordene epoxide; chlordane and nonachlor are apparently not metabolized in these systems. In animals, heptachlor is metabolized to heptachlor epoxide, which is well retained in lipid tissue. Chlordane in animals is metabolized to oxychlordane, which is also stored with no further metabolism. Nonachlor is apparently not metabolized and is accumulated by animals.

Food residues have been measured in the FDA Total Diet Program as well as in the USDA and FDA Food Surveillance Program. The latter programs are designed to sample foods shipped in interstate commerce. In the total diet study, chlordane and oxychlordane were found only at the lower limit of detection (0.003 ppm in the total diet), if at all, but in the surveillance survey residues with a somewhat higher detection limit were found in root vegetables, meat, and fish in 7 to 17 percent of the samples. Heptachlor was rarely detected, and heptachlor epoxide was found sporadically in dairy products, meat, fish, and poultry at the lower detection limit of 0.003 ppm in the total diet survey. However, in the Food Surveillance Program, heptachlor epoxide residues greater than 0.03 ppm were found in 19 percent of red meat samples, 17 percent of poultry, and 14 percent of dairy products. Based on these data, it is estimated that human consumption of heptachlor epoxide is on the order of 1.0 μg/day for adults in marketed foods of widespread distribution in the United States. Oxychlordane, heptachlor, chlordane, and nonachlor appear sporadically in foods. Chlordane and nonachlor are also found in freshwater fish. Consumption by sport fishermen may be as high as 2.2 μg/day.

During 1970 and 1972, two national ambient air monitoring surveys sampled pesticides at a number of sites across the United States. Heptachlor, chlor-

dane, heptachlor epoxide, and oxychlordane (in order of decreasing frequency) were detected. Chlordane and heptachlor were detected in a study of suburban areas in 1976. The average air concentration of heptachlor in the community with the highest reading was 6.3 ng/m^3. Heptachlor, and to a lesser extent chlordane and heptachlor epoxide, are widespread in ambient air, with typical mean concentrations on the order of 0.5 ng/m^3. The amounts of chlordane and heptachlor in air above treated fields were reported to be as high as 16 ng/m^3, even 3 weeks after treatment. Chlordane concentrations as high as 72 ppm have been found in house dust for up to 4 years after fumigation treatment.

The data available for residues in human tissue are more extensive and reliable than the data for food residues. The principal source of this data has been the National Human Monitoring Program conducted between 1970 and 1974 (U.S. EPA, 1977). Heptachlor epoxide was found in 97 percent of approximately 7,000 samples of human tissue tested. Similarly, oxychlordane was found in approximately 97 percent of 5,000 samples, and about 98 percent of 755 samples showed residues of trans-nonachlor. The geometric mean levels of heptachlor, oxychlordane, and nonachlor are about 0.085, 0.11, and 0.11 ppm, respectively.

There is some evidence that chlordane may undergo sensitized photolysis in aquatic media. The direct photolysis of chlordane in ambient aquatic media without a sensitizer; oxidation processes involving singlet oxygen and peroxy/radical; hydrolysis; and biodegradation in ambient aquatic media are not likely to be significant fate-determining processes. The three processes that are likely to determine the fate of this compound in aquatic media are evaporation, sorption, and bioaccumulation. The evaporative half-life of this compound from 1 m depth of water was estimated to be 28 to 33 hours. Thus, although the evaporation from aquatic media may not be rapid, it could be significant in the absence of any other faster processes. Chlordane is expected to be significantly

sorbed from the aquatic phase to suspended particles and sediment, which may play a significant role in determining its aquatic fate.

Heptachlor may undergo significant photolysis in ambient aquatic media, since laboratory studies have demonstrated photolytic decomposition in 1 week and complete decomposition in 2 weeks. The singlet oxygen reaction with heptachlor in aquatic media may be significant, with the half-life of heptachlor for this reaction estimated to be about 1 day. The hydrolysis of heptachlor in aquatic media is also an important process, with an estimated hydrolytic half-life in the range of 1 to 3 days. The volatilization half-life of heptachlor from aquatic media is estimated to be 2 to 10 days from pond, river, and lake water. Although most estimates indicate that the evaporative half-life of heptachlor in aquatic media appears to be longer than that of chlordane (2 to 10 days vs. 1 to 2 days), Huang (1970) reported that the evaporation rate of heptachlor from aquatic media is faster than for chlordane. Heptachlor may be significantly sorbed onto suspended particles and sediments present in aquatic media, which may play a significant role in determining the fate of heptachlor in aquatic media. The biodegradation rate of heptachlor in aquatic media is slower than the rate of hydrolysis.

The fate of heptachlor epoxide in aquatic media has not been comprehensively studied. Photolysis, oxidation, or hydrolysis are not expected to be significant fate-determining processes for heptachlor epoxide in aquatic media. Biodegradation also does not appear to be a significant process. The two processes that are likely to determine the fate of heptachlor epoxide in aquatic media are volatilization and sorption. The rate of volatilization of heptachlor epoxide from aquatic media is much slower than for heptachlor. However, it is difficult to make a reasonable estimate of the evaporative half-life for this compound because of the lack of appropriate data. The sorption of this

compound on suspended particulate matter or sediment may be a moderately significant process (U.S. EPA, 1985a).

In summary, exposure to chlordane and heptachlor can occur to a variety of human populations through direct use of the materials or indirectly through their entry into environmental pathways. Potentially exposed populations consist of workers in manufacturing and formulating plants; field and home applicators of the pesticides; and the general population through atmospheric, aquatic, or food residues.

4. HAZARD IDENTIFICATION

4.1. METABOLISM AND PHARMACOKINETICS

Chlordane and heptachlor are related bicyclic chlorinated hydrocarbons. In fact, heptachlor is a minor metabolite of chlordane. Chlordane has two isomers, cis and trans, and the chemical structures of chlordane and heptachlor are given in Figure 1. As there are virtually no data on the metabolism and pharmacokinetics of chlordane and heptachlor in humans, the review in this section consists of animal studies only.

4.1.1. Absorption

4.1.1.1. Chlordane--Quantitative data on the absorption of chlordane from the gastrointestinal tract of laboratory animals were not available. However, the systemic toxicity of the insecticide to laboratory animals following oral, dermal, and inhalation exposure indicates that some absorption does take place. That chlordane is absorbed can also be inferred from excretion data. Chlordane consists of a mixture of components, including the cis- and trans-isomers. A high purity (98+ percent) formulation of the insecticide, designated HCS-3260, contains cis- and trans-chlordane in a 3:1 ratio. Sprague-Dawley rats (one of each sex) received a single dose of 0.05, 0.2, or 1.0 mg ^{14}C-HCS-3260/kg body weight (bw) by gavage, or a single oral dose of each ^{14}C-labeled isomer at 0.2 mg/kg bw (Barnett and Dorough, 1974). Elimination of radioactivity in the urine over 7 days was 6 percent for females and 2 percent for males following ^{14}C-HCS-3260 treatment. In other studies, following treatment with cis- and trans-chlordane-^{14}C, female rats eliminated 8.5 and 5 percent of the administered radioactivity, respectively. These results indicate that at least 2 to 8.5 percent of the administered chlordane dose was absorbed by the gastrointestinal tract of rats. Based upon a study in which a male rabbit was given

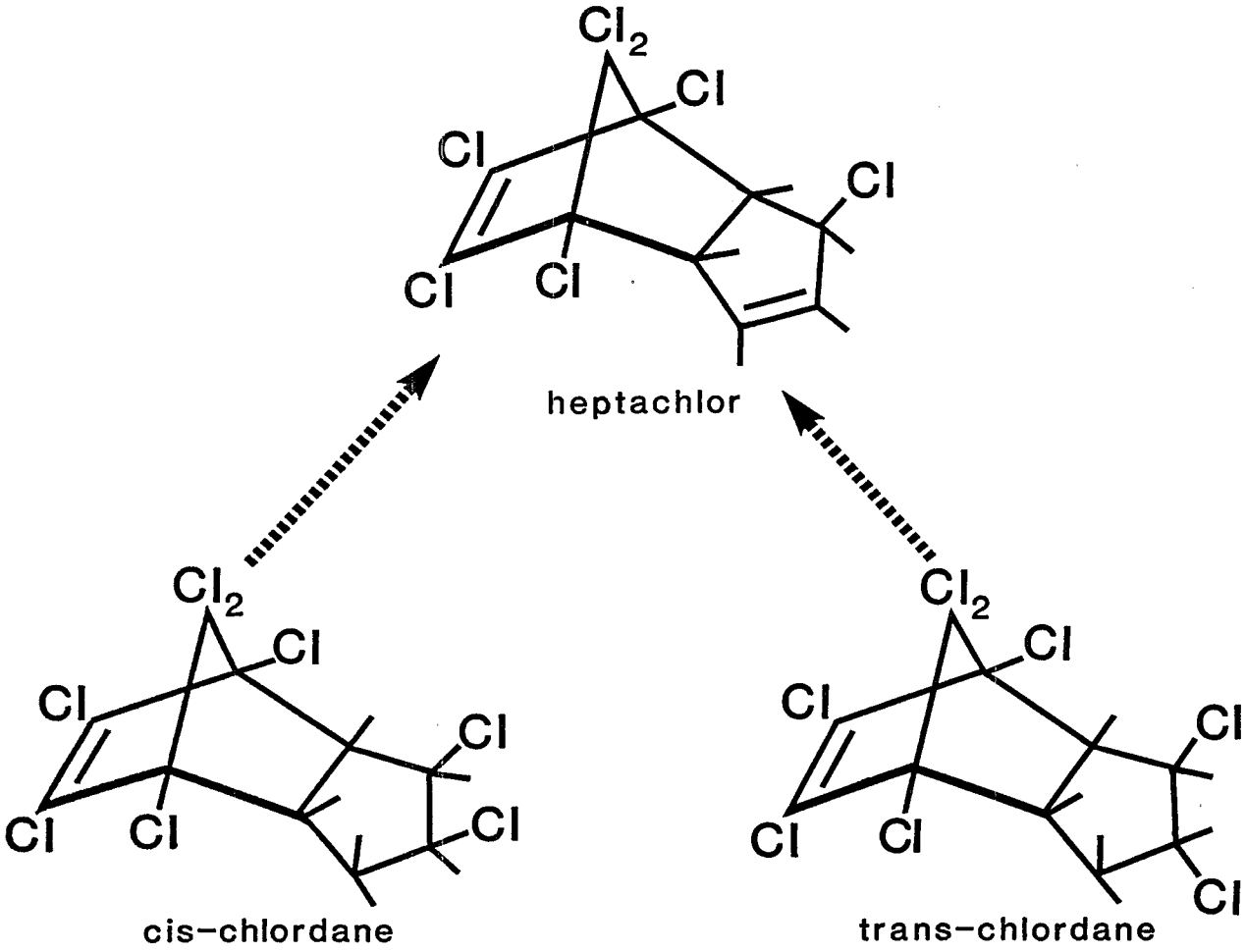


Figure 1. The structures of chlordane and heptachlor.

^{14}C-HCS-3260 in a dietary concentration of 25 ppm (25 mg/kg diet) for 2 days, 33 percent of the radioactivity was excreted in the urine and 21 percent in the feces 24 hours after dosing (Barnett and Dorough, 1974). Thus, it would appear that at least 33 percent of the chlordane was absorbed by the rabbit as compared with only 2 to 8.5 percent for the rat after oral administration.

Pulmonary absorption of an unspecified amount of ^{14}C-chlordane (11,500 dpm/μg) in 20 μL ethanol administered as an aerosol intratracheally to female Sprague-Dawley rats was measured by Nye and Dorough (1976). No intact ^{14}C-chlordane was detected in exhaled air. A peak blood concentration of radioactivity of approximately 4 percent of the applied dose was reached in <5 minutes.

Ambrose et al. (1953b) reported in an abstract that dermal application of 50 mg chlordane/kg bw was more toxic to rats when the chemical was applied in cottonseed oil rather than in ethyl alcohol, indicating greater absorption with the lipophilic vehicle.

4.1.1.2. Heptachlor--Limited information was found on the absorption of heptachlor following ingestion by animals. However, the systemic toxicity of heptachlor following oral, dermal, or inhalation exposure is an indication of the absorption of the insecticide. The U.S. EPA (1980b) reviewed an abstract of a Soviet study (Mizyukova and Kurchatov, 1970) and reported that heptachlor administered intragastrically in a single oral dose of 120 mg/kg bw to rats was detected in blood within 0.5 to 1 hour of administration.

The absorption of heptachlor following inhalation exposure also has not been well studied. From July 1, 1972, to October 4, 1972, Arthur et al. (1975) placed 10 rabbits of each sex in open-air cages so that they were exposed to the ambient air of Stoneville, MS, an area where insecticides had been heavily used. Control groups of male and female rabbits (10 each) were housed in a

room at Mississippi State University, a low pesticide use area. The average level of heptachlor epoxide (heptachlor was either not measured or not detected) in Stoneville air was 1.86 ng/m^3; the air at Mississippi State was not sampled. Heptachlor epoxide residue levels in adipose tissue of the test rabbits averaged 0.039 ppm, as compared with 0.016 ppm in controls ($p < 0.001$). The average respiratory intake of heptachlor epoxide was calculated as 0.002 μg/day for rabbits in the Stoneville area.

4.1.2. Tissue Distribution

4.1.2.1. Chlordane--The tissue distribution of ^{14}C-HCS-3260, cis- or trans-chlordane-^{14}C, and the metabolite oxychlordane in male and female rats following treatment with single oral doses was compared by Barnett and Dorough (1974). At 1 day following doses of 0.05 to 2.0 mg/kg bw of the respective compounds, the concentrations of radioactive equivalents in brain, muscle, liver, and kidney were generally low (0.00 to 0.08 ppm), while the concentrations of radioactivity in fat were somewhat higher (the average for all treatments was approximately 0.47 ppm). Male and female rats treated with 0.1 mg ^{14}C-HC-3260/kg bw had higher tissue residue levels of radioactive equivalents in liver (0.50 ppm), kidney (0.26 ppm), and fat (3.71 ppm for females, 2.58 ppm for males). In general, female rats accumulated greater concentrations of radioactivity in fat than male rats after treatment with any preparation. At 7 days after dosing with 1.0 mg ^{14}C-HCS-3260/kg bw, radioactivity in all tissues declined; radioactivity in fat declined to 2.0 ppm for females and 1.19 ppm for males. Slightly more radioactivity was present in rat tissues following oral doses of trans- as compared with cis-chlordane-^{14}C.

When ^{14}C-HCS-3260 was administered to male rats in the diet at 5 ppm (5 mg/kg diet) for 56 days, the tissue distribution of radioactive equivalents was 0.42, 0.91, 0.55, 0.68, and 14.73 ppm for muscle, brain, kidney, liver,

and fat, respectively (Barnett and Dorough, 1974). After discontinuing treatment for 28 and 56 days, the concentration of radioactivity in fat declined to 3.67 and 2.49 ppm, respectively. Radioactivity was still detected in other tissues 56 days after treatment was terminated. Greater accumulation of radioactivity in all tissues resulting from the absorption of transrather than from cis-chlordane-^{14}C occurred in female rats treated with 25 ppm (25 mg/kg diet). Analysis of the nature of the radioactivity revealed that approximately 30 to 60 percent of the radiocarbon was associated with oxychlordane.

Ambrose et al. (1953a) found that the perirenal fat of male rats contained 43, 41, and 81 ppm of chlordane residues following feeding for 5, 148, and 407 days, respectively, of a diet containing 320 ppm (320 mg/kg diet). The fat of female rats contained approximately twice the values for males.

Residues of parent isomers and oxychlordane in adipose tissue from male and female Holtzmann rats maintained on diets containing either 50 to 220 ppm (50 to 200 mg/kg diet) cis- or trans-chlordane, 100 ppm (100 mg/kg diet) of fixed ratios of the isomers from 9:1 trans:cis to 1:9, or 50 ppm (50 mg/kg diet) technical chlordane for 15 days were determined by Street and Blau (1972). Adipose tissue of female rats fed trans-chlordane at 50, 100, and 200 mg/kg diet contained approximately 6, 10, and 23 μg/g lipid of transchlordane, respectively. Much greater concentrations of oxychlordane than trans-chlordane were stored in adipose tissue by females (approximately 104, 202, and 471 μg/g lipid) and males (approximately 5, 15, and 22 μg/g lipid) after being fed trans-chlordane. The feeding of cis-chlordane likewise resulted in greater fat storage of the metabolite than of the parent isomer in both male and female rats; however, the ratio oxychlordane:isomer was reduced. Female rats fed 50 ppm technical grade chlordane (50 mg/kg diet) stored approximately 7 times more oxychlordane than the parent compound in adipose tissue. The results of feed-

ing ratios of cis- and trans-chlordane indicated that oxychlordane accumulation was additive for each isomer.

Polen et al. (1971) also detected oxychlordane in the fat depots of rats, dogs, and pigs maintained on diets containing individual isomers or technical chlordane. Rats received dietary concentrations of cis-, trans-, or technical grade chlordane at 0 to 150 ppm (0 to 150 mg/kg diet) for 1 year; dogs received technical grade chlordane at 3 or 30 ppm (3 or 30 mg/kg diet) for 2 years; and pigs were fed diets containing the cis- or trans-isomer at 300 ppm (300 mg/kg diet) for 90 days. The respective levels of oxychlordane in the fat of these species were 0.2 to 150, 3 to 24, and 36 to 90 ppm, respectively.

Rabbits received trans-chlordane-^{14}C daily per os in doses of 14.3 mg/rabbit/day for 10 weeks (Poonawalla and Korte, 1971). Two weeks after treatment was discontinued, low levels of radioactivity were detected in kidney (0.05 percent of administered dose), liver (0.52 percent), heart (0.09 percent), lung (0.04 percent), spleen (0.03 percent), testes (0.03 percent), and brain (0.04 percent) tissue. Higher levels were found in adipose tissue (2.59 percent in abdominal fat, 1.53 percent in subcutaneous fat) and in muscle (5.68 percent). Barnett and Dorough (1974) found that the tissues of a rabbit fed ^{14}C-HCS-3260 (25 mg/kg diet for 2 days) contained ^{14}C-oxychlordane and ^{14}C-dichlorochlordene.

The distribution of radioactivity in the tissues of rabbits administered cis- or trans-chlordane-^{14}C orally in four doses, one capsule given every 4 days containing 100 mg per capsule, were as follows: fat > kidney > muscle > liver > brain (cis-); kidney > fat > liver > muscle > brain (trans-) (Balba and Saha, 1978). The majority of the radioactivity in the tissues was associated with oxychlordane, regardless of which isomer was administered.

The mobilization of chlordane or its metabolites from adipose tissue of

rats after food deprivation was studied by Ingle (1952). Chlordane was removed from the diet after 80-week maintenance of 16 rats on a diet containing chlordane at 150 ppm (150 mg/kg diet). At weekly intervals, two male and two female rats were fasted for 48 hours and observed for symptoms of toxicity. After 4 weeks, symptoms such as tremors and hyperactivity were no longer observed.

The distribution of radioactivity (expressed as percent of administered radioactivity/tissue) in selected tissues of rats following the previously described intratracheal administration of ^{14}C-chlordane (Nye and Dorough, 1976) was 23.9 percent in lung, 19.6 percent in liver, 0.3 percent in kidney, and 0.1 percent in the bladder and its contents.

4.1.2.2. Heptachlor--An abstract of a Soviet study (Mizyukova and Kurchatov, 1970) reported that following a single intragastric dose of 120 mg/rat heptachlor to female rats, heptachlor was detected in blood, liver, kidney, and adipose tissue within 1 hour. After 4 hours, heptachlor epoxide, a metabolite of heptachlor, was detected in blood, liver, and fat and persisted in the adipose tissue for 3-6 months.

Radomski and Davidow (1953) studied the tissue distribution of heptachlor epoxide in rats and dogs following oral administration of heptachlor. Nine rats of either sex received dietary concentrations of heptachlor at 30 to 35 ppm (30 to 35 mg/kg diet) for 2 months. Heptachlor epoxide levels in the fat of six female rats averaged 384 μg/g tissue, whereas fat in male rats contained an average of only 43 μg/g tissue. Much lower levels of heptachlor epoxide were detected in liver (0.4 to 33 μg/g), kidney (0 to 21 μg/g), and muscle (0 to 27 μg/g) tissue. None was detected in brain tissue. A similar pattern of distribution was observed in three female dogs that received 1 mg heptachlor/kg bw in capsules daily for 12 to 18 months. Fat contained an average heptachlor epoxide concentration of 636 μg/g; liver, 36 μg/g; kidney,

7.5 µg/g; and muscle, 13 µg/g. Again, no heptachlor epoxide was detected in the brain. Small amounts of unchanged heptachlor were detected in the fat of dogs when higher levels of the insecticide were administered. Administration of 1 mg heptachlor/kg bw to four dogs resulted in no detectable heptachlor in fat after 26 weeks, while three of eight dogs, surviving 6 to 18 weeks, dosed with 3 mg/kg bw daily had <1 µg heptachlor/g fat tissue. A daily dose of 5 mg/kg bw resulted in an average concentration of heptachlor in fat of 6.5 µg/g. This dose was fatal to the dogs within 2 to 11 weeks.

Radomski and Davidow (1953) also determined the rate of accumulation and disappearance of heptachlor epoxide in rats (three rats/sex/group) fed a diet containing 30 ppm (30 mg/kg diet) heptachlor. One group was sacrificed each week for 12 weeks. Female rats accumulated heptachlor epoxide to a maximum level of approximately 225 µg/g fat in 8 weeks, as compared with only 50 µg/g fat in males. When the test diet was discontinued, heptachlor epoxide was no longer detected at 8 weeks for female fat tissue and 6 weeks for male fat tissue. Further tests established that the maximum dietary level at which fat storage did not occur was 0.3 mg/kg diet for male rats and 0.1 mg/kg diet for female rats.

Although Radomski and Davidow (1953) failed to detect heptachlor epoxide in rat and dog brain tissue after heptachlor treatment, Yamaguchi et al. (1979), using a gas chromatographic (GC) method, detected 3.15 ppm heptachlor epoxide in brain tissue of rats 5 hours after an intraperitoneal injection of 200 mg heptachlor/kg bw.

4.1.3. Metabolism

4.1.3.1. Chlordane--The metabolism of chlordane has been well studied in rats and rabbits *in vivo* and *in vitro*. Oxychlordane was a metabolite of both cis- and trans-chlordane (Street and Blau, 1972). A metabolic pathway, based upon

in vitro studies with rat liver homogenates, was proposed in which either isomer is dehydrogenated to 1,2-dichlorochlordene with subsequent epoxidation to oxychlordane. In these experiments, trans-chlordane was converted to oxychlordane at a sevenfold greater rate than was the cis-isomer.

Barnett and Dorough (1974) isolated seven radioactive metabolites (in addition to the respective unchanged parent compounds) in the feces of rats that had been administered cis- or trans-chlordane-^{14}C or the 3:1 mixture of cis- and trans-chlordane, designated as ^{14}C-HCS-3260, either as single oral doses (0.2 mg/kg bw) or by continuous feeding (5 mg/kg diet of ^{14}C-HCS-3260 for 56 days or 25 mg/kg diet cis- or trans-chlordane-^{14}C for 14 days). These metabolites, analyzed by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC), were tentatively identified as hydroxychlordane; chlordene chlorohydrin; monochloro and dihydroxy derivatives of chlordane; cis- and/or trans-dihydroxychlordane derivatives; a trihydroxylated chlordene; and a conjugated form of a hydroxylated chlordane metabolite. No oxychlordane or dichlorochlordene was detected in feces; however, oral administration of oxychlordane resulted in fecal excretion of unchanged oxychlordane. The nature of urinary metabolites was essentially the same as fecal metabolites in rats fed HCS-3260 in dietary concentrations of 25 mg/kg diet; however, oxychlordane was also present. The 24-hour feces of a rabbit fed ^{14}C-HCS-3260 at 25 mg/kg diet for 2 days contained the same fecal metabolites found in the rat, although the amounts of unchanged isomers were greater. The urine of the rabbit contained a greater percentage of the conjugated hydroxylated metabolites than did the urine of rats.

In rabbits, Balba and Saha (1978) identified the following urinary metabolites of cis-chlordane: 1-hydroxy-2-chlorochlordene, trans-chlordene chlorohydrin, and 1-hydroxychlordene. Urinary metabolites in rabbits of trans-chlor-

dane were 1-hydroxy-2-chlorochlordene, 1,2-dichlorochlordene, trans-chlordene chlorohydrin, and 3-hydroxychlordane. The metabolism of cis- and trans-chlordane-^{14}C was also studied in rats *in vivo* and *in vitro* by Tashiro and Matsumura (1977). Male rats maintained on diets containing cis- or trans-chlordane-^{14}C in a concentration of 100 ppm (100 mg/kg diet) for 4 weeks excreted 13 metabolites from cis- and 14 metabolites from trans-chlordane in the feces. The fecal metabolites of both isomers, identified by TLC and GLC, included heptachlor, 1,2-dichlorochlordene, oxychlordane, 1-hydroxy-2-chlorochlordene, 1-hydroxy-2-chloro-2,3-epoxychlordene, chlordene chlorohydrin, monohydroxy dihydrochlordene, 1,2-dihydroxychlordene, and trihydroxydihydrochlordene. These metabolites were found in different proportions, depending upon the administered isomer. The only urinary metabolite identified from both cis- and trans-chlordane was a glucuronide conjugate of 1-hydroxydihydrochlordene. The *in vitro* incubation of the isomers with rat liver microsomes and cofactors resulted in the same metabolites qualitatively.

Brimfield et al. (1978) also studied the *in vitro* metabolism of the pure cis- and trans-isomers of chlordane by microsomal preparations from Sprague-Dawley rats fed the individual isomers in dietary concentrations of 100 ppm (100 mg/kg diet) for 9 days. Incubation of cis-chlordane-induced enzymes with cis-chlordane resulted in the following metabolites, identified by GC-MS: cis-chlordane, dichlorochlordene, oxychlordane, dihydroheptachlor, hydrochlordene, and 1-chloro-2-hydroxydihydrochlordene. Similar products from trans-chlordane were identified; however, heptachlor rather than dihydroheptachlor was formed, as was hydroxychlordane. A metabolic pathway, based upon reductive dechlorination via dihydroheptachlor to dihydrochlordene, was proposed. According to this scheme, these molecules can also be hydrolyzed, desaturated, and epoxidized. In the Brimfield et al. (1978) proposal, oxychlordane is an

end point, whereas in the scheme proposed by Tashiro and Matsumura (1977), oxychlordane undergoes further hydroxylation and reduction. Many of the proposed intermediates in the Brimfield scheme have not actually been found in this or other studies (Brimfield et al., 1978).

Therefore, using the in vivo data by Barnett and Dorough (1974) and Tashiro and Matsumura (1977) as the primary references along with supporting in vitro data, the metabolic pathways proposed by Tashiro and Matsumura (1977) best fit the data reported. Chlordane is dehydrogenated to dichlorochlordene, which is epoxidized at carbons 2 and 3 and hydroxylated at carbon 1 (Route A). An additional route whereby chlordane is hydroxylated at carbon 1 to yield 1-hydroxydihydrochlordene was also proposed (Route B). Heptachlor is a minor metabolite of both cis- and trans-chlordane. These pathways are presented in Figure 2.

4.1.3.2. Heptachlor--Davidow and Radomski (1953) first identified heptachlor epoxide in the adipose tissue of dogs that had been treated with daily oral doses of 1-3 mg heptachlor/kg bw in corn oil. They concluded that the metabolite arose by the epoxidization of heptachlor. Subsequently, these investigators (Radomski and Davidow, 1953) also isolated heptachlor epoxide, identical to the compound found in dogs, from the adipose tissue of male and female rats maintained on diets containing 30 to 35 ppm (30 to 35 mg/kg diet) heptachlor.

In an abstract of a Soviet study, Ermakov (1977) reported that heptachlor, administered to rats and rabbits in single oral doses of 28-50 mg/kg bw, was metabolized by reactions involving hydrolysis, hydroxylation, epoxidation, dehydrogenation, and molecular rearrangement, but details as to intermediate structures were not given in the abstract.

The most extensive investigation of the metabolic fate of heptachlor in rats was performed by Tashiro and Matsumura (1978) in vivo and in vitro. Over

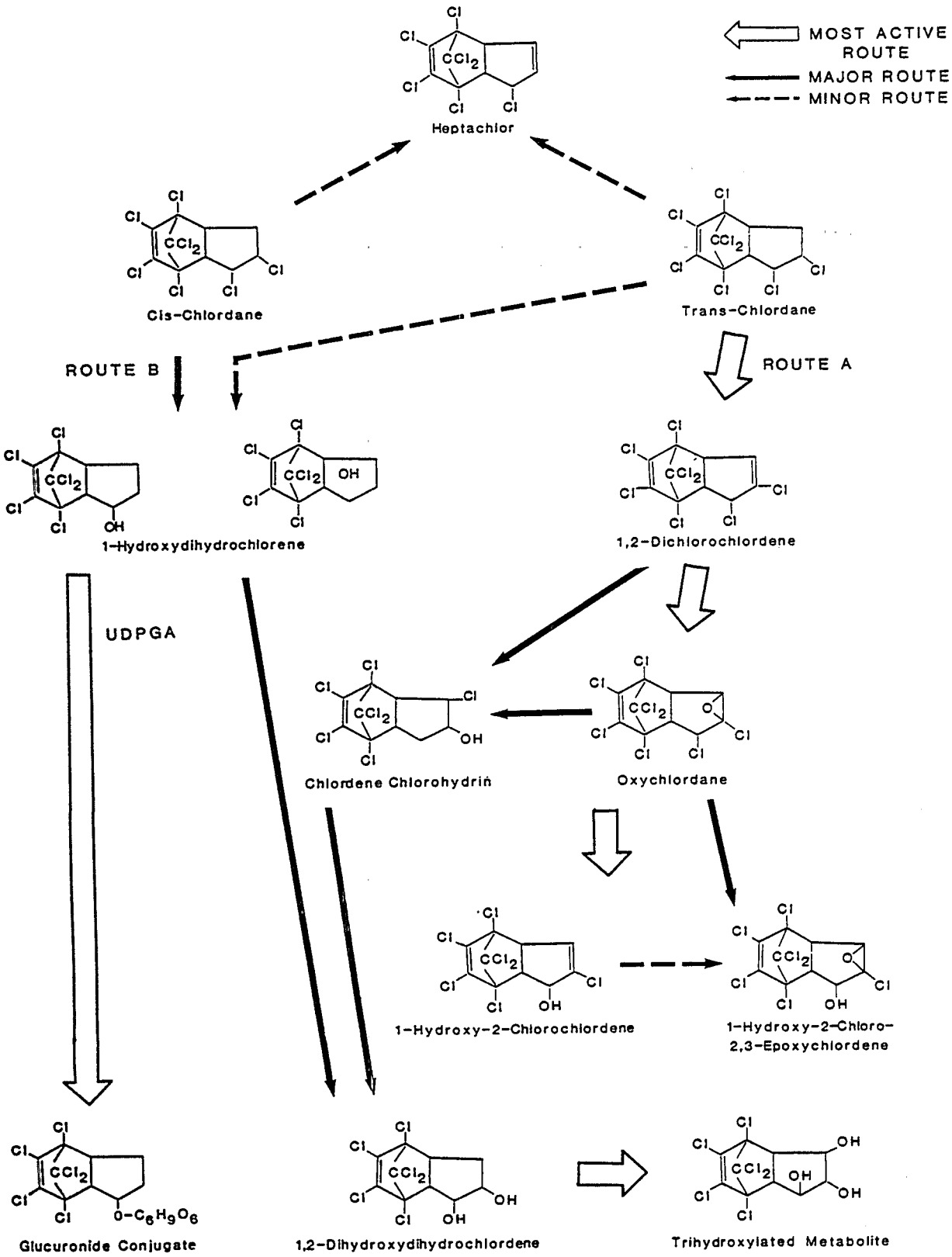


Figure 2. Proposed metabolic pathways of chlordane in rats.

SOURCE: Tashiro and Matsumura, 1977.

a 10-day period following a single oral unspecified dose of ^{14}C-heptachlor (position of label not specified) in corn oil, rats excreted >50 percent of the administered radioactivity in feces, and <5 percent in urine. The relative abundance of fecal metabolites, expressed as percent of ^{14}C-compounds, were as follows: unchanged heptachlor, 26.2 percent; heptachlor epoxide, 13.1 percent; 1-hydroxychlordene, 19.5 percent; 1-hydroxy-2,3-epoxychlordene, 17.5 percent; 1,2-dihydroxydihydrochlordene, 3.5 percent; and two unnamed metabolites, one of which accounted for 19 percent of the radioactivity, the other for <0.1 percent. The latter metabolite was designated as H-2 by these authors, and its structure is identical to that proposed by Matsumura and Nelson (1971) for what they called the fecal metabolite of heptachlor epoxide. The in vitro metabolism of ^{14}C-heptachlor by rat liver microsomal preparations resulted in the following relative abundance of metabolites, expressed as percent of the total ^{14}C-compounds: heptachlor, 4.4 percent; heptachlor epoxide, 85.8 percent; 1-hydroxychlordene, 2.9 percent; 1-hydroxyepoxychlordene, 3.0 percent; 1,2-dihydroxydihydrochlordene, 0.9 percent; and an unknown, 3.0 percent (Tashiro and Matsmura, 1978). No H-2 fecal metabolite was detected. The identity of the metabolites was confirmed by TLC analysis and comparison with authentic standards of fecal metabolites from rats fed diets containing 100 ppm (100 mg/kg diet) heptachlor for 4 weeks.

Matsumura and Nelson (1971) administered heptachlor epoxide to four rats in dietary concentrations of 10 ppm (10 mg/kg diet) for 30 days. The authors estimated that each rat consumed 5 mg of heptachlor epoxide over the test period and excreted 950 μg of a fecal metabolite (see Figure 3) and 66 μg of heptachlor epoxide in the feces.

