United States
Environmental Protection
Agency

Office of Health and
Environmental Assessment
Washington DC 20460

EPA/600/6-87/006
August 1987

Research and Development

EPA

Carcinogenicity Assessment of Aldrin and Dieldrin

EPA/600/6-87/006
August 1987

CARCINOGENICITY ASSESSMENT OF ALDRIN AND DIELDRIN

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.

1.1.1.2.2. Dieldrin. Twelve carcinogenicity bioassays of dieldrin have been conducted with mice; all but one were judged adequate in design. Seven strains of mice were used: C_3HeB/Fe, C_3H, CBA/J, Swiss-Webster, CF_1, $B6C3F_1$, and C57BL/6J. In all studies, either benign or malignant liver tumors were observed. The authors of six of these studies indicated that the tumors were malignant (hepatocellular carcinomas), whereas in five others the tumors were diagnosed as hepatomas. In three of these five, a pathology reevaluation was performed by other pathologists who classified the lesions as being malignant. In three studies, all with CF_1 mice, many of the hepatocellular carcinomas had metastasized to the lung. In addition, in one study, a significant ($p < 0.05$) reduction in latency period was observed. In one of the studies with CF_1 mice, a slight but significant ($p < 0.05$) increase in the incidences of pulmonary adenomas and carcinomas, lymphoid tumors, and other tumors was seen in female mice at 1 ppm. The doses used in the bioassays ranged from 0.1 to 20 ppm with dietary administration for 18 to 24 months.

Seven long-term carcinogenicity studies were conducted with rats, three of which were considered adequate carcinogenicity assays. The others suffered mainly from too few animals, too high mortality, too short a duration, and/or inadequate pathology examination or reporting. Four strains were used: Carworth, Osborne-Mendel, Holtzman, and Fischer 344. Doses ranged from 0.1 to 285 ppm in the diet generally for 2 years or more. Although liver pathology was generally associated with exposure to the chlorinated insecticides, there was no firm evidence of a carcinogenic response in any of the studies.

1.1.1.3. Mutagenicity--

1.1.1.3.1. Aldrin. An evaluation of mutagenicity is hampered by a paucity of data on point mutations and cytogenetics. However, the available data indicate that aldrin is probably not mutagenic in procaryotes and does not induce DNA

damage or clastogenic effects. A report of induced unsceduled DNA synthesis (UDS) was discounted due to technical deficiencies.

1.1.1.3.2. Dieldrin. The data are insufficient to establish a complete genetic toxicology profile. However, the results of 13 studies support the conclusion that dieldrin is not mutagenic in procaryotes. However, two studies reported positive results, one with *S. typhimurium* and the other with Chinese hamster V79 cells. Both, however, were seriously flawed so that the data are of questionable value. However, based on these data, dieldrin is classified as a presumptive mutagen for *S. typhimurium* and for Chinese hamster V79 cells. Dieldrin was reported to be weakly clastogenic in mammalian somatic cells and nonclastogenic in male germinal cells. There is no evidence for genotoxicity in yeast or primary rat and mouse hepatocytes or for primary DNA damage in bacteria. A positive result of UDS in SV-40 transformed cells is questionable based upon numerous technical deficiencies in the study.

1.1.1.4. Structural Relationship--Three compounds, structurally related to aldrin and dieldrin, have induced malignant liver tumors in animals. Chlordane, heptachlor, and chlorendic acid have produced liver tumors in mice, and chlorendic acid has also produced liver tumors in rats.

1.1.2. Quantitative Analysis

1.1.2.1. Aldrin--Three data sets are suitable for quantitative risk estimation: male and female C_3H mice, and male $B6C3F_1$ mice. The most sensitive sex and strain tested is female C_3H mice. From these the potency can be estimated at 23 per mg/kg/day.

The most sensitive species tested is mice. There are three potency estimates, ranging from 23 down to 12 per mg/kg/day, with a geometric mean of 17 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 17 per mg/kg/day.

These estimates are plausible upper bounds for the increased cancer risk from aldrin, meaning that the true risk is not likely to exceed these estimates and may be lower. These estimates have been calculated using the Agency's preferred methodology in the absence of specific physiologic, metabolic, or kinetic information: the linearized multistage model with doses scaled according to relative body surface area. Other assumptions about the dose-response model or the interspecies dose adjustments may result in lower estimates.

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 aldrin is 6.2×10^3 per mmol/kg/day. This places aldrin in the most potent quartile of suspect carcinogens ranked by the Carcinogen Assessment Group (CAG).

1.1.2.2. Dieldrin--Thirteen data sets are suitable for quantitative risk estimation. The most sensitive sex and strain tested is male CF_1 mice. From these the potency can be estimated at 55 per mg/kg/day. This estimate may, however, be misleading because 100% of the treated animals developed cancer. The second most sensitive estimate is 28 per mg/kg/day.

The most sensitive species tested is mice. There are 13 potency estimates, ranging from 55 down to 7 per mg/kg/day, with a geometric mean of 16 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 16 per mg/kg/day.

These estimates are plausible upper bounds for the increased cancer risk from dieldrin, meaning that the true risk is not likely to exceed these estimates and may be lower. These estimates have been calculated using the Agency's preferred methodology in the absence of specific physiologic, metabolic, or kinetic information: the linearized multistage model with doses scaled accord-

ing to relative body surface area. Other assumptions about the dose-response model or the interspecies dose adjustments may result in lower estimates.

The molecular potency index for dieldrin is 6.1×10^3 per mmol/kg/day. This places dieldrin in the most potent quartile of suspect carcinogens ranked by the CAG.

1.2. CONCLUSIONS

1.2.1. Aldrin

For aldrin, the finding of hepatocellular carcinomas in male $B6C3F_1$ mice and the finding of hepatomas in C_3H and C_3HeB/Fe mice, which were later diagnosed as hepatocellular carcinomas, in both males and females, constitute as a first approximation, "sufficient" evidence of carcinogenicity in animals, according to criteria in the EPA's Guidelines for Carcinogen Risk Assessment. However, the Guidelines call for a careful analysis of the nature of the mouse liver tumor only response to ascertain whether there is a sufficiently strong reason to downgrade the evidence to "limited." This downgrading was found not to be appropriate since the tumor increases were not marginal in male $B6C3F_1$ mice (which have a high spontaneous incidence of liver tumors), there was a dose-related increase in the proportion of tumors that were malignant, and the response occurred in both males and females. In addition, the response occurs in C_3H and C_3HeB/Fe mouse strains with a low spontaneous incidence of liver tumors, which is a response not subject to the downgrading factors.

One factor which would argue for a "limited" classification is that the two C_3H strains are genetically closely related and the $B6C3F_1$ strain derives half of its gene pool from these strains. However, the fact that the epoxide metabolite, dieldrin, produces liver tumors in seven different mouse strains indicates strongly that the carcinogenic effect of aldrin is not limited to a restricted genetic range of mice. Although in rats, one adequate study with

aldrin and three adequate studies with dieldrin were negative or equivocal, the multiplicity of mouse strains responding is considered to be enough for classification of "sufficient" evidence in animals.

In the absence of adequate evidence in humans, the sufficient animal evidence amounts to an overall classification of B2, or "probable" carcinogen for aldrin.

For aldrin, the carcinogenic potency, obtained by averaging estimates from the most sensitive species tested (mice), is 17 per mg/kg/day. The potency using the most sensitive sex and strain is 23 per mg/kg/day. These are plausible upper bounds for the increased cancer risk from aldrin, meaning that the true risk is not likely to exceed these estimates and may be lower. These estimates have been calculated using the Agency's preferred methodology in the absence of specific physiologic, metabolic, or kinetic information: the linearized multistage model with doses scaled according to relative body surface area. Other assumptions about the dose-response model or the interspecies dose adjustment may result in lower estimates. The molecular potency index for aldrin is 6.2×10^3 per mmol/kg/day. This places aldrin in the first (most potent) quartile of suspect carcinogens ranked by the CAG.

1.2.2. Dieldrin

For dieldrin, the mouse liver tumor response in seven mouse strains (C_3HeB/Fe, C_3H, CBA/J, CF_1, Swiss-Webster, $B6C3F_1$, and C57BL/6J) along with the appearance of pulmonary adenomas and carcinomas and lymphoid tumors in one strain (CF_1) would justify the preliminary classification of dieldrin as having "sufficient" evidence in animals. Based on the nature of the response there is no reason for downgrading the classification to "limited," since the carcinoma response is unmistakably strong, it occurs in both males and females, and at both high and low doses. The occurrence in several strains implies that it is

not a genetically isolated finding. The carcinogenicity studies in rats for dieldrin were considered negative. However, the ability of most studies to detect carcinogenicity was compromised due to too few animals, too high mortality, and too short duration. Although liver pathology was associated with exposure to the chlorinated insecticides, there was no firm evidence of a carcinogenic response. In the absence of adequate evidence, the sufficient animal evidence amounts to an overall classification of "B2" or probable carcinogen for dieldrin.

For dieldrin, the carcinogenic potency, obtained by averaging estimates from the most sensitive species tested, is 16 per mg/kg/day. The potency using the most sensitive sex and strain, is 55 per mg/kg/day. This estimate may, however, be misleading because 100% of the exposed animals developed cancer. The second most sensitive estimate is 28 per mg/kg/day. These are plausible upper bounds for the increased cancer risk from dieldrin, meaning that the true risk is not likely to exceed these estimates and may be lower. These estimates have been calculated using the Agency's preferred methodology in the absence of specific physiologic, metabolic, or kinetic information: the linearized multistage model with doses scaled according to relative body surface area. Other assumptions about the dose-response model or the interspecies dose adjustment may result in lower estimates. The molecular potency index for dieldrin is 6.1×10^3 per mmol/kg/day. This places dieldrin in the first (most potent) quartile of suspect carcinogens ranked by the CAG.

Although there is always uncertainty in extrapolating potency from animals to humans, our confidence in these animal potency estimates is relatively high. This confidence is based not only on the tight clustering of aldrin and dieldrin potency estimates, but on other factors as well. The potency estimates for aldrin are consistent with the potency estimates for dieldrin, one of its

metabolites. In addition, aldrin's potency (and dieldrin's as well) is based in part on studies of mice with low background tumor rates. A study of female C3HeB/Fe mice, for example, saw the tumor incidence increase from 4% in controls to 85% in the treated group. Potencies derived from high-background strains are consistent with potencies from low-background strains. Furthermore, potencies derived from mouse liver tumors are consistent with potencies derived from rat tumors for two other related substances, chlordane and heptachlor epoxide. Thus, mouse liver tumors can provide a basis for estimating cancer potency in humans, recognizing the current issues associated with use of this type of data and attendant uncertainties in a risk estimate based upon it.