Brooks et al. (1968, 1970) investigated the in vitro metabolism of heptachlor epoxide by pig liver microsomes. The product, formed upon incubation at

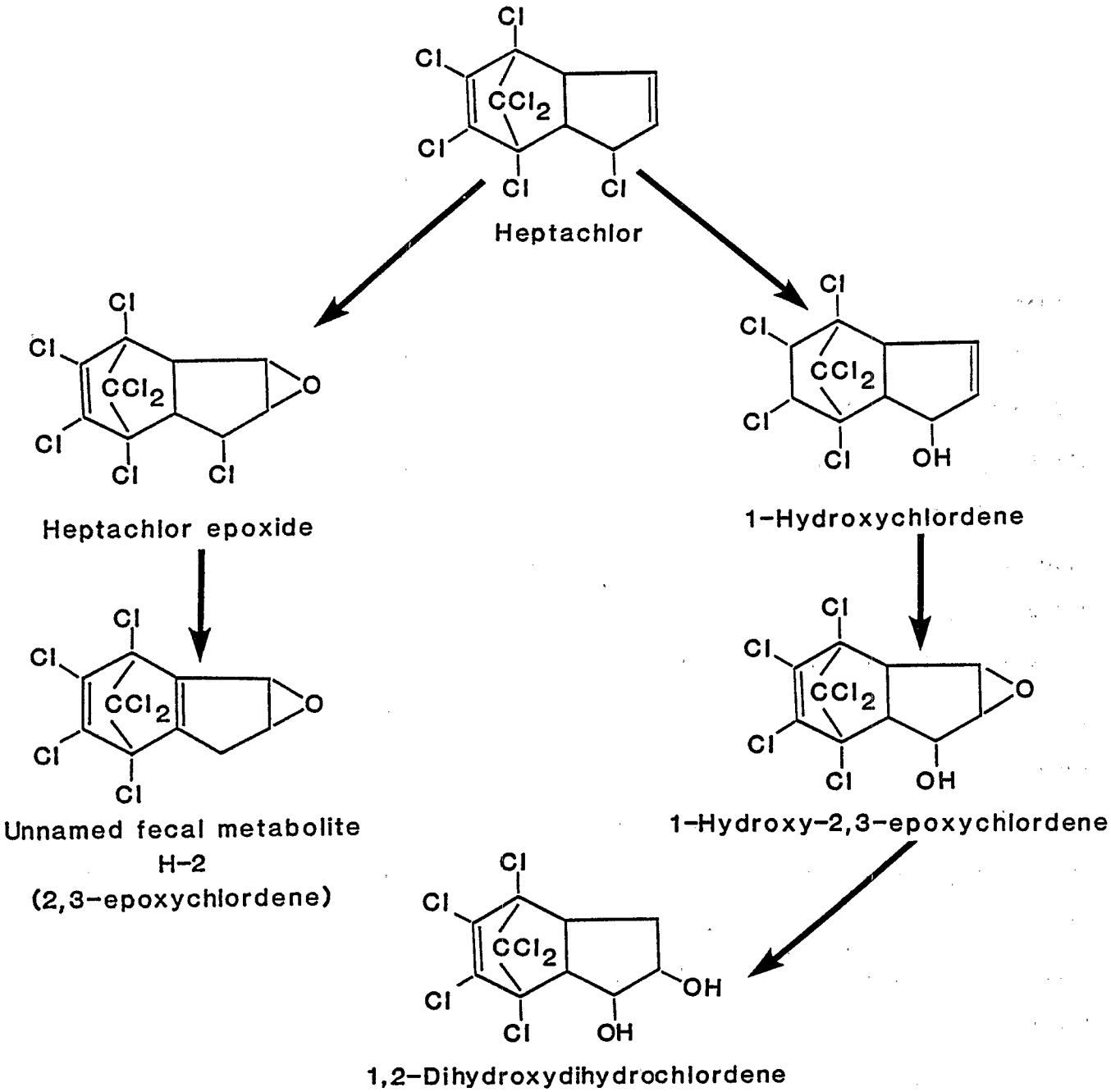
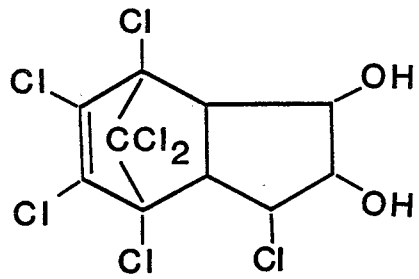


Figure 3. Proposed metabolic pathway of heptachlor in rats.

SOURCE: Tashiro and Matsumura, 1978.

45°C for 60 hours, was identified as heptachlor epoxide diol:



Incubation of heptachlor epoxide with rabbit microsomes also resulted in the formation of heptachlor epoxide diol as well as another unidentified product. The *in vivo* product, 2,3-epoxychlordene, was not formed or identified in these *in vitro* preparations.

Therefore, the metabolic pathway of heptachlor in rats, as proposed by Tashiro and Matsumura (1978), is presented in Figure 3.

4.1.4. Excretion

4.1.4.1. Chlordane--In the experiments of Barnett and Dorough (1974), >90 percent of the administered radioactivity was excreted over 7 days by rats that were given single oral doses (0.2 mg/kg diet) of cis- or trans-chlordane-^{14}C or ^{14}C-HCS-3260. Females excreted 6 percent of the radioactivity in the urine, while males excreted only 2 percent. At higher doses (0.5 or 1.0 mg/kg diet) of HCS-3260, the pattern of elimination was essentially the same. Females excreted slightly, but not significantly, more of the cis- than the trans-isomer. When ^{14}C-HCS-3260 was fed in the diet for 56 days, fecal elimination as measured by radioactivity was 70 percent for the 1 mg/kg diet level, 75 percent for the 5 mg/kg diet level, and 80 percent for the 25 mg/kg diet level, indicating possible decreased absorption; the investigators made no mention of possible biliary excretion. Elimination of cis-chlordane (75 percent) was greater than trans-chlordane (65 percent) following 14 days of dietary levels of the isomers at 25 mg/kg diet.

Tashiro and Matsumura (1977) reported similar results for elimination from rats treated with single oral doses of cis- (5.4 mg/kg bw) and trans-chlordane-^{14}C (9.7 mg/kg bw) in corn oil. The total 7-day elimination of radioactivity from cis- and trans-chlordane was 85 and 66 percent of the administered dose, respectively. The 24-hour total excretion was 59 percent for cis- and 27 percent for trans-chlordane.

Rabbits excreted 18 percent of an orally administered single dose of 200 mg/kg bw of chlordane in the urine collected over 16 days when organic chlorine contents were measured (Stohlman et al., 1950). The peak urinary elimination of organic chlorine occurred within 2 days and amounted to 9 percent of the dose.

Poonawalla and Korte (1971) observed appreciable urinary excretion of radioactivity by rabbits that received 14.3 mg/rabbit of trans-chlordane-^{14}C daily for 10 weeks. At the end of this period, approximately 70 percent of the daily dose had been eliminated: 22.7 percent was excreted in the feces, 30 percent of which was unchanged trans-chlordane, and 47 percent was excreted as urinary metabolites. In agreement with these results, excretion of radioactivity by one rabbit 24 hours after termination of feeding 25 ppm (25 mg/kg diet) of HCS-3260-^{14}C for 2 days was 21 percent in feces and 33 percent in urine (Barnett and Dorough, 1974).

Balba and Saha (1978) also observed appreciable urinary excretion of radioactivity of cis- or trans-chlordane-^{14}C by rabbits that were treated with either isomer orally at a dose of 100 mg/rabbit in a capsule every 4 days up to 400 mg/rabbit; however, in this study, urinary excretion did not exceed fecal excretion. For the cis-isomer, 48.5 percent and 28.4 percent of the radioactivity were eliminated in the feces and urine, respectively. For the trans-isomer, fecal and urinary excretion were 46.1 percent and 35.8 percent,

respectively.

When an unspecified amount of chlordane-^{14}C in 20 µL of ethanol was administered intratracheally to female Sprague-Dawley rats, elimination of radioactivity was primarily in the feces (Nye and Dorough, 1976). After a lag period of 2 days during which <20 percent of the dose was eliminated, fecal excretion rose to approximately 50 percent by day 4 and 56 percent by day 6. Urinary excretion over 6 days amounted to 12 percent of the dose. No radioactivity was detected in the expired air of these animals.

4.1.4.2. Heptachlor--Tashiro and Matsumura (1978) reported that rats excreted >50 percent of the administered single oral unspecified dose of radioactivity from ^{14}C-heptachlor in the feces over 10 days. Urinary excretion of radioactivity accounted for <5 percent of the administered amount.

In an abstract of a Soviet study (Ermakov, 1977), it was reported that 16 to 40 percent of the orally administered dose of heptachlor (28 to 50 mg/kg bw to rats and rabbits was excreted unchanged in the feces and that heptachlor epoxide was excreted over at least a 12-month period. Further details were not given. As reported in an abstract of another Soviet study (Mizyukova and Kurchatov, 1970), and as reviewed by U.S. EPA (1980b), heptachlor and its metabolites were excreted mainly in the feces of rats within 5 days of an intragastric dose of 120 mg/kg bw.

Each of four male albino rats excreted an average of 950 µg of the fecal metabolite of 99 percent pure heptachlor epoxide during 30 days of maintenance on a diet containing 10 ppm (10 mg/kg diet) heptachlor epoxide (each rat consumed approximately 5 mg over 30 days) (Matsumura and Nelson, 1971). Approximately 66 µg of unchanged heptachlor epoxide was also excreted in the feces.

4.1.5. Species Differences

Some 30 to 40 percent of the 186 NCI chemicals tested in both rats and mice

were found to be positive in one species and negative in the other (Gold et al., 1984). Of these, approximately the same number were rat-positive and mouse-negative as were mouse-positive and rat-negative. However, a review of the bioassays of chlorinated hydrocarbons reveals a preponderance of mouse-positive and rat-negative studies. As such, are chlorinated hydrocarbons more toxic in mice than rats; initiators in mice but not in rats; or are there other differences that lead to the apparent discrepancy between rats and mice for carcinogenicity studies of chlorinated hydrocarbons?

In the selection of the laboratory animal species for a carcinogenesis bioassay, rats and mice have generally been used because of their size, lifespan, and the fact that these species are reliable predictors of human carcinogens for at least some chemicals. As the need to extrapolate from animals to humans has increased, along with the pressure to calculate the risk of exposure, the selection of species and the dosages continues to be discussed. As late as 1976, when the NCI guidelines were published (Sontag et al., 1976), considerable discussion of the dosage centered on the "maximum tolerated," and only the notion of the importance of metabolism data in relation to target concentrations was provided. Recently, Haseman (1985) focused on dose selection for NTP studies and restated the possible importance of pharmacokinetic and metabolism studies. Much of the discussion of metabolism data centers on qualitative similarities to humans and the possible consequences of metabolic overload.

In actuality, very little metabolism data are available in both rats and mice for any class of chemicals, including chlorinated hydrocarbons. The metabolism of chlordane and heptachlor has been studied in rats but not in mice; however, there is considerable evidence that significant species differences exist with regard to other substances. Such differences could be directly related to the carcinogenic response in rats and mice.

There have been a number of reports that emphasize metabolic differences among laboratory animal species (Brodie, 1962; Forrest and Aber, 1968; NAS, 1969; Oehme, 1970; Hanley et al., 1970; Conney et al., 1974; Zapp, 1977). Table 2 shows the large range of metabolic differences that can occur among animal species and humans.

TABLE 2. SPECIES DIFFERENCES IN THE METABOLISM OF THREE CHEMICALS

	Metabolic half-life (min)		
Species	Hexabarbital	Antipyrene	Aniline
Mouse	19	11	35
Rat	140	141	71
Guinea pig	--[a]	110	45
Rabbit	60	63	35
Dog	260	107	167
Human	360	600	--

[a]Data not available.

SOURCE: Adapted from Conney et al., 1974.

Similarly, a 200-fold difference was noted among species to elicit a pharmacologic response of ICI 33,828 on the pituitary, while the maximum plasma concentration was identical in all species tested, including humans. In addition, consideration of the rate and pattern of metabolism is of great importance (NAS, 1969). These types of data have recently led investigators to place greater emphasis on pharmacokinetic and metabolism data (Neal, 1983; NTP, 1984; Bernstein et al., 1985).

The use of pharmacokinetic data in estimating the human inhaled dose from oral data for laboratory animals has been reported (Cavender and Salasin, 1982).

Although considerable data for rats were available, very little data for mice were found for chlorinated hydrocarbons. Recently, Prout et al. (1985) published data for rats and mice for trichloroethylene, and Tables 3 and 4 present pharmacokinetic data for two strains of rats and two strains of mice. Of particular importance is the difference in urinary excretion and exhaled parent compound. The mouse apparently has a greater capacity for metabolizing trichloroethylene, which may lead to higher concentrations of important intermediates or metabolites than are possible in the rat. In this case, the parent compound is exhaled over time in the rat but not in the mouse. For compounds that are readily stored in fat depots, they may be metabolized over days and weeks. These data emphasize the fact that, although the relative proportion of metabolites is similar for the two species, there are considerable differences in the quantitative data, especially at the high dosages. Unfortunately, similar data do not exist for chlordane, heptachlor, or heptachlor epoxide. However, these data do emphasize the fact that the pharmacokinetic and metabolic differences may be quite different for the two species and may relate to the "sensitivity" of mice and/or the "resistance" of rats to these chlorinated hydrocarbons.

4.2. TOXIC EFFECTS

The toxic effects of chlordane/heptachlor were not addressed in a previous report of the Carcinogen Assessment Group (U.S. EPA, 1977). Thus, the key studies are reviewed here.

4.2.1. Human Studies

Data describing effects in humans range from information collected through clinical case studies and human monitoring data to epidemiologic studies. The three effects seen most frequently in clinical case studies are central nervous system (CNS) effects, blood dyscrasias, and neuroblastomas. Eleven case studies

TABLE 3. ELIMINATION OF A SINGLE ORAL DOSE OF ^{14}C-TRICHLOROETHYLENE IN OSBORNE-MENDEL RATS AND B6C3F1 MICE

Dose (mg/kg)	Species[a]	Time (hr)	% Dose excreted					
			Urine	Feces	CO_2	Unchanged	Carcass[b]	Total
10	Rat	0-24	66.3 ± 1.8	5.5 ± 0.9	7.5 ± 0.7	1.3 ± 0.4		
	Mouse		55.3 ± 4.7	15.5 ± 3.1	9.4 ± 1.1	3.9 ± 3.1		
	Rat	24-72	3.1 ± 0.8	2.8 ± 0.9	2.7 ± 0.2	0.0	8.4 ± 0.4	97.5 ± 1.8
	Mouse		4.1 ± 0.4	1.7 ± 0.8	2.3 ± 0.7	0.0	4.8 ± 1.0	97.1 ± 1.9
500	Rat	0-24	41.2 ± 1.4	2.1 ± 0.8	2.6 ± 0.1	42.7 ± 5.1		
	Mouse		59.2 ± 4.8	12.1 ± 1.0	6.9 ± 0.5	6.0 ± 1.5		
	Rat	24-72	2.4 ± 0.8	2.2 ± 0.6	1.1 ± 0.2	0.0	3.8 ± 0.5	98.1 ± 3.8
	Mouse		4.5 ± 2.6	2.4 ± 0.4	2.0 ± 0.2	0.0	4.4 ± 0.3	97.6 ± 2.4
1000	Rat	0-24	29.2 ± 0.8	1.7 ± 0.5	2.1 ± 0.3	56.2 ± 0.8		
	Mouse		41.8 ± 0.8	13.0 ± 9.5	6.0 ± 0.7	17.5 ± 8.2		
	Rat	24-72	1.9 ± 0.2	1.4 ± 0.6	0.8 ± 0.3	0.0	2.4 ± 0.4	95.7 ± 0.5
	Mouse		4.6 ± 1.1	2.0 ± 1.2	1.6 ± 0.4	0.0	8.6 ± 9.2	95.2 ± 1.2
2000	Rat	0-24	13.2 ± 3.3	2.2 ± 0.6	1.0 ± 0.1	77.8 ± 2.6		
	Mouse		43.7 ± 3.7	15.8 ± 2.0	6.0 ± 0.4	13.6 ± 4.2		
	Rat	24-72	0.8 ± 0.1	1.2 ± 0.5	0.4 ± 0.1	0.0	1.3 ± 0.01	98.0 ± 5.9
	Mouse		3.8 ± 0.6	3.4 ± 0.7	1.8 ± 0.1	0.0	3.8 ± 0.5	91.9 ± 2.6

NOTE: Values shown are means ± SD.
[a]Four animals per group.
[b]Residue remaining in carcass at 72 hr.

SOURCE: Adapted from Prout et al., 1985.

TABLE 4. ELIMINATION OF A SINGLE ORAL DOSE OF ^{14}C-TRICHLOROETHYLENE IN ALDERLEY PARK WISTAR RATS AND SWISS-WEBSTER MICE

Dose (mg/kg)	Species[a]	Day	% Dose excreted					
			Urine	Feces	CO_2	Unchanged	Carcass[b]	Total
10	Rat	1	54.6 ± 5.0	15.5 ± 2.9	7.4 ± 1.4	1.0 ± 0.5		
	Mouse		56.5 ± 5.4	13.2 ± 6.2	11.4 ± 1.1	1.0 ± 0.6		
	Rat	2	2.9 ± 0.4	7.2 ± 4.7	1.9 ± 0.4	0.0		
	Mouse		2.1 ± 0.6	1.8 ± 0.3	2.3 ± 0.2	0.0		
	Rat	3	0.8 ± 0.2	1.4 ± 0.9	0.2 ± 0.2	--	10.1 ± 1.3	102.9 ± 4.3
	Mouse		0.6 ± 0.1	0.6 ± 0.2	0.8 ± 0.2	--	5.8 ± 0.8	96.1 ± 1.6
500	Rat	1	24.9 ± 4.0	8.8 ± 1.3	3.7 ± 1.0	49.9 ± 4.2		
	Mouse		52.6 ± 5.8	12.1 ± 4.8	7.5 ± 0.8	12.5 ± 5.3		
	Rat	2	1.2 ± 0.1	3.2 ± 0.6	0.5 ± 0.2	0.0		
	Mouse		2.6 ± 0.8	3.4 ± 2.0	1.6 ± 0.3	0.0		
	Rat	3	0.4 ± 0.2	0.5 ± 0.06	0.2 ± 0.08	--	5.4 ± 0.8	98.7 ± 2.7
	Mouse		0.5 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	--	4.6 ± 0.7	98.9 ± 6.0
1000	Rat	1	17.1 ± 3.3	6.6 ± 2.7	4.8 ± 1.7	52.1 ± 6.5		
	Mouse		51.7 ± 9.2	10.8 ± 1.8	6.6 ± 1.8	16.0 ± 9.8		
	Rat	2	1.2 ± 0.3	5.5 ± 2.8	0.9 ± 0.3	0.0		
	Mouse		3.2 ± 2.5	3.0 ± 1.1	1.2 ± 0.9	0.0		
	Rat	3	0.4 ± 0.1	0.8 ± 0.7	0.6 ± 0.3	--	5.4 ± 1.4	95.3 ± 1.7
	Mouse		0.7 ± 0.4	1.1 ± 0.6	0.9 ± 0.3	4.0 ± 1.2	4.0 ± 1.2	99.2 ± 4.9

NOTE: Values shown are means ± SD.
[a]Four animals per group.
[b]Residue remaining in carcass at 72 hr.

SOURCE: Adapted from Prout et al., 1985.

describing CNS effects following oral, dermal, or inhalation exposure to technical grade chlordane are described in the literature. Six of these case studies involve oral exposure to chlordane. Effects ranged from irritability, salivation, labored respiration, muscle tremors, and convulsions to death. Derbes et al. (1955) described the death of a 32-year-old woman who ingested 104 mg/kg of chlordane in a dust formulation. Findings at autopsy included cerebral congestion and edema and lower nephron nephrosis. Convulsions were exhibited before death. Convulsions and vomiting accompanied other oral ingestions of chlordane (Dadey and Kammer, 1953; Lensky and Evans, 1952; Aldrich and Holmes, 1969; and Curley and Garrettson, 1969). The other five reports involving CNS effects involve dermal or a combination of dermal and inhalation exposure. Although dose level could not be quantified for these exposures, two resulted in death. Other CNS effects include convulsions, lethargy, and dizziness (Kutz et al., 1983; Derbes et al., 1955; Furie and Trubowitz, 1976; Klemmer et al., 1977; and Barnes, 1967).

Eight cases of blood dyscrasias have been reported following exposure to chlordane or heptachlor: four aplastic anemias (Infante et al., 1978; Klemmer et al., 1977), one refractory megaloblastic anemia (Furie and Trubowitz, 1976), one acute stem cell leukemia (Infante et al., 1978), one acute lymphoblastic leukemia (Infante et al., 1978), and one acute myelomonocytic leukemia (Infante et al., 1978). Muirhead et al. (1959) and Loge (1965) reported 5 and 12 cases of aplastic anemia, respectively, associated with either chlordane; a combination of chlordane and heptachlor; or chlordane, heptachlor, and other chemicals. The American Medical Association's Council on Drugs (Anonymous, 1962) stated that a "specific cause-effect relationship exists" between exposure to chlordane and resulting blood dyscrasias.

Infante et al. (1978) described five cases of neuroblastoma in children

with a pre- and/or postnatal history of exposure to technical grade chlordane. This is consistent with documented transplacental transfer of chlordane in humans (Curley et al., 1969; Wasserman et al., 1972, 1974; Zavon et al., 1969).

Savage et al. (1973, 1981) and Strassman and Kutz (1977), in results from human monitoring studies, reported levels of heptachlor epoxide and oxychlordane, metabolites of heptachlor and chlordane, respectively. Savage et al. (1981) found detectable levels of heptachlor epoxide in 67 percent of 1,436 human milk samples from nursing mothers at 163 hospitals stratified by geographic location. Oxychlordane was reported in 74 percent of the samples. Samples collected from the southeastern United States tended to have a higher percentage of samples with detectable levels of each metabolite than other regions of the country. Human milk samples of 54 women in Hawaii were collected during 1979 and 1980 (Takei et al., 1983). Oxychlordane was detected in 100 percent of the samples, with a mean of 0.068 ppm and a maximum value of 0.55 ppm. Heptachlor epoxide was also detected in all samples, with a mean of 0.036 ppm and a maximum value of 0.068 ppm.

Data from the EPA's National Human Monitoring Program show that levels of oxychlordane and heptachlor epoxide in human adipose tissue have remained constant over the years 1970 to 1983 (U.S. EPA, 1985a). The geometric mean of oxychlordane from 1972 to 1983 (excluding 1982) was 0.11 ppm with a maximum mean of 0.09 in 1981. Levels of heptachlor epoxide ranged from a geometric mean of 0.07 ppm in 1972 and 1977-79 to 0.09 ppm in 1970-71, 1973, 1981, and 1983, with a mean of 0.08 ppm for all years (excluding 1982).

4.2.2. Laboratory Animal Studies

4.2.2.1. Chlordane--Symptoms of acute chlordane intoxication include CNS stimulation, as evidenced by irritability, tremors, and convulsions (U.S. EPA, 1985a). When chlordane was given orally to rats at a dose of 25 mg/kg bw/day

for 15 days, it had no toxic effects, whereas 50 mg/kg bw or more resulted in toxic effects and death; cumulative toxic effects were observed (Ambrose et al., 1953a). The toxic effects of chlordane in rats include CNS stimulation, stomach ulcers, inflammation of the intestine, nephritis, hepatitis, an increase in liver weight, coma, and death (Boyd and Taylor, 1969).

In a review by Fouts (1970), chlordane induced hepatic drug-metabolizing enzymes in all species examined. Estradiol-17B and estrone metabolism were also stimulated by chlordane pretreatment in mice and rats, respectively (Welch et al., 1971).

Osborne-Mendel rats were fed diets containing chlordane for 80 weeks and held until week 109. Decreased body weight gain was noted in males receiving 407 ppm (approximately 20.3 mg/kg bw) in the diet and in females receiving 242 ppm (approximately 12.1 mg/kg bw). Tremors were noted in the females at week 44, and the animals that survived to termination were in poor condition (NCI, 1977a).

B6C3F1 mice were fed diets containing chlordane for 80 weeks and held until weeks 90-91. No body weight effects were noted, but tremors were observed in female mice at week 20. Alopecia was noted in male and female mice, and a hunched appearance was noted in a few mice. Abdominal distension was observed in all groups but was predominant in the females. Increased mortality incidence was noted for male mice fed 29.9 ppm (approximately 1.5 mg/kg bw) ($p \leq 0.02$) and 56.2 ppm (approximately 2.8 mg/kg bw) ($p \leq 0.01$). No mortality differences were noted for female mice (NCI, 1977a).

In an unpublished study by Velsicol Chemical Corporation (1984), Wistar rats and cynomolgus monkeys were exposed to 0, 0.1, 1.0, or 10 mg/m^3 for 90 days. Some of the low-exposure group rats were held for 17 days after exposure as a recovery group. No effects were noted on mortality, body weight, food

consumption, ophthalmoscopy, pulmonary function, rectal temperature, hematology, or urinalysis for either rats or monkeys. Blood levels of calcium, cholesterol, and glutamine dehydrogenase and liver cytochrome P-450 levels were increased in male and/or female rats. Increased organ weights were noted for male and/or female rats for the brain, liver, kidneys, and thyroids, while there was a decrease in adrenal weight in female rats. For monkeys, liver and thyroid weights were increased but not statistically significantly different from control group values. For microscopic pathology, only adaptive liver changes were significantly different for rats and monkeys. Male rats also exhibited an increase in the height of the follicular epithelium of the thyroid. Based on these results, the NOEL for chlordane in rats and monkeys was 0.1 mg/m^3 and the LOEL was 1.0 mg/m^3.

In an earlier unpublished study by Velsicol Chemical Corporation (1983b), ICR mice were fed 0, 1, 5, or 12.5 ppm chlordane (approximately 0, 0.15, 0.71, or 1.79 mg/kg bw) in the diet for 2 years. In mice receiving 5 or 12.5 ppm, liver changes were noted, including increased levels of serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT); hepatocellular swelling and necrosis; and increased liver weight in both male and female mice. The NOEL for chlordane was 1 ppm and the LOEL was 5 ppm.

In another unpublished study by Velsicol Chemical Corporation (1983a), Fischer 344 rats were fed diets containing 0, 1, 5, or 25 ppm chlordane (approximately 0, 0.05, 0.25, or 1.25 mg/kg bw) for 130 weeks. There was a significant increase in liver weight for females receiving 5 or 25 ppm at weeks 26 and 52, but not at week 130. Liver weight was significantly increased in males receiving 5 or 25 ppm at week 130, but not at weeks 26 or 52. An increased incidence of hepatocellular swelling was noted in males receiving 1, 5, or 25 ppm. Based on these results, the NOEL for female rats was 1 ppm but was

not established for male rats. The LOEL for female rats was 5 ppm and for male rats was 1 ppm.

An additional unpublished chronic study was performed in dogs by Wazeter (1967, cited in Vettorazzi, 1975). The dogs were maintained for 2 years on diets containing chlordane at levels of 0, 0.3, 3, 15, or 30 ppm (approximately 0, 0.008, 0.075, 0.375, or 0.75 mg/kg bw). The effects observed were increased liver weight and histologic changes in the liver. A scientific review panel for WHO/FAO examined this study and concluded that no effects were observed at exposures of 3 mg/kg diet or less. No other data on the experimental design or results of this study were found.

Mice maintained for 4 months on a diet containing 100 ppm chlordane (approximately 14.3 mg/kg bw) had decreased viability of offspring (Deichmann and Keplinger, 1966). Chlordane decreased fertility in male and female rats (Ambrose et al., 1953a) and in female mice (Welch et al., 1971). Ambrose et al. (1953b) reported reduced survival of offspring for rats fed 640 and 1280 ppm chlordane (approximately 32 and 64 mg/kg bw).

4.2.2.2. Heptachlor--Symptoms of acute heptachlor intoxication include tremors, convulsions, paralysis, and hypothermia (U.S. EPA, 1985a). Daily oral administration of 2 or 5 mg/kg bw heptachlor for 78-86 days to pigs, sheep, and rats induced hepatic necrosis and synthesis of smooth endoplasmic reticulum. Rats were the most sensitive species (Halacka et al., 1975). Chronic administration of intramuscular injections in rats with daily doses of 3 and 15 mg/kg bw heptachlor or 1 and 5 mg/kg bw heptachlor epoxide decreased liver size but had no effect on other tissues. Certain hepatic and renal gluconeogenic enzymes were stimulated (Kacew et al., 1973).

Shain et al. (1977) found that feeding of heptachlor (99.8 percent) to male Sprague-Dawley rats for 90 days at an average dose of 1.29 mg/kg bw/day

significantly reduced the food consumption and mean body weight gain in a subgroup of 12 randomly selected rats. In another subgroup of 22 rats, body weight was not reduced and no changes were observed in testicular or ventral prostate. Histologic examination of the rats was not performed, since the study was designed to determine the effect of heptachlor on prostate homeostasis. Rats were castrated 24 hours before sacrifice. Cytoplasmic, but not nuclear, androgen receptor site content of the ventral prostate was significantly increased. Ventral prostate protein content was reduced to 13 percent of control levels, and cell number was reduced to 18 percent of control.

In an unpublished report by IRDC (1973b), a 25:75 mixture of heptachlor: heptachlor epoxide was administered to groups of 100 male and 100 female CD-1 mice in the diet at 0, 1, 5, and 10 ppm (approximately 0, 0.14, 0.71, and 1.43 mg/kg bw) for 18 months. An interim sacrifice of 10 mice/sex/group was performed at 6 months. Female mice at the 10 mg/kg diet level had decreased body weight gain; otherwise, no other effect on body weight or food consumption was observed. Mean liver weight increased significantly in a dose-related manner in treated females and males at 6 and 18 months. This increase was more marked in males. Survival, although underestimated due to the interim sacrifice, was 29 percent for males and 30 percent for females at the highest dose level and 51-66 percent for all other groups, including controls. Increased incidence of hepatocytomegaly in all treated female and male groups occurred in a dose-related manner. The incidence of hyperplastic nodules, as reported by IRDC (1973a), was dose-related at the two highest levels for males and females and was highly significant ($p < 1 \times 10^{-9}$). Reevaluation of histologic slides by six other pathologists resulted in several different interpretations; however, the overall result was a decreased incidence of hyperplasia in favor of a greater incidence of carcinoma. Further discussion of this study is presented

in the Carcinogenicity section.

Davis (1965) administered 0 and 10 ppm (approximately 0 and 1.43 mg/kg bw) heptachlor or heptachlor epoxide to groups of 100 male and 100 female C3H mice for 2 years. For heptachlor, low survival was observed for treated (30 percent) as well as control (34 percent) mice. For heptachlor epoxide, survival was 9.5 percent. A twofold increase over controls in the incidence of hepatic hyperplasia and of benign tumors was observed for both compounds, although reevaluation of the slides by four other pathologists changed the interpretation in favor of a greater number of hepatomas.

Reuber (1977a, 1978), using slides from the study of Davis (1965), found hepatic vein thrombosis and cirrhosis among the heptachlor and heptachlor epoxide (10 ppm in the diet) treated mice. These conditions were not observed in any of the 127 control slides available for review. For heptachlor-treated mice, 13 percent (10 percent of males, 15 percent of females) had hepatic vein thrombosis and 6 percent had venous occlusion with recent liver infarcts. Thrombosis of the cardiac atrium was also present in some mice with hepatic vein thrombosis. The incidence of cirrhosis was 2/86 treated males and 5/77 treated females. For heptachlor epoxide, 10 percent of treated mice (7 percent of males, 11 percent of females) had hepatic vein thrombosis and 9 percent had venous occlusion. Cardiac atrium thrombosis was present in some mice. The incidence of cirrhosis was 12/78 treated males and 15/81 treated females. In addition, liver carcinomas were also observed in the treated mice.

In the NCI (1977b) bioassay of the carcinogenicity of heptachlor, groups of 50 male and 50 female Osborne-Mendel rats and groups of 50 B6C3F1 mice of each sex were maintained on diets containing technical grade heptachlor for 80 weeks plus 21 and 10 weeks of observation for rats and mice, respectively. Controls for the rats consisted of 10 matched and 60 pooled untreated rats

(controls from concurrent and recent bioassays of other related compounds) per sex. For male mice, 20 matched and 100 pooled controls were used, and for female mice, 10 matched and 80 pooled controls were used. Male rats were fed 38.9 or 77.9 ppm (approximately 1.95 or 3.90 mg/kg bw) and female rats were fed 25.7 or 51.3 ppm (approximately 1.29 or 2.57 mg/kg bw) while male mice were fed 6.1 or 13.8 ppm (approximately 0.87 or 1.97 mg/kg bw) and female mice were fed 9.0 or 18.0 ppm (approximately 1.29 or 2.57 mg/kg bw).

For rats, mean body weights of high-dose males and females were consistently depressed, especially in males. The low-dose groups had growth rates similar to controls. Adverse clinical signs such as loss of body weight, rough and discolored hair, and palpable masses developed in treated and untreated groups. In some females from both dose groups, vaginal bleeding developed after 80 weeks. A dose-related but not statistically significant increase in mortality was observed for treated males. The increase was statistically significant for treated female groups. Histopathologic signs of aging, such as chronic nephritis, biliary hyperplasia, and chronic prostatitis, were observed with about equal frequency in all control and treated groups. The incidences of follicular and C-cell hyperplasia in thyroid glands of treated and control groups were observed to be non-treatment-related.

The results in mice indicated no differences in body weight gains among groups. Sores and hair loss occurred in treated and control mice during the first year. Abdominal distention and hair loss were prevalent in high-dose females. Adverse clinical signs developed in mice of all groups. Male mortality was unaffected; however, there was a significant dose-related increase in mortality for treated female groups. Hepatocytomegaly and diffuse hyperplasia occurred infrequently in individuals of all groups.