2. INTRODUCTION

Aldrin and dieldrin were first synthesized in the laboratory in 1948 with commercial production in the United States first reported in 1950 (IARC, 1974a, b). Aldrin and dieldrin are organochlorine pesticides extensively used in the 1960s and early 1970s for a large variety of pesticidal uses. Most uses were banned in the United States in 1974 under the Federal Insecticide, Fungicide, and Rodenticide Act (39 FR 37246, October 18, 1974). Uses were also restricted in many other countries at about the same time. The approved uses in the United States now are mainly for termite control by direct soil injection and for nonfood seed and plant treatment. Production of aldrin and dieldrin in the United States was also discontinued along with the restrictions of 1974, so that virtually all aldrin and dieldrin now used in the United States is imported. Exposure of humans has been primarily from dermal and inhalation exposure related to the application as pesticides; however, due to the persistence and bioaccumulation of dieldrin, considerable exposure has occurred through ingestion of contaminated water and food products. In contrast, little human exposure from environmental sources of aldrin occurs, as it is readily converted to dieldrin by direct epoxidation in the environment. During the early 1970s, nearly all humans sampled had measurable tissue levels of dieldrin and, due to its stability and persistence, dieldrin is still detected in humans. However, the levels are considerably lower. Aldrin and dieldrin are usually considered together, as they are structurally-related cyclodiene insecticides, have similar uses, and because aldrin is readily epoxidized to dieldrin in the body or the environment.

It is the purpose of this report to review the currently available literature on the toxicity and carcinogenicity of both aldrin and dieldrin and conduct

an assessment as to the potential carcinogenicity risk to humans. The Carcinogen Assessment Group (CAG) conducts a "weight-of-evidence" evaluation that weighs data concerning innate biological activity including metabolism, toxic effects as related to duration and levels of exposure, results of mutagenicity and other short-term tests, long-term animal bioassays, and, where available, data derived from epidemiologic studies. Since mutagenicity is considered as one major mechanism for cancer induction, those studies are considered supportive to the *in vivo* animal or human data.

Following a review of the data, those data most relevant and amenable to mathematical extrapolation are selected for the risk assessment. Several mathematical models, as described in EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986) are used. On the basis of the "weight of evidence" and risk extrapolation, the substance is classified as a potential human carcinogen according to the EPA guidelines.

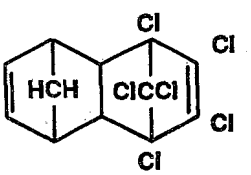
3. GENERAL BACKGROUND INFORMATION

3.1. CHEMICAL AND PHYSICAL PROPERTIES

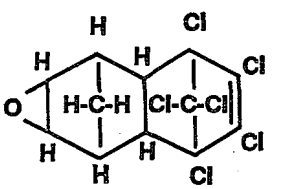
Those properties most relevant to carcinogenesis and human exposure are listed in Table 3-1.

3.1.1. Identification

Aldrin



Dieldrin



	Aldrin	Dieldrin
Molecular Formula:	$C_{12}H_8Cl_6$	$C_{12}H_8Cl_6O$
Molecular Weight:	364.93	380.93
CAS Number:	309-00-2	60-57-1

3.1.2. Synonyms (IARC, 1974a, b)

3.1.2.1. Aldrin--

1,2,3,4,10,10-hexachloro-1,4,4a,8,8a-hexahydro-endo,exo-1,4:5,8-dimethanonapthalene;

hexachlorohexahydro-endo-exo-dimethanonaphthalene;

1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-endo-1,4,-exo-5,8-dimethanonaphthalene;

TABLE 3-1. CHEMICAL AND PHYSICAL PROPERTIES OF ALDRIN AND DIELDRIN

Physical properties	Aldrin	Dieldrin
Boiling point	Decomposes	Decomposes
Melting point	104°-105.5°C (technical grade 49°-60°C)	176°-177°C
Specific gravity	NF	1.75
Vapor pressure	7.5×10^{-5} mmHg at 20°C 1.4×10^{-4} mmHg at 25°C	3.1×10^{-6} mmHg at 20°C 7.78×10^{-7} mmHg at 25°C
Solubility	27 µg/L at 27°C in water	186 µg/L at 25°C in water
	Soluble in most organic solvents	Soluble in acetone and benzene
Evaporation Half-life ($t_{1/2}$) from water	185 hr at 25°C and 1 m depth	12,940 hr at 25°C and 1 m depth
Log octanol/water partition coefficient	3.01	NF
Odor threshold	0.017 mg/kg water	0.041 mg/kg water
Conversion factor	1 ppm = 14.96 mg/m^3 at 25°C and 760 mmHg	1 ppm = 15.61 mg/m^3 at 25°C and 760 mmHg

NF = not found.

1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanonaphthalene;

1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-exo-1,4-endo-5,8-dimethanonaphthalene

Compound 118 ENT 15,949

HHDN

3.1.2.2. Dieldrin--

endo-exo isomer of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-dimethanonaphthalene

1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-exo-1,4:5,8-dimethanonaphthalene

1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-1,4-exo-5,8-dimethanonaphthalene

1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahyd ro-1,4-endo-exo-5,8-dimethanonaphthalene

1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene

1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene

1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-exo-5,8-endo-dimethanonaphthalene

Compound 497 ENT 16,225

HEOD

3.2. USES

The only known uses for aldrin and dieldrin are as insecticides. According to the IARC (1974a, b), it was estimated that 80% of the combined U.S. production of aldrin and dieldrin was used on corn crops and that about 10% was used for termite control. In the early 1970s, aldrin and dieldrin were approved for use in the United States on over 40 agricultural crops and for soil treatment around certain fruits, nuts, and vegetables.

Most uses in the United States were banned by the EPA in 1974. While aldrin and dieldrin are approved for use on non-food seed and plants, the Chemical Economics Handbook (1985) reported that current use is for termite control exclusively, and that all quantities used are imported. The use of aldrin and dieldrin has also been banned or highly restricted in many other countries (IARC, 1974a, b).

3.3. ROUTES AND PATTERNS OF EXPOSURE

Exposure to aldrin and dieldrin can occur by virtually all major routes, including inhalation, skin absorption, or by ingestion of contaminated food or water.

The potential for inhalation exposure is greatest for pesticide applicators or residents in buildings where termite treatment occurs. However, during the period when there was extensive use of these pesticides, atmospheric pollution was fairly common. In 1976, more than 85% of atmospheric air samples tested by the EPA contained aldrin or dieldrin with levels as high as 2.8 ng/m^3 resulting in an intake of up to 0.098 µg/day (U.S. EPA, 1985). In the IARC (1974a, b) review, the intake by occupationally exposed workers was estimated to range from 0.72 to 1.10 mg/person/day compared to 0.025 mg/person/day for the general population during 1967 and 1968.

Estimates of potential dermal exposure during orchard spraying ranged from 14.2 to 15.5 mg/hour, and the potential respiratory exposure was estimated to be 0.03 to 0.25 mg/hour from 1963 to 1967.

Aldrin and dieldrin are widely applied to vast areas of agricultural land and aquatic areas in the United States and in most parts of the world until government restrictions were imposed. As a result, both fresh and marine waters were contaminated, with exposure via ingestion of water being an important route of exposure.

Aldrin itself is readily converted to dieldrin. Dieldrin is stable, highly persistent in the environment, lipophilic in nature, and accumulates in the food chain. Due to these factors, the levels of aldrin in water and foods have been low, with those for dieldrin considerably higher and more persistent. Weaver et al. (1965) found dieldrin in all major U.S. river basins more often than any other pesticide and at a mean concentration of 7.5 ng/L. In 1976, dieldrin was present in many fresh waters in the United States at even higher concentrations, ranging from 5 to 396 ng/L in surface waters and from 1 to 7 ng/L in drinking water. A 1975 survey in the United States found dieldrin in 117 of 715 water samples. Aldrin was also detected at concentrations of 15 to 18 ng/L (U.S. EPA, 1976). Harris et al. (1977) reported concentrations of 1 ng/L in Seattle, Washington, and Cincinnati, Ohio; 2 ng/L in Miami, Florida, and Ottumwa, Iowa; and as high as 50 ng/L in New Orleans, Louisiana.

It has been estimated by MacKay and Wolkoff (1973) that dieldrin has by far the longest half-life of the chlorinated hydrocarbons in water 1 meter (m) in depth, calculating a half-life for aldrin and dieldrin of 10.1 days and 723 days, respectively, compared to 3.5 days for DDT and 289 days for lindane. This long half-life in water combined with the potential for bioconcentration by aquatic organisms further enhances the hazard of these two pesticides (Wurster, 1971).

The EPA (1980) estimated that 99.5% of all human beings in the United States had dieldrin residues in their tissues. Wurster (1971) claimed that these residue levels were mainly due to contamination of foods of animal origin. The overall concentration of dieldrin in the diet in the United States was calculated by Epstein (1976) to be approximately 43 ng/g of food consumed.

4. HAZARD IDENTIFICATION

4.1. METABOLISM AND PHARMACOKINETICS

Aldrin and dieldrin are related pesticides, and in biological systems, aldrin is rapidly converted to its epoxide, dieldrin. For all practical purposes, therefore, the kinetic functions and metabolism of these chlorinated cyclodienes are identical. Part of the confusion regarding the metabolism of these chemicals resides in the nomenclature. There are two systems that number the ring carbons differently. The Chemical Abstracts Services (CAS) and International Union of Pure and Applied Chemistry (IUPAC) systems define the chemicals as derivatives of octahydromethanonaphthalene, while the von Baeyer-IUPAC system defines the chemicals as derivatives of tetracyclo[6.2.1.13,6,92,7]-dodec-9-ene. Figure 4-1 shows the structures of aldrin for the two systems. For this discussion, the von Baeyer-IUPAC system is used.

4.1.1. Absorption

4.1.1.1. Aldrin--Quantitative data on the absorption of aldrin from the gastrointestinal tract of animals are not available. However, metabolic studies indicate that aldrin is absorbed slowly via the portal venous system following oral administration. A time-course study of the distribution and metabolism of aldrin in the liver, kidneys, stomach, and small intestine of neonate (24-hour-old) Sprague-Dawley rats following a single oral dose was studied by Farb et al. (1973). The concentration of aldrin in the liver increased to about 13% of the dose during the first 72 hours after dosing, whereas in the stomach and small intestine, aldrin was detected through the sixth day after dosing.

Dermal absorption has not been specifically investigated in laboratory animals, but acute and subchronic dermal toxicity studies indicate that percutaneous absorption does occur. Moreover, the rate of absorption is apparently

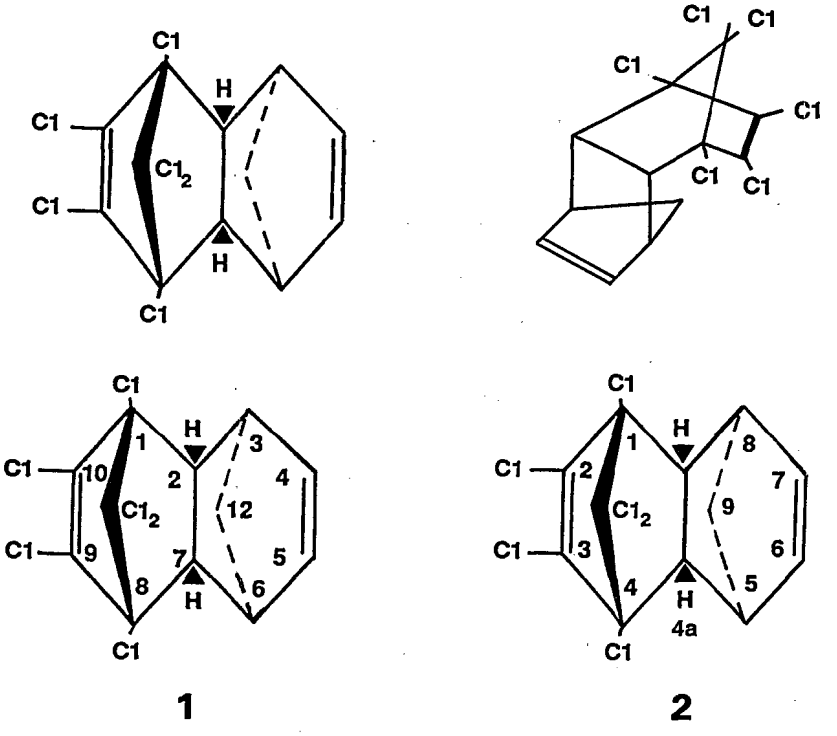


Figure 4-1. Chemical structure and numbering system of aldrin: (1) the von Baeyer-IUPAC system, and (2) CAS and IUPAC systems.

affected by the vehicle used, such that the application of aldrin in Ultrasene or oil is several-fold more toxic to rabbits than the application of aldrin as a dry powder (Treon and Cleveland, 1955).

In humans, dermal absorption of aldrin was reported to be 7.8% $\pm$ 2.9% of the applied dose in acetone over a period of 5 days (Feldmann and Maibach, 1974). However, the accuracy of this value is questionable because the dose used in both the topical and intravenous (control study used to correct the data of the topical application study) studies was only 1 μCi/subject, and excretion was only determined in the urine and accounted for only 2% to 5% of the dose following injection. In addition there was a large variability among the subjects. Moreover, the rate of percutaneous absorption may be affected by the amount of adipose fat in and/or the metabolizing capability of the skin.

The absorption of aldrin following inhalation exposure has been demonstrated in humans (Beyermann and Eckrich, 1973; Bragt et al., 1984); about 20% to 50% of the inhaled aldrin vapor was retained. However, the data presented by Beyermann and Eckrich (1973) were inadequate, and an unpublished study by Bragt et al. (1984) was not available for review. Animal studies were not available, but the uptake, metabolism, and disposition of aldrin by isolated, perfused rabbit lungs was reported by Mehendale and El-Bassiouni (1975). Recirculating and single-pass experiments were conducted with an artificial medium perfused through the pulmonary artery of ventilated lungs. Aldrin was taken up by simple diffusion from the vascular space, and then quickly diffused into the intercellular and intracellular spaces. Aldrin was then metabolized at a slower rate to dieldrin in the endoplasmic reticulum of the lung; metabolism was dose dependent, and up to 70% of the aldrin was epoxidized in 1 hour at low doses ($< 4 \times 10^{-6}$ M). Dieldrin was then distributed into two pools, a saturable and a nonsaturable pool. An equilibrium was established quickly with respect

to the net uptake of dieldrin or aldrin. After steady-state conditions were established, the disappearance of aldrin from the perfusate was attributed to its metabolic turnover rather than to further uptake by the lung. There was no evidence for irreversible binding or potential for long-term storage of aldrin or dieldrin in the lung; they apparently can be completely effluxed from the lung by simple diffusion into the blood.

4.1.1.2. Dieldrin--Quantitative data on the absorption of dieldrin from the gastrointestinal tract of animals has not been reported. Absorption through the gastrointestinal tract depends upon the vehicle used (Heath and Vandekar, 1964). Metabolic studies indicate that absorption is slow and initially occurs via the portal venous system. Immediately following a single oral dose of [^{36}Cl]- or [^{14}C]-dieldrin to rats, little if any radioactivity was found in the lymph system (Heath and Vandekar, 1964; Iatropoulos et al., 1975). Large concentrations of radioactivity were still present in the stomach and small intestine 24 to 48 hours after dosing, whereas concentrations in the liver decreased. The subsequent redistribution of dieldrin among body tissues (from about 12 hours after dosing) apparently involves the lymphatic system (Iatropoulos et al., 1975).

The *in situ* absorption of [^{14}C]-dieldrin across the isolated small intestine of rats also occurred at a slow rate. About 8% and 15% of the [^{14}C] was absorbed after 1- and 5-hour incubations, respectively (Tanaka et al., 1981).

The absorption of dieldrin through the skin occurs at levels great enough to cause toxic effects, but quantitative data are not available. Most of the dieldrin absorbed through the skin of guinea pigs, dogs, and monkeys is accumulated in the subcutaneous fat (Sundaram et al., 1978a, b).

Studies on the absorption of dieldrin following inhalation exposure were not available. The absorption of dieldrin by isolated perfused rabbit lungs was studied by Mehendale and El-Bassiouni (1975). The uptake of dieldrin was rapid in the lung and there was no evidence for long-term storage. Apparently, dieldrin can be completely effluxed from the lung by simple diffusion into the blood.

The absorption of dieldrin in humans is expected to resemble that observed in animals. In one survey conducted by Stacey and Tatum (1985) in Perth, Australia, the effect of treating homes with organochlorine pesticides was correlated with pesticide residue levels in human breast milk. Fourteen donors provided milk on a monthly basis for a 1-year period. There was a strong correlation between the dieldrin levels in the milk and the treatment of the houses with aldrin.

4.1.2. Tissue Distribution

4.1.2.1. Aldrin--There is no direct information on the distribution or elimination of aldrin by humans following its transfer from the gastrointestinal tract, or skin, into the circulating blood. In humans, aldrin is rarely if ever found in human blood or other tissues, except in cases of acute poisoning by accidental or intentional ingestion of massive doses.

A number of animal studies have been conducted which yield information on tissue distribution. In one study by Farb et al. (1973), the concentrations of aldrin in the liver decreased from 13% at 6 hours to 3% and 0.1% of the dose after 24 and 72 hours, respectively. The only metabolite detected in aldrin-treated animals was dieldrin, which was detected in the liver as early as 2 hours after dosing. The concentration of dieldrin increased, reaching a maximum of 31% of the administered dose at 18 hours, then rapidly decreased to less than 10% at 48 hours. Dieldrin concentrations in the liver decreased slowly

between 48 and 96 hours, leveling off at about 5% of the dose at days 4 to 7. In the kidneys, unknown metabolite A and dieldrin were detected 2 and 6 hours after dosing and through day 10; the distribution of metabolite A and dieldrin in the stomach and small intestine was similar to that observed in the kidneys (Farb et al., 1973).

The distribution of [^{14}C] in the carcass, fat, and other tissues of male rats given daily oral doses of [^{14}C]-aldrin (4.3 μg/rat) for 3 months accouted for 7.2% of the total administered dose. The [^{14}C] stored in rats, calculated as the difference between administered and excreted [^{14}C], reached maximum levels (steady-state) at 53 days. The estimated amount of [^{14}C] stored at this time was 0.13 to 0.15 ppm of total rat weight (Ludwig et al., 1964). Residues dropped to about 0.37% 82 days after the final dose, but the rates of elimination were not determined (Table 4-1). Most of the administered [^{14}C] was excreted in the feces and urine.

4.1.2.2. Dieldrin --The tissue distribution of dieldrin following ingestion was investigated in two studies by Deichmann et al. (1969, 1975). In the first, Deichmann et al. (1969) determined the concentrations of dieldrin in the fat, liver, and blood of male beagle dogs given oral doses of aldrin in corn oil at 0.6 mg/kg/day, 5 days/week for 10 months. The mean dieldrin concentration in blood during months 4 to 10 of dosing did not show any specific pattern and ranged between 0.1 and 0.22 ppm. During the following 12 months, after dosing was terminated, dieldrin concentrations gradually dropped to about 0.075 ppm. Fat and liver biopsies showed increasing concentrations of dieldrin in these tissues during months 1 to 10 of dosing, and at 10 months, the levels reached about 70 and 20 ppm, respectively. When aldrin administration was discontinued, dieldrin concentrations gradually decreased, and by month 12, had dropped to 25 and 6 ppm in fat and liver, respectively.

TABLE 4-1. DISTRIBUTION OF RADIOACTIVITY IN MALE RATS 1 AND 82 DAYS AFTER DOSING WITH [^{14}C]-ALDRIN FOR 3 MONTHS[a]

	Percent of [^{14}C] administered dose after final dose	
	Day 1	Day 82 (12 weeks)
Total excretion:		
Feces	80.80	90.25
Urine	7.90	9.29
Carcass	3.60	0.21
Fat	1.77	0.11
Other tissues	1.83	0.05

[a]Mean value from two rats.

SOURCE: Ludwig et al., 1964.

In the second study, Deichmann et al. (1975) measured the concentration of dieldrin in the abdominal fat and total extractable lipids from mice fed diets containing 5 or 10 ppm aldrin for seven generations, except for the F_4 generation. For each generation, residues were determined in the parents after 260 $\pm$ 15 days on the test diets. All groups of animals in the F_1, F_2, and F_3 generations showed significant increases in dieldrin retention. These concentrations in mice were generally related directly to increases in total body lipids, and were higher in females than in males. Dieldrin concentrations in total lipids from animals of the P_1, F_1, F_2, and F_3 generations receiving 10 ppm of aldrin ranged between 115 and 121 ppm in males and 149 and 159 ppm in females.

Tissue distribution of [^{14}C] following oral distribution of [^{14}C]-dieldrin to rats in a single dose indicated that the initial rapid uptake of [^{14}C] by the liver during the first 12 hours after dosing is followed by a biphasic decrease and redistribution of dieldrin and/or its metabolites among body tissues. Redistribution of radioactivity suggests the accumulation and storage of unchanged dieldrin in body fat (Hayes, 1974; Iatropoulos et al., 1975). Hayes (1974) reported that the concentrations of dieldrin in fat increased to about 30% of the dose (10 mg/kg) by 8 hours after dosing and continued to increase, reaching about 50% of the dose by 24 to 48 hours. Although residues in the kidneys, brain, and muscle attained maximum levels about 2 to 4 hours after dosing (0.08% to 4% of the dose), these concentrations remained at the same levels 48 hours after dosing and then gradually decreased at an unspecified rate (Hayes, 1974). There is some evidence that [^{14}C] residue levels in the brain, blood, liver, and subcutaneous fat and the profile of metabolites in urine and feces are similar and independent of the dose following oral or intraperitoneal administration of [^{14}C]-dieldrin to rats (Lay et al., 1982).

Sex differences have been noted in the tissue distribution of [^{14}C] resi-

dues in rats 9 days after administration of a single oral dose at about 0.108 and 0.153 mg/kg in males and females, respectively. Greater [^{14}C] residue levels were found in the muscle, fat, and liver of females when compared to males, whereas residues in the kidneys and stomach were greater in males than in females. In addition, all of the radioactivity in female tissues except the stomach and small intestine contained unchanged dieldrin. In contrast, male tissues contained various amounts of metabolites (< 1.0% to 85.6% of extractable radioactivity), namely trans-6,7-dihydroxydihydroaldrin, 9-hydroxydieldrin, dicarboxylic acid, and pentachloroketone (PCK) (Figure 4-2) (Matthews et al., 1971).