Witherup et al. (1955) studied the effects of heptachlor on groups of 20

male and 20 female CF rats administered dietary concentrations of 0 to 10 ppm (approximately 0 to 1.43 mg/kg bw) for 110 weeks. Mortality among test groups was not dose-related. Loss of body weight, decreased food consumption, and increased liver weight were noted among treated males but not among females. These signs were marked for males fed 10 ppm. Liver lesions described as "chlorinated hydrocarbon" type and considered to be nonneoplastic were observed in 17 percent of the males and 50 percent of the females at 10 ppm, and in 38 percent of the males and 17 percent of the females at 7 ppm. At dose levels at or below 5 ppm diet, none of these lesions was observed.

In a later study, Witherup et al. (1959) studied the effects of heptachlor epoxide on groups of 25 male and 25 female CFN rats administered the compound in the diet for 108 weeks. The dietary concentrations ranged from 0.5 to 10 ppm (approximately 0.07 to 1.43 mg/kg bw). Control rats received heptachlor epoxide-free diets. No differences were observed with respect to food consumption or growth rate. There was a dose-related increase in liver weight in females. Hepatic cell vacuolization occurred in treated males. Hepatocytomegaly, degeneration, and regeneration in unspecified groups were reported. Mortality was higher than the control level for all treated groups, but a dose-response relationship was not observed.

Jolley et al. (1966) administered a 75:25 mixture of heptachlor:heptachlor epoxide to groups of 25 female CD rats in the diet at concentrations of 5 to 12.5 ppm (approximately 0.25 to 0.625 mg/kg bw) for 2 years. A group of 54 rats received diets free of insecticide. Mortality was increased in a somewhat dose-related way. Comprehensive histologic evaluations were performed. Spontaneous lesions and tumors were present in all groups and included multiple cell-type hypertrophy, telangiectasia in the anterior pituitary, and adrenal hypertrophy. The incidence of hepatocytomegaly was increased over con-

trol levels at 7.5, 10.0, and 12.5 ppm.

Mestitzova (1967) found that heptachlor (98.1 percent) administered in the diet at a dose at 6 mg/kg bw for 52 weeks resulted in markedly decreased litter sizes, decreased lifespan of pups, and the development of cataracts in parent rats and pups. It is unclear whether the 6 mg/kg bw dose was administered daily or whether it represented a total dose consumed over 1 year. In the former case, the dose would be quite high, roughly corresponding to a concentration of 120 mg/kg diet, assuming that a rat consumes 5 percent daily of its body weight as food. In the latter case, the dose would be quite low, resulting in a daily average of approximately 0.016 mg/kg bw/day.

Injection of 1.5 mg/egg heptachlor resulted in a 12 percent reduction in hatchability but no abnormal chicks (Smith et al., 1970). However, it was toxic to sea urchins and many abnormal embryos were induced (Bresch and Arendt, 1977).

4.3. MUTAGENICITY

Evaluations of the published mutagenicity studies on chlordane and heptachlor have recently been completed by the Office of Pesticide Programs (OPP). The OPP memoranda of November 1986 containing descriptions of the studies and evaluations are appended to this document. Presented below are the overall interpretations of the data extracted from the OPP chlordane and heptachlor reports, respectively.

4.3.1. Chlordane

A total of 25 published articles containing information on 34 assays testing technical- or commercial-grade chlordane were reviewed. Approximately two-thirds of the studies were judged unacceptable or inconclusive because of study deficiencies or inadequate reporting of critical information required for independent verification of results. While the remaining nine studies were adequate

to satisfy minimal data requirements for the genetic end points routinely requested by OPP (gene mutation, chromosome damage, and DNA repair), they do not provide a fully comprehensive data set upon which to definitively assess the mutagenic potential of chlordane. The following general conclusions on the mutagenicity of chlordane are warranted:

1. Neither technical- nor commercial-grade chlordane appears to be mutagenic in bacterial assays.
2. Although negative responses have been obtained in some less well-established mammalian cell systems, chlordane and/or one of its isomers have induced positive results without activation in other mammalian cell mutation systems. However, in these positive studies testing in the presence of S-9 activation was not conducted. Hence, the question of whether chlordane induces gene mutations in mammalian cells still needs to be clarified.
3. Chlordane was negative in mouse dominant lethal assays and in an *in vitro* mammalian cell cytogenetics assay; however, there are no data for *in vivo* somatic cytogenetic end points.
4. Chlordane was reported negative in *in vitro* DNA repair assays using mouse, rat, and hamster hepatocytes.
5. Positive effects of chlordane have been reported for sister chromatid exchange (SCE) in fish and human cells in culture and for gene conversion in yeast. All of these assays have had limited utility in genetic toxicology, and the paucity of data for more traditional assays precludes the incorporation of these findings into an overall classification of the mutagenetic potential of chlordane.

4.3.2. Heptachlor

Eighteen published articles containing information on 32 assays were

reviewed for genotoxic effects of heptachlor/heptachlor epoxide. Again, the majority of the assay reports were judged inadequate or inconclusive for reasons mentioned previously for chlordane. The results of these assays, however, were not significantly different from the results in the 10 assays judged to be acceptable. Following are the general conclusions on the mutagenicity of heptachlor.

1. Neither heptachlor nor heptachlor epoxide appears to induce gene mutations in bacteria or mammalian cells in culture.
2. Both heptachlor and heptachlor epoxide were negative in mouse dominant lethal assays. While one study did report an increase in resorptions and "abnormal mitoses" in a three-generation reproduction test, this was only reported as an abstract, and was therefore judged inconclusive. The absence of data for either *in vitro* or *in vivo* direct cytogenetic effects precludes a definitive statement about the clastogenic potential of heptachlor.
3. Heptachlor was negative in *in vitro* DNA repair assays using mouse, rat, and hamster hepatocytes, and in bacterial DNA damage tests using repair-deficient strains.

4.4. CARCINOGENICITY

4.4.1. Animal Studies

4.4.1.1. Chlordane--Chlordane has been studied in four mouse and four rat long-term carcinogenesis bioassays. Tables 5 and 6 present a summary of the experimental design and tumor results. Each study is described in more detail in the following sections.

4.4.1.1.1. Studies with Mice.

4.4.1.1.1.1. Becker and Sell (1979). A 90:10 mixture of chlordane/heptachlor was fed to an unspecified number of male C57B1/6N mice at concentrations

TABLE 5. SUMMARY OF MOUSE DIETARY CARCINOGENICITY TESTS FOR CHLORDANE

Strain	No./sex	Dose (ppm)	Duration of exposure/ observation	Tumor results	Reference source
C57B1/6N	Not specified	25 or 50 ppm	18 mo	Hepatocellular carcinomas in 27% (16) of the survivors. No other information on tumor incidences and time of death was presented.	Becker and Sell, 1979
CD-1	100/M & F	5, 25, or 50 ppm	18 mo	Liver nodules at 25 and 50 ppm (IRDC); hepatocellular carcinomas at 25 and 50 ppm (Reuber)	IRDC, 1973a; U.S. EPA, 1985b
B6C3F1	50/M & F	29.9 & 56.2-M 30.1 & 63.8-F	80 wk plus 10 wk observation	Hepatocellular carcinomas in both males and females	NCI, 1977a
ICR	80/M & F	1, 5, or 12.5 ppm	24 mo	Hepatocellular adenomas and hemangiomas in 12.5 ppm males; nontumor liver lesions in females at 5 and 12.5 ppm and males at 5 ppm	RIASBT, 1983a

TABLE 6. SUMMARY OF RAT DIETARY CARCINOGENICITY TESTS FOR CHLORDANE

Strain	No./sex	Dose (ppm)	Duration of exposure/ observation	Tumor results	Reference source
Albino	3-5/M & F	10, 20, 40, 80, 160, 320 640, 1280 ppm	400 days	No treatment-related tumors; liver lesions at 80 ppm and above	Ambrose et al., 1953a
Osborne-Mendel	20/M & F	5, 10, 30, 150, or 300 ppm	2 yr	High toxicity at 150 and 300 ppm; no treatment-related tumors	Ingle, 1952
Osborne-Mendel	50/M & F	203.5 and 407 ppm-M; 120.8 and 241.5 ppm -F	80 wk plus 29 wk observation	Toxicity in all groups; high mortality in females. Neoplastic nodules of liver in low-dose females; possible thyroid tumors in both males and females	NCI, 1977a
Fischer 344	80/M & F	1, 5, or 25 ppm	130 wk	Hepatocellular adenomas in high-dose males; nonneoplastic lesions of liver at all doses in males and at high dose in females	RIASBT, 1983b

of 25 or 50 ppm (approximately 3.57 or 7.14 mg/kg bw) for 18 months. Specific information as to treatment and observation periods and time of death was not provided. The C57B1/6N mouse rarely develops spontaneous liver lesions and in a group of 200 control mice no liver tumors or nodular lesions were found over 18 months of observation. In mice receiving the chlordane/heptachlor diet, many liver lesions were seen, including both benign proliferative lesions and hepatocellular carcinomas. Of those surviving to the end of the experiment, 27 percent (16 mice) had primary hepatocellular carcinomas, with the first appearing at 36 weeks. No other information was presented as to early deaths and associated tumor incidences. An even greater number of mice had benign proliferative lesions. Data relating tumor incidence to dose were not available. Elevated levels of alpha-fetoprotein were associated with animals with primary hepatocellular carcinomas. Cells from the carcinomas grew when transplanted, producing tumors that were histologically similar to the primary hepatocellular carcinoma. Cells from the benign lesions did not grow when transplanted. The authors concluded that the benign lesions did not appear to be premalignant lesions.

In a companion study, acetylaminofluorene was administered to the same strain of mice (C57B1/6N) at 0.045 or 0.03 percent diet. After 61 weeks, seven (18 percent) of the survivors had liver carcinomas, and three showed growth at 2.4 to 4 months when transplanted. On a comparison basis, chlordane was considered to be a more potent carcinogen than acetylaminofluorene. Another key finding in this study was that chlordane induced hepatocellular carcinomas in a strain of mice that does not spontaneously develop hepatocellular carcinomas.

4.4.1.1.1.2. <u>The International Research and Development Corporation (IRDC, 1973a)</u>. IRDC, under contract to the Velsicol Chemical Corporation, administered analytical grade chlordane in the diet at concentrations of 0, 5,

25, or 50 ppm (approximately 0, 0.71, 3.57, or 7.14 mg/kg bw) for 18 months to groups of 100 male and 100 female CD-1 mice. The mice were 6 weeks of age when exposure began. A 6-month interim sacrifice of 10 mice/sex/group did not reveal compoundrelated lesions. No effect of chlordane on body weight gain or food consumption was observed. However, the survival was greatly reduced at the high-dose levels. A large number of animals were also lost due to autolysis. Only about one-half of the mice were histologically examined. A dose-related increased incidence of hepatocytomegaly was seen in all treated female and male groups. The incidence of hyperplastic liver nodules, as reported by IRDC (1973a), was dose-related at the two highest dose levels ($p < 0.01$). For males, the incidences were: 1/47 (2 percent), 34/52 (65 percent), and 38/50 (76 percent) at 0, 25, and 50 ppm, respectively, whereas for females, the incidences were 0/57 (0 percent), 32/51 (63 percent), and 36/51 (71 percent), respectively. The number of hepatomas was higher, although not statistically at $p = 0.05$, in the 25 ppm group (12) vs. that in the control (5) or 5 ppm groups (6). Only four hepatomas were diagnosed at 50 ppm; however, early deaths and a large amount of autolysis markedly reduced the number of mice at risk. Dr. Reuber (U.S. EPA, 1985b) reexamined the IRDC slides and found highly significant ($p < 1 \times 10^{-9}$) incidences of hepatic carcinoma rather than hyperplastic nodules at the 25 and 50 mg/kg diet levels. Three other independent pathologists (Drs. R. Squire, H. Stewart, and H. Popper) examined subsets of the same slides examined by Reuber and were in close agreement with the diagnosis. Table 7 presents a breakdown of liver lesions as diagnosed by Reuber. The incidences of hepatic carcinoma, as determined by Reuber, are presented in Table 8, along with those found in the NCI study to be discussed next.

4.4.1.1.1.3. National Cancer Institute (1977a). In the NCI bioassay, groups of 50 male and 50 female B6C3F1 mice were fed chlordane consisting of

TABLE 7. INCIDENCE OF LIVER LESIONS AND TUMORS IN CD-1 MICE FOLLOWING DIETARY ADMINISTRATION OF CHLORDANE

Dose (ppm)	Sex	No. of mice examined	No. of mice exhibiting liver lesions and tumors[a]				
			H	N	SC	LC	TC
0	M	33	20(61)	1(3)	0	3(9)	3(9)
	F	45	26(58)	0	0	0	0
5	M	55	34(62)	6(11)	3(5)	2(4)	5(9)
	F[b]	61	32(52)	1(2)	0	0	0
25	M	52	7(13)	3(6)	9(17)	32(62)	41(79)
	F	50	13(26)	4(8)	7(14)	25(50)	32(64)
50	M	39	7(18)	0	4(10)	28(72)	32(82)
	F	37	11(30)	0	2(5)	24(65)	26(70)

[a]H = hyperplasia; N = nodules; SC = small carcinomas (including hyperplastic nodules with focal carcinomas); LC = large carcinomas $\geq$ 5 mm; TC = total carcinomas.
[b]Two liver sarcomas diagnosed in the 5 ppm female group.

SOURCE: IRDC, 1973a; breakdown of liver lesions diagnosed by Reuber (U.S. EPA, 1985b).

TABLE 8. INCIDENCE OF HEPATOCELLULAR CARCINOMA IN MICE FOLLOWING CHRONIC DIETARY ADMINISTRATION OF CHLORDANE

Dose (ppm)	No. of tumor-bearing mice/no. of mice examined (% Positive)	
	Male	Female
Strain CD-1[a]		
0	3/33 (9%)	0/45 (0%)
5	5/55 (9%)	0/61 (0%)
25	41/52 (79%)	32/50 (64%)
50	32/39 (82%)	26/37 (70%)
Strain B6C3F1[b]		
0 (pooled)	17/92 (18%)	3/78 (4%)
0 (matched)	2/18 (11%)	0/19 (0%)
29.9	16/48 (33%)	NA[c]
30.1	NA	3/47 (6%)
56.2	43/49 (88%)	NA
63.8	NA	34/49 (69%)

[a]IRDC, 1973a; tumor incidence as determined by Reuber. Tumor incidences significantly different from controls for 25 and 50 ppm in the diet ($p < 1 \times 10^{-9}$) (U.S. EPA, 1985b).
[b]NCI (1977a); dose-related trend significant at $p < 0.0001$.
[c]NA = Not applicable.

71.7 percent cis-chlordane, 23.1 percent trans-chlordane, 0.3 percent heptachlor, 0.6 percent nonachlor, 1.1 percent hexachlorocyclopentadiene, and 0.25 percent chlordene isomers in the diet for 80 weeks, at two doses--a predicted maximum tolerated dose (MTD) and 1/2 MTD. This was followed by a 10-week observation period. As upward or downward adjustments were made in dose levels, the doses are expressed as time-weighted average (TWA) concentrations. The TWA concentrations for male mice at the high dose and low dose were 29.9 and 56.2 ppm (approximately 4.27 and 8.03 mg/kg bw), respectively, and 30.1 and 63.8 ppm (approximately 4.3 and 9.11 mg/kg bw), respectively, for female mice. Controls consisted of 20 matched control mice of each sex and 100 and 80 pooled male and female control mice, respectively. The results revealed no differences in body weight gain among groups. Tremors were observed in high-dose males and females after 20 weeks. A dose-related increase in mortality was seen in treated males, but not in females. A statistically higher ($p < 0.001$) incidence of hepatocellular carcinoma was found in both males and females (Table 8).

4.4.1.1.1.4. Research Institute for Animal Science in Biochemistry and Toxicology, Japan (RIASBT, 1983a). In this study, conducted for the Velsicol Chemical Corporation (1983b), technical grade chlordane (distribution of isomers not specified) was fed to groups of 80 male and 80 female ICR mice at levels of 0, 1, 5, or 12.5 ppm (approximately 0, 0.14, 0.71, of 1.79 mg/kg bw) for a period of 24 months.

Each group (sex and dose level) consisted of 80 mice of which 8 were sacrificed at 52 weeks. There was no apparent effect of dosing on survival or body weight gain. The mean liver weight was significantly increased for the eight males receiving 12.5 ppm sacrificed at 52 weeks, and the liver-to-body weight ratios were significantly increased for all dosed groups of males when compared with controls. At terminal sacrifice (104 weeks), the mean weight

and organ-to-body weight ratio of the liver were statistically significantly increased in both males and females receiving 12.5 ppm. In addition, the liver-to-body weight ratios of females receiving 1 and 5 ppm chlordane were statistically significantly greater than in controls.

A significant increase ($p < 0.001$) in the incidence of hepatocellular adenoma and hemangioma of the liver was found in the 12.5 ppm male group in animals dying between 19 and 24 months or at terminal sacrifice, as described in Table 9. There was no increase in hepatic tumors in female mice. Other than for the liver tumors in male mice, there were no significant differences in tumors at other sites related to chlordane exposure.

In addition to liver tumors, there were remarkable increases in liver lesions. There were also significant increases ($p < 0.01$) in the incidence of hepatocellular swelling, degeneration, and necrosis in males at 5 and 12.5 ppm and a significant increase ($p < 0.01$) in fatty degeneration at 12.5 ppm. A significant increase ($p < 0.001$) in hepatocellular swelling and degeneration was found in females at doses of 5 and 12.5 ppm. In summary, these liver lesions were observed after 12 months: dose-related increased incidences of hepatocellular swelling and degeneration; fatty degeneration; and necrosis in the livers of males, with a less distinct trend in females.

4.4.1.1.2. Studies with Rats.

4.4.1.1.2.1. Ambrose et al. (1953a, b). In this study, technical grade chlordane was fed to groups of three to five male and female albino rats for 400 days at dietary levels of 0, 10, 20, 40, 80, 160, 320, 640, or 1280 ppm (approximately 0, 0.5, 1.0, 2.0, 4.0, 8.0, 32.0, or 64.0 mg/kg bw). Increased mortality was observed in the 640 or 1280 ppm groups, and retarded growth was observed in all animals at $\geq$320 ppm. No growth retardation was observed in female rats fed $\leq$160 ppm or in male rats fed 10 or 80 ppm. Significantly

TABLE 9. NEOPLASTIC AND/OR TOXIC LESIONS OF THE LIVER IN ICR MICE FED CHLORDANE

	Males/dose (ppm)				Females/dose (ppm)			
	0	1	5	12.5	0	1	5	12.5
No. of tissues examined	71	71	72	72	72	72	71	72
Hepatocellular adenoma	13	13	15	28[a]	1	1	3	1
Hepatocellular adenoma - carcinoma	3	3	7	9	0	0	0	1
Hemangioma	4	1	8	14[a]	0	2	1	0
Hepatocellular swelling and degeneration	5	8	58[a]	59[a]	3	2	24[a]	59[a]
Fatty degeneration	2	0	3	9[b]	1	2	9	9
Necrosis	6	7	23[b]	19[a]	14	8	15	20

[a]Statistically significantly different from control value ($p < 0.001$).
[b]Significantly different from control value ($p < 0.01$).

SOURCE: Velsicol Chemical Corporation, 1983b.

enlarged livers and other liver changes were found in male and female rats fed chlordane at $\geq$ 80 ppm, and pathologic changes in the liver was occasionally found in male rats fed 40 ppm. No treatment-related increase in tumors was found. The study duration (400 days) is considered too short and the number of animals too small for this to be a valid carcinogenicity study.

4.4.1.1.2.2. Ingle (1952). Six groups of 20 male and 20 female Osborne-Mendel rats were fed chlordane for up to 2 years at dietary dose levels of 5, 10, 30, 150, or 300 ppm (approximately 0.25, 0.5, 1.5, 7.5, or 15.0 mg/kg bw). Marked toxicity was encountered at 300 ppm in both males and females. This included high mortality, reduced growth rates, eye and nose hemorrhaging, and severe histopathologic damage to the liver, kidneys, heart, adrenals, lungs, myocardium, and spleen. At 150 ppm, similar but less severe effects were seen. The effects at 30 ppm included inducible tremors and slight liver damage. At 10 ppm, only minor liver damage, such as occasional hepatocytomegaly and mild bile duct hyperplasia, was seen. No symptoms of toxicity, gross or histopathologic changes in the liver, kidneys, lungs, pancreas, testes, ovaries, heart, or spleen were noted at 5 ppm. No treatment-related tumor incidence was found.

4.4.1.1.2.3. National Cancer Institute (1977a). In the NCI carcinogenicity study, groups of 50 male and 50 female Osborne-Mendel rats were fed chlordane (94.8 percent pure) in the diet for 80 weeks, at two dose levels--a predicted MTD and 1/2 MTD. This was followed by a 29-week observation period. As upward or downward adjustments were made in dose levels, the doses are expressed as TWA concentrations. The TWA concentrations for male rats at the low dose and high dose were 203.5 and 407 ppm (approximately 10.2 and 20.4 mg/kg bw), respectively, for males, and 120.8 and 241.5 ppm (approximately 6.04 and 12.08 mg/kg bw), respectively, for females. Ten rats of each sex served as matched controls, and 60 rats of each sex served as pooled controls. Complete

necropsies and histologic examinations were performed, except in the cases of a few spontaneous deaths.

The mean body weight of high-dose males and females was consistently lower than controls. All treated groups had symptoms of toxicity, including loss of body weight, rough and discolored hair, palpable masses, and tumors, that became progressively worse as the study continued. Among female rats, there was a highly significant ($p = 0.003$) dose-related increase in mortality. Mortality was not significantly increased for male rats. Only two hepatocellular carcinomas were observed, one in a low-dose male and one among the pooled controls. A significant ($p < 0.05$) increase in neoplastic nodules of the liver was seen in the low-dose females but not in the high-dose females or in either the high- or low-dose males. A dose-related trend ($p < 0.05$) was found for neoplastic lesions (adenomas and carcinomas) of the thyroid glands (follicular cell and C-cells) for females when compared with the matched controls. However, the results were ambiguous and internally inconsistent. NCI discounted the importance of these findings because the incidences were comparatively low and/or were known to be variable in populations of control rats.

In a more recent review of tumors in Osborne-Mendel rats in the NCI studies (over 900 of each sex), Goodman et al. (1980) presented data indicating 7.1 percent follicular cell tumors in control males and 3.4 percent in control females. These data provide additional support for NCI's decision to discount the importance of apparent increase in thyroid tumors. A highly significant dose-related increase in the incidence of fibrous histiocytoma ($p = 0.0007$) was observed for male rats. This was based on an increase only in the high-dose male group (7/44) as compared to 1/44, 0/8, and 2/58 for the low-dose, matched control, and pooled control groups, respectively. The investigators discounted this finding because they did not believe these lesions to be treatment-related,

as they had occurred spontaneously throughout the bioassay program. All other tumors were common for this strain of rat, and were not treatment-related.

4.4.1.1.2.4. The Research Institute for Animal Science in Biochemistry and Toxicology, Japan (RIASBT, 1983b). In this study, conducted for the Velsicol Chemical Corporation, chlordane (distribution of isomers not specified) was fed to groups of 80 male and 80 female Fischer 344 rats at levels of 0, 1, 5, or 25 ppm (approximately 0, 0.5, 0.25, or 1.25 mg/kg bw) for a period of 130 weeks. Each group (sex and dose level) consisted of 80 rats, of which subsets of eight rats were sacrificed and studied at 26 and 52 weeks. The dose levels were set on the basis of a pilot study in which groups of 5 male and 5 female Fischer 344 rats were fed diets containing 0, 50, 100, 200, 400, or 800 ppm technical grade chlordane for 4 weeks. Hepatocellular swelling and fatty degeneration in the liver were found in both male and female rats at 50 ppm, the lowest dose tested. One ppm was set as the no-effect level, based on an 18-month study in mice (provided by Velsicol) in which changes in the liver were evidenced at 5 ppm chlordane.

In the 130-week study, there were no dose-related effects on mortality, food consumption, water consumption, hematology, clinical chemistry, or urinalysis. Virtually all of the toxic effects were restricted to the liver.

The weight of the liver in females receiving 25 ppm was significantly increased at weeks 26 and 52 but not at week 130, whereas in males receiving 5 and 25 ppm, the liver weight was increased at week 130 but not at the 26- or 52-week sacrifice.

At necropsy, enlargement of the liver was noted in 19 control males and 19, 26, and 32 males dosed at 1, 5, and 25 ppm, respectively. Table 10 presents the tumor and nontumor lesions of the liver. There was a significant increase in adenomas of the liver in males receiving 25 ppm as compared to controls, but

TABLE 10. LIVER TUMORS AND NONNEOPLASTIC LESIONS IN FISCHER 344 RATS FED CHLORDANE[a]

	Males/Dose (ppm)				Females/Dose (ppm)			
	0	1	5	25	0	1	5	25
No. of tissues examined	64	64	64	64	64	64	64	64
Hepatocellular adenoma	1	1	3	9[b]	0	2	0	0
Mesenchymoma	0	3	1	2	0	0	0	0
Hepatocellular swelling	5	15[c]	14	42[b]	7	2	8	38[b]
Hepatocellular necrosis	3	13[c]	11	27[b]	0	0	1	1
Hepatocellular fatty degeneration	26	15[c]	19	22	20	16	19	2
Focal hepatocellular hyperplasia	5	7	3	11	5	10	1	1

[a]The animals sacrificed at weeks 26 and 52 are not included.
[b]Significantly different from control value ($p < 0.001$).
[c]Significantly different from control value ($p < 0.05$).

SOURCE: Velsicol Chemical Corporation, 1983a.

no corresponding effect occurred in females. All of these tumors were found after 104 weeks (mean time to tumor death was 121.8 weeks). There was also a significant increase in fibroadenomas of the mammary gland in females receiving 1 ppm as compared to controls but no significant increase at 5 or 25 ppm.

Nonneoplastic lesions occurred frequently. There was a dose-related increase in the incidence of hepatocellular swelling and necrosis in male rats. When compared to controls, the incidence of hepatocellular swelling was significantly increased in all dosed males, and the incidence of hepatocellular necrosis was significantly increased in males receiving 1 and 25 ppm. The incidence of hepatocellular swelling was significantly higher in females receiving 25 ppm than in controls. There was also an increase in focal hepatocellular hyperplasia in males receiving 25 ppm, but the increase was not significantly different compared to controls. Most of these lesions of the liver occurred after 78 weeks of the study. A slight increase in nonneoplastic liver lesions was seen in the 26- and 52-week sacrifice groups (Table 11).

Chlordane was considered positive for oncogenicity by the authors, since the incidence of hepatic adenomas was significantly increased ($p < 0.001$) in males in the 25 ppm group (9/64 versus 1/64 in controls). The historical incidence of this tumor in F344/CRJ rats for the testing laboratory was 2.5 percent in males and 2.3 percent in females. The control incidence in this study was 1.6 percent.

An independent review of the liver histopathology, conducted by Dr. Gary M. Williams, differed somewhat in that three of the neoplasms of the liver, identified as adenomas by the report authors, were diagnosed as carcinomas by Dr. Williams. In addition, three neoplasms (adenomas) were found that were not diagnosed by the testing laboratory (Table 12). It was noted that only two slices of liver, one from the median lobe and one from the left lobe, were

TABLE 11. LIVER LESIONS IN FISCHER 344 RATS FED CHLORDANE FOR 26 OR 52 WEEKS[a]

Lesion	Males/Dose (ppm)				Females/Dose (ppm)			
	0	1	5	25	0	1	5	25
26 weeks								
Hepatocellular fatty degeneration	0	0	0	1	0	0	1	0
Focal necrosis	0	0	0	0	0	1	0	0
Bile duct proliferation	0	0	0	1	1	0	0	1
52 weeks								
Hepatocellular fatty degeneration	0	2	0	1	0	0	0	0
Hepatocellular swelling	0	0	0	2	0	1	0	3
Focal necrosis	0	0	1	1	0	1	0	1
Bile duct proliferation	0	0	0	0	0	0	1	0
Small granuloma	0	0	0	0	0	0	1	2

[a]Livers from eight rats/group were examined.

SOURCE: Velsicol Chemical Corporation (1983a).

TABLE 12. LIVER NEOPLASMS IN MALE FISCHER 344 RATS FED CHLORDANE[a]

Neoplasm	Dose (ppm)			
	0	1	5	25
Adenoma	1[b]	1	4	8[b]
Carcinoma	1[b]	0	0	2[b]
Total neoplasms	2	1	4	10[c]

[a]Pathology by Gary M. Williams, M.D., dated March 9, 1984.
[b]One animal had both an adenoma and carcinoma.
[c]Significantly different from control value ($p \leq 0.05$).

SOURCE: Velsicol Chemical Corporation (1983a).

taken from rats without grossly observed tumors, and Dr. Williams felt that the number of neoplasms might have increased had more sections been evaluated. In his opinion, the small increase in benign liver neoplasms was considered as weak evidence for the oncogenicity of chlordane in rats.

The increased incidence of mammary fibroadenomas in females receiving 1 ppm chlordane was not considered to be compound/dose related because mammary fibroadenomas were absent in females dosed at higher levels.

Nonneoplastic changes in the liver of both males and females were increased in dosed animals compared to controls. The principal changes consisted of hepatocellular swelling and necrosis. The liver changes were accompanied by an increase in liver weights in males receiving 5 and 25 ppm at 130 weeks and in females receiving 25 ppm at weeks 26 and 52.

It was concluded that, under the conditions of the study, technical grade chlordane caused a significant increase in the incidence of benign hepatocellular tumors when fed at a level of 25 ppm in the diet to male F344 rats for 130 weeks. There was also a significant increase in nonneoplastic lesions of the liver in both male and female rats; namely, an increased incidence of hepatocellular swelling in males receiving 1, 5, or 25 ppm and in females receiving 25 ppm. In addition, there was a significant increase in hepatocellular necrosis in males receiving 1 or 25 ppm, but no corresponding effect occurred in females. The histologic changes in the liver were accompanied by increased liver weights in males at 130 weeks (5 and 25 ppm groups) and females receiving 25 ppm at weeks 26 and 52. A NOEL and LOEL for chronic toxicity in females based on nonneoplastic changes in the liver are 5 ppm and 25 ppm, respectively; the LOEL in males is 1 ppm.

4.4.1.1.3. Other Species.

4.4.1.1.3.1. Wazeter (1967). In this unpublished study, Wazeter, as cited

in Vettorazzi (1975), performed a chronic study with dogs that were fed a diet containing chlordane at levels of 0, 0.3, 3, 15, or 30 ppm (approximately 0, 0.008, 0.075, 0.375, or 0.75 mg/kg bw) for 2 years. Increased liver weight and histologic changes in the liver were reported along with a NOEL of 3 ppm. No tumors were reported. The study duration (2 years) is considered inadequate for a carcinogenicity assay in dogs.

4.4.1.2. Heptachlor--Heptachlor has been studied in three mouse and five rat long-term carcinogenesis bioassays; Tables 13 and 14 present a summary of the experimental design and tumor results for these studies. One long-term chronic study using dogs has also been conducted. These studies are described in more detail in the following sections.

4.4.1.2.1. Studies with Mice.

4.4.1.2.1.1. The Food and Drug Administration (Davis, 1965)--The carcinogenicity of heptachlor and heptachlor epoxide (purity not specified) was studied using groups of 100 male and 100 female C3H mice fed a diet mixture containing 0 or 10 ppm (approximately 1.43 mg/kg bw) for 2 years. Survival was generally low: 50 percent of the controls, 30 percent of the heptachlor-treated, and 9.5 percent of the heptachlor epoxide-treated mice survived the 2-year period; early deaths were due to tumors. FDA pathologists found a two-fold increase in benign liver lesions (hepatic hyperplasia and benign tumors) in the treated animals over the controls, although the incidence of malignant liver tumors was less (Table 15).

A reevaluation was performed by Reuber (1977b), resulting in a change in diagnosis for many benign tumors to liver carcinomas. Hepatic carcinomas in the treated groups were generally large, especially in the epoxide groups, and were frequently multiple, in contrast to carcinomas in control groups, which were smaller and solitary. The incidences of liver carcinoma are presented in Table 16.

TABLE 13. SUMMARY OF MOUSE DIETARY CARCINOGENICITY TESTS FOR HEPTACHLOR AND HEPTACHLOR EPOXIDE

Strain	No./sex	Dose	Duration of exposure/ observation	Turmor results	Reference
C3H	100/M & F	10 ppm	2 yr	Benign liver tumors (FDA); hepatocellular carcinomas in both males and females (Reuber)	FDA (Davis, 1965); U.S. EPA, 1985b
B6C3F1	50/M & F	6.1 and 13.8 ppm-M; 9 and 18 ppm-F	80 wk plus 10 wk observation	Hepatocellular carcinomas in both males and females	NCI, 1977b
CD-1	100/M & F	1, 5, and 10 ppm	18 mo observation	Nodular hyperplasia at 5 and 10 ppm in both males and females (IRDC)	IRDC, 1973b
				Hepatocellular carcinomas at 5 and 10 ppm in both males and females (Reuber)	Reuber, as cited in U.S. EPA, 1985b

TABLE 14. SUMMARY OF RAT DIETARY CARCINOGENICITY TESTS FOR HEPTACHLOR AND HEPTACHLOR EPOXIDE

Strain	No./sex	Dose	Duration of exposure/ observation	Tumor results	Reference
Wistar	95/M & F no. of each sex in dose group not specified	10 mg/kg (5 doses)	5 daily doses then observation for 106-110 wk	No treatment-related increase in tumors	Cabral et al., 1972
Osborne-Mendel	50/M & F	38.9 and 77.9 ppm-M; 25.7 and 51.3 ppm-F	80 wk plus 30 wk observation	No liver tumors; neoplastic nodules in treated and control groups; thyroid tumors in females	NCI, 1977b
CD	25/M & F	5, 7.5, 10, and 12.5 ppm	2 yr	No increase in tumors; some liver lesions at 7.5 ppm and above	Kettering (Jolley et al., 1966)
CF	20/M & F	1.5, 3, 5, 7, and 10 ppm	110 wk	No increase in tumors; liver lesions at 7 and 10 ppm	Kettering (Witherup et al., 1955)
CFN	25/M & F	0.5, 2.5, 5.0, 7.5, and 10 ppm	108 wk	No dose-related increase in tumors (Kettering); slight increase in liver nodules and carcinomas (Reuber)	Kettering (Witherup et al., 1959); U.S. EPA, 1985b

TABLE 15. INCIDENCES OF LIVER LESIONS IN C3H MICE TREATED WITH HEPTACHLOR OR HEPTACHLOR EPOXIDE

Dose group	Hepatic hyperplasia	Benign liver tumors only	Malignant liver tumors only
Controls	38/200	30/200	21/200
Heptachlor (10 ppm)	108/200	51/200[a]	10/200
Heptachlor epoxide (10 ppm)	65/200	85/200[b]	13/200

[a]Statistically significant ($p < 0.0064$).
[b]Statistically significant ($p < 10^{-9}$).