Differences among species in tissue distribution of [^{14}C] and/or metabolites have also been reported for CFE rats and CF_1 and LACG mice (Hutson, 1976). Although residues in only three tissues were determined, in general, [^{14}C] residue concentrations in liver (about 1.5 ppm) and fat (12 to 14 ppm) were greater in mice than in rats, whereas [^{14}C] residue concentrations in kidneys (3.5 ppm) were much greater in rats. Unchanged dieldrin was the major constituent in the livers, with concentrations in mice (0.8 to 1 ppm) being 8- to 10-fold those in rats. Relatively higher concentrations of 9-hydroxydieldrin were found in the livers of mice (0.27 to 0.51 ppm) and PCK in the kidneys of rats (2.48 ppm). Fat tissues in rats and mice contained only dieldrin. In addition, the data indicated that a relatively small amount of dieldrin may be available at any given time for hepatic metabolism and enterohepatic circulation in rats. Pretreatment of animals with dieldrin for 28 days prior to dosing with [^{14}C]-dieldrin did not greatly alter the distribution and levels of radioactivity in the tissues, but the concentrations of total (labeled and unlabeled) dieldrin (as noted by GLC) and its metabolites increased, especially in mice tissues (Hutson, 1976).

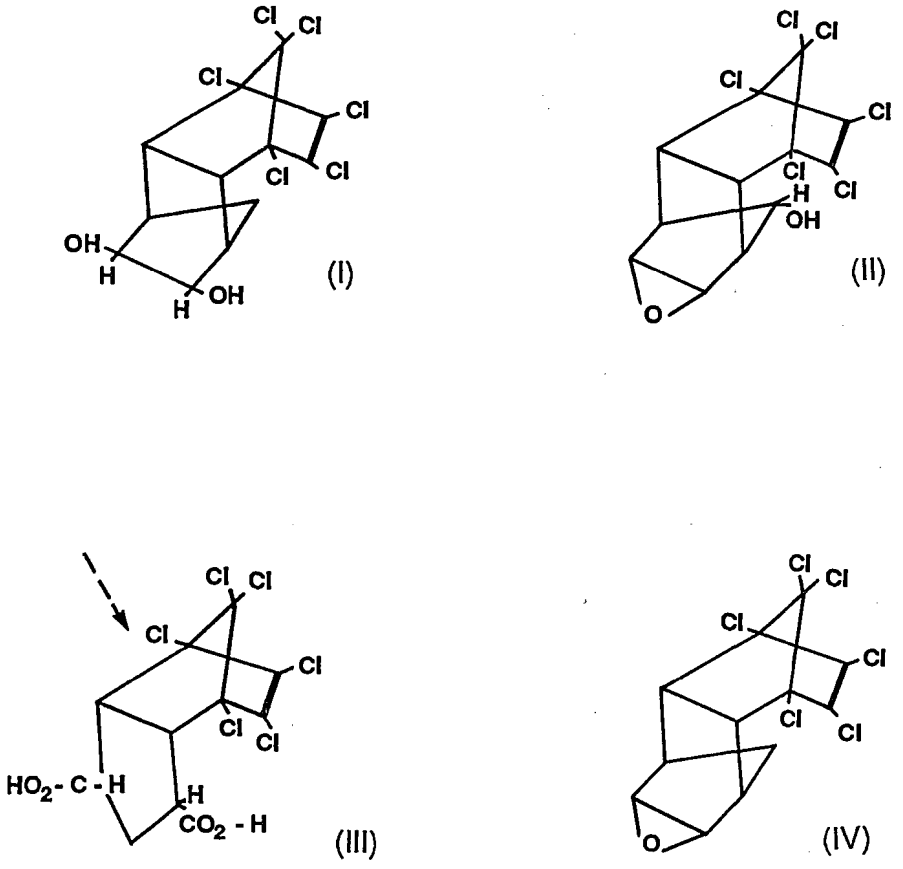


Figure 4-2. Chemical structures of dieldrin metabolites: trans-6,7-dihydroxy-dihydroaldrin (I), 9-hydroxydieldrin (II), dicarboxylic acid (III), pentachloroketone (IV).

Following repeated oral dosing (2 to 104 weeks), an equilibrium or steady state was reached between the intake, storage, and excretion of dieldrin in various strains of rats and beagle dogs (Deichmann et al., 1968; Walker et al., 1969; Barron and Walton, 1971; Davison, 1973). Steady-state kinetics were determined by measuring, at various time intervals, either total radioactivity excreted or that found in fat, blood, and liver. In rats, a steady state was reached 4 to 39 weeks after receiving diets containing various sublethal concentrations of dieldrin (0.04 to 50 ppm). Equilibrium was reached earlier in rats receiving relatively higher doses of dieldrin, which the authors speculated as possibly associated with the induction of microsomal metabolism. In dogs, an approximate plateau in blood residue levels was noted after 12 to 18 and 18 to 30 weeks in dogs receiving 0.005 and 0.05 mg/kg, respectively (Walker et al., 1969). Statistically significant relationships were found between the concentrations of dieldrin in the blood, fat, liver, and brain of rats and dogs receiving dieldrin orally at various concentrations (rats, 0, 0.1, 1, or 10 ppm in the diet; dogs, 0, 0.005, or 0.05 mg/kg for 2 years [Walker et al., 1969]).

The steady-state concentration in adipose tissue of male Osborne-Mendel rats placed on diets containing 25 ppm of dieldrin for 8 weeks was reported to be 50 mg/kg (50 ppm) dieldrin. The elimination of dieldrin residues from fat was rapid when the rats were subsequently placed on untreated diets, the estimated half-life being about 4 to 5 days (Barron and Walton, 1971). The pharmacokinetics of dieldrin elimination from the liver, brain, blood, and adipose tissue of Carworth Farm E male rats placed on diets containing 10 ppm dieldrin for 8 weeks was studied by Robinson et al. (1969). Dieldrin storage in the four tissues was as follows: adipose tissue >> liver > brain > blood. The half-lives of dieldrin in the liver were estimated to be 1.3 and 10.2 days for the initially rapid and subsequently slower phases of elimination, respectively.

Similar values were estimated for blood. The estimated half-lives of dieldrin in adipose tissue and brain were 10.3 and 3 days, respectively. Based on similar mathematical equations, Moriarty (1975) estimated a half-life of 10.3 days for dieldrin in plasma. However, the data may be confounded by the fact that the estimated half-life for plasma residues also included some measurement for residues present in the plasma lipid fraction.

Sex differences were noted in steady-state tissue residues between male and female Sprague-Dawley and Carworth Farm E rats (Walker et al., 1969; Davison, 1973) but not in beagle dogs (Walker et al., 1969). The [^{14}C] residue levels in the whole carcass of female Sprague-Dawley rats were 3 to 4 times those found in males (Davison, 1973), whereas dieldrin residues in blood, fat, and liver of female Carworth Farm E rats were 2- to 10-fold those of males (Walker et al., 1969).

In rhesus monkeys fed daily diets containing 2 ppm of dieldrin for 260 days, daily excretion never equaled the daily dose administered. By extrapolation, it was estimated that a steady state would have been reached after 350 to 400 days of dosing. In one male and one female monkey sacrificed after 260 days of dosing, fat contained the highest concentrations of [^{14}C] residues (54.4 and 6.1 ppm dieldrin, respectively). Lesser levels were found in other tissues. Animals subsequently placed on untreated diets for 130 days showed a marked decrease in tissue residue levels. However, appreciable amounts remained in the fat (21.4 and 13 ppm in males and females, respectively). Fat extracts contained only unchanged dieldrin (Muller et al., 1979).

The concentrations of dieldrin found in the liver of male rhesus monkeys (0.22 to 23.25 ppm) fed diets containing various concentrations of dieldrin (0 to 5 ppm) for 69 to 74 months were dose dependent (Wright et al., 1978). Most of the radioactivity was located in the liver microsomes. The authors suggested

that rhesus monkeys and humans exhibited similar pharmacokinetics and had considerably greater concentrations of dieldrin in the fat, liver, and blood when compared to rodents receiving similar or higher doses of dieldrin, thus indicating a slower metabolic clearance in primates than in rodents.

While there is little information on long-term retention of dieldrin, the U.S. EPA National Human Adipose Tissue Survey (U.S. EPA, 1985) reported geometric-mean levels of dieldrin in human adipose tissue from 1970 to 1983 excluding 1980 and 1982. These mean values ranged from a maximum of 0.22 ppm in 1971 to a minimum value of 0.05 ppm in 1981. There was a trend toward lower levels over time.

4.1.3. Metabolism

4.1.3.1. Aldrin--Following absorption from the gastrointestinal tract, aldrin is readily epoxidized in mammals to dieldrin (Figure 4-3). Epoxidation of aldrin to dieldrin has also been demonstrated in plants, insects, and microorganisms (Soto and Deichmann, 1967; Brooks and Harrison, 1966). The *in vivo* epoxidation of aldrin to dieldrin in mammals and detection of dieldrin body fat was first reported by Winteringham and Barnes (1955) in experiments with mice. Similar findings were reported for other species receiving unlabeled aldrin, including beef and dairy cattle, pigs, sheep, rats, and poultry (Bann et al., 1956), or [^{36}Cl]-aldrin, in rats and pigs (Hunter et al., 1960).

The epoxidation of aldrin to dieldrin is catalyzed by an enzyme (or enzymes) found in the hepatic microsomes; the enzyme has been called aldrin epoxidase. Further metabolism of dieldrin is relatively slow and, consequently, aldrin has been used as a substrate to measure the epoxidase activity in many *in vitro* studies. Aldrin epoxidation by hepatic microsomes from pigs was reported by Brooks and Harrison (1966). Dieldrin was readily formed when aldrin was incubated with the microsomes in a phosphate buffer for 10 minutes

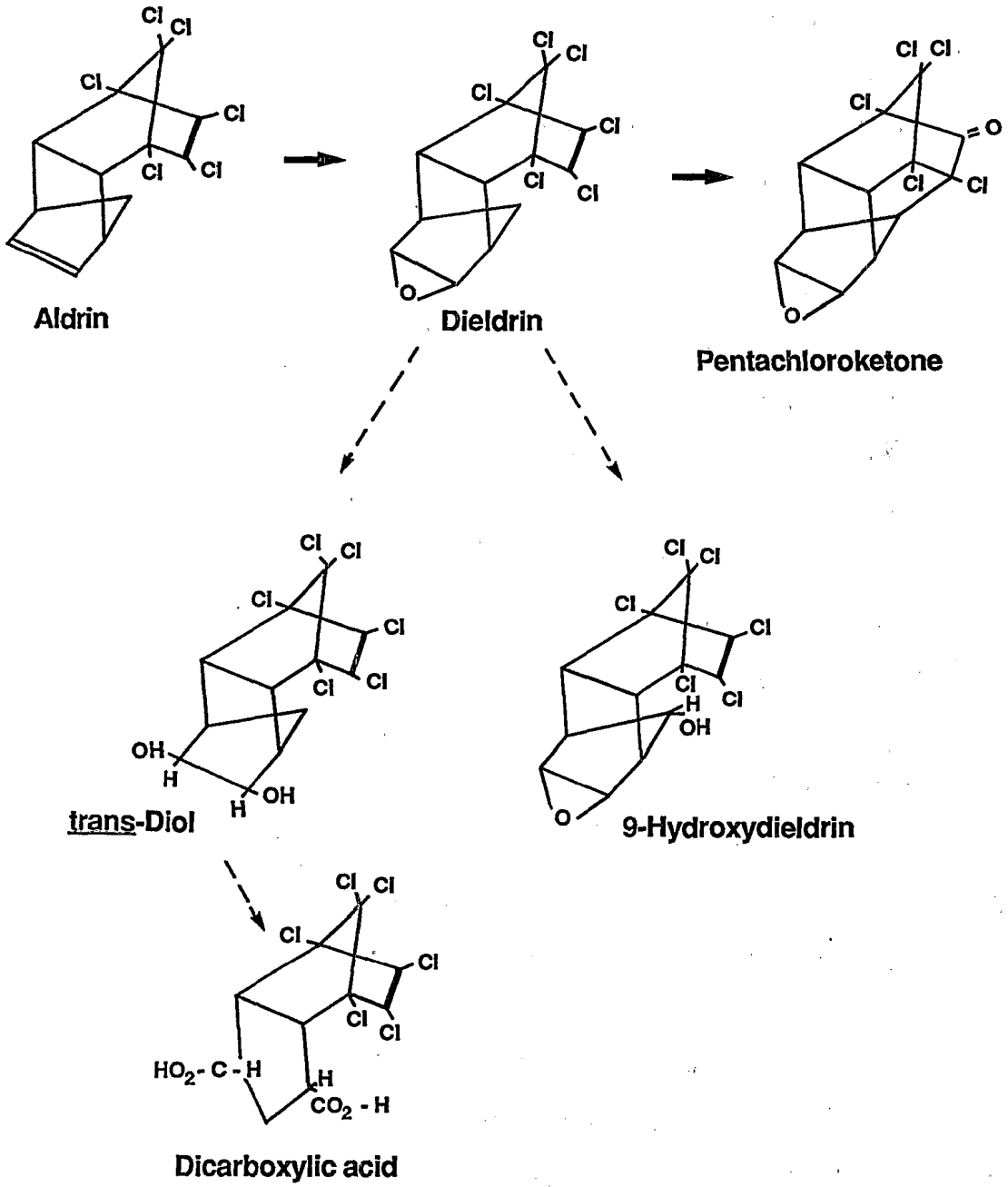


Figure 4-3. Metabolic pathways of aldrin.

at 30°C in the presence of a NADPH-generating system.

Wolff et al. (1980) studied the epoxidation of aldrin in a reconstituted enzyme system using two purified isozymes of cytochrome P-450. Dieldrin formation was faster (apparent K_m of 7 $\pm$ 2 mM) when cytochrome P-450, isolated from hepatic microsomes of phenobarbital-induced rats, was the enzyme used than when the 3-methylcholanthrene-inducible form, P-448, was used (apparent K_m of 27 $\pm$ 7 mM).

Aldrin epoxidation occurs readily with five purified cytochrome P-450 isozymes from (uninduced) male ICR mouse liver (Levi and Hodgson, 1985). The rate of dieldrin formation ranged between 5.1 and 11 nmol dieldrin formed/nmol cytochrome P-450/30 minutes, with cytochrome P-450A1 being the most active. Davies and Keysell (1983) demonstrated that the rate of aldrin epoxidation by hepatic microsomal preparations from Wistar rats was sex dependent, being faster in males than in females. The apparent K_m and V_{max} for hepatic cytochrome P-450 from females were 78.8 mM and 0.39 nmol/nmol cytochrome P-450/minute, respectively. These values were 28% higher and 94% lower, respectively, than hepatic cytochrome P-450 from males.

In humans, aldrin epoxidation occurs readily *in vitro* upon incubation with liver microsomes. The rate of aldrin epoxidation by liver microsomes from 28 subjects was directly related to the cytochrome P-450 content (McMannus et al., 1984).

4.1.3.2. *Dieldrin*--The metabolic pathways of dieldrin in laboratory animals are presented in Figure 4-4. The only monohydroxy derivative detected, 9-hydroxydieldrin, may be conjugated with glucuronic acid. The 6,7-*trans*dihydroxydihydroaldrin is a biotransformation product of the epoxy group and may be conjugated with glucuronic acid or oxidized to the tricyclic dicarboxylic acid. Pentachloroketone (PCK) is one of the few examples known of a metabolite of a

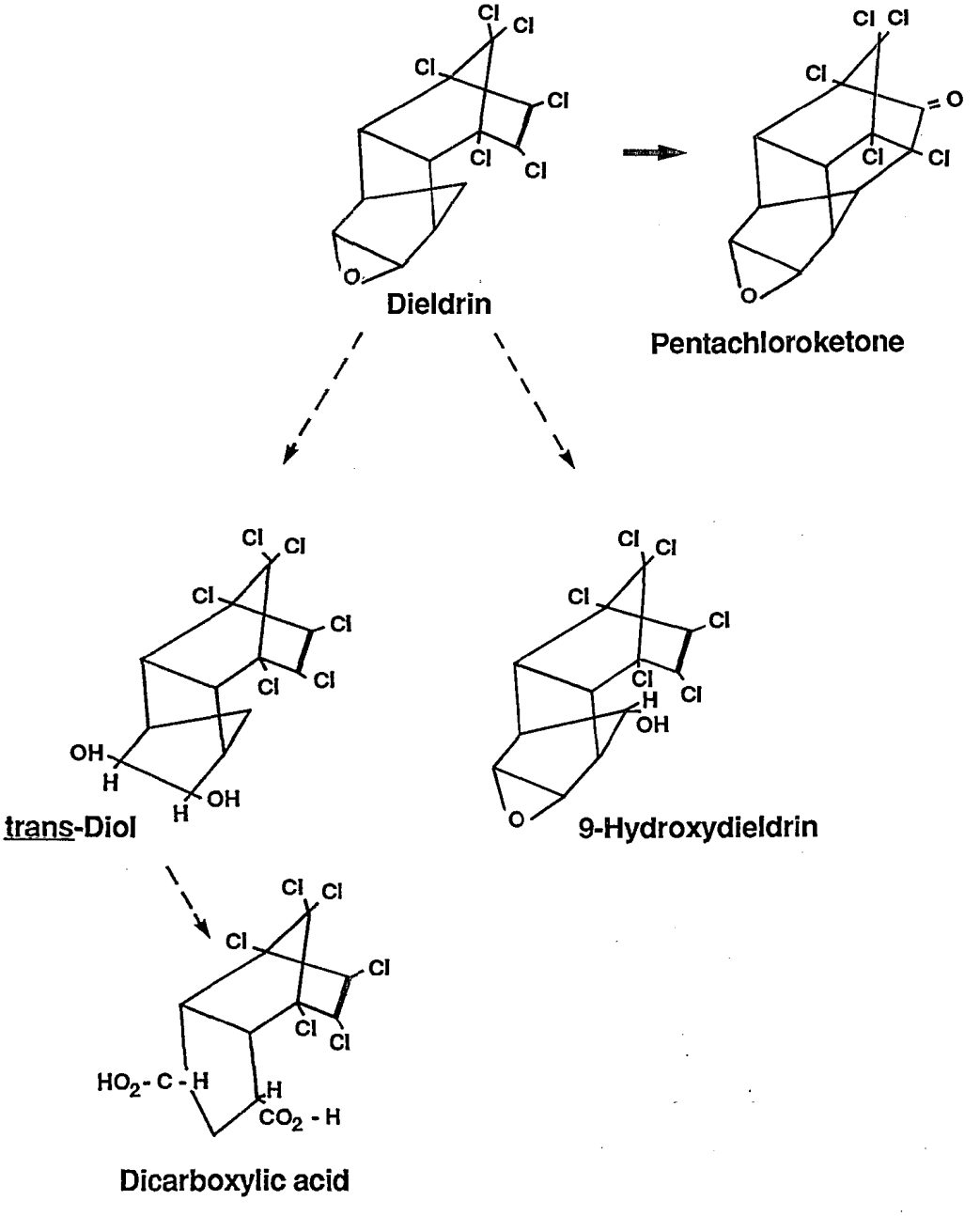


Figure 4-4. Metabolic pathways of dieldrin.

foreign compound that is formed by a chemical skeletal rearrangement (Figure 4-4).

Dieldrin is hydroxylated to 9-hydroxydieldrin by liver microsomal mono-oxygenase from rats, and the reaction is inhibited by the addition of the mono-oxygenase inhibitor sesamex (Matthews and Matsumura, 1969). Its isomer anti-9-hydroxydieldrin is neither an in vitro nor an in vivo metabolite, which may be due to the inaccessibility of the anti C-12 hydrogen of dieldrin to oxidative attack by the mono-oxygenase. Further, oxidation of 9-hydroxydieldrin to 9-ketodieldrin apparently does not occur, since oxidation would also involve attack of the inaccessible anti C-9 hydrogen atom (Bedford and Hutson, 1975).

The 9-hydroxydieldrin glucuronide is formed both in vitro and in vivo. It has been identified in the bile of dosed rats (Heath and Vandekar, 1964; Chipman and Walker, 1979), but is extensively deconjugated in the intestine and excreted in the feces in the free form (Bedford and Hutson, 1975; Hutson, 1976). However, 9-hydroxydieldrin has also been found in the urine of monkeys (Muller et al., 1975). The 9-hydroxydieldrin glucuronide is formed rapidly in vitro from dieldrin (Hutson, 1976) or 9-hydroxydieldrin (Matthews et al., 1971) upon incubation with liver microsomes from rat and uridine diphosphoglucuronic acid. The rate of conversion from dieldrin was 0.0028 nmol/min/mg protein (Hutson, 1976), whereas about 67% of the 9-hydroxydieldrin was converted to the conjugate (Matthews et al., 1971). The hydroxyl conjugate has also been isolated and identified in mouse urine (Hutson, 1976).