SOURCE: Davis, 1965.

TABLE 16. INCIDENCES OF HEPATOCELLULAR CARCINOMA IN C3H MICE TREATED WITH HEPTACHLOR OR HEPTACHLOR EPOXIDE

Dose group	Males	Females
Controls	22/78 (28%)	2/54 (4%)
10 ppm heptachlor	64/87 (73%)	57/78 (73%)
10 ppm heptachlor epoxide	73/79 (92%)	77/81 (95%)

SOURCE: Davis, 1965 (as diagnosed by Reuber, 1977b).

Reuber's findings yielded a highly significant ($p = 5 \times 10^{-8}$ to $<1 \times 10^{-9}$) increase in incidences of liver carcinoma in both sexes for heptachlor and heptachlor epoxide. Four other independent pathologists (Drs. R. Squire, R. Sternberg, H. Stewart, and G. Williams) reviewed a sample of 19 slides and generally concurred in the Reuber diagnoses (U.S. EPA, 1985b).

In addition to liver tumors, Reuber also diagnosed nontumor liver lesions, primarily hyperplasia, nodules, hepatic vein thrombosis, and cirrhosis, in the heptachlor and heptachlor-epoxide-treated mice.

4.4.1.2.1.2. National Cancer Institute (1977b). In the NCI carcinogenicity bioassay conducted at Gulf South Research Institute, technical grade heptachlor (approximately 73 percent heptachlor, 22 percent trans-chlordane, and 5 percent nonochlor) was fed to groups of 50 B6C3F1 mice of each sex for 80 weeks, followed by an observation period of 10 weeks. The TWA high-dose and low-dose concentrations were 6.1 and 13.8 ppm (approximately 0.87 and 1.97 mg/kg bw) for males and 9.0 and 18.0 ppm (approximately 1.29 and 2.57 mg/kg bw) for females, respectively. Controls consisted of 20 male and 10 female matched control mice, and 100 male and 80 female pooled control mice.

Hepatocellular carcinoma was the most frequently observed neoplasm. The incidence in high-dose males was significantly higher ($p = 0.001$) when compared with matched controls, while the incidence in low-dose males was comparable to that of the control groups. The difference in hepatocellular carcinoma incidence between high-dose females and matched controls was also significant ($p < 0.005$), and there was a highly significant ($p < 0.0001$) dose-related increase in the incidence of hepatocellular carcinomas for female mice due to the difference between the high- and low-dose groups. Other tumor types were observed with low frequency among all groups. The incidence of hepatocellular carcinoma in mice is presented in Table 17.

TABLE 17. INCIDENCE OF HEPATOCELLULAR CARCINOMA IN B6C3F1 MICE FOLLOWING CHRONIC DIETARY EXPOSURE TO HEPTACHLOR/CHLORDANE MIXTURE[a]

Groups (ppm diet)	Incidence[a]
Males	
0 (pooled)	17/92 (18%)
0 (matched)	5/19 (26%)
6.1	11/46 (24%)
13.8	34/47 (72%)[b]
Females	
0 (pooled	3/78 (4%)
0 (matched)	2/10 (20%)
9.0	3/47 (6%)
18.0	30/42 (71%)[c]

[a]Incidence expressed as $\frac{\text{No. of tumor-bearing mice}}{\text{No. of tissues examined}}$

[b]Statistically different from matched controls ($p = 0.0001$); also dose-related increase in male mice ($p < 0.0001$).

[c]Statistically different from matched controls ($p < 0.005$); also dose-related increase in female mice ($p < 0.0001$).

SOURCE: NCI, 1977b.

There were also many nodules in the mice that did not have liver carcinomas.

4.4.1.2.1.3. International Research and Development Corporation (1973b). IRDC, under contract with Velsicol Chemical Corporation, fed a 25:75 mixture of heptachlor:heptachlor epoxide in the diet, at concentrations of 1, 5, or 10 ppm (approximately 0.14, 0.71, or 1.43 mg/kg bw) for 18 months to groups of 100 male and 100 female CD-1 mice, starting at 7 weeks of age. Similar control groups were fed an insecticide-free diet. The incidences of hepatomas were lower among the higher dose groups than in the 1 mg/kg diet group and controls. However, the incidences of nodular hyperplasia (Table 18) were highly significant at 5 and 10 ppm in both males and females when compared with controls. Upon reexamination of the slides, Reuber diagnosed more hepatic carcinomas and less hyperplasia and hyperplastic nodules (U.S. EPA, 1985b). Five other pathologists (Drs. J. Rust, P. Newberne, R. Squire, H. Stewart, and G. Williams) examined a portion of the slides and agreed with Reuber that the incidence of hepatic carcinoma was considerably underdiagnosed in the original analysis (U.S. EPA, 1985b). The incidences of liver carcinoma as diagnosed by Reuber are presented in Table 19.

4.4.1.2.2. Studies with Rats.

4.4.1.2.2.1. Cabral et al. (1972). Heptachlor (96.8 percent pure) in corn oil was administered by gavage to Wistar rats in five doses of 10 mg/kg bw each on alternate days starting at 10 days of age. The heptachlor-treated group contained 95 Wistar rats, 7 of which died before weaning. The controls consisted of 19 males and 27 females treated with corn oil alone. Many rats were lost because of high mortality of both treated and control rats and to an interim sacrifice at 60 weeks. Twenty-nine females and 30 males remained after 60 weeks and comprised the carcinogenicity test groups. All surviving rats were sacrificed at 106 to 110 weeks. There was no indication of a treat-

TABLE 18. INCIDENCE OF NODULAR HYPERPLASIA IN CD-1 MICE EXPOSED TO HEPTACHLOR/HEPTACHLOR EPOXIDE (25:75) MIXTURE

Dose (ppm)	Males	Females
0	0/50 (0%)	1/67 (1%)
1	2/53 (4%)	0/63 (0%)
5	24/57 (42%)	9/56 (16%)
10	53/69 (77%)	28/46 (61%)

SOURCE: IRDC, 1973b.

TABLE 19. INCIDENCE OF HEPATIC CARCINOMA IN CD-1 MICE FOLLOWING CHRONIC DIETARY EXPOSURE TO HEPTACHLOR EPOXIDE (25:75)[a]

Groups (ppm diet)	Incidence[b]
Males	
0	0/62 (0%)
1.0	2/68 (3%)
5.0	18/61 (26%)
10.0	52/80 (65%)[c]
Females	
0	6/76 (8%)
1.0	1/70 (10%)
5.0	6/65 (9%)
10.0	30/57 (53%)[c]

[a]Based on Reuber's reevaluation of IRDC (1973b) slides (U.S. EPA, 1985b).
[b]Incidence expressed as $\frac{\text{No. of tumor-bearing mice}}{\text{No. of tissues examined}}$
[c]Significantly different from control value ($p \leq 0.001$).

ment-related increase in tumors.

4.4.1.2.2.2. Kettering Laboratory (Jolley et al., 1966). In this study, a 75:25 mixture of heptachlor:heptachlor epoxide was administered to groups of 25 female CD rats in the diet at concentrations of 5, 7.5, 10, and 12.5 ppm (approximately 0.25, 0.375, 0.5, and 0.625 mg/kg bw) for 2 years. A dose-related increase in mortality was observed. A comprehensive histological evaluation revealed spontaneous tumors, such as mammary adenomas or fibroadenomas, with random frequency among treatment and control groups. No malignant lesions of the liver were observed, although hepatocytomegaly was increased at 7.5, 10, and 12.5 ppm.

4.4.1.2.2.3. National Cancer Institute (1977b). In the NCI bioassay conducted at Gulf South Research Institute, technical grade heptachlor was fed for 80 weeks to groups of 50 male and 50 female Osborne-Mendel rats at a time-weighted average dietary level of 38.9 or 77.9 ppm (approximately 1.95 or 3.90 mg/kg bw) for males and 25.7 or 51.3 ppm (approximately 1.29 or 2.57 mg/kg bw) for females. Animals were maintained for an additional 30 weeks on a heptachlor-free diet. Ten rats of each sex served as the matched controls, and 60 rats of each sex served as pooled controls.

Overall, the incidence of neoplastic liver lesions was somewhat greater in the heptachlor-treated groups than that observed after chlordane treatment. However, no hepatocellular carcinomas were observed in any of the rats, with one cholangiocarcinoma diagnosed in one low-dose male. Neoplastic nodules were observed in all treated and control groups, with no statistically significant ($p \leq 0.05$) dose-related trend. The incidences are presented in Table 20. A statistically significant exact test for a dose-related trend ($p < 0.002$) was found for follicular-cell carcinomas of the thyroid of females, but not in males, when they were combined with adenomas. However, this finding was dis-

counted by NCI because the incidences of carcinomas were low and because of the variability of thyroid tumors in control rat populations. The more recent review by Goodman et al. (1980) of historical control tumor incidences in the Osborne-Mendel rat used in the NCI studies lends further support to the NCI decision.

TABLE 20. INCIDENCES OF NEOPLASTIC NODULES IN OSBORNE-MENDEL RATS FOLLOWING CHRONIC DIETARY EXPOSURE TO HEPTACHLOR[a]

Groups (ppm diet)	Incidence[b]
Males	
0 (pooled)	2/58 (3%)
0 (matched	1/10 (10%)
38.9	3/44 (7%)
77.9	6/49 (12%)
Females	
0 (pooled)	5/59 (8%)
0 (matched)	1/10 (10%)
25.7	9/48 (19%)
51.3	5/46 (11%)

[a]Not significant ($p \leq 0.05$) by either exact test or life-table adjustment.
[b]Incidence expressed as $\frac{\text{No. of nodule-bearing rats}}{\text{No. of tissues examined}}$

SOURCE: NCI, 1977a.

4.4.1.2.2.4. Kettering Laboratory (Witherup et al., 1955). In this first Kettering study, heptachlor (purity not specified) was administered to groups of 20 male and 20 female CF rats at dietary levels of 1.5, 3, 5, 7, and 10 ppm (approximately 0.075, 0.15, 0.25, 0.35, and 0.5 mg/kg bw) for 110 weeks.

Similar groups of 20 rats served as controls. Benign and malignant tumors were randomly distributed among test and control groups, with greater incidences observed for females, especially at 5 and 7 ppm. Liver lesions, described as the "chlorinated hydrocarbon" type, were observed with high incidence at 7 and 10 ppm in the diet, but no liver lesions were found at lower dose levels. The authors did not believe that the liver lesions were neoplastic. The analysis indicated that the incidence of tumors in treated rats was not significantly different from control incidence.

4.4.1.2.2.5. Kettering Laboratory (Witherup et al., 1959). In the second Kettering study, heptachlor epoxide (purity not specified) was administered in the diet to groups of 25 male and 25 female CFN rats at concentrations of 0.5, 2.5, 5.0, 7.5, and 10 ppm (approximately 0.025, 0.125, 0.25, 0.375, and 0.5 mg/kg bw) for 108 weeks. Similar groups of controls were maintained on heptachlor epoxide-free diets. In the Kettering analysis, malignant and benign tumors occurred randomly among the test groups and were not related to heptachlor epoxide treatment. A reexamination of the histologic slides was conducted by two pathologists, Drs. M. Reuber and G. Williams. Dr. Reuber concluded that the incidence of hepatic carcinomas was significantly increased above control incidence at 5 and 10 ppm in female rats. Dr. Williams found more hepatic nodules at the 10 ppm level in males. Table 21 presents a summary of Reuber's and Williams' diagnoses of liver carcinomas and nodules. Three other pathologists reviewed the Kettering studies and also diagnosed more carcinomas than reported by Witherup (U.S. EPA, 1985b).

4.4.1.2.3. Studies with Dogs.

4.4.1.2.3.1. Kettering Laboratory (U.S. EPA, 1977). In this study, groups of two males and three females were exposed to dietary doses of 0, 0.5, 2.5, 5, and 7.5 ppm heptachlor epoxide (purity not indicated) for 60 weeks, at

TABLE 21. INCIDENCE OF HEPATIC CARCINOMA AND NEOPLASTIC NODULES IN CFN RATS FOLLOWING CHRONIC DIETARY EXPOSURE TO HEPTACHLOR EPOXIDE[a]

Dose (ppm)	Reuber		Williams	
Males	Carcinomas	Nodules	Carcinomas	Nodules
0	1/24 (4%)	6/24 (25%)	0/24 (0%)	0/24 (0%)
0.5	1/22 (5%)	6/22 (27%)	1/23 (4%)	0/23 (0%)
2.5	0/19 (0%)	7/19 (37%)	0/21 (0%)	0/21 (0%)
5.0	1/23 (4%)	9/23 (39%)	1/22 (5%)	0/22 (0%)
7.5	1/25 (4%)	2/25 (8%)	0/25 (0%)	1/25 (4%)
10.0	4/22 (18%)	7/22 (32%)	1/22 (5%)[b]	4/22 (18%)[b]
Females				
0	0/17 (0%)	7/17 (41%)	0/17 (0%)	4/17 (24%)
0.5	3/22 (14%)	8/22 (36%)	0/20 (0%)	5/20 (25%)
2.5	3/18 (17%)	4/18 (22%)	0/18 (0%)	2/18 (11%)
5.0	7/22 (32%)[c]	10/22 (45%)[c]	1/22 (5%)	2/22 (9%)
7.5	3/21 (14%)	14/21 (67%)	0/23 (0%)	5/23 (22%)
10.0	5/19 (26%)[c]	8/19 (42%)[c]	1/19 (5%)	1/19 (5%)

[a]Incidence is expressed as $\frac{\text{No. of tumor-bearing rats}}{\text{No. of rats examined}}$

[b]Statistically significant ($p = 0.019$) for combined incidence of carcinomas and nodules (U.S. EPA, 1985b).

[c]Statistically significant ($p = 0.05$) for combined incidence of carcinomas and nodules (U.S. EPA, 1985b).

SOURCE: Witherup et al., 1959. Data from the Reuber/Williams diagnoses presented in U.S. EPA, 1985b.

which time they were sacrificed and autopsied. No tumors were reported. While the liver weights of both males and females tended to increase logarithmically in proportion to the amounts of heptachlor epoxide in the diet, only one male at the highest dose had observable hepatic damage. The damage was characterized by cloudy swelling of the cells with slight clumping of the cytoplasm. The study duration (60 weeks) is considered too short and the number of animals too small for this to be a valid carcinogenicity study.

4.4.2. Epidemiologic Studies

4.4.2.1. Infante et al. (1978)--Infante et al. (1978) presented data on five case reports of neuroblastoma and six case reports of aplastic anemia and acute leukemia. Between December 1974 and February 1976, 14 cases of neuroblastoma were diagnosed at a single pediatric hospital. Of these, a history of exposure to toxic agents indicated that five had previous exposure to chlordane. The cases ranged in age from 2 years, 8 months to 6 years, 5 months. Exposure in each case was due to the use of chlordane in the home due to pest infestation. In two of the five cases, the first exposure occurred during the first trimester of pregnancy in the mother. One of these included additional exposure to a halothane-nitrous oxide anesthetic during the first month of pregnancy. In the remaining three cases, exposure occurred after birth. Two of the five cases had postnatal exposure to X-rays. The authors stated that of the other nine cases admitted to the hospital during this time period, a history of exposure to chlordane was not known.

According to the authors, three cases of aplastic anemia and three cases of leukemia were reported "at several hospitals in the past several years." The three aplastic anemias were in males with exposure to chlordane in the home. In each case, the man had actually applied the chemical himself. In two cases, exposures to other toxic agents, including other insecticides,

paints, thinners, and varnishes were reported. The ages of the three men were 15, 28, and 68.

Of the three leukemia cases, only one was reported to have been exposed to other toxic substances besides chlordane. This 23-year-old man was employed by a lawn care company for which he sprayed lawns with Banvel D, diazinon, and 2,4-D, as well as chlordane. The second case was a 9-year-old girl who had been exposed to chlordane annually since birth through the use of chlordane in the home to treat termites. The third leukemia case was a 37-year-old male who had used chlordane around his house for a period of 10 years.

The authors reported on several case studies of exposure to chlordane or heptachlor. As no control group was used as a comparison population, these data provide inadequate information with which to assess the association of cancer risk and chlordane or heptachlor exposure.

4.4.2.2. Wang and MacMahon (1979a)--A retrospective cohort mortality study of 16,126 professional pesticide applicators was undertaken to study the mortality patterns of these workers. The cohort was selected from three pest control companies with offices in over 40 states. Personnel records for all persons in job categories potentially involving exposure to various pesticides and employed between January 1, 1967 and June 30, 1976, were reviewed for two of the companies. For the third company, employee records were not available for the period prior to January 1, 1968; therefore, this date was used as the earliest date of eligibility for this company, with a closing date of December 31, 1976. From 44,083 records, 16,126 subjects were identified who met the following criteria: (1) male, (2) employed for 3 months or more, and (3) social security number, date of birth, and employment dates were also available. Of those not eligible, a sample of 4,000 were examined. Eleven percent had been excluded because they were female, 71 percent because they were employed for

less than 3 months, and 18 percent because of missing identifying information.

Individual follow-up was not attempted. Identifying information for the 16,126 eligible subjects was submitted to the Social Security Administration (SSA). The SSA identified 311 deaths, of which 269 death certificates were obtained from the states or registration areas. The authors stated that because there was no reason to believe that the 42 deaths for which death certificates could not be found were distributed differently than those for which certificates were found, observed numbers were "corrected" by distributing the 42 cases according to the distribution of obtained death certificates and adding the results to the observed values for those with obtained death certificates.

In order to develop some measurement for intensity of exposure to pesticides, job titles were classified as either normal, moderate, or heavy exposure. Particular interest was focused on those applicators exposed to chlordane and heptachlor by conducting separate analyses for persons ever holding jobs as termite control operators, a job category likely to have exposure to these two pesticides. If a worker held more than one job he was classified under the category of exposure corresponding to the job held for the longest period of time.

Expected numbers of deaths by cause were calculated by means of a computer program by Munson (1974), using national mortality rates of white males specific for 5-year categories of age and calendar year. SMRs were calculated along with 95 percent confidence intervals by an extension of the Cornfield method. The authors presented SMRs based on the corrected number of observed deaths. For this review, SMRs based on the true numbers of observed deaths were calculated whenever possible. Based on the corrected observed numbers, the SMR for all causes was 84 (observed = 311, 95 percent CI 75-94). The SMR for all malignant neoplasms was 83, which was statistically nonsignificant. Increased

SMRs were observed for skin cancer (SMR = 173, statistically nonsignificant) and for bladder cancer (SMR = 277, corrected observed = 3.5, 95 percent CI 101-761). Statistically significant SMR deficits were observed for malignant neoplasms of the digestive organs and peritoneum (SMR = 46, corrected observed = 6.9, 95 percent CI 22-95), respiratory system diseases (SMR = 29, corrected observed = 4.6, 95 percent CI 12-70), diseases of the digestive system (SMR = 55, corrected observed = 11.6, 95 percent CI 31-98), and all other causes (SMR = 355, corrected observed = 15, 95 percent CI 33-90).

SMRs for various specific causes of death using the true number of observed deaths can be calculated and are presented here. For the total cohort they are as follows: SMR for all causes of death is 84 (observed = 269); for malignant neoplasms, the SMR is 75 (observed = 47); for cancer of the digestive organs and peritoneum, the SMR is 40 (observed = 6); for skin cancer, the SMR is 150 (observed = 3); for bladder cancer, the SMR is 231 (observed = 3); for non-malignant diseases of the respiratory system, the SMR is 25 (observed = 4); and for non-malignant diseases of the digestive system, the SMR is 48 (observed = 10).

Analyses were also conducted for those workers classified as termite control operators, since they were thought to have had the most exposure to chlordane and heptachlor. SMRs were presented for termite control operators and for all other applicators for various causes of death. These SMRs were based on corrected observed values. The SMR for all causes of death for the termite control operators was 92 and for all other applicators was 78. For malignant neoplasms, the SMR for both groups was 83. Elevated SMRs were observed for each group for cancer of the skin (SMR for termite control operators = 148, SMR for all other applicators = 187), and cancer of the bladder (SMR for termite control operators = 215, SMR for all other applicators = 187). None of

the above-mentioned SMRs was statistically significant. The authors stated that the only significant SMRs ($p < 0.05$) observed for termite control operators were for cancer of the digestive organs (SMR = 20, corrected observed = 1.2) and for cerebrovascular disease (SMR = 39, corrected observed = 2.4). The authors also stated that there were no significant findings in the "all other applicator" group, nor were there significant differences in SMRs between the two groups. (The method used to determine significant differences between the two groups was not reported.) A nonsignificant increase in lung cancer was restricted to the "all other applicator" group (SMR = 131, corrected observed = 16.9). It is possible to calculate SMRs using the true number of observed deaths for cancer of the skin and bladder. For skin cancer, the SMR for termite control operators was 120, and for all other applicators was 167. For bladder cancer, the SMR for termite control operators was 200, and for all other applicators was 250.

Workers were classified according to intensity of exposure into three groups: minimal, intermediate, and highest exposure. None of the causes of death exhibited an increase in SMR with an increase in intensity of exposure. In fact, SMRs based on corrected observed values for lung cancer and skin cancer tended to decrease with intensity of exposure. For lung cancer SMRs of 138, 120, and 87 were observed for minimal, intermediate, and highest exposure, respectively, and for skin cancer SMRs of 198, 187, and 138 were observed, respectively. The authors did not discuss the statistical significance of these findings.

A latency analysis was performed for lung cancer. However, few workers were followed for more than 10 years since first employment, and none of the lung cancer deaths had been followed for that length of time. Positive trends toward an increase in lung cancer deaths occurred as the period of latency

increased.

There are several limitations to this study. The major limitation is the distribution of deaths without death certificates according to the distribution of those with death certificates. If the age distribution (or the distribution of any other disease-related variable) of the group without death certificates had been different from that of the group with death certificates, the cause-specific death distribution could have been different, and could have resulted in inaccurate calculation of observed numbers.

There was also no quantitative information available on levels of exposure, and duration of exposure could only be assessed for lung cancer because of small numbers of observed cases. It is not possible to assess risks for chlordane and heptachlor exposure independently of the risks from other possible exposures. While the termite control applicators may have had a greater likelihood of chlordane and heptachlor exposure than other applicators, their exposure to other pesticides cannot be ignored.

The authors did not individually follow up each cohort member. The only method used to ascertain vital status was a search through SSA records. Thus, the number of deaths may have been underreported. There was no control of confounding factors such as smoking and alcohol consumption. This study provides inadequate evidence on the carcinogenicity of chlordane and heptachlor.

4.4.2.3. Wang and MacMahon (1979b)--A retrospective cohort mortality study was undertaken in two chlordane and heptachlor manufacturing plants between 1946 and 1976. Personnel records were available for 951 workers who had ever worked at a chlordane production plant in Marshall, IL, and for 1425 workers who had ever worked at a heptachlor and endrin plant in Memphis, TN before the spring of 1976. Of these, 1,403 subjects were identified who met the following criteria: (1) male, (2) who had worked for more than 3 months, and (3) "adequate"

identifying information was available. Of the 973 excluded, 7 percent were excluded because they were female, 64 percent because they worked less than 3 months, and 29 percent because identifying information was not available.

The SSA identified 104 deaths in the study cohort through the end of 1975. Nine additional deaths, not identified by SSA, were discovered during the conduct of a separate study (reference not reported) that individually followed up terminated workers from these plants. Death certificates were obtained from the appropriate states for the 113 deaths (98 percent of those ascertained) and were coded according to the 8th revision of the ICD by one of the authors (Wang). Cause-specific SMRs were calculated by means of Munson's (1974) computer program, using national mortality rates for white males by age and calendar year in 5-year groups. For the computation of person-years, the beginning dates were January 1, 1946 for the Marshall plant and January 1, 1952 for the Memphis plant, or at the end of 3 months of employment if that date was later than the appropriate date above. The authors calculated 95 percent confidence intervals by an iterative method based on mid-p values.

The authors attempted to correlate the intensity of exposure with mortality experience. Complete occupational histories were not available for each worker, and serum levels of pesticides actively used in 1975 and 1976 did not correlate with a classification of presumed exposure based on job category; however, no data were presented. Thirty-four percent of the study cohort had less than 10 years duration of follow-up, 25 percent had 20 to 29 years of follow-up, and 11 percent had more than 29 years of follow-up.

The overall SMR for all causes of death was 72 (observed = 113, 95 percent CI 59-86), confirming the healthy worker effect. A deficit was seen in the SMR for all cancers (SMR = 82), but this was not statistically significant. Statistically nonsignificant deficits were observed for malignant neoplasms: for

cancer of the digestive organs and peritoneum, the SMR was 82; for lymphatic and hematopoietic cancers the SMR was 30; and for all other cancers combined the SMR was 45. A statistically nonsignificant excess was observed for lung cancer; the SMR was 134 ($p > 0.05$). The only significantly elevated SMR was for cerebrovascular disease (SMR = 183; observed = 17, 95 percent CI 110-287). Only one death was attributed to liver cancer: an 81-year-old man who died in 1958 and had worked for the company for 5 years beginning in 1944. A significant deficit was observed for ischemic heart disease (SMR = 69, observed = 37, 95 percent CI 49-94).

Because the SMR for lung cancer was elevated (SMR = 134), though not statistically significant, the distribution of lung cancer deaths across other variables was investigated. For workers less than 35 years old at entry into occupation and less than 50 years old at observation, the authors noted that the difference between observed and expected (5 and 1.2, respectively) was statistically significant ($p < 0.01$). The relationship between duration of employment and duration of follow-up or latency for lung cancer was studied. The numbers were small and no overall pattern was observed. However, the results indicate that there was a statistically significant deficiency (p-value not reported) in lung cancer deaths (observed = 1, expected = 4) among those exposed for 20 or more years. Conversely, among those employed for 10 to 19 years and followed for 10 to 19 years, there was an excess risk (observed = 6, expected = 2.1).

There are limitations to this study. There was no information available on levels of exposure or duration of exposure. It is not possible to assess risks for chlordane and heptachlor exposure independently of the risks of endrin exposure, a pesticide also manufactured at the Memphis plant.

The cohort included all plant employees, including those with little

potential for exposure, such as office workers; and therefore, the risk of cancer may have been underestimated for those directly involved in chlordane and heptachlor manufacture. While the period of follow-up was long, the size of the cohort was very small. Thus, the study had very little power to detect a real difference if one was present.

The authors did not individually follow up each cohort member. Therefore, the number of deaths may have been underreported. Excluded from the cohort were 282 individuals with missing data, representing about 29 percent of the final cohort population. The authors provide no data with which to assess the impact of excluding these workers from the cohort; however, this may be a random occurrence. If this assumption is true, then their exclusion poses no bias to the estimates of risk.

There was no control of confounding variables such as smoking. However, a significantly low SMR was observed for ischemic heart disease, a disease for which smoking is a well-documented risk factor. The SMR for other respiratory diseases was also low, though not significantly. This result suggests that the cohort in question smoked at levels below that of males nationally, and provides additional evidence that the reported excess incidence of lung cancer (SMR = 134) may have been an occupationally related increase. Finally, race was assumed to be white for all study subjects. As no deaths among non-whites were observed, this assumption may be a valid one. Thus, this study provides inadequate evidence for the carcinogenicity of chlordane and heptachlor.

4.4.2.4. Ditraglia et al. (1981)--A retrospective cohort mortality study of employees at four organochlorine pesticide manufacturing plants was undertaken. All workers (race and sex not specified) who had at least 6 months of employment in pesticide manufacture prior to December 31, 1964 were included in the study population. Vital status was determined for each worker as of December

31, 1976, through the SSA, State motor vehicle offices, U.S. Postal Mail Corrective Services, and "other" sources.

Four separate cohorts representing four pesticide plants comprised the study population. Only one plant had manufactured chlordane and had done so since 1946. No other pesticides were manufactured at this plant, but other chemical products manufactured there included chlorine and dicyclopentadiene. The cohort at plant 1 consisted of 327 individuals, representing 8,354 person-years of observation. Three percent were lost to follow-up. Plant 2 had manufactured heptachlor since 1951. Endrin was also produced at this plant in addition to chlorine, chlorendic anhydride, hexachlorocyclopentadiene, and vinyl chloride. The cohort at this plant consisted of 305 workers (5,672 person-years of observation), and 5 percent were lost to follow-up. The two remaining plants were not involved in the production of chlordane or heptachlor and, thus, results are not discussed here for these two plants. The chlordane and heptachlor plants had previously been studied by Wang and MacMahon (1979b), who combined the two plant populations into a single cohort with a longer period of follow-up in comparison to the follow-up period reported in this study (Ditraglia et al., 1981).

Death certificates for all known decedents were obtained and coded by a nosologist to the ICD-A in effect at the time of death. Those with an unknown vital status were assumed alive as of December 31, 1976. SMRs were calculated by using the U.S. white male age-, calendar time-, and cause-specific mortality rates. Statistical significances between the observed and expected values were tested with the Poisson distribution. Confidence intervals were presented for the SMR estimates, but the method of calculation was not reported by the authors.

Statistically significant ($p < 0.05$) deficits were observed for all causes of death at the chlordane plant (SMR = 68, observed = 59, 95 percent CI 52-87)

and at the heptachlor plant (SMR = 66, observed = 24, 95 percent CI 42-98). For deaths due to malignant neoplasms, the chlordane plant had an SMR of 69, and the heptachlor plant had an SMR of 91, none being statistically significant. At the chlordane plant, risks were elevated for stomach cancer (SMR = 303), rectal cancer (SMR = 178), pancreatic cancer (SMR = 110), and respiratory system cancer (SMR = 110). None of these risks was statistically significant. At the heptachlor plant, excess risk was observed for intestinal cancer (SMR = 175), respiratory system cancer (SMR = 122), and cancer of the bladder and urinary system (SMR = 606). These risks were also not statistically significant.

An analysis by latency was also carried out. SMRs were calculated for deaths due to all malignant neoplasms according to latency, which was defined as the number of years from date of first employment. Latency was categorized into three time periods: (1) less than 10 years since first employed, (2) 10 to 19 years since first employed, and (3) 20 or more years since first employed. In the chlordane plant, SMRs of 66, 90, and 60 were reported for all malignant neoplasms for the three latency time periods, respectively. The individual point estimates were not significant. There were no observed deaths in the less than 10-year latency period in the heptachlor plant. The SMR for all malignant neoplasms for the 10- to 19-year latency period was 91, and for the 20 or more year period the SMR was 162. These were not statistically significant; however, there may have been a positive trend of increasing risk with increasing latency at the heptachlor plant.

This study revealed excess risk of cancer at various tissue sites for all workers at these two plants. While none of the SMRs was statistically significant, the SMR of 303 (observed = 3) for stomach cancer in the chlordane plant may be important. However, there are limitations to this study.

No information on quantitative exposures were provided. It was not pos-

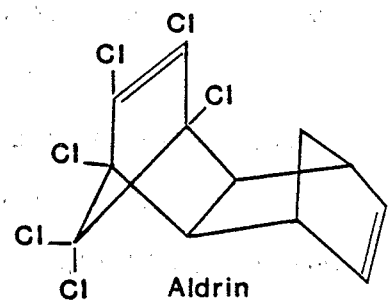
sible to assess the effects of chlordane or heptachlor independently of the other toxic chemicals at the two plants, some of which are known to be carcinogenic. No attempt was made to exclude or adjust for the effects of sex or race, nor was there an effort to control for other confounding variables such as smoking or alcohol consumption. However, the size of the study populations would preclude any real analysis of these variables. Workers with little or no occupational exposure to these chemicals (i.e., office workers, etc.) were included in the cohort. Thus, risks may have been underestimated for workers involved in the day-to-day manufacture of chlordane or heptachlor. The size of the study population was small in spite of follow-up periods of 25 years or more. Thus, the power of this study to detect a statistically significant result is limited. This study provides inadequate evidence to link chlordane or heptachlor exposure to cancer.

4.5. STRUCTURE-ACTIVITY RELATIONSHIPS

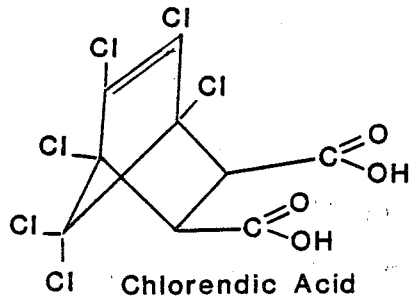
Seven chemicals were identified as important in the analysis of the strucure-activity relationships of chlordane and heptachlor for carcinogenicity. These chemicals are aldrin, chlorendic acid, dieldrin, endrin, endosulfan, hexachlorocyclopentadiene, and isodrin; their structures are presented in Figure 4.

4.5.1. Aldrin

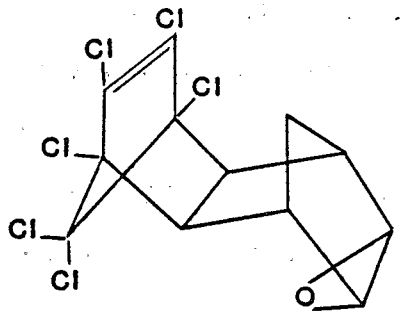
4.5.1.1. Evidence for Carcinogenicity - Human Exposure (IARC, 1982)--Specific mention of aldrin in analytic epidemiologic studies is limited to reports of the follow-up of a cohort of men employed in its manufacture at a plant where dieldrin, and later endrin and telodrin, were also manufactured. In the most recent report, of 166 men exposed to these compounds for more than 4 years and for 15 or more years before the end of follow-up, two cases of cancer were observed. No estimate was provided of the expected number of cancers, and the



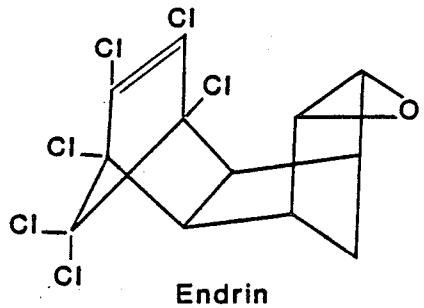
Aldrin



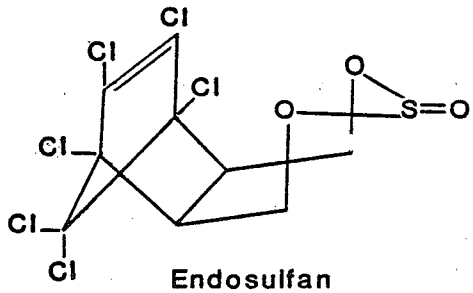
Chlorendic Acid



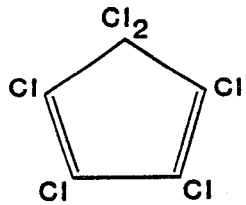
Dieldrin



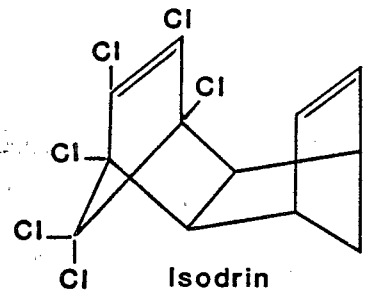
Endrin



Endosulfan



Hexachlorocyclopentadiene



Isodrin

Figure 4. Chemicals selected for structure-activity analysis.

description of follow-up was limited. These data are inadequate to assess the carcinogenicity of aldrin in humans.

4.5.1.2. Evidence for Carcinogenicity - Animal Studies (IARC, 1982)--Aldrin has been tested by the oral route in mice and rats. It was carcinogenic in mice at 10 ppm in the diet, producing malignant liver neoplasms: three studies in rats were negative or equivocal and one was inadequate due to decreased survival rates. These data provide sufficient evidence for the carcinogenicity of aldrin.

4.5.1.3. Evidence for Activity in Short-Term Tests (IARC, 1982)--Aldrin did not produce damage to *Escherichia coli* plasmid DNA and was not mutagenic to *Salmonella typhimurium* or to yeast. It did not induce recessive lethal mutations in *Drosophila melanogaster*. Aldrin induced DNA repair in cultured human fibroblasts and lymphocytes, but it did not elicit unscheduled DNA synthesis in cultured rat hepatocytes. It produced chromosomal aberrations in bone-marrow cells of rats and mice exposed *in vivo*, but it did not elicit a positive response in the mouse bone-marrow micronucleus test. No data on human cell lines or lymphocytes were available. These data are inconclusive in assessing the carcinogenicity of aldrin.

4.5.2. Chlorendic Acid

4.5.2.1. Evidence for Carcinogenicity - Human Exposure (NTP, 1985)--Chlorendic acid is manufactured in an essentially closed system. Although this procedure would seem to minimize human exposure, there are no published data on the level of occupational exposure to chlorendic anhydride or chlorendic acid.

4.5.2.2. Evidence for Carcinogenicity - Animal Studies (NTP, 1985)--In an NTP bioassay, there was clear evidence of carcinogenicity for chlorendic acid in male F344/N rats as shown by increased incidences of neoplastic nodules of the liver and acinar cell adenomas of the pancreas. Increased incidences of alve-

olar/bronchiolar adenomas and preputial gland carcinomas may also have been related to the administration of chlorendic acid. There was evidence of carcinogenicity for chlorendic acid in female F344/N rats as shown by increased incidences of neoplastic nodules and of carcinomas of the liver. There was evidence of carcinogenicity for chlorendic acid in male B6C3F1 mice as shown by increased incidences of hepatocellular adenomas and hepatocellular carcinomas. There was no evidence of carcinogenicity for chlorendic acid in female B6C3F1 mice given chlorendic acid in the diet at concentrations of 620 or 1,250 ppm for 103 weeks. These data provide sufficient evidence for the carcinogenicity of chlorendic acid.

4.5.2.3. Evidence of Activity in Short-Term Tests (NTP, 1985)--Chlorendic acid was not mutagenic in strains TA100, TA98, TA1535, or TA1537 of *Salmonella typhimurium* in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or male Syrian hamster liver S9 when tested according to the preincubation protocol. Chlorendic acid was mutagenic in the L5178Y/TK^{+}/$^{-}$ mouse lymphoma cell forward mutation assay in the absence of S9 and was not tested in the presence of S9. There was no mutagenic response in the absence of severe toxicity. The toxicity curve was sharp, going from relative total growth of 74 percent to 5 percent at doses of 1,600 to 1,700 µg/mL. With such a sharp toxic response, the increase in mutant count and mutant frequency was observed only at the higher dose; this response was replicated in another experiment. A response in which only one toxic response is positive raises the question of whether the mutagenic response is an indirect mechanism in which the mutation is not due to the direct interaction of the chemical with DNA. This assay, as performed, does not answer this question. These data are inconclusive in assessing the carcinogenicity of chlorendic acid.

4.5.3. Dieldrin

4.5.3.1. Evidence for Carcinogenicity - Human Exposure (IARC, 1982)--Mean tissue levels of dieldrin were elevated in one autopsy study of 50 cancer patients and 42 comparison subjects. Mean serum levels were also reported to be elevated in eight cancer patients compared with seven controls. Follow-up of some 233 workmen (166 employed for more than 4 years and followed for 15 or more years) showed two cancer deaths at a plant where aldrin and dieldrin, and later endrin and telodrin, were manufactured. These data are inadequate to assess the carcinogenicity of dieldrin.

4.5.3.2. Evidence for Carcinogenicity - Animal Studies (IARC, 1982)--Dieldrin is carcinogenic to mice, producing benign and malignant liver neoplasms in many strains following oral administration. Studies in rats are negative or equivocal. No carcinogenic effect was observed in feeding studies with hamsters. Feeding studies with dogs and monkeys were considered to be inadequate for a carcinogenicity evaluation. These data provide sufficient evidence for the carcinogenicity of dieldrin.

4.5.3.3. Evidence for Activity in Short-Term Tests (IARC, 1982)--Dieldrin did not produce DNA breaks in an *Escherichia coli* plasmid. It did elicit DNA repair in cultured human fibroblasts and human lymphocytes, but it did not do so in cultured rat hepatocytes and did not produce DNA damage in cultured Chinese hamster cells. It was not mutagenic to *Salmonella typhimurium*, to yeast, to *Drosophila melanogaster* (recessive lethals) or to the wasp, *Bracon hebetor*. In a limited study, it was weakly mutagenic to Chinese hamster V79 cells. It produced chromosomal aberrations in human embryonic lung cells *in vitro* and chromosomal damage to mouse bone-marrow cells *in vivo* in one study but not in another, and not in Chinese hamster bone-marrow cells. It did not induce cell transformaton *in vitro* (in BHK cells). It was negative in the mouse dominant-

lethal test and did not induce mitotic gene conversion in yeast in a host-mediated assay. No data on human cell lines or lymphocytes were available. These data are inconclusive in assessing the carcinogenicity of dieldrin.

4.5.4. Endrin

4.5.4.1. Evidence for Carcinogenicity - Human Exposure (IARC, 1974)--A study was conducted in 1968 of 233 workers employed in a factory that had been manufacturing aldrin and dieldrin since 1954-1955, endrin since 1957, and telodrin during 1958-65. Lengths of exposure ranged from 4 to 13.2 years (average, 7.6 years). One hundred and eighty-one workers were still employed by the same firm at the time of the study, and their average age was 41 years (range, 22 to 64). Only two deaths had occurred, and one was due to stomach cancer. The 52 workers who had left the company were the subject of a subsequent report. Average age at the time of this survey was 47.4 years (range, 29 to 72), average occupational exposure was 6.6 years (4.0 to 12.3), and average time since end of exposure was 7.4 years (4.5 to 16). Only one death was recorded, and this had not been caused by cancer. These data are inadequate to assess the carcinogenicity of endrin.

4.5.4.2. Evidence for Carcinogenicity - Animal Studies (IARC, 1982)--There was no evidence of carcinogenicity in B6C3F1 mice or Osborne-Mendel rats fed endrin, although there was early mortality in both species. Another rat study was inadequate. These data are inadequate to assess the carcinogenicity of endrin.

4.5.4.3. Evidence for Activity in Short-Term Tests (NTP, 1985)--Endrin did not cause mutations in NTP *Salmonella* mutagenicity tests (NTP, 1985). These data are inadequate to assess the carcinogenicity of endrin.

4.5.5. Endosulfan

4.5.5.1. Evidence for Carcinogenicity - Human Exposure (NCI, 1978)--Specific mention of endosulfan in analytic epidemiologic studies was not found. Medical

reports mention fainting, convulsions, and unconsciousness following exposure to endosulfan; however, recovery was rapid. These data are inadequate to assess the carcinogenicity of endosulfan.

4.5.5.2. Evidence for Carcinogenicity - Animal Studies (NCI, 1978)--In an NCI bioassay, no conclusion was drawn on the carcinogenicity of endosulfan for male rats or male mice because of early deaths in these groups. Under the conditions of the bioassay, endosulfan was not carcinogenic in female Osborne-Mendel rats or in female B6C3F1 mice. These data are inadequate to assess the carcinogenicity of endosulfan.

4.5.5.3. Evidence for Activity in Short-Term Tests (NTP, 1985)--No evidence of the mutagenicity of endosulfan was noted in the standard Ames test and in the mouse micronucleus test. These data are inadequate to assess the carcinogenicity of endosulfan.

4.5.6. Hexachlorocyclopentadiene

4.5.6.1. Evidence of Carcinogenicity - Human Exposure (U.S. EPA, 1984)--Although there is human experience with respect to mortality, there is only limited information on the morbidity results in those exposed to hexachlorocyclopentadiene (HEX). Acute inhalation produces a high prevalence of headaches and severe irritation of the eyes, nose, throat, and lung. Dermal contact can cause severe burns. Epidemiologic studies have generally shown no significant differences in mortality between workers exposed to HEX in the workplace and the general population. Although a significant excess of deaths from cerebrovascular disease was reported in one study, the deaths showed no consistent pattern with respect to duration of employment or follow-up. Current human exposure is limited to improper handling and disposal and proximity to either manufacturing sites utilizing HEX or disposal sites. These data are inadequate to assess the carcinogenicity of HEX.

4.5.6.2. Evidence of Carcinogenicity - Animal Studies (U.S. EPA, 1984)--*In vivo* bioassays have not been conducted; however, an inhalation bioassay of HEX has been scheduled by NTP.

4.5.6.3. Evidence for Activity in Short-Term Tests (U.S. EPA, 1984)--The available evidence suggests that HEX is not a mutagen. Negative mutagenicity results were obtained in bacteria, liver epithelial cells, Drosophila, mouse lymphoma cells, and in the mouse dominant lethal test. In addition, HEX did not induce unscheduled DNA synthesis in rat hepatocytes. HEX was not a carcinogen *in vitro* in transformation assays using BALB/3T3 cells. These data are inadequate to assess the carcinogenicity of HEX.

4.5.7. Isodrin

4.5.7.1. Evidence of Carcinogenicity - Human Exposure (HSDB, 1985)--No data of occupational exposure to this isomer of aldrin were found.

4.5.7.2. Evidence of Carcinogenicity - Animal Studies (HSDB, 1985)--The toxicity of isodrin is stated to be twice that of aldrin (species tested and parameters examined were not specified). No other data on the carcinogenicity of isodrin were available.

4.5.7.3. Evidence for Activity in Short-Term Tests (HSDB, 1985)--Isodrin is converted to endrin by liver microsomes, and endrin did not cause mutations in NTP Salmonella mutagenicity tests. These data are inadequate to assess the carcinogenicity of isodrin.

5. RISK ESTIMATION FROM ANIMAL DATA

5.1. SELECTION OF DATA

For some chemicals, several studies in different animal species, strains, and sexes at several doses and different routes of exposure may be available. A choice must be made as to which data sets should be used to quantify human risk by low-dose extrapolation. The following procedure was used to make this choice. The animal studies are evaluated qualitatively to assure that only properly conducted studies are used. The tumor incidence data were separated according to organ sites and tumor types. The data sets used in the model are the ones in which the tumor incidence is statistically significantly higher in at least one test dose level as compared to controls and/or where the tumor incidence rate shows a significant trend with respect to dose level. Both biological and statistical considerations have been used to select the most appropriate data sets.

Because humans may be as sensitive as the most sensitive animal species, potency estimates obtained from the most sensitive species tested can be averaged to estimate potency for the general population. Because some subpopulations may be more sensitive than the general population, the potency estimate from the most sensitive sex and strain tested is also presented. This approach is consistent with EPA's guidelines for carcinogen risk assessment (U.S. EPA, 1986).

5.1.1. Chlordane

As described in section 4.4, four data sets showed a significant increase in hepatocellular carcinomas in treatment groups compared to controls in mice. These are male and female mice in the IRDC study, and male and female mice in the NCI study. Tables 22-25 give tumor incidence data for these studies. In

TABLE 22. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF CHLORDANE FROM HEPATOCELLULAR CARCINOMAS IN FEMALE MICE

Compound:	Analytical grade chlordane
Species, strain, sex:	Mouse, CD-1, female
Body weight:	0.030 kg (assumed)
Length of experiment:	19-1/2 months
Length of exposure:	18 months
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	2.98 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0	0	0/45
5	0.65	0.052	0/61
25	3.25	0.260	32/50
50	6.50	0.520	26/37

SOURCE: IRDC, 1973a.

TABLE 23. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF CHLORDANE FROM HEPATOCELLULAR CARCINOMAS IN MALE MICE

Compound:	Analytical grade chlordane
Species, strain, sex:	Mouse, CD-1, male
Body weight:	0.030 kg (assumed)
Length of experiment:	19-1/2 months
Length of exposure:	18 months
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	4.74 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0	0	3/33
5	0.65	0.052	5/55
25	3.25	0.260	41/52
50	6.50	0.520	32/39

SOURCE: IRDC, 1973a.

TABLE 24. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF CHLORDANE FROM HEPATOCELLULAR CARCINOMAS IN MALE MICE

Compound:	Technical grade chlordane
Species, strain, sex:	Mouse, B6C3F1, male
Body weight:	0.030 kg (assumed)
Length of experiment:	90 weeks
Length of exposure:	80 weeks
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	0.76 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0	0	2/18
29.9	3.9	0.31	16/48
56.2	7.3	0.58	43/49

SOURCE: NCI, 1977a.

TABLE 25. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF CHLORDANE FROM HEPATOCELLULAR CARCINOMAS IN FEMALE MICE

Compound:	Technical grade chlordane
Species, strain, sex:	Mouse, B6C3F1, female
Body weight:	0.030 kg (assumed)
Length of experiment:	90 weeks
Length of exposure:	80 weeks
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	0.25 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0	0	0/19
30.1	3.9	0.31	3/47
63.8	8.3	0.66	34/49

SOURCE: NCI, 1977a.

rats, only the RIASBT study from Japan showed a significant increase in tumors. The incidence of hepatocellular carcinomas in male rats as reevaluated by Williams was significantly increased in the high-dose group when compared to controls. Table 26 gives the tumor incidence for this study. These five data sets were used to quantify human risk.

5.1.2. Heptachlor/Heptachlor Epoxide

Eight data sets showed significant increases in the incidence of hepatocellular carcinomas in treated groups compared to controls. Tables 27-34 present the tumor incidence for these data sets. In rats, a significant increase in hepatocellular carcinomas was diagnosed by Reuber. Incidence data are shown in Table 35. These studies were used to quantify the carcinogenic risk due to heptachlor/heptachlor epoxide exposure.

5.2. CHOICE OF EXTRAPOLATION MODELS

Mathematical models are used to estimate human excess cancer risks associated with exposure to a given chemical. The models are fitted to tumor incidence data from animal bioassays, and an extrapolation is made to low doses to estimate human risk. The animal bioassay data used to predict human cancer risk from exposure to chlordane and heptachlor are described in the previous section.

Although no single mathematical model is recognized as the most appropriate, some mechanisms of the process are known. If one accepts the linearity of tumor formation and if the carcinogenic agent of interest acts in the same manner as carcinogenic agents acting in the background, then the added effect of the carcinogen on tumor formation at low doses is linear. On this basis, a model that is linear at low doses is appropriate.

The linearized multistage model is used in this assessment to calculate human risk. This model takes the following form:

TABLE 26. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF CHLORDANE FROM LIVER ADENOMAS AND CARCINOMAS IN MALE RATS

Compound:	Technical grade chlordane
Species, strain, sex:	Rat, F344, male
Body weight:	0.35 kg
Length of experiment:	130 weeks
Length of exposure:	130 weeks
Tumor site and type:	Liver, adenoma and carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	1.11 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0	0	1/64
1	0.05	0.17	1/64
5	0.25	0.85	4/64
25	1.25	4.25	9/64

SOURCE: RIASBT, 1983b.

TABLE 27. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF HEPTACHLOR FROM HEPATOCELLULAR CARCINOMAS IN MALE MICE

Compound:	Heptachlor
Species, strain, sex:	Mouse, C3H, male
Body weight:	0.030 kg (assumed)
Length of experiment:	24 months
Length of exposure:	24 months
Tumor site and type:	Liver carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	12.4 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0.00	0.000	22/78
10	1.43	0.108	64/87

SOURCE: Davis, 1965, as diagnosed by Reuber, 1977b.

TABLE 28. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF HEPTACHLOR FROM HEPATOCELLULAR CARCINOMAS IN FEMALE MICE

Compound:	Heptachlor
Species, strain, sex:	Mouse, C3H, female
Body weight:	0.030 kg (assumed)
Length of experiment:	24 months
Length of exposure:	24 months
Tumor site and type:	Liver carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	14.9 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0.00	0.000	2/54
10	1.43	0.108	57/78

SOURCE: Davis, 1965, as diagnosed by Reuber, 1977b.

TABLE 29. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF HEPTACHLOR FROM HEPATOCELLULAR CARCINOMAS IN MALE MICE

Compound:	Technical grade heptachlor
Species, strain, sex:	Mouse, B6C3F1, male
Body weight:	0.030 kg (assumed)
Length of experiment:	90 weeks
Length of exposure:	80 weeks
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	2.79 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0	0	5/19
6.1	0.79	0.063	11/46
13.8	1.79	0.14	34/47

SOURCE: NCI, 1977b.

TABLE 30. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF HEPTACHLOR FROM HEPATOCELLULAR CARCINOMAS IN FEMALE MICE

Compound:	Technical grade heptachlor
Species, strain, sex:	Mouse, B6C3F1, female
Body weight:	0.030 kg (assumed)
Length of experiment:	90 weeks
Length of exposure:	80 weeks
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	0.83 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0	0	2/10
9.0	1.17	0.094	3/47
18.0	2.34	0.18	30/42

SOURCE: NCI, 1977b.

TABLE 31. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF HEPTACHLOR EPOXIDE FROM HEPATOCELLULAR CARCINOMAS IN MALE MICE

Compound:	Heptachlor epoxide
Species, strain, sex:	Mouse, C3H, male
Body weight:	0.030 kg (assumed)
Length of experiment:	24 months
Length of exposure:	24 months
Tumor site and type:	Liver carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	27.7 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0.00	0.000	22/78
10	1.43	0.108	73/79

SOURCE: Davis, 1965, as diagnosed by Reuber, 1977b.

TABLE 32. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF HEPTACHLOR EPOXIDE FROM HEPATOCELLULAR CARCINOMAS IN FEMALE MICE

Compound:	Heptachlor epoxide
Species, strain, sex:	Mouse, C3H, female
Body weight:	0.030 kg (assumed)
Length of experiment:	24 months
Length of exposure:	24 months
Tumor site and type:	Liver carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	36.2 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0.00	0.000	2/54
10	1.43	0.108	77/81

SOURCE: Davis, 1965, as diagnosed by Reuber, 1977b.

TABLE 33. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF HEPTACHLOR EPOXIDE FROM HEPATIC CARCINOMAS IN FEMALE MICE

Compound:	25:75 mixture of heptachlor/heptachlor epoxide
Species, strain, sex:	Mouse, CD-1, female
Body weight:	0.030 kg (assumed)
Length of experiment:	19 months, 3 weeks
Length of exposure:	18 months
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	1.04 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0	0	6/76
1	0.13	0.01	1/70
5	0.65	0.052	6/65
10	1.30	0.10	30/57

SOURCE: IRDC, 1973b, as reevaluated by Reuber.

TABLE 34. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF HEPTACHLOR EPOXIDE FROM HEPATIC CARCINOMAS IN MALE MICE

Compound:	25:75 mixture of heptachlor/heptachlor epoxide
Species, strain, sex:	Mouse, CD-1, male
Body weight:	0.030 kg (assumed)
Length of experiment:	19 months, 3 weeks
Length of exposure:	18 months
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	6.48 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0	0	0/62
1	0.13	0.010	2/68
5	0.65	0.052	18/68
10	1.30	0.10	52/80

SOURCE: IRDC, 1973b, as reevaluated by Reuber.

TABLE 35. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF HEPTACHLOR EPOXIDE FROM HEPATIC CARCINOMAS IN FEMALE RATS

Compound:	Heptachlor epoxide
Species, strain, sex:	Rat, CFN, female
Body weight:	0.350 kg (assumed)
Length of experiment:	108 weeks
Length of exposure:	108 weeks
Tumor site and type:	Liver, carcinoma
Route, vehicle:	Oral, diet
Human potency (q_1^*):	5.76 per mg/kg/day

Experimental animal dose (ppm)	Average animal dose (mg/kg/day)	Equivalent human dose (mg/kg/day)	Tumor incidence No. responding/ No. examined
0	0	0	0/17
0.5	0.025	0.0043	3/22
2.5	0.125	0.021	3/18
5.0	0.250	0.043	7/22
7.5	0.375	0.064	3/21
10.0	0.500	0.085	5/19

SOURCE: Witherup et al., 1959, as reevaluated by Reuber.

$$P(d) = 1-\exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)]$$

where

$$q_i \geq 0,\ i = 0, 1, \ldots, k \text{ dose levels and } d = \text{dose}.$$

Cancer risks can also be estimated using models such as the probit, logit, Weibull, one-hit, and gamma multihit. The linearized multistage model leads to an upper limit on risk.

5.3. INTERSPECIES DOSE CONVERSION

The low-dose extrapolation process involves the use of animal tumor incidence data to predict human cancer risk. Differences exist between humans and experimental test animals with respect to lifespan, body size, and pharmacokinetic mechanisms. Hence, animal doses need to be converted to equivalent human doses. The conversion accounts for noncontinuous feeding, differences in lifetime, and size differences. In the absence of information to the contrary, this assessment uses the surface-area correction recommended by EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986). Therefore, a factor of the cube root of the ratio of the animal to human body weights is applied to animal doses to calculate equivalent human doses. This equation is

$$DH = DA\ (WA/70)^{1/3}$$

where

DH = equivalent human dose (mg/kg/day)

DA = animal dose (mg/kg/day)

WA = weight of animal (kg): for mice, 0.03 kg; for rats, 0.35 kg

70 = weight of a human (kg)

In the absence of information on human absorption, tissue distribution, metabolism, and excretion, this assessment makes no adjustment for potential differences between animals and humans.

5.4. ESTIMATION OF CARCINOGENIC POTENCY

Estimates of carcinogenic potency can be obtained by fitting the linearized multistage model, described in Section 5.2, to each data set described in Section 5.1. Table 36 summarizes 14 potency estimates obtained in this way.

5.4.1. Chlordane

Five data sets involve chlordane: male and female CD-1 mice, male and female B6C3F1 mice, and male F344 rats. The most sensitive sex and strain tested is male CD-1 mice. From these, the potency is estimated at 4.7 per mg/kg/day.

The most sensitive species tested is mice. There are four potency estimates, ranging from 4.7 down to 0.25 per mg/kg/day, with a geometric mean of 1.3 per mg/kg/day. This geometric mean from mice is consistent with the potency estimate from rats of 1.1 per mg/kg/day. Because humans may be as sensitive as the most sensitive animal species, the potency for the general population is estimated at 1.3 per mg/kg/day.

These estimates are plausible upper bounds for the increased cancer risk from chlordane, meaning that the true risk is not likely to exceed these estimates and may be lower. These estimates supersede the potency of 1.61 per mg/kg/day previously calculated by the EPA (U.S. EPA, 1980a).

The molecular potency index, which is the potency expressed in terms of molecular weight, has been used to rank suspect carcinogens according to potency. The index is computed by multiplying the general-population potency by the molecular weight. The molecular potency index for chlordane is 5.2×10^2 per mmol/kg/day. This places chlordane in the upper middle quartile of

TABLE 36. HUMAN POTENCY ESTIMATES BY CHEMICAL

Chemical	Sex, species, strain	Tumor site, type	Potency $(mg/kg/day)^{-1}$	Reference
Chlordane	Male mice CD-1	Liver, carcinoma	4.74	IRDC (1973a)
Chlordane	Female mice CD-1	Liver, carcinoma	2.98	IRDC (1973a)
Chlordane	Male mice $B6C3F_1$	Liver, carcinoma	0.76	NCI (1977a)
Chlordane	Female mice $B6C3F_1$	Liver, carcinoma	0.25	NCI (1977a)
Chlordane	Male rats F344	Liver, adenoma and carcinoma	1.11	RIASBT (1983b)
Heptachlor	Male mice C3H	Liver, carcinoma	12.4	Davis (1965)/ Reuber
Heptachlor	Female mice C3H	Liver, carcinoma	14.9	Davis (1965)/ Reuber
Heptachlor	Male mice $B6C3F_1$	Liver, carcinoma	2.79	NCI (1977b)
Heptachlor	Female mice $B6C3F_1$	Liver, carcinoma	0.83	NCI (1977b)
Heptachlor epoxide	Male mice C3H	Liver, carcinoma	27.7	Davis (1965)/ Reuber
Heptachlor epoxide	Female mice C3H	Liver, carcinoma	36.2	Davis (1965)/ Reuber
Heptachlor epoxide	Female mice CD-1	Liver, carcinoma	1.04	IRDC (1973b)/ Reuber
Heptachlor epoxide	Male mice CD-1	Liver, carcinoma	6.48	IRDC (1973b)/ Reuber
Heptachlor epoxide	Female rats CFN	Liver, carcinoma	5.76	Witherup et al. (1959)/Reuber

suspect carcinogens ranked by CAG.

The unit risk in air, which is the potency expressed in terms of $\mu g/m^3$ ambient air concentration, is used to estimate risk when exposures are expressed as air concentrations. The unit risk in air is the increased cancer risk to a person who throughout life breathes air contaminated with 1 $\mu g/m^3$ of a pollutant. With a linear dose-response curve, risks at any concentration can be computed by multiplying the unit risk by the concentration. The unit risk is computed by converting a 1 $\mu g/m^3$ concentration to a mg/kg/day dose and then multiplying by the potency. For a 70-kg person who breathes 20 m^3 a day, an air concentration of 1 $\mu g/m^3$ is equivalent to a dose of:

$$(1\ \mu g/m^3)\ (10^{-3}\ mg/\mu g)\ (20\ m^3/day)\ /\ (70\ kg) = 2.9 \times 10^{-4}\ mg/kg/day.$$

Multiplying this dose by the potencies calculated above gives unit risks of 3.7×10^{-4} per $\mu g/m^3$ for the general population and 1.3×10^{-3} per $\mu g/m^3$ for sensitive subpopulations.

The unit risk in water, which is the potency expressed in terms of $\mu g/L$ drinking water concentrations, is used to estimate risk when exposures are expressed as water concentrations. The unit risk in water is the increased cancer risk to a person who throughout life drinks water contaminated with 1 $\mu g/L$ of a pollutant. With a linear dose-response curve, risks at any concentration can be computed by multiplying the unit risk by the concentration. The unit risk is computed by converting a 1 $\mu g/L$ concentration to a mg/kg/day dose and then multiplying by the potency. For a 70-kg person who drinks 2 L a day, a water concentration of 1 $\mu g/L$ is equivalent to a dose of:

$$(1\ \mu g/L)\ (10^{-3}\ mg/\mu g)\ (2\ L/day)\ /\ (70\ kg) = 2.9 \times 10^{-5}\ mg/kg/day.$$

Multiplying this dose by the potencies calculated above gives unit risks of 3.7×10^{-5} per μg/L for the general population and 1.3×10^{-4} per μg/L for sensitive subpopulations.

5.4.2. Heptachlor

Four data sets involve heptachlor: male and female C3H mice, and male and female B6C3F1 mice. The most sensitive sex and strain tested is female C3H mice. From these, the potency is estimated at 14.9 per mg/kg/day.

The most sensitive species tested is mice. There are four potency estimates, ranging from 14.9 down to 0.83 per mg/kg/day, with a geometric mean of 4.5 per mg/kg/day. Because humans may be as sensitive as the most sensitive animal species, the potency for the general population is estimated at 4.5 per mg/kg/day.

These estimates are plausible upper bounds for the increased cancer risk from heptachlor, meaning that the true risk is not likely to exceed these estimates and may be lower. These estimates supersede the potency of 3.37 per mg/kg/day previously calculated by the EPA (U.S. EPA, 1980b).

The molecular potency index for heptachlor is 1.7×10^{3} per mmol/kg/day. This places heptachlor in the upper middle quartile of suspect carcinogens ranked by the CAG.

The unit risk in air is 1.3×10^{-3} per μg/m^3 for the general population and 4.3×10^{-3} per μg/m^3 for sensitive subpopulations. The unit risk in water is 1.3×10^{-4} per μg/L for the general population and 4.3×10^{-4} per μg/L for sensitive subpopulations.

5.4.3. Heptachlor Epoxide

Five data sets involve heptachlor epoxide: male and female C3H mice, male and female CD-1 mice, and female CFN rats. The most sensitive sex and strain tested is female C3H mice. From these the potency is estimated at 36.2 per

mg/kg/day.

The most sensitive species tested is mice. There are four potency estimates, ranging from 36.2 down to 1.0 per mg/kg/day, with a geometric mean of 9.1 per mg/kg/day. This geometric mean from mice is consistent with the potency estimate from rats of 5.8 per mg/kg/day. Because humans may be as sensitive as the most sensitive animal species, the potency for the general population is estimated at 9.1 per mg/kg/day.

These estimates are plausible upper bounds for the increased cancer risk from heptachlor epoxide, meaning that the true risk is not likely to exceed these estimates and may be lower. These estimates supersede the potency of 57.86 per mg/kg/day previously calculated by the EPA (U.S. EPA, 1985a).

The molecular potency index for heptachlor epoxide is 3.5×10^3 per mmol/kg/day. This places heptachlor epoxide in the most potent quartile of suspect carcinogens ranked by CAG.

The unit risk in air is 2.6×10^{-3} per $\mu g/m^3$ for the general population and 1.0×10^{-2} per $\mu g/m^3$ for sensitive subpopulations. The unit risk in water is 2.6×10^{-4} per $\mu g/L$ for the general population and 1.0×10^{-3} per $\mu g/L$ for sensitive subpopulations.

6. SUMMARY

6.1. QUALITATIVE DATA

6.1.1. Human Studies

6.1.1.1. Case Reports--There were 11 case reports involving CNS effects where the author looked at the toxic effects of chlordane/heptachlor, eight case studies involving blood dyscrasias, and five case studies of neuroblastomas in children with pre-/postnatal exposure to chlordane or heptachlor. The blood dyscrasias in children included four cases of aplastic anemia and one case each of refractory megaloblastic anemia, acute lymphoblastic leukemia, acute stem-cell leukemia, and acute myelomonocytic leukemia.

6.1.1.2. Epidemiologic Studies--Three epidemiologic studies of workers exposed to chlordane and/or heptachlor have been reported. One of these studies, conducted in chlordane/heptachlor applicators, was considered inadequate in sample size and duration of follow-up since initial exposure. However, this study showed increased mortality from bladder cancer (SMR = 277, $p < 0.05$). A second study showed an increased mortality from lung cancer (SMR = 134), but the increase was not statistically significant. The mortality from cerebrovascular disease was statistically significant (SMR = 183, $p \leq 0.05$). Of the 1,043 men involved in the study, only one liver cancer was reported. The third study involved 2,141 workers exposed to organochlorine pesticides. One of the four plants involved in pesticide manufacture produced chlordane and one produced heptachlor. The SMR for malignant neoplasms was 69 at the chlordane plant and 91 at the heptachlor plant. There was an excess risk for cancer in various tissues; none was statistically significant. The last two studies were carried out in chlordane/heptachlor manufacturing plants.

All of these studies have several limitations. Neither the quantitative nor length of exposure histories are available for chlordane/heptachlor for the populations studied. They were also exposed to other pesticides and chemicals. Adjustments for these other chemical exposures and other confounding factors, like smoking and alcohol consumption, were not considered in any of these studies. All of the study populations were small. In the pesticide applicator study, individual follow-up was not undertaken and the data were missing on 10.3 percent of the decedents reported by the Social Security Administration.

Because of these methodological limitations and the limited data, it is difficult to establish either a negative or positive association between chlordane/heptachlor and carcinogenicity. Hence, these studies are considered inadequate epidemiologic evidence.