Dieldrin can also be metabolized by epoxide hydratase to form 6,7-trans-dihydroxydihydro-aldrin, which was the first metabolite to be isolated and identified from rabbits (Korte and Arent, 1965). However, Matthews and McKinney (1974) reported that the cis-diol is also formed in vitro from dieldrin by rat liver microsomes. Apparently, the cis-diol can be metabolized

further to other metabolites or to the trans-diol by means of a microsomal epimerase, whereas the trans-diol formed in vitro is not metabolized further. It was suggested that the formation of the cis- and the trans-diol may occur via a hydroxyketodihydro intermediate (Matthews and McKinney, 1974; Bedford and Hutson, 1975). The compound 6,7-trans-dihydroxydihydroaldrin is further oxidized to aldrin dicarboxylic acid (ADA) or conjugated to glucuronic acid (Figure 4-4). Elimination of the ADA occurs mainly in the urine of mice and rats (Baldwin et al., 1972; Hutson, 1976) and in the feces of rats (Hutson, 1976), whereas the diol conjugate has been identified in urine from rabbits and monkeys (Muller et al., 1975, 1979). The diol glucuronide is also formed in vitro upon incubation of 6,7-trans-dihydroxydihydroaldrin and hepatic microsomal preparations from rabbits or rats in the presence of uridine diphosphoglucuronic acid and NADPB. About 40% to 50% of the substrate is converted to the conjugate (Matthews and Matsumura, 1969).

PCK, also known as Klein's metabolite, is a major urinary metabolite in male rats, but it is only found in trace amounts in the urine of male mice (Matthews et al., 1971; Baldwin et al., 1972; Hutson, 1976). PCK is also found in trace amounts in the urine of female rats (Matthews et al., 1971). It has also been identified in the urine of rats fed diets containing aldrin (Klein et al., 1968). PCK is also formed in vitro by hepatic microsomes and its production is inhibited by sesamex (Matthews and Matsumura, 1969). The mechanism of formation of this skeletal rearrangement product has been the subject of much speculation. Bedford and Hutson (1975) suggested that PCK is the product of rearrangement of the same intermediate that leads to 9-hydroxy-dieldrin (Figure 4-4).

4.1.3.2.1. Dieldrin Metabolism in Humans. Very little is known about the metabolism of dieldrin in humans. Routes of exposure for dieldrin handlers are

by inhalation, ingestion, and dermal. For the general population, the major source of dieldrin exposure is believed to be the ingestion of contaminated food. It has been shown that exposures due to inhalation of cigarette smoke (Domanski et al., 1977) and through drinking water (Barquet et al., 1981) are relatively insignificant. The absorption of dieldrin following exposure by any of these routes has not been well characterized. Feldmann and Maibach (1974) investigated the percutaneous absorption of 4 mg/cm^2 [^{14}C]-dieldrin applied to the ventral forearms of male volunteers; this concentration represented the amount that would be deposited by a thin film of 0.25% pesticide solution. It was reported that only about 8% of the applied dose was absorbed based on urinary [^{14}C] excretion when compared to the excretion of a known dose administered intravenously. This conclusion, however, is questionable because of the large variability of the data, the small amounts of dieldrin administered, the lower recovery of [^{14}C] in the urine (2% to 5% of the dose) following intravenous injection, and the possible effect on absorption by cutaneous metabolism or adipose tissue content. No human studies were found that examined the absorption of dieldrin via inhalation and only limited information was found on absorption via ingestion.

Most of what is known about the pharmacodynamics of dieldrin in humans following absorption comes from a two-part study (Hunter and Robinson, 1967; Hunter et al., 1969) in which normal male volunteers ingested either 10, 50, or 211 mg of dieldrin per day for 18 to 24 months. The authors reported that dieldrin concentrations in blood and fat increased with increasing dosages of the pesticide. For higher doses of dieldrin, a significant correlation existed between the concentration of dieldrin in the blood and in fat. The concentration of dieldrin in adipose tissue was approximately 156-fold the concentration of dieldrin in blood. The time-dependent increase of dieldrin

concentration in blood and adipose tissue approached an asymptote in an exponential manner, indicating that there is a finite upper limit to the storage of dieldrin by each person that is dependent on that person's daily intake of dieldrin. This upper limit for the storage of dieldrin corresponds to a balance between the amount of dieldrin ingested and the amount eliminated daily, and was observed at about 15 months. The equilibrium value is characteristic of a person and his particular daily intake. At about month 15, dieldrin levels in the blood of the group receiving 211 μg HEOD/day was 0.0143 ppm and the concentration in adipose tissue was 2.12 ppm, giving a fat-to-blood ratio of 148 (Hunter and Robinson, 1967). Measurements of blood dieldrin concentrations for 8 months following the end of dosing on these same volunteers indicated that dieldrin is eliminated at an exponential rate that follows first-order kinetics. The reported half-life for dieldrin elimination was 369 days (Hunter et al., 1969). However, this value is only an estimate based on a limited number of samples, and may be confounded with adventitious ingestion of dieldrin in foodstuffs (estimated at about 14 mg/day).

Moriarty (1975) estimated a half-life of about 2150 days for dieldrin in plasma. It therefore appears that humans, like primates, exhibit a slower metabolic clearance than rodents (Wright et al., 1978).

Additional information on body burdens of dieldrin and its mobilization during times of stress was reported by Hunter and Robinson (1967). In measuring the adipose tissue concentrations of dieldrin, it was found that the body burden of dieldrin is related to the lipid mass of an individual and that for a given chronic dose of dieldrin, a lean person will attain higher relative adipose tissue concentrations of dieldrin but will retain less of the administered dose than a person having more body fat. The authors also concluded that the catabolism of fat resulting from surgery or from sudden weight loss

due to dieting did not lead to significant increases in blood dieldrin levels. Consequently, the body burden of dieldrin in the general population does not constitute a risk for intoxication as a result of tissue mobilization at times of illness or weight loss.

Eckenhausen et al. (1981) measured dieldrin levels in the blood of mothers and their babies, as well as in the mothers' breast milk and placental tissue and the babies' meconium. Measurable levels in all samples were detected. The reported ranges were mothers' blood, < 0.1 to 4.1 ng/mL; umbilical cord blood, < 0.1 to 2.6 ng/mL; babies' blood, < 0.1 to 4.6 ng/mL; breast milk, 1.4 to 3.3 ng/mL; placental tissue, 1.9 ng/g (mean); and meconium, 3.1 ng/g (mean). It was concluded that breast-fed babies did not have higher blood levels of dieldrin than bottle-fed babies, and that the placenta offered only a partial barrier against the transfer of dieldrin from the mother to her baby. The levels of dieldrin present were too low to allow for metabolite analysis.

Dieldrin and/or its metabolites have been detected in human urine (Cueto and Hayes, 1962; Cueto and Biros, 1967) but were not characterized further. Studies on dieldrin and/or its metabolites in human feces other than in meconium have not been found.

4.1.4. Excretion

4.1.4.1. Aldrin--The metabolism and excretion of [^{14}C]-aldrin following daily administration by gavage to male rats at a dose of 4.3 mg/rat for 3 months was studied by Ludwig et al. (1964). The elimination of [^{14}C], expressed as percent of dose administered weekly per rat, increased from 30.5% on the second day of dosing to about 103% during weeks 10 to 12. Most of the [^{14}C] was excreted in the feces; about 48% of the dose given in week 1 was found in the feces and 2% in the urine. For weeks 10 to 12, fecal excretion accounted for 93% to 94% of the weekly dose, whereas the urinary excretion accounted for 8.8%

to 9.1%. It was found that after about 53 days of dosing, the amount of the radioactivity that was administered each day was equal to the amount excreted, so that subsequent doses of aldrin did not result in further accumulation in the body. It should be noted that Quaife et al. (1967) concluded that the above study failed to provide unequivocal evidence for the equilibrium concept (i.e., between intake and storage of aldrin) following chronic feeding of aldrin because only one dose level was used and the equilibrium was established and maintained for only a month before dosing was terminated. Nonetheless, the results provided by Ludwig et al. (1964) indicate that such an equilibrium may indeed be attained, especially since aldrin can induce liver enzymes leading to a higher rate of aldrin metabolism following repeated dosing.

After termination of dosing, the excretion of [^{14}C] decreased rapidly, and about 98% and 99.5% of the total [^{14}C] administered had been eliminated by 6 and 12 weeks after the final dose, respectively. Chromatographic analysis of diethyl ether extracts of urine and methanol extracts of feces, collected at various intervals of the study, revealed the predominant excretion products were hydrophilic metabolites as shown in Table 4-2. Aldrin concentration in urine and feces decreased during the dosing period, whereas dieldrin concentrations were not greatly changed. Although the decrease in aldrin concentrations in urine and feces during the dosing period suggests increased rates of metabolism due to enzyme induction, some of the aldrin and dieldrin present in urine may also have been due to contamination by feces in the metabolism cages (Ludwig et al., 1964).

One of the polar metabolites found in the urine of rats fed diets containing 25 ppm aldrin for an unspecified period of time was identified as 1,1,2,3a,7a-pentachloro-5,6-epoxydecahydro-2,4,7-metheno-3Hcyclopenta[a]-pentalen-3-one (pentachloroketone) (Klein et al., 1968). Pentachloroketone was

TABLE 4-2. RELATIVE CONCENTRATIONS OF EXCRETION PRODUCTS IN MALE RATS FED ALDRIN FOR 12 WEEKS AND OBSERVED FOR AN ADDITIONAL 12 WEEKS

		Compound/Metabolite[a]		
Week	Specimen	Aldrin	Dieldrin	Hydrophilic metabolites
0.29 (Day 2)	Urine	25	10	65
	Feces	45	15	40
3	Urine	2	5-9	89-93
	Feces	15	15	70
12	Urine	1	5-9	90-94
	Feces	10-15	15	70-75
20[b]	Urine	--	3-8	92-97
	Feces	5	20	75

[a]Expressed as percent of radioactivity found in extracts.
[b]Eight-week recovery period after final dose.

also identified in the urine of rats fed diets containing 25 ppm dieldrin (Figure 4-4) (Klein et al., 1968). Other known metabolites of dieldrin, namely 9-hydroxydieldrin, the trans-diol, and dicarboxylic acids, have not been identified in excreta of animals dosed with aldrin. These metabolites should be formed readily. However, neither qualitative nor quantitative data are available.

4.1.4.2. Dieldrin--The excretion of radioactivity following administration of single oral doses of [^{14}C]-dieldrin to rats, mice, monkeys, and chimpanzees is mainly in the feces, accounting for 5% to 62% of the dose (Matthews et al., 1971; Muller et al., 1975; Hutson, 1976), whereas in rabbits, elimination is greater in urine, accounting for 1.5% to 2.2% of the dose (Muller et al., 1975). The ratio of radioactivity excreted in the feces and urine is about 10 to 18 in rats, 18 to 38 in mice, 0.2 in rabbits, 3.6 in monkeys, and 3.8 in chimpanzees. Similar findings were reported following repeated dosing of dieldrin to rabbits twice weekly for 22 weeks for a total dose of about 56 to 58 mg/kg (Korte and Arent, 1965) and to rhesus monkeys receiving 2 ppm daily in the diet for 260 days (Muller et al., 1979). However, the ratio of radioactivity excreted in feces versus urine in monkeys decreased with time of dosing, so that about 40% of the radioactivity was excreted in urine and 60% in the feces after 100 days of dosing.