6.1.2. Animal Studies

6.1.2.1. Chlordane

6.1.2.1.1. Mice. Four chlordane carcinogenesis bioassays in mice have been reported. The strains tested include C57B1/6N, CD-1, B6C3F1, and ICR. In C57B1/6N mice fed 25 or 50 ppm for 18 months, hepatocellular carcinomas were observed in 27 percent (16) of the survivors. This mouse strain rarely develops spontaneous liver lesions. For CD-1 mice fed 5, 25, or 50 ppm for 18 months, liver nodules/hepatocellular carcinomas were observed in the 25 and 50 ppm groups. In B6C3F1 mice fed approximately 30 and 60 ppm for 80 weeks and then held for 10 weeks, hepatocellular carcinomas were observed in both males and females. For ICR mice fed 1, 5, or 12.5 ppm for 24 months, hepatocellular adenomas and hemangiomas were significantly increased ($p < 0.001$) in males receiving 12.5 ppm and nonneoplastic liver lesions were present in males fed 5 ppm and in females fed 5 or 12.5 ppm.

6.1.2.1.2. Rats. Four chlordane carcinogenesis bioassays in rats have been reported. The strains tested include albino, Osborne-Mendel, and Fischer 344. Three of these studies were considered adequate, and one was inadequate. In albino rats fed 10, 20, 40, 80, 160, 320, 640, or 1280 ppm for 400 days, there were no treatment-related tumors. In Osborne-Mendel rats fed 5, 10, 30, 150, or 300 ppm for 2 years, hepatic toxicity was noted at 150 and 300 ppm, but no liver tumors were noted. In Osborne-Mendel rats fed 203.5 or 407 ppm (males) or 120.8 or 241.5 ppm (females), respectively, for 80 weeks and held for an additional 29 weeks, no liver tumors were noted, but thyroid tumors were significantly increased. In light of historical data for Osborne-Mendel rats, the thyroid tumors were not considered to be treatment-related. In Fischer 344 rats fed 1, 5, or 25 ppm for 130 weeks, there was a statistically significant increase in hepatocellular adenomas, which was considered by the authors as weak evidence for carcinogenicity in males fed 25 ppm. Hepatocellular swelling was significant in females fed 25 ppm. The hepatocellular adenomas occurred only in males surviving longer than 104 weeks.

6.1.2.2. Heptachlor/Heptachlor Epoxide

6.1.2.2.1. Mice. Three heptachlor/heptachlor epoxide carcinogenesis bioassays in mice have been reported. The strains studied include C3H, B6C3F1, and CD-1 mice. In C3H mice fed 10 ppm of both heptachlor and heptachlor epoxide for 2 years, benign liver tumors/hepatocellular carcinomas were reported in both male and female mice. Hepatocellular carcinomas in treated groups were generally large and frequently multiple tumors, especially in the epoxide group in respect to the controls. For B6C3F1 mice fed technical grade (containing 22 percent chlordane) at concentrations of 6.1 or 13.8 ppm (males) or 9 or 18 ppm (females), respectively, for 80 weeks and held for an additional 10 weeks, hepatocellular carcinomas were significantly ($p < 0.001$) increased in both male

and female mice. In CD-1 mice fed a mixture of heptachlor epoxide/heptachlor (75:25) at concentrations of 1, 5, or 10 ppm for 18 months, nodular hyperplasia/hepatocellular carcinomas were noted at 5 and 10 ppm in both male and female mice.

6.1.2.2.2. Rats. Five heptachlor/heptachlor epoxide carcinogenesis bioassays in rats have been conducted. The strains of rats studied include Wistar, Osborne-Mendel, CD, and CFN. In Wistar rats given 5 doses of 10 mg/kg bw of heptachlor and held for 106 to 110 weeks, no treatment-related tumors were observed. For Osborne-Mendel rats fed technical grade heptachlor at concentrations of 38.9 or 77.9 (males) or 25.7 or 51.3 (females) ppm, respectively, for 80 weeks and held for 30 weeks, no liver tumors were noted, although neoplastic nodules were found in both treated and control rats. In CD rats fed a mixture of heptachlor/heptachlor epoxide (75:25) at concentrations of 5, 7.5, 10, or 12.5 ppm for 2 years, no liver tumors were noted, although nonneoplastic lesions were noted in the livers of rats fed 7.5, 10, or 12.5 ppm. In one study using CFN rats fed 1.5, 3, 5, 7, or 10 ppm of heptachlor for 110 weeks, the incidence of liver tumors was not statistically different in treated and control animals. In a second study using CFN rats fed 0.5, 2.5, 5, 7.5, or 10 ppm of heptachlor epoxide for 108 weeks, treatment-related liver carcinomas were noted by several pathologists.

6.1.3. Supporting Evidence

6.1.3.1. Mutagenicity--The published literature on mutagenicity tests of chlordane and heptachlor/heptachlor epoxide is quite similar, with most studies reporting results on both chemicals. Generally, the results have indicated that these chemicals are not mutagenic in bacteria or in mammalian cells in culture, and do not induce DNA repair, as measured by unscheduled DNA synthesis in rodent hepatocytes. While dominant lethal tests in mice have been negative

for both chemicals, the absence of direct cytogenetic tests in both germinal and somatic cells precludes a conclusion as to their potential for causing chromosomal aberrations.

6.1.3.2. Structural Relationship--Three compounds, structurally related to chlordane/heptachlor/heptachlor epoxide, have induced malignant liver tumors in animals. Aldrin, dieldrin, and chlorendic acid have produced liver tumors in mice and chlorendic acid has also produced liver tumors in rats.

6.2. QUANTITATIVE ANALYSIS

In the absence of information on human absorption, tissue distribution, metabolism, and excretion, this assessment makes no adjustment for potential differences between animals and humans.

6.2.1. Chlordane

Five data sets involve chlordane: male and female CD-1 mice, male and female B6C3F1 mice, and male F344 rats. The most sensitive sex and strain tested is male CD-1 mice. From these, the potency is estimated at 4.7 per mg/kg/day.

The most sensitive species tested is mice. There are four potency estimates, ranging from 4.7 down to 0.25 per mg/kg/day, with a geometric mean of 1.3 per mg/kg/day. This geometric mean from mice is consistent with potency estimate from rats of 1.1 per mg/kg/day. Because humans may be as sensitive as the most sensitive animal species, the potency for the general population is estimated at 1.3 per mg/kg/day.

These estimates are plausible upper bounds for the increased cancer risk from chlordane, meaning that the true risk is not likely to exceed these estimates and may be lower. These estimates supersede the potency of 1.61 per mg/kg/day previously calculated by the EPA (U.S. EPA, 1980a).

The molecular potency index, which is the potency expressed in terms of molecular weight, has been used to rank suspect carcinogens according to po-

tency. The index is computed by multiplying the general-population potency by the molecular weight. The molecular potency index for chlordane is 5.2×10^2 per mmol/kg/day. This places chlordane in the upper middle quartile of suspect carcinogens ranked by CAG.

6.2.2. Heptachlor

Four data sets involve heptachlor: male and female C3H mice, and male and female B6C3F1 mice. The most sensitive sex and strain tested is female C3H mice. From these, the potency is estimated at 14.9 per mg/kg/day.

The most sensitive species tested is mice. There are four potency estimates, ranging from 14.9 down to 0.83 per mg/kg/day, with a geometric mean of 4.5 per mg/kg/day. Because humans may be as sensitive as the most sensitive animal species, the potency for the general population is estimated at 4.5 per mg/kg/day.

These estimates are plausible upper bounds for the increased cancer risk from heptachlor, meaning that the true risk is not likely to exceed these estimates and may be lower. These estimates supersede the potency of 3.37 per mg/kg/day previously calculated by EPA (U.S. EPA, 1980b).

The molecular potency index for heptachlor is 1.7×10^3 per mmol/kg/day. This places heptachlor in the upper middle quartile of suspect carcinogens ranked by CAG.

6.2.3. Heptachlor Epoxide

Five data sets involve heptachlor epoxide: male and female C3H mice, male and female CD-1 mice, and female CFN rats. The most sensitive sex and strain tested is female C3H mice. From these, the potency is estimated at 36.2 per mg/kg/day.

The most sensitive species tested is mice. There are four potency estimates, ranging from 36.2 down to 1.0 per mg/kg/day, with a geometric mean of

9.1 per mg/kg/day. This geometric mean from mice is consistent with the potency estimate from rats of 5.8 per mg/kg/day. Because humans may be as sensitive as the most sensitive animal species, the potency for the general population is estimated at 9.1 per mg/kg/day.

These estimates are plausible upper bounds for the increased cancer risk from heptachlor epoxide, meaning that the true risk is not likely to exceed these estimates and may be lower. These estimates supersede the potency of 57.86 per mg/kg/day previously calculated by the EPA (U.S. EPA, 1985a).

The molecular potency index for heptachlor epoxide is 3.5×10^3 per mmol/kg/day. This places heptachlor epoxide in the most potent quartile of suspect carcinogens ranked by the CAG.

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APPENDIX A

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: The Mutagenicity Assessment (GENE-TOX PROFILE) of Chlordane - Addendum to the Registration Standard

TB Projects: 0008/1244
Caswell No.: 174

FROM: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)
11-25-86

TO: Henry Spencer, Ph.D.
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and

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THRU: Judy Hauswirth, Acting Head
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Judith W. Hauswirth 11/25/86

The assessment of the mutagenicity of chlordane developed here was generated from our review of the available published literature located by Dynamac Corporation (TB Project 1244), as well as those articles in the files of EMIC (Environmental Mutagen Information Center at Oak Ridge National Laboratory) which were employed for evaluation in Phase I of the Agency's GENE-TOX PROGRAM (Dr. Michael D. Waters, HERL/RTP). A cursory appraisal ("Chlordane/Heptachlor Mutagenicity," memorandum: Mauer to Spencer December 16, 1985--attached as APPENDIX I)

of some of these assays as summarized by the CAG ("Carcinogenicity Risk Assessment of Chlordane and Heptachlor/ Heptachlor Epoxide," DRAFT, December 1985) is also incorporated here, since the conclusions in this more comprehensive assessment corroborate those in that preliminary discussion. There appears to have been no primary gene-tox studies submitted as CBI by registrants.

Technical chlordane (CAS Registry No. 12789-03-06) is a complex mixture of related, chlorinated methanoindane compounds.* Approximately one-third of the published studies available for review could not be interpreted because they did not clearly state the nature, purity, source, and composition of the material tested but used unspecified "chlordane/commercial grades" of the pesticide. Collectively, however, results from over thirty assays reviewed here permit at least a preliminary assessment of the mutagenicity potential of the technical/its principal isomers.

This assessment is organized as follows: A survey and discussion (with summary tabulation), as well as evaluation (for regulatory purposes) of all studies located by both sources (DYNAMAC, EMIC/GENE-TOX PROGRAM) listed according to FIFRA categories of genetic effect assayed (gene mutation, chromosomal effects, primary DNA damage/repair, and other mechanisms); followed by conclusions as well as recommendations both to satisfy regulatory requirements ("data gaps") and to indicate further testing the results from which would be useful for risk assessment.

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* Approximate composition as follows:

- cis (alpha)-chlordane (5103-71-9) 19%
- trans (gamma)-chlordane (57-74-9) 24%
- chlordene isomers 21%
- heptachlor (76-44-8) 10%
- nonachlor (3734-49-4) 7%
- Diels-Adler adduct (68441-65-6) of cyclopentadiene (542-92-7) and pentachlorocyclopentadiene (25329-35-5) 2%
- hexachlorocyclopentadiene (77-47-4) 1%
- octochlorocyclopentadane (287-92-3) 1%
- (miscellaneous constituents) 15%

[AG-chlordane (HCS-3260, Velsicol) is an experimental 3:1 mixture (95%) of cis (α) and trans (γ) chlordane (95% purity) employed is some toxicological assays (e.g., Arnold et al., 1977).]

Although a definitive genetic toxicology profile cannot be established from the published literature reviewed here, the following preliminary conclusions on the mutagenicity of chlordane are warranted:

1. Neither technical chlordane nor any of its constituents appear to be mutagenic in bacterial cells in adequately controlled assays.

2. Although negative in acceptable tests with less well established mammalian cell lines, technical chlordane and/or one of its isomers have induced positive results in assays without activation (not tested with S9) in mammalian cell systems with established data bases. Hence, its potential to induce gene mutation in adequately controlled assays (i.e., with and without metabolic activation in the standard *in vitro* mammalian assays) still needs to be determined.

3. Similarly, adequate germinal assays indicate this organochlorine does not cause gross chromosome damage, but its potential for somatic cytogenetic activity has not been evaluated.

4. The combined results of DNA repair assays indicate that chlordane (the technical and/or one or more of its constituents) is genotoxic in yeast and fish, as well as in some types of mammalian cell systems.

5. There is also evidence suggesting that chlordane may act by epigenetic (e.g., promoter) mechanisms.

MUTAGENICITY OF CHLORDANE

THE DATA BASE

A total of 25 published articles were available for review, containing 34 assays testing technical chlordane/its constitutent isomers as well as commercial preparations (TABLE 1). One-third were judged inadequate (UNACCEPTABLE) to support the negative results reported, because of major deficiencies, and another one-third reporting positive results were declared INCONCLUSIVE because of a combination of procedural/accounting inadequacies. The remaining nine studies satisfy the minimal data requirements for all genetic end-points necessary for regulatory purposes (gene mutation, chromosomal damage, primary DNA repair), but do not provide a comprehensive assessment of genotoxic potential for this pesticide. Further testing is recommended, in order to confirm some suspect positive results, as well as to provide support for assessments in other areas (e.g., evaluation as a suspect carcinogen).

DISCUSSION AND EVALUATION OF ASSAY RESULTS

A. Survey Studies

The published results of studies conducted with chlordane that contained little or no primary data, although individually considered technically inadequate and unacceptable in meeting regulatory requirements, can be used to support the overall conclusions drawn from the acceptable studies discussed below.

As shown in Table 1, chlordane was evaluated in three modified *Salmonella typhimurium* mammalian microsome plate incorporation Ames assays (Wildeman and Nazar 1982; Probst et al. 1981; Gentile et al. 1982). Wildeman and Nazar (1982) reported that a commercial preparation providing 50 ug/plate active ingredient did not induce reversion to histidine prototrophy of *S. typhimurium* TA98 and TA100 either in the absence or the presence of the following activation systems: rat S9, S14 microsome fractions derived from corn or wheat, or combined rat S9 and plant S14 fractions.

Qualitative negative results were also reported by both Probst and associates (1981) over a 1- to 10,000-fold non-activated and S9-activated concentration range of chlordane in the concentration agar gradient test with eight *S. typhimurium* and two *Escherichia coli* strains, and by Gentile et al. (1982) who reported both commercial and technical grade chlordane (tested at unreported concentrations) to be negative in the conventional Ames test using rat S9 and in an Ames assay using corn microsome 1S fractions for activation.

Both Ashwood-Smith and colleagues (1972) and Ficsor (1972) reported negative results in E. coli reverse mutation nonactivated spot tests with chlordane, the former assayed a 1 mg/disc sample of 10% chlordane and the latter tested an unreported dose of 44.4% chlordane.

A reverse mutation assay using Zea mays at the waxy (wx) locus as the target was also conducted by Gentile and associates (1982), but provided only qualitative evidence of a positive response induced by chlordane in a survey gene mutation study. For this experiment, 2.24 kg/hectare commercial grade chlordane was sprayed on mutant seeds in situ prior to emergence of corn seedlings. Plants were allowed to grow 12 to 14 weeks and pollen grains were harvested, dehydrated, and stained. The authors reported a significiant increase ($p < 0.05$) in back-mutations of wx^- to wx^+. Because primary data were not reported, chlordane is classified inconclusive but a presumptive positive mutagen in this assay.

These authors also included the results of a Saccharomyces cerevisiae D4 mitotic gene conversion assay. Log-phase S. cerevisiae cells were exposed for 30 minutes to four doses of commercial and technical grade chlordane over a threefold to hundredfold dilution range in the absence and presence of rat S9 and corn 1S. Commercial grade chlordane was reported positive for unspecified doses in the presence of both rat (S9) and corn (1S) microsome fractions. Technical grade chlordane was active only in the presence of rat S9 activation. Based on this qualitative evidence, chlordane is considered presumptively genotoxic in yeast.

The ability of 0.1 mg/mL chlordane of unreported purity to interact with and damage DNA was also evaluated in purified CoL E1 DNA from E. coli K12 by Griffin and Hill (1978), who reported the assay as negative.

The findings presented in these survey articles did not provide sufficient data to draw unequivocal conclusions. However, the data collectively provide qualitative evidence that chlordane is probably not mutagenic in bacteria, but may be presumptively mutagenic in plants. The data also provide presumptive evidence that chlordane causes mitotic gene conversion in yeast.

B. Primary Studies

The following published results of genetic toxicology assays performed with chlordane contained sufficient primary data for review and evaluation according to FIFRA genetic points.

1. Gene Mutation

a. Bacteria

Chlordane technical as well as trans (gamma) chlordane were selected by the National Toxicology Program for evaluation in the preincubation modification to the Ames test using nonactivation and 10% rat or hamster S9 activation (Haworth et al. 1986; Mortelmans et al. 1986). For both preparations, five nonactivated doses (0.1 to 10 ug/plate) did not induce histidine prototrophy in S. typhimurium TA1535, TA1537, TA98, or TA100; a slight cytotoxic effect was reported for strain TA1537 at 10 ug/plate. In the presence of either hamster or rat S9 activation, compound precipitation was reported at 3.3 and 10 mg/plate. Similarly, neither preparation induced mutants in any strain over the assayed dose range (10 to 1000 ug/plate with strains TA1535 and TA1537 and 100 to 10,000 ug/plate with strains TA100 and TA98) in the presence of either of the two rodent species' S9 microsomal fractions. Based on the findings of this study, both the technical as well as the trans-isomer were considered to be nonmutagenic. The test material was adequately investigated in these properly controlled assays, and these studies are judged acceptable.

b. Mammalian Cells

Three assays evaluated the potential of chlordane to induce gene mutation in mammalian cells (Ahmed et al. 1977a; Telang et al. 1982; Tong et al. 1981). In the preliminary dose selection test Ahmed and associates (1977a) found that nonactivated $\geq$ 100 uM chlordane was cytotoxic to Chinese hamster lung V79 cells. Based on these results, nonactivated 10 uM chlordane was evaluated in the mutagenicity assay. The reported results showed that at cell survival of 44.4%, the selected dose (30 replicate cultures) induced a fifteenfold increase in ouabain-resistant mutants.

However, the study was incomplete because exogenous metabolic activation was not used. These data are needed to establish whether metabolism has an effect on the test material's mutagenic potential. Additionally, the study results are considered inconclusive because:

- Only a single dose of an unspecified preparation of chlordane was evaluated for mutagenicity.
- The presumptive mutagenic response was not confirmed at the concentration range expected to give dose-response data.

- Carbaryl and 2,4-D fluid, which have previously been shown to be either weak mutagens or nonmutagens, were reported to give strong positive responses.

- No positive control was included to ensure an appropriate level of sensitivity.

Although assessed as inconclusive evidence for a positive response, nonactivated chlordane may be classified as an unconfirmed presumptive mutagen in Chinese hamster lung cells.

Telang and colleagues (1982) exposed 4 x 10^5 primary adult rat liver epithelial cells (ARL) to six concentrations of technical chlordane (10^{-3} to 10^{-6} M) in two independent experiments. Chlordane was cytotoxic at 10^{-3} M, but 6-thioguanine-resistant (6-TG^r) mutants were not induced at any dose. Under identical conditions the positive controls, benzo[a]pyrene (B[a]P) or 7,12-dimethylbenz[a]anthracene (DMBA), induced definitive mutagenic responses.

Negative results were also reported by the same laboratory (Tong et al. 1981) in human foreskin fibroblasts (D-550) exposed to nonactivated chlordane at the maximum non-cytotoxic dose (10^{-4} M). When D-550 fibroblasts were also cocultivated with hepatocyte primary cells (HPC), chlordane did not increase the frequency of 6-TG^r mutants in three separate experiments. By contrast, the concurrent positive control (DMBA) induced a significant ($p < 0.05$) mutagenic response in the presence of HPC. Since chlordane was properly evaluated up to cytotoxic levels, the study is acceptable.

The National Toxicology Program (1985) reported forward mutation at the TK locus of L5178Y mouse cells, but only in the absence of metabolic activation.

c. Conclusions for Gene Mutation

Based on the *in vitro* results of two inconclusive mammalian gene mutation assays, chlordane is presumed to be a direct-acting mutagen for Chinese hamster lung cells and mouse lymphoma cells. However, the two acceptable studies submitted in the *in vitro* mammalian cell gene mutation category provide evidence that chlordane was not mutagenic in liver epithelial cells or human foreskin fibroblasts cocultivated with or without HPC. Hence, the relevance of the direct positive responses are unclear until tests are conducted to assay the possibility that chlordane mutagenicity can be either enhanced or detoxified in the presence of exogenous metabolic activity.

2. Chromosomal Aberrations

a. Mammalian Studies

Technical chlordane and its major constituent isomers have been evaluated in dominant lethal assays by two laboratories (Arnold et al. 1977; Epstein et al. 1972).

Arnold et al. showed that single gavage or ip administration of 50 and 100 mg/kg of the technical did not elicit a dominant lethal effect in the offspring of male mice (eight/dose) sequentially mated (three females/mating) for 6 weeks. The negative control groups consisted of eight males/group/route of administration. Measured parameters included pregnancy rates, total implants, number of early deaths, and the embryonic index. The higher dose evaluated (100 mg/kg) was selected based on the results of an acute toxicity study which indicated that 300 and 1000 mg/kg produced mild to severe toxic effects. The same investigators performed additional dominant lethal assays in mice given 50 and 100 mg/kg AG-chlordane (3:1 mixture of alpha and gamma chlordane) orally (gavage) and ip. Dose selection was based on the evidence of mortality/other toxic signs at 300 and 1000 mg/kg by both routes of administration. The dominant lethal assay was performed as described for technical chlordane; results indicated that the alpha and gamma chlordane mixtures were negative.

Although the Arnold et al. data are discussed here, they have not been considered for regulatory purposes because these studies were conducted at IBT, and never validated.

Epstein and associates (1972) also showed that neither technical chlordane nor alpha or gamma chlordane induced dominant lethal effects. In one series of experiments, seven and nine male mice received 48 and 240 mg/kg chlordane, respectively, as single ip injections, and in a second experiment 10 males were administered 50 mg/kg/day by gavage for 5 consecutive days. Four males receiving 240 mg/kg and two animals in the 50 mg/kg gavage group died. All surviving males were sequentially mated (three females/mating) for 8 weeks. Females were sacrificed 13 days after mating and scored for pregnancy, total implants, and early fetal deaths. At the seventh mating interval, early fetal deaths were recorded for 55 percent of the females mated to males in the 50 mg/kg gavage group. An analysis of variance revealed no significant effect ($p < 0.05$). Based on the detailed criteria developed by the authors to evaluate assay validity and positive responses, chlordane was classified as negative. We assess that the study is acceptable.

Epstein et al. also tested for dominant lethal assays with the alpha isomer of technical chlordane ([1] 58 and

290 mg/kg, ip, [2] 42 and 210 mg/kg ip, and [3] 75 mg/kg/day by gavage for 5 days) and gamma chlordane (50 mg/kg/five daily gavage administrations). These additional studies were also reported as negative, an assessment with which we concur that neither alpha or gamma chlordane induced a dominant lethal effect; the studies are acceptable.

In a single in vitro assay, trans-chlordane was reported negative for chromosome aberrations in Chinese hamster ovary (CHO) cells tested up to levels of cytotoxicity (NTP 1985).

b. Conclusions for Chromosomal Aberrations

The results of dominant lethal assays conducted with chlordane, chlordane isomers, and mixtures of chlordane isomers demonstrated that the test material is not clastogenic in male mouse germinal cells. Only one study investigating the potential clastogenic effects of chlordane on somatic cells was located. The reported negative result in vitro requires confirmation in an adequately conducted in vivo assay.

3. DNA Repair

The results of a variety of DNA repair assays conducted with chlordane preparations are variable, but appear to be generally consistent for the specific type of assay. Thus, although considered inconclusive, both assays for gene conversion in Saccharomyces cerevisiae strain D4 recorded presumptively positive results (Gentile et al. 1982; Chambers and Dutta 1976).

Maslansky and Williams (1981) assayed 10^{-1} to 10^{-6} M chlordane for unscheduled DNA synthesis (UDS) in rat, mouse, and hamster primary hepatocytes. Three separate experiments were conducted with each hepatocyte species and 30 morphologically normal cells/experiment/species were counted for nuclear grains at the highest noncytotoxic dose only. Chlordane at $\geq 10^{-4}$ M was cytotoxic for all cell systems; no significant increase in net nuclear grain counts was seen at 10^{-5} M. The positive control, DMBA, gave an appropriate response thereby demonstrating the sensitivity of the test system to detect UDS in rat, mouse, and hamster hepatocytes. The study is considered acceptable and showed that chlordane assayed up to a cytotoxic level was not genotoxic in hepatocytes of three rodent species.

Probst and associates (1981) also reported a negative UDS response in rat hepatocytes. No significant increase in net nuclear grain counts was seen in 20 morphologically normal hepatocytes per dose group exposed for 5 and 18 to 20 hours to the maximum noncytotoxic dose of chlordane (100 nmol/mL). The study appears as have been properly controlled and is acceptable.

Brandt et al. (1972) reported that treatment of nonirradiated and UV-irradiated HeLa cells with 0.04 mM alpha-chlordane did not cause UDS or inhibition of DNA repair. Although reported as negative, the study provided no meaningful data. The sensitivity of this assay is questionable because the positive controls (methyl methanesulfonate and ethyl methanesulfonate) were only marginally active in inducing UDS, and showed no interference with repair replication of the UV-challenged cells. The study is therefore unacceptable.

By contrast to the negative finding in primary rat mouse, and hamster hepatocytes, the UDS assay conducted in SV-40 transformed human fibroblasts (VA-4) by Ahmed et al. (1977b) with nonactivated and rat S9-activated doses of chlordane (1, 10, 100, and 1000 uM) reported a significant increase in UDS over the control ($\bar{p} < 0.05$) at all doses. It should be noted that hydroxyurea treatment was used to block cell entry into scheduled (S-phase) DNA synthesis. Although quantitative data were not presented for the three highest doses, the mean net nuclear grain counts at the lowest dose, 1 uM (11.5 $\pm$ 2.6), was approximately threefold higher than the control value (3.5 $\pm$ 0.9). It is noteworthy that in the presence of S9 activation, no increases in UDS were seen.

To further define the type of repair mechanism elicited by chlordane, these investigators performed a BUdR photolysis assay with 1, 10, and 100 uM chlordane without metabolic activation. The authors concluded based on the size of the repaired region that the damage induced by chlordane is repaired by long (UV-type) excision repair mechanisms. The authors gave the following explanation for the lack of a dose-related increase in induced strand breaks/10^8 Daltons: "With increasing pesticide concentration, a corresponding increase in the number of breaks was not observed. This finding is consistent with other observations and suggestions that excision repair systems responsible for the removal of chemically bound DNA regions are saturated at relatively low concentrations."

However, the lack of primary data for the majority of doses assayed and the inability to determine if the response was dose related preclude acceptance of these results as definitive evidence of a genotoxic response. Further, the findings are suspect for the following reasons:

- SV-40, VA-4 is a virus transformed cell line; and it has been recommended by a panel of experts[1]

1 Mitchell, A.D.; Casciano, D.A.; Meltz, M.L.; Robinson, D.E.; San, R.C.H.; Williams, G.M.; Von Halle, E.S. (1983) Unscheduled DNA synthesis tests, a report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res 123:363-410.

that only nontransformed diploid human cells should be used in this assay.

- The cells were not grown under reduced serum or arginine deprivation conditions in conjunction with hydroxyurea treatment to completely preclude entry of the cells into (S-phase) DNA synthesis, thus increasing the possibility of a false positive result.

- The results were not confirmed.

- The publication did not indicate that the slides were coded to eliminate bias.

- Several agents which have been repeatedly shown to be nongenotoxic or weakly genotoxic (i.e., diquat, 2-4-D fluid, and carbaryl) induced strong UDS responses.

Based on the above rationale we consider this study as inconclusive but classify chlordane as a presumptive but unconfirmed genotoxin for SV-40 transformed human cells. We recommend that these findings be confirmed in a rigorously controlled experiment that conforms to the currently accepted protocols for UDS assays with human cell lines.

Sobti et al. (1983) exposed human lymphoid LAZ-007 cells to nonactivated and rat S9-activated 10^{-4} to 10^{-6} M chlordane for 48 hours and 1 hour, respectively. Although statistically significant increases ($p < 0.01$) in the incidence of sister chromatid exchanges (SCE) were reported at 10^{-5} -S9 and at 10^{-5} and 10^{-6} M +S9, the elevated SCE frequencies were neither dose-related nor twofold at any level. It should be mentioned that experts recommend[2] that for a compound to be considered positive in the SCE assay it should either cause at least a twofold increase over baseline SCE frequencies or demonstrate a three-point, dose-response curve showing a progressive increase over baseline SCE frequencies with at least one SCE value at $p < 0.001$ level. Although the authors concluded that chlordane was genotoxic, the study is considered inconclusive and should be repeated.

Vigfusson and associates (1983) described the *in vivo* exposure of 15 central mudminows (*Umbra limi*)/dose in

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2 Latt, S.A.; Allen, J.; Bloom, S.E.; Carrano, A.; Falke, E; Kram, D.; Schneider, E.; Schreck, R.; Tice, R.; Whitfield, B; Wolff, S. (1981) Sister-Chromatid Exchanges: A Report of the Gene-Tox Program, *Mutat. Res.* 87:17-62.

5 gallon well aerated aquaria (5 fish/aquaria) to four doses of chlordane (5.4×10^{-9} to 5.4×10^{-2} M) for 11 days. The positive findings of this in vivo study provided evidence of a dose-related and highly significant ($p < 0.01$) increase in the frequency of SCE in metaphases harvested from intestinal tissue. At the highest dose, four fish survived treatment and the SCE frequency/cell (19.3 ± 0.51) was approximately 4.3-fold higher than the control. Similarly, 3.8- and 1.7-fold increases were reported at 5.4×10^{-10} and 5.4×10^{-11} M, respectively; the lowest concentration assayed was negative. The sensitivity of the assay was demonstrated by the significant ($p < 0.01$) increase in SCE frequency/cell induced by 5.4×10^{-9} M Mitomycin C. We assessed, therefore, that chlordane is genotoxic in Umbra limi, and the study is acceptable.

Finally, the NTP (1985) reported trans-chlordane induced sister chromatid exchanges in CHO cells cultured in vitro, but only in the absence of metabolic activation. This is further supporting evidence for a direct DNA-damaging effect of the trans-isomer in mammalian cells, and indicates the necessity to evaluate this potential in adequately controlled mammalian test systems which include mammalian activation.

a. Conclusions for DNA Repair

The results of UDS assays appear to indicate that chlordane is not genotoxic in primary mouse, rat, or hamster liver cells. However, nonactivated chlordane was reported to induce a positive genotoxic response in SV-40 transformed human fibroblasts but was negative in the presence of S9 activation. The authors indicated that direct-acting chlordane appeared to elicit the long (UV-type) excision repair mechanisms to remove the lesions. Due to numerous technical deficiencies in this study and the possibility that the results represent a false positive response, it is with reservation that we assess chlordane to be presumptively genotoxic in the absence of S9 activation. These results should be validated by well-conducted studies with adequate controls.

Elevations in SCE frequency were observed in human lymphoid cells following exposure to both S9-activated and non-activated doses, however, the response did not satisfy currently accepted criteria for a positive effect in this system.

The single in vivo DNA repair study reported that chlordane induced SCE in intestinal cells of fish and that the response was dose related.

Based on the results of DNA repair assays we conclude that chlordane is not genotoxic in rodent liver cells

or human lymphoid cells, but is genotoxic in fish. The presumptive direct-acting genotoxic effect on transformed human fibroblasts is unconfirmed.

4. Epigenetic Effects

Telang et al. (1982) evaluated six doses of technical chlordane (10^{-4} to 10^{-7} M) for the potential to inhibit intercellular communication between TG^r mutants of ARL ($HGPRT^-$) and ARL $(HGPRT)^+$ cells. Chlordane was cytotoxic at 10^{-4} M for ARL ($HGPRT^-$) cells. In the presence of $HGPRT^+$ cells, 5×10^{-6} through 1×10^{-7} M, chlordane markedly inhibited the metabolic cooperation between the $HGPRT^-$ -deficient and -competent cells. At 5×10^{-6} M an almost complete blockage of phosphoribosylated TG transfer between the cells was observed as indicated by the > 100 and 86 percent recovery of TG^r mutants cocultivated with 0.75 and 1.25×10^6 $HGPRT^+$ cells. Increased survival was also reported at 10^{-5} and 10^{-6} M chlordane. The specificity of the assay to detect only agents which interfere with cell-to-cell communication (epigenetic) in contrast to chemicals which induce genotoxicity was demonstrated with the lyphophilic procarcinogen/promutagen, B[a]P. Four doses of B[a]P, up to a cytotoxic level, did not increase the recovery of TG^r colonies.

To support the conclusion that chlordane interferes with intracellular communication, the investigators evaluated the effects of chlordane on guanine metabolism of treated ARL cells. At the cytotoxic level and at those concentrations that profoundly affected cell-to-cell communication in the primary assay (10^{-4} to 10^{-6} M), chlordane did not cause an appreciable reduction of [^{14}C] guanine conversion to its principal metabolites. Although a positive control which is known to inhibit cell-to-cell communication, such as DDT, was not included in this experiment, we assess that the findings provide evidence that chlordane interferes with metabolic cooperation suggesting that chlordane may act as a promoter.

Further suggestive evidence for a promoter effect is provided by the study of Brubaker and colleagues (1970), who assessed that trans-chlordane was positive for cell division inhibition but negative for inhibition of DNA synthesis in mouse lymphoma (L5178Y) cells.

OVERALL INTERPRETATION OF STUDY RESULTS

The individual published studies were insufficient to establish a complete genetic toxicology profile for chlordane. When considered collectively, however, some preliminary assessments can be made.

The single bacterial gene mutation assay with primary data and the survey studies with procaryotes show that chlordane is not mutagenic in bacteria. The positive reverse mutation assay using Zea mays provided qualitative evidence of a mutagenic response induced by chlordane in a plant system. Although the study reported no primary data, chlordane is classified as presumptively mutagenic.

Mammalian cell gene mutation assays with chlordane showed that the test material was not genotoxic in ARL cells or human fibroblasts. Although nonactivated chlordane induced an inconclusive, mutagenic response in Chinese hamster V79 cells, the validity of this assay was seriously compromised because of technical deficiencies, the reporting of positive responses with compounds that are either weakly mutagenic or not mutagenic, and the lack of a positive control. Although we classified chlordane as an unconfirmed presumptive positive mutagen for Chinese hamster V79 cells, we have serious reservations about the validity of the study, and recommend that these results be verified in a complete assay (i.e., three or more noncytotoxic doses, with and without metabolic activation and with known mutagenic positive controls).

Although chlordane proved to be nonclastogenic in dominant lethal assays in male mouse germinal cells, confirmation of its potential clastogenicity in somatic cells is needed.

The results of survey studies indicate that chlordane in the presence of rat and plant microsomes is presumptively recombinogenic in yeast. Data from studies with sufficient primary data to draw meaningful conclusions, however, indicate that chlordane is not genotoxic in primary mouse, rat, or hamster hepatocytes. Although elevations in the SCE frequency were seen in human lymphoid cells, the increases were not considered indicative of a genotoxic response. On the other hand, chlordane was reported to induce SCE in central mudminnows, and in CHO cells without activation.

There was a statistically significant increase in UDS reported in SV-40 transformed human fibroblasts exposed to nonactivated doses of chlordane; however, the effect was eliminated in the presence of rat S9 activation. The validity of this assay was seriously compromised by numerous technical deficiencies, strong positive responses by weakly genotoxic or nongenotoxic substances, the lack of positive controls, and the use of a transformed cell line. The UDS assay should be repeated with a nontransformed cell line.

In preliminary results with a novel assay, Telang et al. (1982) reported that chlordane interferes with cellular communication. As enunciated by these authors, the mechanism by which chlordane inhibits such metabolic cooperation may involve intercellular communication mediated by gap junctions and thus, interference with this process could be produced by a variety of

effects on the cell membrane. Since organochlorine pesticides are lipophilic in nature, they consequently tend to accumulate in the lipid layer of the cell membrane, thereby altering membrane functions. Therefore, these authors suggest that accumulation of organochlorine pesticides in the cell membrane could directly interfere with the function of gap junctions.

The combined results of gene mutation assays indicate that chlordane is not mutagenic in bacteria. On the other hand, presumptive (unconfirmed) positive results have been reported in V79 and L5178Y cells with and without activation, as well as in plant assays. DNA repair assays indicate chlordane appears to be not genotoxic in bacteria, rodent hepatocytes and human lymphoid cells, but is an unconfirmed presumptive genotoxin in yeast and transformed human fibroblasts.

Based upon the lack of a definitive genotoxic effect, the positive results in the metabolic cooperation assay, and the evidence that chlordane has properties similar to known promoters, chlordane may act by promotional (epigenetic) mechanisms. We recommend, however, that this indication of promoter activity be corroborated in additional studies.

REGULATORY REQUIREMENTS

The following assays are needed to complete the genetic toxicology requirements for chlordane:

- Gene mutation assays in mammalian cell systems with and without metabolic activation in accordance with acceptable procedures.
- *In vivo* mammalian assays for chromosome aberrations in somatic cells (e.g., rodent bone marrow).
- *In vitro* assays for SCE (with and without activation) in mammalian cell systems.

RECOMMENDATIONS

The following tests are recommended to investigate the potential promotional activity of the test material, according to recognized procedures:[3]

- *In vitro* transformation with promotion assay.
- *In vivo* promoting activity assay.

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3 Weisburger, J.H.; Williams, G.M. (1984) Bioassay of carcinogens: *In vitro* and *in vivo* in *Chemical Carcinogens*, Vol. 2, Univ. of Birmingham, UK, Am. Chem. Soc. Monograph 182, pp. 1323-1373.

MUTAGENICITY TESTING OF CHLORDANE

Genetic Category	Study Type/ Test System	Test Materials: Dose (mg/kg) or Concentration (ug, ppm) Range	Citation	Reported Results	TB Evaluation	Comment
Gene Mutation	Bacterial reversion in S. typhimurium (Ames Test)	[Unspecified "commercial"]: 50 only, +/- rat S9 or corn S14, or both combined	Wildemann/Nazar (1982)	Negative under all conditions	Unacceptable	Only one dose of an unspecified commercial preparation in two strains only. (TA98, TA100)
		[Unspecified]: 1 to 10,000 (gradient), +/- rat S9	Probst et al. (1981)	Negative	Unacceptable	Survey screen of 218 compounds
		Technical: (unreported doses), +/-S9 [Unspecified "Commercial"]	Gentile et al. (1982)	Negative	Unacceptable	Summary qualitative results only with unreported doses of commercial/technical grades
		Technical: (1) 0.1 to 10.0, -S9 (2) 100 to 10,000, +S9 (rat, hamster)	Haworth (1986)	Negative under all conditions up to cytotoxic/ insoluble levels	Acceptable	-----
		Trans-Chlordane: (1) 1 to 10, -S9 (2) 100 to 10,000, +S9 (rat, hamster)	Mortelmans et al. (1986)	Negative under all conditions up to cytotoxic/ unsoluble levels	Acceptable	-----

MUTAGENICITY TESTING OF CHLORDANE

Genetic Category	Study Type/ Test System	Test Materials: Dose (mg/kg) or Concentration (ug, ppm) Range)	Citation	Reported Results	TB Evaluation	Comment
Gene Mutation (Cont'd)	Bacterial reversion in S. typhimurium (Ames Test) (Cont'd)	[Unspecified]: 20 to 100, +/-S9	Simmon et al. (1977)	Positive only in TA100	Inconclusive	Insufficient procedural details (toxicity, etc.) with an unspecified preparation.
		[Unspecified]: (1) 10 to 5000 (2) 1000 to 10,000	Simmon/Tanaka (1977)	Positive in TA100, equivocal in TA98	Inconclusive	Insufficient details with an unspecified preparation.
		[Unspecified]: 5 to 10,000, only with TA98, TA100	Maruyama (1980)	Positive in TA100, negative TA98	Inconclusive	Insufficient details with an unspecified preparation.
	Bacterial reversion in E. coli	[Unspecified]: 1 to 10,000 (gradient), +/- rat S9	Probst et al. (1981)	Negative	Unacceptable	Survey screen.
		Technical: 1000/disc, -S9	Ashwood-Smith et al. (1972)	Negative	Unacceptable	Survey screen without activation in spot tests.
		Commercial (Ortho-Klor 74 Spray): (44% of a commercial prep -S9	Ficsor (1972)	Negative	Unacceptable	Survey screen without activation in spot tests.
	Plant mutation in corn (Zea mays, wx)	Commercial: 2.24 kg/ha sprayed in situ	Gentile et al. (1982)	Positive for pollen grains containing Wx	Inconclusive	Summary results with a commercial preparation.

MUTAGENICITY TESTING OF CHLORDANE

Genetic Category	Study Type/ Test System	Test Materials: Dose (mg/kg) or Concentration (ug, ppm) Range)	Citation	Reported Results	TB Evaluation	Comment
Gene Mutation (Cont'd)	Mammalian cell forward mutation in vitro (V79, oua)	[Unspecified]: 10 uM, -S9 only	Ahmed et al. (1977a)	Positive at single non-toxic dose	Inconclusive	Only one dose without activation
	Mammalian cell forward mutation in vitro (ARL18, HGPRT)	Technical: 10^{-3} to 10^{-6} M (S9 not required)	Telang et al. (1982)	Negative up to cytotoxic doses	Acceptable	-----
	Mammalian cell forward mutation in vitro (D-550, HGPRT)	Technical: 10^{-4} to 10^{-5} M, +/- rat hepatocytes	Tong et al. (1981)	Negative up to cytotoxic doses	Acceptable	-----
	Mammalian cell forward mutation in vitro (L5178Y, TK)	Trans-Chlordane, -S9 only	NTP (1985)	Positive without S-9	Inconclusive	Not tested +S9 activation
Chromosome Aberrations/ Damage	Mouse dominant lethal	Technical: 50, 100 (300, 1000) once, oral, ip. AG-Chlordane: 1X 50, 100 (300, 1000), oral and ip	Arnold et al. (1977)	Negative at nontoxic doses (50, 100 mg/kg) in 6-week matings	Acceptable	----

MUTAGENICITY TESTING OF CHLORDANE

Genetic Category	Study Type/ Test System	Test Materials: Dose (mg/kg) or Concentration (ug, ppm) Range)	Citation	Reported Results	TB Evaluation	Comment
Chromosome Aberrations/ Damage (Cont'd)	Mouse dominant lethal (Cont'd)	Technical: (1) 1 x 48 and 240, ip (2) 5 x 50, gavage CIS (Alpha)-isomer: (1) 1 x 58 and 290, ip (2) 1 x 42 and 210, ip (3) 5 x 75, gavage TRANS (Gramma)-isomer: (1) 5 x 50, gavage	Epstein et al. (1972)	Negative up to toxic levels for all preparations in 8-week matings	Acceptable	-----
	Cytogenetics in mammalian cells *in vitro* (CHO/CA)	Trans-Chlordane: (?) +S-9	NTP (1985)	Negative for CA w/w'out S9	Acceptable	-----
DNA Damage Repair	Yeast gene conversion (*S. cerevisiae* D4)	Technical: Doses not specified, but tested +/- rat S9 and corn 1S)	Gentile et al. (1982)	Positive with activation only	Inconclusive	Summary of qualitative results at unreported doses.
		[Unspecified]: "0.75%"	Chambers/Dutta (1976)	Positive	Inconclusive	Abstract summary only without procedural details
	DNA strand breaks in Col E1 plasmid E. coli K-12 host	[Unspecified]: 100 (-S9)	Griffen/Hill (1978)	Negative	Unacceptable	Single dose in a screening survey of new procedure.

MUTAGENICITY TESTING OF CHLORDANE

Genetic Category	Study Type/ Test System	Test Materials: Dose (mg/kg) or Concentration (ug, ppm) Range)	Citation	Reported Results	TB Evaluation	Comment
DNA Damage Repair (Cont'd)	Unscheduled DNA synthesis (UDS) in primary hepatocytes *in vitro* (hepatocytes from rat, mouse, hamster)	[Unspecified]: 10^{-1} to 10^{-6} M (rat, mouse, hamster)	Maslansky and Williams (1981)	Negative up to cytotoxic doses	Acceptable	-----
		[Unspecified]: 0.5 to 1000 nM/mL (rat only)	Probst et al. (1981)	Negative up to cytotoxic doses	Acceptable	-----
	Inhibition of repair (CsCl density gradient assay) in mammalian cells (HeLa)	Cis (alpha) Chlordane: 0.04 mM, +/- uv (300 ergs/mm^2)	Brandt et al. (1972)	Negative	Unacceptable	Only one dose tested in a questionably sensitive assay
	UDS in transformed (SV-40) human fibroblasts (VA-4)	[Unspecified]: 1, 10, 100, and 1000 uM, +/- rat S9	Ahmed et al. (1977b)	Positive only without activation (+S9, no effect) for long-patch repair	Inconclusive	-----
	Sister-chromatid exchange (SCE) *in vitro* (human lymphoid cells, LAZ-007)	Trans-Chlordane: 10^{-4} to 10^{-6} M, +/- rat S9	Sobti et al. (1983)	Positive at noncytotoxic doses (10^{-5}, 10^{-6} M)	Inconclusive	Non dose-related response in a nonconventional cell line.
	SCE *in vivo* (intestinal cells of exposed central mudminnows, *Umbra limi*)	Technical: 5.4 x 10^{-9} to 5.4 x 10^{-12} M for 11 days in aquaria water	Vigfusson et al. (1983)	Positive, dose-related increase	Acceptable	-----

MUTAGENICITY TESTING OF CHLORDANE

Genetic Category	Study Type/ Test System	Test Materials: Dose (mg/kg) or Concentration (ug, ppm) Range)	Citation	Reported Results	TB Evaluation	Comment
DNA Damage Repair (Cont'd)	SCE *in vitro* (CHO)	Trans-Chlordane: (?) -S9	NTP (1985)	Positive without S9	Inconclusive	Not tested with S9 activation
Other Mechanisms	Metabolic cooperation (cell-to-cell communication) *in vitro* (ARL $HGPRT^-/HGPRT^+$ cells)	Technical: 10^{-4} to 10^{-7} M to mixed cultures	Telang et al. (1982)	Positive for inhibition of intercellular communication	Acceptable	-----
	Inhibition of replication in mammalian cells *in vitro* (L5178Y)	Trans-Chlordane: 4 ug/mL, -S9	Brubaker et al. (1970)	Positive for inhibition of cell division (G2 arrest). Negative for DNA synthesis	N/A	Only one dose tested without activation.

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APPENDIX I

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chlordane/Heptachlor Mutagenicity

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Irv Mauer 12-16-85

TO: Henry Spencer, Ph.D., Pharmacologist
Section 7, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Jane E. Harris, Ph.D.
Section Head, Toxicology Branch
Hazard Evaluation Division (TS-769C)

This is in response to your request for an expeditious appraisal of the positive mutagenicity studies for chlordane and heptachlor/heptachlor epoxide, as reported in the CAG document ("Carcinogenicity Risk Assessment for Chlordane and Heptachlor/Heptachlor Epoxide"). The negative reports listed in that document were also scanned.

Based upon recent Velsicol submissions (Accession Nos. 254320 and 254324) and other information available to me (NTP, EMIC), two general conclusions are warranted from this preliminary assessment (see below for a summary of available data):

1. Although the adequacy of the data base as reported could not be undertaken (e.g., DER's) because of the timely response requested, it appears that all the genetic end-points we require to be assayed (gene mutation, chromosomal aberrations, DNA damage/repair) have been addressed.

exposed central mudminnows (Umbra limi), as well as in CHO cells in culture and in a human lymphoid cell line (one report each). Whereas the positive SCE in fish was recorded at nonlethal concentrations (added to aquarium water), both in vitro studies were only marginally positive (less than twofold above controls) at toxic concentrations. Only one of the four studies assaying for unscheduled DNA synthesis (UDS) in vitro was positive (in VA-4 cells, an SV-transformed human cell line), but only in the absence of metabolic activation (negative with MA). Technical chlordane was negative for UDS in primary hepatocyte cultures from rats, mice, and hamsters (two reports) as well as in a human fibroblast cell line (D-550). Finally, gene conversion was reported in yeast cells (S. cerevisiae D4) exposed to an activation system (negative without), but too few data are included in the article to properly interpret the results.

2. Heptachlor/Heptachlor Epoxide. In contrast to the positive results for (gene) mutagenicity reported for chlordane (both technical and reagent grades), the available data for heptachlor and its epoxide is consistently negative (three Ames and/or E. coli, one B. subtilis rec assay, 1 ARL-HGPRT, two Drosophila SLRL, 1 HPC/UDS). Reversions to histidine prototrophy in Salmonella TA-1535 and 100 under activation conditions to an unstated dose range, as well as to the wild-type (nonwaxy) phenotype in corn pollen grains were reported by the same investigator who found "commercial" chlordane positive.

Adequate reports on dominant-lethal assays in mice were negative, but an abstract from a meeting reported positive results for both germinal (DLT) and somatic (bone marrow) chromosome damage in rats fed 1 and 5 ppm of an unstated formulation of heptachlor for three generations. A Russian study also reported positive chromosome damage in bone marrow cells from "white male mice" treated i.p. with "heptachlor" (also of unstated source and purity) at a single dose level stated to be "4% of the LD_{50}." Too few procedural details are included in these "positive" studies to interpret the results reported.

As with chlordane, negative UDS results have been reported for heptachlor technical in primary rodent hepatocytes (two reports), but a positive recorded in VA-4 cells (virus-transformed human fibroblasts) for both the technical and epoxide, but only with metabolic

2. Consistent with the results of mutagenicity testing previously reported for other agents of this chemical class (organochlorines such as Lindane, chloroform, Inter alia), the mutagenic potential of these chemicals are low to unsubstantial. Hence, from the available data it would appear their oncogenic potential derives from mechanisms other than "genetic initiation" (direct interaction with DNA). [There is suggestive evidence in mammalian systems (the few promotion assays available) of an "epigenetic" mode of action for these chemicals.]

Survey of Available Literature

1. Chlordane-technical (but not reagent-grade alpha-chlordane, gamma-chlordane, and gamma-chlordene) was positive in adequate bacterial (Ames) assays (three positive, one negative), but consistently so only in the most sensitive strain (TA-100), and only at very high concentrations (5000 ug/plate and above). Testing for mutagenicity in mammalian cells in culture (four reports) revealed inconsistent results (two positive, two negative), due in part to the different test systems employed. The two negatives were reported for HGPRT in ARL (adult rat liver) and for both thioguanine and diphtheria-toxin resistance in V79 (Chinese hamster lung) cells. One of the positives was for ouabain resistance in V79 cells, but reported for only a single concentration of an unstated formulation, at less than 50 percent cell survival; the second was found in non-activated mouse lymphoma cells (L5178Y-TK), but the study is incomplete since metabolic activation was not employed.

   The plant systems may activate chlordane to mutagenic derivatives is suggested by a single article reporting both gene conversion in Saccharomyces cerevisiae D4 cultures exposed to a reagent grade (presumably alpha-chlordane according to the CAS Number stated), and reversion to wild type in pollen grains from the homozygous "waxy" strain W22 of Zea mays (corn) exposed to a "commercial-grade formulation." However, too few procedural details were reported to adequately interpret these results.

   Although reportedly negative for gross chromosomal damage in vivo (two mouse DLT's) and in vitro (CHO cells), technical chlordane or one of its principal components (alpha-chlordane) apparently has DNA-damaging activity, as revealed in a single study reporting increased dose-dependent sister-chromatid exchanges (SCE) in intestinal cells sampled from

activation (in contrast to chlordane, positive only in the absence of activation). [The mutagenicity data base for heptachlor epoxide is less than adequate to satisfy FIFRA guidelines.]

Discussion

Recent studies have suggested that organochlorines (Lindane, chlordane, heptachlor, inter alia) do not interact directly with DNA (i.e., are not "genotoxic"), but rather act "epigenetically" by mechanisms affecting cell membrane permeability and/or following an irreversible "initiating event" (i.e., are "promoters" ensuring survival of preexisting transformed cells). The following citations employing chlordane heptachlor available for this "quick-and-dirty" review (and listed in the CAG document) are consistent with this suggestion:

1. Inhibition of metabolic cooperation in mixed cultures consisting of thioguanine-resistant and TG-sensitive cells (several reports from both Williams' and Trosko's labs).

2. The putative positive UDS results in cells already "initiated" (e.g., the SV-40 transformed cell line, VA-4).

3. Inhibition of DNA synthesis and/or cell cycle mechanisms by severely toxic concentrations leading to perturbation of repair (increased UDS, SCE).

cc: Dr. Amy Rispin
Science Intergration Staff
Hazard Evaluation Division (TS-769C)

PRELIMINARY EVALUATION OF REPORTED POSITIVE STUDIES

Compound	Test Material	Assay	Test System	Dose/Conc. Range	Reference	TB Evaluation	Comments
CHLORDANE	Technical	Gene Mutation	Ames (all)	20-100	Simmon *et al.* (77)	ACCEPT.	POS. only in TA 100
	Technical	Gene Mutation	Ames (all)	a) 10-5000 b) 1000-50,000	Simmon and Tanaka (77)	ACCEPT.	a) EQUIV in TA 98 b) POS. in TA 100
	Technical	Gene Mutation	Ames (TA 98, 100)	5-10,000	Maruyama (80)	ACCEPT.	POS. in TA 100; NEG. in TA 98
	Reagent (alpha)	Gene Mutation	Sacch. - D4	(Unstated)	Gentile *et al.* (82)	INCONCL.	POS. only + S9; too few data
	"Commercial"	Gene Mutation	*Z. mays* Pollen	(Unstated)	*Ibid*	INCONCL.	Tech not tested; too few data
	(Unstated)	Ouabain resist.	V79 cells	(Only 1 dose reported)	Ahmed *et al.* (77b)	INCONCL.	"Weak" at < 50% survival; few data
	(Unstated)	Gene Mutation	ARL/HGPRT	10^{-3}-10^{-6} M	Telang *et al.* (82)	ACCEPT.	NEG. for HGPRT, but POS. for promotion
	Technical	*In vivo* SCE	Mudminnow	$5.4/10^{-12}$-10^{-9}	Vigfusson *et al.* (83)	ACCEPT.	Dose-dependent POS.
	Reagent (alpha)	*In vitro* SCE	LAZ-007 cells	10^{-6}-10^{-3} M	Sobti *et al.* (83)	INCONCL.	< 2-fold increase; no dose response
	(Unstated)	*In vitro* UDS	VA-4 cells	1,10,100,1000 uM	Ahmed *et al.* (77a)	ACCEPT.	POS. only without S9
	Reagent (gamma)	Cell cycle inhib.	L5178Y cells	4 ug/ml	Brubaker *et al.* (70)	INCONCL.	NEG. for DNA, POS. for G2 arrest; only 1 dose
	Reagent (alpha)	*In vitro* CA/SCE	CHO cells	(Unstated)	NTP (85)	INCONCL.	NEG. for CA; POS. for SCE
	Reagent	Gene Mutation	L5178Y/TK	(Multiple)	NTP (85)	INCONCL.	Not tested with S9

PRELIMINARY EVALUATION OF REPORTED POSITIVE STUDIES (cont'd)

Compound	Test Material	Assay	Test System	Dose/Conc. Range	Reference	TB Evaluation	Comments
HEPTACHLOR	Technical	Gene Mutation	Ames (all)	(Unstated)	Gentile *et al.* (82)	INCONCL.	POS. in TA 1535/100, but only with S9
	(Unstated)	Gene Mutation	ARL/HGPRT	10^{-7} to 10^{-4} M	Telang *et al.* (82)	ACCEPT.	NEG. for HGPRT; POS. for promotion
	(Unstated)	*in vitro* UDS	VA-4 cells	100,1000 uM	Ahmed *et al.* (77a)	INCONCL.	POS. only with S9, but no values for UDS
	Epoxide	*in vitro* UDS	VA-4 cells	10,100,1000 uM	Ibid.	INCONCL.	POS. only with S9, but no values for UDS
	(Unstated)	*in vivo* CA	Mouse BM	5 mg/kg	Markarjan (66)	INCONCL.	Too few procedural details; 1 dose
	(Unstated)	DLT/BM-CA	Rat repro. study	1, 5 ppm	Carey (74)	INCONCL.	[Abstract]
	Technical	*in vitro* CA/ SCE	CHO cells	(Multiple)	NTP (85)	INCONCL.	POS. for both CA and SCE, but too few details available

APPENDIX B

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: The Mutagenicity Assessment (Gene-Tox Profile) of Heptachlor/Heptachlor Epoxide: Addendum to the Registration Standard

TB Projects: 0007
1244

Caswell Nos. 474
474F

FROM: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Irving Mauer 11-25-86

TO: Henry Spencer, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769C)

and

Amy S. Rispin, Ph.D.
Science Integration Staff
Hazard Evaluation Division (TS-769C)

THRU: Judy Hauswirth, Ph.D., Acting Head
Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Judith W. Hauswirth 11/25/86

The attached assessment of the mutagenicity of heptachlor* was developed from reviews of the available published literature located by Dynamac Corporation, as well as articles filed with

* Technical heptachlor is a ternary mixture of related chlorinated methanoindene compounds, typically containing 72 to 73 percent 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanol-1H-indene (CAS Registry No. 76-44-8) as principal active ingredient, 18 to 20 percent trans (gamma) and 2 to 3 percent cis (alpha)-chlordane (CAS No. 57-74-9), and 5 percent nonachlor (CAS No. 3734-49-4), plus about 1 to 2 percent other manufacturing impurities. Heptachlor is readily metabolized in mammals to the epoxide (2,3,4,5,6,7,7-heptachloro-1a,1b,5,5a,6,6a-hexahydro-2,5-methano-2H-ideno(1,2-b) oxirene---CAS No. 1024-57-3).

the Environmental Mutagen Information Center (EMIC, Oak Ridge National Laboratory) for evaluation in Phase I of the Agency's GENE-TOX PROGRAM. A preliminary appraisal of some of these studies** as summarized by the Carcinogen Assessment Group (CAG)*** is also incorporated, since the conclusions in that preliminary discussion are consistent with those derived from this more comprehensive evaluation. There appears to have been no submissions of primary gene-tox (mutagenicity) studies by registrants (Caswell No. 474).

As provided previously for the Registration Standards of other organochlorine insecticides (aldrin/dieldrin), this mutagenicity assessement is organized as follows: A discussion and evaluation (for regulatory purposes) of the data base located by both major sources (DYNAMAC; EMIC/GENE-TOX PROGRAM), tabulated according to the FIFRA categories of genetic effect (gene mutation, chromosomal damage, primary DNA damage/repair, and other mechanisms), followed by our conclusions, as well as recommendations to satisfy both regulatory requirements ("data gaps") and to indicate further testing the results from which would be useful for risk characterization.

Although a definitive genetic toxicology profile cannot be established from the individual published studies reviewed here, collectively they permit the following preliminary conclusions:

1. Neither heptachlor nor heptachlor epoxide appears to be mutagenic in microbial or mammalian cells.

2. Although negative for chromosome damage in germinal assays, the potential for somatic cytogenetic activity has not been adequately evaluated.

3. The combined results of DNA repair assays indicate that heptachlor is not genotoxic in bacterial, yeast, or mammalian cells.

4. There is evidence suggesting that these organochlorines act by epigenetic (e.g., promoter) rather than established genotoxic mechanisms.

In addition to our previous cursory assessment (Appendix I), a listing of published articles cited is attached as Appendix II.

Attachments

** "Chlordane/Heptachlor Mutagenicity," memorandum: Mauer to Spencer, December 16, 1985 (attached as Appendix I)

*** Carcinogen Assessment Group: "Carcinogenicity Risk Assessment for Chlordane and Heptachlor/Heptachlor Epoxide" (DRAFT, December, 1985).

MUTAGENICITY OF HEPTACHLOR

The Data Base

Eighteen published articles were located for review, reporting the results of 32 assays with technical heptachlor/heptachlor epoxide (or other formulations), distributed according to genetic endpoint as follows:

Genetic Endpoint	Test System	Chemical	
		Heptachlor	H. epoxide
Gene Mutation	Bacterial	12	5
	Mammalian cell	1	-
	in vivo	2	2
Chromosome Aberration	*in vitro*	-	-
	in vivo	4	2
DNA Damage/ Repair	*in vitro*	6	1
	in vivo	-	-
Other Mechanisms	*in vitro*	1	-
	in vivo	2	-

Fifteen (47%) of these reports are inadequate (judged UNACCEPTABLE) to support the negative results reported, because of one or more of the following deficiencies:

- No primary data presented, and/or only qualitative assessments (+/-);
- Preliminary screening surveys or new techniques, with insufficient details/procedures;
- Only one dose or insufficient (nontoxic) doses tested in incomplete assays;
- Assays with no positive controls to assure sensitivity of the test system to respond; and
- Inappropriate procedures/test systems.

However, these studies were considered to corroborate the conclusions from the minority of sufficiently adequate assays

(10, or 31%) judged ACCEPTABLE. An equal number of assays were judged "inconclusive" because they reported unconfirmed (presumptively) positive results which could not be satisfactorily interpreted, due to inadequate procedures or controls, and/or the reporting of qualitative assessments in assays compromised by conflicting variables. Some of these studies are useful, however, in directing attention to further testing necessary to establish the mechanism(s) of action possessed by these organochlorines, discussed more fully below.

Since the available data base is incomplete in some of the genetic endpoints (as indicated in the above summary table), further testing is recommended (see below) in order to satisfy both the regulatory requirements for continued registration of heptachlor formulations and to provide approaches to risk characterization.

Discussion and Evaluation of Assay Results

The results of all published studies conducted with technical heptachlor/heptachlor epoxide, as well as other formulations of this organochlorine insecticide, are summarized below in tabular form (following this discussion).

A. Survey Studies

As indicated in the summary table, a number of these reports are considered technically inadequate and unacceptable in meeting regulatory requirements, but tend to support the overall conclusions drawn from fully adequate studies discussed below.

Thus, as reported by Moriya *et al*. (1983), concentrations up to 5000 *ug*/plate of heptachlor and the oxidation product, heptachlor epoxide, were negative for mutagenicity in both the *Salmonella typhimurium*/mammalian microsome (Ames test) and the *Escherichia coli* tryptophan reversion assays. Similarly, qualitative negative results were reported over a onefold to 10,000-fold nonactivated and S9 activated dose range of heptachlor in the concentration agar gradient test with eight *S. typhimurium* and two *E. coli* strains (Probst *et al*. 1981). In an unactivated spot test, 20 *ug*/disc of heptachlor was also negative with four *S. typhimurium* and two *E. coli* strains (Shirasu *et al*. 1976).

Gentile *et al*. (1982) reported that technical but not commercial grade heptachlor induced a 2.3-fold and 1.6-fold increase in histidine revertants of *S. typhimurium* TA100 and TA98, respectively, in the presence of rat S9 activation and at the reported highest active dose (10 *ug*/mL). Although reported as positive, the results are considered inconclusive evidence for a positive response for the following reasons: the data presented did not include the concentrations or the number of revertants at

doses lower than 10 ug/mL to support a dose-related increase; moreover, the increase with TA100 was only slightly higher than 2-fold at the highest dose, and the background spontaneous frequency for TA98 (112) was excessive.

The same authors also reported that 889 uM technical heptachlor induced a 2.3-fold increase in revertents in S. typhimurium TA1535 cultures exposed to a 1S fraction derived from corn microsomes; this finding was also considered inconclusive evidence for a positive response for the same reasons as above.

In the same article, Gentile and associates also reported positive results in a reverse mutation assay using commercial grade heptachlor on Zea mays at the waxy (wx) locus. In this study, 1.12 kg/hectare of the formulation was sprayed in situ on mutant seeds (wx^-/wx^-) prior to the emergence of corn seedlings. Plants were allowed to grow 12 to 14 weeks and pollen grains were harvested, dehydrated, and stained. The authors reported a significant increase ($p < 0.05$) in back mutations of wx^- to wx^+. However, because essential primary data were not present, the results are considered inconclusive, but heptachlor is listed as a presumptive mutagen in this study.

Four survey articles evaluated the potential of heptachlor to interact with and damage DNA (Shirasu et al. 1986; Gentile et al. 1982; Griffin and Hill 1978; Seiler 1977). When 20 ug/disc of non-activated heptachlor were placed on a Petri dish, Shirasu and associates recorded no preferential inhibition of Bacillus subtilis repair-deficient M45 relative to the repair-proficient wild-type, H17. Similarly, Griffin and Hill reported that 0.1 mg/mL heptachlor did not induce singlestrand breaks or alkali-labile sites in purified Col E1 DNA derived from E. coli K12, and the Gentile group reported that four doses of technical or commercial grade heptachlor over a threefold to hundredfold dilution range, with or without rat liver S9 or corn 1S microsomal fractions, were not recombinogenetic in Saccharomyces cerevisiae D4.

Finally, Seiler (1977) reported that there was no increased inhibition of testicular DNA synthesis in mice exposed by gavage to 40 mg/kg heptachlor. Although the author provided data that indicate a high rate of detection of mutagens/carcinogens in the inhibition of testicular DNA synthesis assay, several well documented genotoxic/carcinogenic agents gave a negative response in this test system (ethylenethiourea, aminotriazole, maleic hydrazide, and isoniazid). Hence, interpretation of a negative result in this assay is unclear.

Although individually the data provided by these survey articles were insufficient to form definitive conclusions and thus unacceptable in meeting regulatory requirements, nevertheless the data collectively provide evidence that heptachlor is not

mutagenic in bacteria. In addition, apparently heptachlor does not cause other genotoxic responses in bacteria or yeast. On the other hand, the ability of heptachlor to induce a mutagenic response in corn is not clearly established.

B. Primary Studies

A respectable number of genotoxic assays with heptachlor did contain sufficient primary data for review and evaluation according to FIFRA genetic points.

1. Gene Mutation

a. Bacteria

Heptachlor/heptachlor epoxide were assayed in three Ames tests. Marshall et al. (1976) showed that both heptachlor and heptachlor epoxide were cytotoxic at doses that exceeded 2500 ug/plate in the absence of metabolic activation provided by rat S9, and 1000 ug/plate in the presence of rat S9 activation, but neither agent was mutagenic over decreasing twofold dilutions ranging from 1000 to 50 ug/plate. Although the more sensitive S. typhimurium TA98 and TA100 were not included in the assays, the study is acceptable, since the study was properly controlled in that heptachlor and the epoxide product were adequately tested in S. typhimurium TA1535, TA1536, TA1537, and TA1538.

Glatt et al. (1983) evaluated six half-log dilutions (3000-10 ug/plate) of heptachlor epoxide with or without rat S9 activation in the Ames assay (strains TA1535, TA1537, TA1538, TA98, and TA100) and found that the test material was insoluble at concentrations of 300 ug/plate or greater. Their results further showed that the epoxidation product of heptachlor was negative. By contrast, the authors clearly demonstrated the sensitivity of the test system to detect epoxides of several potent polycyclic aromatic hydrocarbon carcinogens.

In an ancillary assay reported in the same publication, Glatt and associates assayed the same six selected doses of S9-activated heptachlor epoxide (10 to 3000 ug/plate) using TA98 in the presence of the epoxide hydrolase inhibitor and glutathione depletor, 1,1,1-trichloropropene-2,3-oxide (TCPO). Under these conditions, heptachlor epoxide was not mutagenic. Since the study was well designed, properly controlled, and heptachlor epoxide was assayed up to the limit of solubility, the study is acceptable.