Although Muller et al. (1975) did not find a sex-related difference in the amounts of radioactivity excreted in feces and urine of male and female rats and mice, Matthews et al. (1971) reported that metabolism and excretion of dieldrin were 3 to 4 times more rapid in male (about 31% of the dose) than in female (about 10% of the dose) rats. The difference was attributed to the greater ability of males to metabolize dieldrin to its more polar metabolites. The total concentration of dieldrin excreted in the feces of male and female

rats 9 days after dosing was 0.65% and 2.45% of the dose, respectively, whereas 9-hydroxydieldrin accounted for about 21.5% and 5.76& of the dose in males and females, respectively. Most of the radioactivity extracted from urine (50% to 65%) in males was PCK and traces of 6,7-trans-dihydroxydihydroaldrin, whereas in females the diol accounted for most of the extractable radioactivity (5% to 25%).

Species differences have been reported for the excretion of dieldrin and/or its metabolites between male CFE rats and male CF_1 and LACG mice (Baldwin et al., 1972; Hutson, 1976). Eight days after a single oral dose (3 mg/kg), fecal elimination of [^{14}C] was slightly greater and faster in rats (62.4% of the dose) than in mice (27.2% and 51.4% of the dose) (Hutson, 1976). Rats excreted 9-hydroxydieldrin as the major component in the feces, together with the diol and aldrin-derived dicarboxylic acid (ADA), whereas unchanged dieldrin was the major component of [^{14}C] excreted by mice with lesser amounts of 9-hydroxydieldrin, the diol, and ADA (Baldwin et al., 1972; Hutson, 1976). Rats and mice excreted unchanged dieldrin, ADA, and PCK in urine; however, PCK was a major urinary metabolite in rats, whereas it was only present in minute trace amounts in mouse urine. Another difference between rats and mice was the excretion of a polar complex of five metabolites by mice (about 50% of the urinary [^{14}C]). One of these metabolites was 9-hydroxydieldrin glucuronide, present at about 0.1% of the dose in both strains of mice (Hutson, 1976).

In a comparative study conducted by Muller et al. (1975), unchanged dieldrin was found in the feces, but not in the urine, of male and female mice (5.5% and 3.2%, respectively), rats (0.8% and 2.8%, respectively), and rabbits (0.3% and 0.5%, respectively), male rhesus monkeys (9.0%), and a female chimpanzee (3.2%). The animals were dosed with [^{14}C]-dieldrin at 0.5 mg/kg and excreta was collected for 10 days. The major metabolite found in rats, monkeys,

and the chimpanzee was 9-hydroxydieldrin, whereas the diol was the major metabolite found in mice and rabbits. However, the concentrations of each metabolite in the feces and urine were not specified.

The major metabolite excreted in the urine of rabbits (sex not specified) receiving repeated oral doses of [^{14}C]-dieldrin was 6,7-trans-dihydroxydihydroaldrin and accounted for about 86% of the total urinary radioactivity (Korte and Arent, 1965). In monkeys fed diets containing 2 ppm of [^{14}C]-dieldrin for 260 days, 9-hydroxydieldrin and unchanged dieldrin were the major compounds excreted in the feces. As the concentration of [^{14}C] excreted in the urine increased with the duration of dosing, the diol and, subsequently, 9-hydroxydieldrin and the diol conjugate were found. At the end of the 360-day experiment, the proportions of dieldrin and its metabolites, expressed as percent of total [^{14}C] excreted, were: dieldrin, 24%; 9-hydroxydieldrin, 52%; 6,7-dihydroxyhydroaldrin, 8%; and the diol conjugate, 14% (Muller et al., 1979).

Total elimination of [^{14}C] following intraperitoneal or intravenous injection of [^{14}C]-dieldrin to male rats was roughly equal to or less than that observed following oral dosing, and occurred primarily in the feces (about 90%) (Cole et al., 1970; Lay et al., 1982). Elimination in the feces occurs via the bile, since most of the [^{14}C]-dieldrin administered intraperitoneally or intravenously is excreted via the bile within 7 days (Heath and Vandekar, 1964; Cole et al., 1970). Excretion in bile of cannulated male rats reached maximal rates about 0.5 to 5 hours (Chipman and Walker, 1979; Tanaka et al., 1980). The rate of [^{14}C] elimination in the bile increased threefold following pretreatment of rats with phenobarbital (Chipman and Walker, 1979) and was three times as rapid in perfused livers from males when compared to females (Klevay, 1970).

4.2. TOXIC EFFECTS

4.2.1. Human Studies

4.2.1.1. Aldrin--The acute lethal dose of aldrin in humans by the oral route presumably is in the range of other mammals, 20 to 95 mg/kg (Hodge et al., 1967). This is consistent with the lethal dose of 70 mg/kg as reported by Hayes (1971) although Jager (1970) reported the acute lethal dose to be about 5 g/kg. It has also been reported that humans recovered following exposure to aldrin. The signs of acute poisoning are primarily related to the central nervous system, with hyperexcitability, convulsions, depression, and death. Based on data from studies on monkeys, Hodge et al. (1967) estimated that a prolonged oral intake of aldrin at 17.5 mg/day would endanger the life to a 70-kg human, whereas 3.5 mg/day could be tolerated.

There have only been a few cases of severe aldrin poisoning reported. The first was reported by Spiotti (1951), who described a case in which a 23-year-old man intentionally ingested an amount of aldrin equivalent to 25.6 mg/kg body weight. Convulsions began in about 20 minutes, and abnormal electroencephalographic (EEG) tracings revealing generalized cerebral dysrhythmia were seen shortly thereafter. By 5 months the rhythm was essentially normal and the man recovered with no apparent lasting effects.

The World Health Organization (1969), as reported by Hayes (1982), published data on 58 cases of poisoning in Kenya resulting from consumption of seed grain treated with aldrin, sometimes in combination with other pesticides. None of the cases involved convulsions; however, in five of the severe cases, effects on the pupils were observed.

A few reports have documented toxicity following prolonged or chronic exposure. Again the predominant effects were related to the central nervous system. In India, 12 cases of neurotoxicity resulted from repeated consumption of wheat

over a 6- to 12-month period when aldrin powder and gammexane power had been accidentally mixed with the wheat. Convulsions were included among the reported clinical symptoms. The EEG tracings were consistent with a diagnosis of organochlorine insecticide poisoning (Gupta, 1975).

Jager (1970) reported the effects of aldrin and dieldrin following chronic exposure from monitoring workers at the Shell Chemical Company. He reported that 33.2 mg/kg/day was tolerated by workers for up to 15 years. Above this level, some individuals showed signs of intoxication including hyperexcitability and convulsions.

Hoogendam et al. (1962) reported the results of a study designed to develop a useful test for measuring possible subclinical intoxication of workers who had been exposed to aldrin, dieldrin, and endrin at a pesticide manufacturing and formulating plant in the Netherlands. Prior to this study, convulsions followed by abnormal EEGs were observed at the plant following acute or prolonged exposures. In this study, EEGs were made of 122 exposed workers who were compared to 122 male office workers used as controls. Twenty-five of the exposed workers and eleven of the control group exhibited EEG abnormalities. The incidence in the exposed workers was significantly different than controls by chi-square test ($p < 0.05$).

4.2.1.2. Dieldrin--Dieldrin was also reported to be highly toxic by ingestion, inhalation, and skin absorption, with a tolerance of 0.25 mg/m^3 of air (Hawley, 1981). The majority of the data on the toxic effects of dieldrin in humans are the result of accidental intoxication or by adults with suicidal intent. The onset of clinical intoxication is almost always rapid. As in the case of aldrin, the acute lethal dose is estimated to be in the range of 20 to 95 mg/kg (Hodge et al., 1967). Princi (1954) reported survival of one man who ingested a dose of about 44 mg/kg.

More recently, however, Hayes (1982) reported that ingested dosages of about 10 mg/kg of dieldrin resulted in fatalities. Acute intoxication was usually followed by a brief period of excitation or drowsiness, muscle twitching, convulsions, and coma. Most subjects regained consciousness and recovered completely. Death, when it did occur, was usually prognosticated clinically by evidence of hypothermia.

Garrettson and Curley (1968) reported an accidental poisoning in which a 2-year-old girl and her 4-year-old brother ingested a 5% solution of dieldrin. Generalized convulsions began within 15 minutes after ingestion, and the young girl died before medical treatment arrived. The older child sustained convulsive seizures for 7-1/2 hours although he eventually recovered.

Hayes (1975) previously had discussed a series of sublethal cases of occupational poisoning by dieldrin that occurred in various countries. In 90 cases, the individuals showed full epileptoid convulsions, and 11 suffered recurrence of illness up to several months after the last exposure. The occurrence of illness among the sprayers was proportional to the intensity and duration of occupational exposure.

Van Raalte (1977) reported 32 clinical cases of intoxication following dieldrin exposure during a 13-year period (1954-1962). Convulsions were observed in 19 of the cases.

Other than the neurological manifestations, effects on other organ systems have rarely been reported. However, Hamilton et al. (1978) reported a case of dieldrin-induced immunohemolytic anemia observed in Iowa. The person had hemolytic anemia with a positive direct antiglobin (Coombs) test and a positive Ham test in the blood serum. The serum contained antibodies selectively active against erythrocytes coated with dieldrin.

Versteeg and Jager (1973) discussed studies at a pesticide plant in the

Netherlands. Of 233 long-term workers, no permanent adverse effects were observed.

4.2.2. Laboratory Animal Studies

4.2.2.1. Acute Studies--The lethal oral dose (LD_{50}) of aldrin/dieldrin for most species ranges from 3 to 100 mg/kg. This includes mice, rats, hamsters, guinea pigs, dogs, rabbits, monkeys, and humans (Hodge et al., 1967; RTECS, 1985). With both aldrin and dieldrin, the signs of acute toxicity are primarily related to the central nervous system (CNS). The acute effects upon the CNS include intoxication, hyperactivity, hypersensitivity to auditory and tactile stimuli, hyperexcitability, loss of appetite (anorexia) and body weight, hyperpnea, salivation, tremors, depression, convulsions, coma, and ultimate death (Borgmann et al., 1952; Hodge et al., 1967).

4.2.2.2. Subchronic Studies--In short-term (subchronic) feeding studies with mice and rats, the major toxic effects exerted by aldrin/dieldrin are mortality, depressed body weight, increase in liver-to-body weight ratio, and histopathologic changes in the liver known as "chlorinated hydrocarbon insecticide rodent liver (CHIRL)." CHIRL lesions consist of enlarged centrilobular hepatic cells with increased cytoplasmic oxypillia and peripheral migration of the basophilic granules. These lesions are only seen in the liver of rodents exposed to chlorinated pesticides (Borgmann et al., 1952; Treon and Cleveland, 1955; Hodge et al., 1967; Fitzhugh et al., 1964; NCI, 1978b).

Treon and Cleveland (1955) reported that dogs were more susceptible than rats to the toxic effects of aldrin and dieldrin. Diets containing 20 to 50 ppm aldrin were given to the dogs 5 or 6 days per week. The dogs died after a period of 2 days to 3.2 months. When dieldrin was administered at the same dose level, fatalities occurred in a period of 5 days to 1.3 months. Both aldrin and dieldrin induced diffuse degenerative changes in the brain, liver,

and kidneys of these dogs.