As part of the National Toxicology Program, heptachlor was selected for evaluation in the preincubation modification to the Ames test with strains TA1535, TA1537, TA98, and TA100 (NTP, 1983). Preliminary studies showed that this compound precipitated at doses exceeding 1000 ug/plate. Two independent assays were conducted with five nonactivated doses (0.3 to 1000 ug/plate in

experiment 1 and 0.1 to 10 ug/plate in experiment 2). No increase in His^+ colonies of any strain was reported for either study. Five doses (100 to 10,000 ug/plate) were used for the S9-activated preincubation assays; samples were preincubated for 20 minutes with the appropriate strain and either 10 percent rat or hamster S9 cofactor mixes. In two independent assays, S9-activated heptachlor was not mutagenic. Thus, heptachlor appears to have been adequately tested up to an insoluble level in a properly controlled study. Therefore, this study is acceptable, and we conclude that heptachlor is nonmutagenic in this preincubation modification of the Ames assay.

Finally, van Dijck and van der Voorde (1976) found that isomer A of technical heptachlor epoxide was negative in seven Ames strains of S. typhimurium capable of detecting base-substitution, frameshift, and differential toxicity, when tested up to cytotoxic doses in the absence and presence of mouse mammalian activation (S9) systems.

b. Mammalian Cells

Telang et al. (1982) exposed 4 x 10^5 primary adult rat liver epithelial cells (ARL) to six concentrations of heptachlor (10^{-3} to 10^{-6} M) in two independent experiments. Heptachlor was cytotoxic at 10^{-3} M but at no dose was there an increase in the frequency of 6-thioguanine-resistant mutants ($6\text{-}TG^r$). By contrast, the positive controls, benzo[a]pyrene (B[a]P) or 7,12-dimethylbenz-[a]anthracene (DMBA), induced definitive mutagenic responses. This study was judged acceptable and the results demonstrated that heptachlor is not mutagenic in mammalian liver cells.

c. In Vivo

The negative sex-linked lethal result reported for both heptachlor and its epoxide in Drosophila (Benes and Sram, 1969) is discounted because of the low dosage necessary in testing this organochlorine insecticide.

d. Conclusions for Gene Mutation

Heptachlor and/or the oxidation product, heptachlor epoxide, were extensively investigated in S. typhimurium using nonactivated and rat and/or hamster S9-activation conditions. Uniformly, the results indicated that heptachlor was not mutagenic in bacteria. In the only published mammalian cell gene mutation study available for review, the results were also negative. No mammalian cell assays were conducted using the cell lines for which large data bases exist (mouse lymphoma, Chinese hamster ovary cells, or V79), and we recommend that at least one such assay be conducted to confirm the negative result found in primary ARL cells.

2. Structural Chromosome Aberrations

a. Somatic Cells

Only two studies assaying the potential clastogenic (chromosome-breakage) effects of heptachlor on somatic cells were located for review, both considered inconclusive, although reporting presumptively positive results.

In a study reported only as an abstract of a presentation at a meeting of the European Environmental Mutagen Society, Cerey and associates (1973) claimed that 1 and 5 mg/kg/day heptachlor (of unstated purity and source) caused increased "abnormal mitoses" in the mouse bone marrow cells of the F2 and F3 generations of a dietary reproduction study, but provided no other details. The same incompletely reported study also stated that "increased resorptions" ("dominant lethals"?) were found. In the second study, Markaryan (1966) reported that the intraperitoneal injection of "0.0052 mg/g heptachlor" (stated to represent "4% of the LD_{50}" for mice) produced "nuclear disturbances" and unspecified "breakage" in bone marrow cells of an unstated number of mice. In addition to inappropriate sampling techniques employed by this investigator, no attempt was made to differentiate the purely cytological effects of treatment from the standard categories of cytogenetic damage, hence the positive result claimed cannot be properly interpreted.

b. Germinal Cells

In the mouse dominant lethal assay performed by Arnold et al. (1977) a 25:75% blend of heptachlor:heptachlor epoxide was administered once by gavage (7.5 and 15 mg/kg) or i.p. (7.5 and 15 mg/kg) to eight male mice per experimental dose; two control groups (eight males/group) were used for each treatment group. The authors reported no significant increase in dominant lethal indices over a 6-week consecutive mating sequence (three untreated females/male/week). The parameters measured were pregnancy rates, total implants, number of early deaths, and the embryonic index.

No toxic signs were seen during the main study; however, preliminary acute toxicity studies performed by these investigators showed that the heptachlor blend administered either by gavage or i.p. was toxic at 30 and 100 mg/kg. Although the study was performed without a concurrent positive control, the study is acceptable and showed that the maximum nontoxic dose of the heptachlor:heptachlor epoxide mixture did not induce a dominant lethal effect.

In an earlier study Epstein et al. (1972) tested a total of 174 materials, which included heptachlor and heptachlor epoxide, in mouse dominant lethal assays. Groups of seven and nine male

mice received 4.8 and 24 mg/kg heptachlor, respectively, by i.p. injection and other groups of 12 animals each were dosed by gavage for 5 consecutive days with 5 and 10 mg/kg/day. The concurrent negative control group consisted of 10 males. Following dosing, the males were sequentially mated (three untreated females) for 8 weeks. Females were sacrificed 13 days after mating and were scored for pregnancy, total implants, and early fetal deaths. Based on the detailed criteria developed by the authors to evaluate assay validity and positive responses, heptachlor was assessed to be negative. Although no pharmacotoxic responses were reported for the i.p. dosed groups, two of the males that received either 5 or 10 mg/kg of test material by gavage died. Therefore, heptachlor did not induce dominant lethal effects in mice when assayed up to a toxic dose level, and since all appropriate controls were included, the study is acceptable.

Heptachlor epoxide (6 and 30 mg/kg, i.p. and 8 mg/kg, p.o. x5) was also investigated by Epstein and associates. No toxic response was reported for the i.p.-dosed animals; however, 4 to 9 males exposed by gavage died. No dominant lethal effects among offspring of females mated with the heptachlor epoxide-treated males were observed. Since heptachlor epoxide was also assayed up to a toxic dose, the study is acceptable.

c. Conclusions for Structural Chromosomal Aberrations

Only inconclusive evidence for heptachlor to induce chromosomal aberration in somatic cells was available for review. On the other hand, in two adequate mouse dominant lethal assays, neither the parent nor epoxidation product induced major chromosome aberrations in male germinal cells.

3. Other Mutagenic Mechanisms

a. DNA Repair in Mammalian Cells

Maslansky and Williams (1981) assayed 10^{-1} to 10^{-6} M heptachlor for unscheduled DNA synthesis (UDS) in rat, mouse, and hamster primary hepatocytes. In three separate experiments, 30 morphologically normal cells/experiment/species were scored for nuclear grains at the highest noncytotoxic dose only. Heptachlor at $\geq 10^{-4}$ M was cytotoxic in all of these cell systems and there were no significant increases in net nuclear grain counts at 10^{-5} M heptachlor. The positive control, DMBA, adequately demonstrated the sensitivity of the test system to detect UDS in rat, mouse, and hamster hepatocytes. The study is acceptable and showed that heptachlor assayed up to a cytotoxic level was not genotoxic in rodent hepatocytes.

Probst et al. (1981) confirmed the lack of heptachlor-induced UDS esponse in rat hepatocytes. No significant increase

in net nuclear grain counts was seen in 20 morphologically normal hepatocytes exposed to the maximum noncytotoxic dose of heptachlor (10 nmol/mL).

In contrast to the negative results in primary rodent hepatocytes, Ahmed et al. (1977) reported qualitative evidence of a UDS response in a virus (SV-40) transformed human fibroblast cell line (VA-4) exposed to S9-activated doses of heptachlor (100 and 1000 uM) and heptachlor epoxide (10, 100, and 1000 uM). It should be noted that hydroxyurea treatment was used to block cell entry into scheduled (S-phase) DNA synthesis. The lack of primary data and the inability to determine if the response was dose related, however, precludes acceptance of these data as definitive evidence of a genotoxic response. These data are suspect for the following reasons:

1. The SV-40, VA-4 is a transformed cell line; a panel of experts[1] has recommended that only nontransformed diploid human cells be used in this assay.

2. The cells used in this study were not grown under reduced serum or arginine deprivation conditions in conjunction with hydroxyurea to ensure complete blockage of S-phase synthesis, increasing the possibility of a false positive response.

3. The length of exposure in the presence of S9 activation (8 hours) was twice the recommended exposure time;[1] hence, the increases in UDS may have been related to the cytotoxicity of the S9 fraction.

4. Several agents that were also demonstrated to induce strong responses in this study, i.e., Diquat, 2,4-D fluid, and carbaryl, have been reported elsewhere to be nongenotoxic or weakly genotoxic.

5. The results were not confirmed.

6. The slides were not reported as being coded to eliminate bias.

Based on this rationale, this study is considered equivocal, but both heptachlor and heptachlor epoxide may be classified as

1 Mitchell, A.D.; Casciano, D.A.; Meltz, M.L.; Robinson, D.E.; San, R.H.C; Williams, G.M.; Von Halle, E.S. Unscheduled DNA synthesis tests, a report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 123(1983): 363-410.

inconclusive presumptive genotoxins for SV-40 transformed human cells.

b. Inhibition of Spindle Apparatus

Markaryan (1966) reported that anaphase and early telophase bone marrow cells harvested from male mice exposed to 0.0052 mg/g heptachlor for 21 hours had increased frequencies of "nuclear disturbances, total chromosome rearrangements (chromosome and chromatid brides and fragments), and adhesions." Based on standard procedures practiced by experts in this assay, however, the study should be considered inconclusive as a positive response for the following reasons:

(1) The method used to prepare anaphase and telophase plates (slices of bone marrow stained with acetocarmine) has a tendency to increase the number of artifacts and lends itself to a planar effect (causing chromosomes to be excluded from a field being viewed); this makes it difficult to distinguish between true chromosomal rearrangements and artifacts.

(2) The number of animals investigated (three male mice) is an inadequate sample population.

(3) The inclusion of cytotoxic effects (vacuolation, karyopyknosis, and karyolysis) in the total percent nuclear disturbances is an indication that severe cytotoxicity occurred at the assay dose, which was stated to be only approximately 4 percent of the LD_{50}.

(4) The authors did not report that the slides were coded.

Since the study results cannot be properly interpreted, the study is considered inconclusive, but presumed positive until refuted in an adequate assay.

c. Conclusions for Other Genetic Mechanisms

Published studies that evaluated the potential of heptachlor to interact with and damage DNA provides evidence that the test material appears to be inactive in rat, mouse, or hamster primary hepatocyte cultures. Based on a single inconclusive and unconfirmed positive in VA-4 cells, both heptachlor and heptachlor epoxide are listed, with reservations, as presumptively genotoxic in human cells. The results of the spindle mechanism/inhibition assay performed in mouse bone marrow cells with heptachlor could not be interpreted.

4. Epigenetic Effects

Telang et al. (1982) evaluated six concentrations of heptachlor (10^{-4} to 10^{-7} M) for the potential to inhibit intercellular communication in mixed cultures of ARL ($HGPRT^-$) 6-TG^r mutant and normal ARL ($HGPRT^+$) cells. In a preliminary dose-selection test, heptachlor was cytotoxic at 10^{-4} for ARL ($HGPRT^-$) cells. In the presence of $HGPRT^+$ cells, 1×10^{-6} to 1×10^{-7} M heptachlor markedly inhibited the metabolic cooperation between the HGPRT-deficient and HGPRTcompetent cells. At 1×10^{-6} M, an almost complete blockage of phosphoribosylated TG transfer between the cells was observed as indicated by the 140 percent and 95 percent recovery of 6-TG^r mutants cocultivated with 0.75 and 1.25×10^6 $HGPRT^+$ cells as compared to the controls. Increased survival of 6-TG^r mutants was recorded at the remaining doses.

The specificity of the assay to detect only agents which interfere with cell-to-cell communication in contrast to chemicals which induce a genotoxic effect was demonstrated with the lipophilic procarcinogen/promutagen B[a]P. Four doses of B[a]P up to a cytotoxic dose did not increase the recovery of TG^r colonies.

To support the conclusion that heptachlor interferes with intercellular communication, the authors also evaluated the effects of heptachlor on guanine metabolism of ARL cells, dosed at both the cytotoxic level and at those concentrations that profoundly affected cell-to-cell communication in the primary assay (10^{-4}-10^{-6} M). Heptachlor did not cause appreciable reductions of [^{14}C]guanine conversion to its principal metabolites, and thus such conversion is apparently not related to intercellular communication.

5. Transformation Assays

No studies assaying the potential for heptachlor or heptachlor epoxide to induce neoplastic transformation in mammalian cells were located.

Overall Conclusions/Recommendations

Although adequate studies are still required in some areas, the evaluation of the available data in published literature on heptachlor and its epoxide indicates that neither directly interferes with DNA or chromosomes, i.e., not mutagenic in the sense of initiating genetic effects likely to be transmitted. Thus, sufficient evidence exists to conclude that neither possesses mutagenic activities in bacteria but further testing is needed to confirm the negative result for heptachlor in other than the single mammalian cell system tested, as well as to assay the

epoxide in at least one sensitive test system. Additional studies are also necessary to resolve presumptively positive mutagenic activities under specialized conditions, e.g., in plant systems.

At least two adequate germinal assays indicate that neither organochlorine poses serious risks of transmissible chromosomal aberrations, but the positive results in inadequate somatic chromosomal studies mandate further testing for this endpoint. Finally, while confirmation of the negative results for DNA damage/repair in microbial and mammalian cells is available, comparable assays with the epoxide are recommended to assess its potential, especially for UDS in mammalian systems.

On the other hand, consistent with genetic toxicology assessments prepared for other members of this class (e.g., aldrin/dieldrin, lindane), these organochlorines probably act by epigenetic mechanisms likely to promote/sustain cellular processes initiated by other agencies, thus indirectly affecting primary genetic mechanisms. Such activity is indicated by inadequate but presumptively positive unconfirmed studies for mitotic spindle effects, inhibition of both cell-to-cell communication and DNA synthesis, as well as the apparent potentiation of repair processes in transformed (i.e., initiated) mammalian cells.

In order to affirm the absence of a potential for direct genotoxic activity, and to complete regulatory requirements, the following additional testing is required:

1. Mammalian cell gene mutation assay with established test systems, e.g. mouse lymphoma (L5178Y/TK), or Chinese hamster (CHO/V79/HGPRT) cells *inter alia*, specifically comparing activation systems (S9) derived from rat vs. mouse (or hamster) liver microsomes; and

2. Somatic cell cytogenetic assays, either *in vitro* or *in vivo*.

Further, to confirm the potential activity of these chemicals in indirect (epigenetic) processes[2-5] the following are recommended:

1. Adequately controlled promotion assays, e.g., in cell lines already initiated (by viral transformation), or exposed to known active chemical initiators.

2. Mammalian cell transformation in systems with an established data base, e.g., C3H 10 T1/2, BALB 3T3, *inter alia*.

3. Assays for mitotic spindle effects (*in vitro* or *in vivo*), and/or involving other cellular mechanisms (e.g., oncogene activation), *inter alia*.

4. Assays which can distinguish effects on replicative S-phase (scheduled) DNA synthesis from UDS, e.g., in primary hepatocytes from several species (mouse vs. rat/hamster).

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2 Malcolm, A.R.; Mills, L.J.; McKenna, E.J. Inhibition of metabolic cooperation between Chinese hamster V79 cells by tumor promoters and other chemicals. In: Cellular Systems for Toxicity Testing, eds. Williams, G.M.; Dunkel, V.C.; Ray, V.A.; *Ann.*, *N.Y. Acad. Sci.* 407(1983):448-450.

3 Williams, G.M. Classification of genotoxic and epigenetic hepatocarcinogens using liver culture assays. *Ann. N.Y. Acad. Sci.* 349(1980):272-282.

4 Williams, G.M. Genotoxic and epigenetic carcinogens: Their identification and significance, *Ann., N.Y. Acad. Sci* 407(1983): 328-333.

5 Weisburger, J.H; Williams, G.M. Bioassay of carcinogens: *In vitro* and *in vivo* in Chemical Carcinogens, Vol. 2, Univ. of Birmingham, UK Am. Chem. Soc. Monograph 192 (1984) pp 1323-1373.

TABLE:

MUTAGENICITY TESTING OF TECHNICAL HEPTOCHLOR (H) AND HEPTACHLOR EPOXIDE (HE)

Genetic Category	Study Type/ Test System	TEST MATERIAL: Dose (mg/kg) or Concentration (ug,ppm) Range	Citation	Reported Results	TB Evaluation	Comment
Gene Mutation	Reversions in bacterial cells (S. typhimurium Ames Assays)	H/HE: 1 to 5000,+/-S9	Moriya et al. (1983)	Negative	Unacceptable	Survey screen
		H: 1-10,000, +/-S9	Probst et al. (1981)	Negative	Unacceptable	Survey screen
		H: 20/disc, -S9 only	Shirasu et al. (1976)	Negative	Unacceptable	Survey screen, at only one dose.
		H: (Dose not stated), +/- rat S9	Gentile et al. (1982)	Positive in TA100; eqivocal in TA98 at 10 ug/ml, +S9 only Negative -S9	Inconclusive	Positive reported at only one dose
		"Commercial": (Doses not stated), +/- S9		Negative	Unacceptable	Commercial preparation tested at unstated doses
		H: (Doses not stated), + corn 1S only		Positive in TA1535 at 10 ug	Inconclusive	Positive reported at only one dose.
		"Commercial": (Doses not reported), + corn 1S only		Negative	Unacceptable	Commercial preparation tested at unstated doses.
		HE (isomer A): 1 and 1000 ug, +/- mouse S9	van Dijck and van der Voorde (1976)	Negative (1000 ug dose was cytotoxic)	Acceptable	

MUTAGENICITY TESTING OF TECHNICAL HEPTOCHLOR (H) AND HEPTACHLOR EPOXIDE (HE)

Genetic Category	Study Type/ Test System	TEST MATERIAL: Dose (mg/kg) or Concentration (ug,ppm) Range	Citation	Reported Results	TB Evaluation	Comment
Gene Mutation (Cont'd)	Reversions in bacterial cells (S. typhimurium Ames Assays) (Cont'd)	H/HE (analyt): 50 to 1000, +/-S9	Marshall et al. (1973)	Negative up to cytotoxic level (1000)	Acceptable for strains tested.	Not tested in TA98, TA100.
		HE (analyt): 30 to 3000, +/- S9 in all strains; +S9 +/-TCPO in TA98.	Glatt et al. (1983)	Negative under all conditions up to insoluble levels.	Acceptable	---
		H: 0.3 to 1000, -S9; 100 to 10,000, +S9 (rat, hamster)	NTP (1983); Haworth (1986)	Negative up to insoluble levels.	Acceptable	---
	Reversion in bacterial cells (E. coli try⁻)	H/HE: 1 to 5000, +/-S9	Moriya et al. (1983)	Negative	Unacceptable	Survey screen
		H: 1 to 10,000, +/-S9	Probst et al. (1981)	Negative	Unacceptable	Survey screen
		H: 20/disc, -S9 only	Shirasu et al. (1976)	Negative	Unacceptable	Survey screen at only one dose.
	Reversion in pollen grains of plants exposed in situ (Zea mays)	"Commercial": 1.12 kg/hect. (sprayed prior to seedling emergence)	Gentile et al. (1982	Positive (increase in pollen grains with Wx)	Inconclusive	Only qualitative results reported.
	Forward mutation in mammalian cells in vitro (ARL, HGPRT)	H: 10^{-3} to 10^{-6}M (S9 not required)	Telang et al. (1982)	Negative up to cytotoxic levels	Acceptable	---
	Sex-linked recessive lethals (SLRL) in Drosophila	H: 1x5 ug/mL, ip HE: 1x2.5 ug/mL, ip	Benes/Sram (1969)	Negative	Unacceptable	Inappropriate assay, only one dose.

MUTAGENICITY TESTING OF TECHNICAL HEPTOCHLOR (H) AND HEPTACHLOR EPOXIDE (HE) CONT'D

Genetic Category	Study Type/ Test System	TEST MATERIAL: Dose (mg/kg) or Concentration (ug,ppm) Range	Citation	Reported Results	TB Evaluation	Comment
Chromosome Aberrations (Damage)	Rodent (mouse) dominant lethal	H+HE (25:75) blend: 1x7.5 and 15, oral and i.p.	Arnold et al. (1977)	Negative at nontoxic doses in 6-wk matings	Acceptable	---
		H: (a) 1x4.8 and 24, (b) 5x5 and 10, oral	Epstein et al. (1972)	Negative up to toxic dose	Acceptable	---
		HE: (a) 1x6 and 30, i.p. (b) 5x8, oral		Negative up to toxic dose	Acceptable	---
		H: 1 and 5 feeding over three generations	Cerey et al. (1973)	Positive for increased resorptions	Inconclusive	Reported as an abstract only.
	Bone marrow cytogenetics in mice	H: 1 and 5 feeding in F2 and F3		Positive for increased "abnormal mitosis"	Inconclusive	Reported as an abstract only.
		H: "0.0052 mg/g," i.p.	Markaryan (1966)	Positive at "4% of LD_{50}"	Inconclusive	Inappropriate sampling technique; cytological effects included.
DNA Damage/ Repair	Diffferential toxicity in bacteria (*B. subtilis* rec)	H: 20/disc, -S9 only	Shirasu et al. (1976)	Negative in spot tests	Unacceptable	Survey screen
	DNA strand breaks in bacterial plasmid (Col E1 from *E. coli* K12)	H: 0.1 ug/mL	Griffen/Hill (1978)	Negative at one dose tested	Unacceptable	Survey screen

MUTAGENICITY TESTING OF TECHNICAL HEPTOCHLOR (H) AND HEPTACHLOR EPOXIDE (HE) CONT'D

Genetic Category	Study Type/ Test System	TEST MATERIAL: Dose (mg/kg) or Concentration (ug,ppm) Range	Citation	Reported Results	TB Evaluation	Comment
DNA Damage/ Repair (Cont'd)	Gene conversion in yeast (S. cerevisiae D4)	"Commercial": (Doses not stated), +/- rat S9	Gentile et al. (1982)	Negative	Unacceptable	Qualitative summary only with commercial formulation
		H: (Doses not stated), +/- rat S9		Negative	Unacceptable	Qualitative summary only at unreported doses
	Unscheduled DNA synthesis (UDS) in mammalian cells in vitro (rodent hepatocytes)	H: 10^{-1} to 10^{-6} M (no S9 required) in rat, mouse, and hamster HPC	Maslansky and Williams (1981)	Negative up to cytotoxic doses	Acceptable	---
		H: 0.5 to 1000 nM/mL	Probst et al. (1981)	Negative up to cytotoxic doses	Acceptable	---
	UDS in transformed (SV-40) human cells (VA-4)	H: 100 and 1000 u M, +/- rat S9	Ahmed et al. (1977)	Positive at both doses, +S9 only	Inconclusive	Qualitative results only reported; no evidence of dose-response.
		HE: 10, 100, and 1000 uM, +/- S9		Positive at all doses, +S9 only	Inconclusive	Qualitative results only reported; no evidence of dose-response.
Other Mechanisms	In vivo inhibition of DNA synthesis (mouse testes)	H: 1x40, oral	Seiler (1977)	Negative	(N/A)	Screening survey of a new procedure
	In vivo mitotic spindle inhibition (mouse BM)	H: 1x5.2, i.p. (reported as 0.0052 mg/g "4% of LD_{50}")	Markaryan (1966)	"Positive" for "nuclear disturbances," breakage	Inconclusive	Inadequate methods; data include cytotoxicity.
	Metabolic cooperation (cell-to-cell communication) inhibition in vitro (ARL, HGPRT +/ HGPRT⁻ cultures)	H: 10^{-4} to 10^{-7} M	Telang et al. (1982)	Positive up to cytotoxic level (10^{-4} M)	Acceptable	---

APPENDIX I

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chlordane/Heptachlor Mutagenicity

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Ing Mauer 12-16-85

TO: Henry Spencer, Ph.D., Pharmacologist
Section 7, Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Jane E. Harris, Ph.D.
Section Head, Toxicology Branch
Hazard Evaluation Division (TS-769C)

This is in response to your request for an expeditious appraisal of the positive mutagenicity studies for chlordane and heptachlor/heptachlor epoxide, as reported in the CAG document ("Carcinogenicity Risk Assessment for Chlordane and Heptachlor/Heptachlor Epoxide"). The negative reports listed in that document were also scanned.

Based upon recent Velsicol submissions (Accession Nos. 254320 and 254324) and other information available to me (NTP, EMIC), two general conclusions are warranted from this preliminary assessment (see below for a summary of available data):

1. Although the adequacy of the data base as reported could not be undertaken (e.g., DER's) because of the timely response requested, it appears that all the genetic end-points we require to be assayed (gene mutation, chromosomal aberrations, DNA damage/repair) have been addressed.

2. Consistent with the results of mutagenicity testing previously reported for other agents of this chemical class (organochlorines such as Lindane, chloroform, inter alia), the mutagenic potential of these chemicals are low to unsubstantial. Hence, from the available data it would appear their oncogenic potential derives from mechanisms other than "genetic initiation" (direct interaction with DNA). [There is suggestive evidence in mammalian systems (the few promotion assays available) of an "epigenetic" mode of action for these chemicals.]

Survey of Available Literature

1. Chlordane-technical (but not reagent-grade alpha-chlordane, gamma-chlordane, and gamma-chlordene) was positive in adequate bacterial (Ames) assays (three positive, one negative), but consistently so only in the most sensitive strain (TA-100), and only at very high concentrations (5000 ug/plate and above). Testing for mutagenicity in mammalian cells in culture (four reports) revealed inconsistent results (two positive, two negative), due in part to the different test systems employed. The two negatives were reported for HGPRT in ARL (adult rat liver) and for both thioguanine and diphtheria-toxin resistance in V79 (Chinese hamster lung) cells. One of the positives was for ouabain resistance in V79 cells, but reported for only a single concentration of an unstated formulation, at less than 50 percent cell survival; the second was found in non-activated mouse lymphoma cells (L5178Y-TK), but the study is incomplete since metabolic activation was not employed.

   The plant systems may activate chlordane to mutagenic derivatives is suggested by a single article reporting both gene conversion in Saccharomyces cerevisiae D4 cultures exposed to a reagent grade (presumably alpha-chlordane according to the CAS Number stated), and reversion to wild type in pollen grains from the homozygous "waxy" strain W22 of Zea mays (corn) exposed to a "commercial-grade formulation." However, too few procedural details were reported to adequately interpret these results.

   Although reportedly negative for gross chromosomal damage in vivo (two mouse DLT's) and in vitro (CHO cells), technical chlordane or one of its principal components (alpha-chlordane) apparently has DNA-damaging activity, as revealed in a single study reporting increased dose-dependent sister-chromatid exchanges (SCE) in intestinal cells sampled from

exposed central mudminnows (Umbra limi), as well as in CHO cells in culture and in a human lymphoid cell line (one report each). Whereas the positive SCE in fish was recorded at nonlethal concentrations (added to aquarium water), both in vitro studies were only marginally positive (less than twofold above controls) at toxic concentrations. Only one of the four studies assaying for unscheduled DNA synthesis (UDS) in vitro was positive (in VA-4 cells, an SV-transformed human cell line), but only in the absence of metabolic activation (negative with MA). Technical chlordane was negative for UDS in primary hepatocyte cultures from rats, mice, and hamsters (two reports) as well as in a human fibroblast cell line (D-550). Finally, gene conversion was reported in yeast cells (S. cerevisiae D4) exposed to an activation system (negative without), but too few data are included in the article to properly interpret the results.

2. Heptachlor/Heptachlor Epoxide. In contrast to the positive results for (gene) mutagenicity reported for chlordane (both technical and reagent grades), the available data for heptachlor and its epoxide is consistently negative (three Ames and/or E. coli, one B. subtilis rec assay, 1 ARL-HGPRT, two Drosophila SLRL, 1 HPC/UDS). Reversions to histidine prototrophy in Salmonella TA-1535 and 100 under activation conditions to an unstated dose range, as well as to the wild-type (nonwaxy) phenotype in corn pollen grains were reported by the same investigator who found "commercial" chlordane positive.

Adequate reports on dominant-lethal assays in mice were negative, but an abstract from a meeting reported positive results for both germinal (DLT) and somatic (bone marrow) chromosome damage in rats fed 1 and 5 ppm of an unstated formulation of heptachlor for three generations. A Russian study also reported positive chromosome damage in bone marrow cells from "white male mice" treated i.p. with "heptachlor" (also of unstated source and purity) at a single dose level stated to be "4% of the LD_{50}." Too few procedural details are included in these "positive" studies to interpret the results reported.

As with chlordane, negative UDS results have been reported for heptachlor technical in primary rodent hepatocytes (two reports), but a positive recorded in VA-4 cells (virus-transformed human fibroblasts) for both the technical and epoxide, but only with metabolic

activation (in contrast to chlordane, positive only in the absence of activation). [The mutagenicity data base for heptachlor epoxide is less than adequate to satisfy FIFRA guidelines.]

Discussion

Recent studies have suggested that organochlorines (Lindane, chlordane, heptachlor, inter alia) do not interact directly with DNA (i.e., are not "genotoxic"), but rather act "epigenetically" by mechanisms affecting cell membrane permeability and/or following an irreversible "initiating event" (i.e., are "promoters" ensuring survival of preexisting transformed cells). The following citations employing chlordane heptachlor available for this "quick-and-dirty" review (and listed in the CAG document) are consistent with this suggestion:

1. Inhibition of metabolic cooperation in mixed cultures consisting of thioguanine-resistant and TG-sensitive cells (several reports from both Williams' and Trosko's labs).

2. The putative positive UDS results in cells already "initiated" (e.g., the SV-40 transformed cell line, VA-4).

3. Inhibition of DNA synthesis and/or cell cycle mechanisms by severely toxic concentrations leading to perturbation of repair (increased UDS, SCE).

cc: Dr. Amy Rispin
Science Intergration Staff
Hazard Evaluation Division (TS-769C)

PRELIMINARY EVALUATION OF REPORTED POSITIVE STUDIES

Compound	Test Material	Assay	Test System	Dose/Conc. Range	Reference	TB Evaluation	Comments
CHLORDANE	Technical	Gene Mutation	Ames (all)	20-100	Simmon *et al.* (77)	ACCEPT.	POS. only in TA 100
	Technical	Gene Mutation	Ames (all)	a) 10-5000 b) 1000-50,000	Simmon and Tanaka (77)	ACCEPT.	a) EQUIV in TA 98 b) POS. in TA 100
	Technical	Gene Mutation	Ames (TA 98, 100)	5-10,000	Maruyama (80)	ACCEPT.	POS. in TA 100; NEG. in TA 98
	Reagent (alpha)	Gene Mutation	Sacch. - D4	(Unstated)	Gentile *et al.* (82)	INCONCL.	POS. only + S9; too few data
	"Commercial"	Gene Mutation	*Z. mays* Pollen	(Unstated)	*Ibid*	INCONCL.	Tech not tested; too few data
	(Unstated)	Ouabain resist.	V79 cells	(Only 1 dose reported)	Ahmed *et al.* (77b)	INCONCL.	"Weak" at < 50% survival; few data
	(Unstated)	Gene Mutation	ARL/HGPRT	10^{-3}-10^{-6} M	Telang *et al.* (82)	ACCEPT.	NEG. for HGPRT, but POS. for promotion
	Technical	*In vivo* SCE	Mudminnow	$5.4/10^{-12}$-10^{-9}	Vigfusson *et al.* (83)	ACCEPT.	Dose-dependent POS.
	Reagent (alpha)	*In vitro* SCE	LAZ-007 cells	10^{-6}-10^{-3} M	Sobti *et al.* (83)	INCONCL.	< 2-fold increase; no dose response
	(Unstated)	*In vitro* UDS	VA-4 cells	1,10,100,1000 uM	Ahmed *et al.* (77a)	ACCEPT.	POS. only without S9
	Reagent (gamma)	Cell cycle inhib.	L5178Y cells	4 ug/ml	Brubaker *et al.* (70)	INCONCL.	NEG. for DNA, POS. for G2 arrest; only 1 dose
	Reagent (alpha)	*In vitro* CA/SCE	CHO cells	(Unstated)	NTP (85)	INCONCL.	NEG. for CA; POS. for SCE
	Reagent	Gene Mutation	L5178Y/TK	(Multiple)	NTP (85)	INCONCL.	Not tested with S9

PRELIMINARY EVALUATION OF REPORTED POSITIVE STUDIES (cont'd)

Compound	Test Material	Assay	Test System	Dose/Conc. Range	Reference	TB Evaluation	Comments
HEPTACHLOR	Technical	Gene Mutation	Ames (all)	(Unstated)	Gentile *et al.* (82)	INCONCL.	POS. in TA 1535/100, but only with S9
	(Unstated)	Gene Mutation	ARL/HGPRT	10^{-7} to 10^{-4} M	Telang *et al.* (82)	ACCEPT.	NEG. for HGPRT; POS. for promotion
	(Unstated)	*in vitro* UDS	VA-4 cells	100,1000 uM	Ahmed *et al.* (77a)	INCONCL.	POS. only with S9, but no values for UDS
	Epoxide	*in vitro* UDS	VA-4 cells	10,100,1000 uM	*ibid.*	INCONCL.	POS. only with S9, but no values for UDS
	(Unstated)	*in vivo* CA	Mouse BM	5 mg/kg	Markarjan (66)	INCONCL.	Too few procedural details; 1 dose
	(Unstated)	DLT/BM-CA	Rat repro. study	1, 5 ppm	Carey (74)	INCONCL.	[Abstract]
	Technical	*in vitro* CA/ SCE	CHO cells	(Multiple)	NTP (85)	INCONCL.	POS. for both CA and SCE, but too few details available

APPENDIX II

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