Keane and Zavon (1969) demonstrated a direct relationship between dieldrin levels in the blood and the occurrence of intoxication. Repeated daily oral administration of 0.2, 1, or 2 mg/kg dieldrin to mongrel dogs was continued until intoxication (between days 18 and 85) occurred. A direct relationship between the dieldrin concentration in the blood and severity of clinical signs of intoxication was observed; on the first day of muscle spasms, the average concentration of dieldrin in the blood was 0.38 to 0.50 mg/mL.

Burchfield et al. (1976) examined the effects of dieldrin exposure upon the primate brain by measuring the EEG changes in rhesus monkeys. Dieldrin was administered to groups of three monkeys either as a single "large dose" of 4 mg/kg intravenously or a series of 10 "small doses" of 1 mg/kg intramuscularly at weekly intervals. The results indicated that both single exposure and a series of subclinical exposures to dieldrin altered the frequency spectrum of the spontaneous EEG for up to 1 year.

Singh and Jha (1982) drenched four female goats each day with aldrin at the rate of 2.5 mg/kg body weight for 22 days. The control group consisted of three goats that received no treatment. On study days 16, 20, and 22, an increase in the serum alkaline phosphatase level of treated goats was observed; however, the increase was not statistically significant when compared to the controls. The raised values of inorganic phosphorus on days 20 and 22 were statistically different from control values. No significant change in the serum calcium level was observed during the study. Microscopic examination of the costochondral junction revealed a drastic reduction in the width of proliferating, maturing, and degenerating cartilage cells during the aldrin intoxication.

4.2.2.3. Chronic Studies--In long-term noncarcinogenic feeding studies, both aldrin and dieldrin have been tested at different dose levels in various strains of mice and rats. The overall no-effect level in long-term feeding studies in rats is 0.5 ppm of either aldrin or dieldrin. At feeding levels of 1 ppm and above, an increasing, dose-related hepatomegaly and histologic changes in the liver characterized as CHIRL occurred. At levels of 10 ppm and above, typical signs of organochlorine toxicity occurred such as irritability, tremors, and convulsions.

The long-term feeding studies conducted with aldrin and dieldrin in mice, rats, and dogs are summarized in Table 4-3 (aldrin) and Table 4-4 (dieldrin).

To date, there has been only one long-term study reported on either aldrin or dieldrin in Syrian golden hamsters. Cabral et al. (1979) conducted a lifetime feeding study in hamsters with dieldrin. Male and female hamsters were fed a diet containing 25, 60, or 180 mg/kg for up to 120 weeks. A decreased survival for females at week 70 was observed. Both males and females at the low and high doses exhibited a marked retardation of growth. A dose-related increase in the incidence of hepatic cell hypertrophy in treated hamsters was observed.

There has been one report on the effects of dieldrin on rhesus monkeys. Groups of five male rhesus monkeys were given diets containing 0, 0.1, 0.5, 1, 1.75, or 5 ppm (0.0002 to 0.07 mg/kg body weight) dieldrin for approximately 6 years. After two monkeys in the 5 ppm group had died, the level of exposure to the remaining three animals was reduced to 2.5 and later to 1.75 ppm. Subsequently, one of these animals had its dieldrin intake progressively increased until, at the end of the second year, it was receiving dieldrin at the initial dietary concentration of 5 ppm. Clinical and hematologic examinations, liver and kidney function studies, urinalysis, and pathology revealed no abnormalities.

TABLE 4-3. TOXICITY OF ALDRIN FOLLOWING LONG-TERM FEEDING TO MICE, RATS, AND DOGS

Species	Strain	No./Sex	Dose	Exposure	Effects	Reference
Mouse	C3HeB/Fe	100/M,F	10 ppm	2 yr	Reduced lifespan; enlarged mesenteric lymph node	Davis and Fitzhugh (1962)
Mouse	C3HeB/Fe	100/M,F	10 ppm	2 yr	Reduced lifespan; increase in benign hyperplasia and hepatomas	Davis (1965)
Mouse	C3HeB/Fe	100/M,F	10 ppm	2 yr	Hepatic vein thrombosis	Rueber (1977)
Mouse	B6C3F1	50/M 50/F	4, 8 ppm 3, 6 ppm	80 wk 80 wk	Hyperexcitability Reduced lifespan	NCI (1978a)
Rat	Sprague-Dawley	10/M,F	5, 10, 50, 100, 150 ppm	2 yr	Increased mortality; increased liver/body wt. ratio; CHIRL[a] changes in the liver at the 100 and 150 ppm levels.	Borgmann (1952)
Rat	CF-1	40/M,F	2.5, 12.5, 25 ppm	2 yr	Increased liver/body wt. ratios in males at 2.5 level and in females at 12.5 level; CHIRL at all dose levels.	Treon and Cleveland (1955)
Rat	Osborne-Mendel	12/M,F	0.5, 2, 10 50, 100, 150 ppm	2 yr	A dose-related increase in mortality at the 50, 100, or 150 ppm levels; increased liver/body wt. ratio (not dose related); CHIRL was observed at all levels; hemorrhagic and/or distended urinary bladder associated with nephritis was observed in male rats at the 100 and 150 ppm levels; gross enlargement of the liver in rats 50 ppm and above.	Fitzhugh et al. (1964)
Rat	Holtzman	15/-[b]	250 ppm	375 days	Liver cell necrosis; nuclear hypertrophy; abnormal mitosis.	Song and Harville (1964)
Rat	Osborne-Mendel	30/M,F	5 ppm	2 yr	None	Deichmann et al. (1967)

(continued on the following page)

TABLE 4-3. (continued)

Species	Strain	No./Sex	Dose	Exposure	Effects	Reference
Rat	Osborne-Mendel	50/M,F	20, 30, 50 ppm	31 mo	Dose-related tremors and clonic convulsions particularly in females; reduced lifespan for females at 50 ppm; increased liver/body wt. ratio in males at 30 and 50 ppm; a moderate increase (not dose-related) in the incidence of hepatic centrilobular cloudy swelling and necrosis was observed in both sexes.	Deichmann et al. (1970)
Rat	Sprague-Dawley	50/F	20, 50 ppm	2 yr	Reduced survival at 50 ppm	Deichmann (1974)
	Osborne-Mendel	50/F	20, 50 ppm	2 yr	Reduced survival at 50 ppm	
Rat	Osborne-Mendel	50/F	30, 60 ppm	74-80 wk	Organchlorine intoxication (tremors, convulsions, hyper-excitability) and reduced body weights were observed during the second year.	NCI (1978a)
Rat	Osborne-Mendel	12/M,F	0.5, 2, 10 50, 100, 150 ppm	2 yr	Decreased survival in rats fed 50 ppm and higher, with 33% at 150 ppm dead at the end of 1 year; female rats at 150 ppm died from renal and hepatic necrosis; male rats showed a higher incidence of chronic nephritis at 50 ppm and above.	Rueber (1980)
Dog	Beagle	2/M,F	1, 3 ppm	16 mo	Hepatomegaly; local hyalin (droplet) degeneration of hepatocytes; vacuolization of kidney tubules and liver changes in the 3 ppm females; increase in liver/body wt. ratios at the 3 ppm level.	Treon and Cleveland (1955)

(continued on the following page)

TABLE 4-3. (continued)

Species	Strain	No./Sex	Dose	Exposure	Effects	Reference
Dog	Mongrel	2/M,F	0.2, 0.5, 1, 2	25 mo	Mortality occurred in dogs at the 2 and 5 mg/kg level during weeks 2-5; marked weight loss; common lesions observed were fatty degeneration of the liver, fat in the renal tubules, and reduced erythroid cells in the bone marrow.	Fitzhugh et al. (1964)
Dog	Beagle	6/M	0.6 mg/kg	10 mo	Hyperexcitability, tremors and weight loss; cloudy swelling and fatty degeneration in the liver and hypertrophy of hepatocytes.	Deichmann et al. (1969)

[a]CHIRL = chlorinated hydrocarbon insecticide rodent liver.
[b]Data not given.

TABLE 4-4. TOXICITY OF DIELDRIN FOLLOWING LONG-TERM FEEDING TO MICE, RATS, AND DOGS

Species	Strain	No./Sex	Dose	Exposure	Effects	Reference
Mouse	C3HeB/Fe	100/M,F	10 ppm	2 yr	Reduced lifespan	Davis and Fitzhugh (1962)
Mouse	C3HeB/Fe	100/M,F	10 ppm	2 yr	None	Davis (1965)
Mouse	CF-1	125/M,F	0.1, 1	2 yr	None	Walker et al. (1972)
		200/M,F	10 ppm	2 yr	Decreased survival rate; palpable intra-abdominal masses after 9 months; half the males and females died or were sacrificed by the 15th month.	
Mouse	CF-1	30/M,F	10 ppm	2 yr	Increased mortality and enlarged liver in both sexes	Thorpe and Walker (1973)
Mouse	CO-1	100/M,F	0.15-15 ppm	25 mo	Time- and dose-related hepatic nodules	Benitz et al. (1977)
Mouse	C3HeB/Fe	100/M,F	10 ppm	2 yr	Hepatic vein thrombosis	Reuber (1977)
Mouse	B6C3F1	50/M,F	2.5, 5 ppm	80 wk	Tremors, irritability, abdominal distension	NCI (1978b)
Rat	CF-1	20/M,F	2.5, 12.5, 25 ppm	2 yr	Increase in liver/body wt. ratio in both sexes at 2.5 ppm; CHIRL[a] at all levels	Treon and Cleveland (1955)
Rat	Osborne-Mendel	12/M,F	0.5, 2, 10, 50, 100, and 150 ppm	2 yr	Dose-related increase in mortality at 50 ppm and higher; liver/body wt. ratio was increased in males at 10 ppm and above; CHIRL was observed in all treatment groups; hemorrhagic and/or distended urinary bladder associated with nephritis was observed at 100 and 150 ppm; gross enlargement of the liver in all rats fed 50 ppm and above.	Fitzhugh et al. (1964)

(continued on the following page)

TABLE 4-4. (continued)

Species	Strain	No./Sex	Dose	Exposure	Effects	Reference
Rat	CF	25/M,F	0.1, 1, 10 ppm	2 yr	Irritability, tremors, and convulsions; increase in liver to body wt. ratio in the 1 and 10 ppm females; CHIRL observed at the 10 ppm; chronic nephritis and myocardial fibrosis in all groups.	Walker et al. (1969)
Rat	Osborne-Mendel	50/M,F	20/30, 50 ppm	29 mo	Dose-related tremors and clonic convulsions especially in females; lower survival for females at 30 and 50 ppm; significant increase in liver/body wt. ratio at 30 ppm; moderate (not dose related) increase in the incidence of hepatic centrilobular cloudy swelling and necrosis in both sexes at all levels.	Deichmann et al. (1970)
Rat	Fischer-344	24/M,F	2, 10, 50 ppm	2 yr	Central nervous system disorders (convulsions, tremors, nervous behavior) in high-dose males and females at 80 weeks.	NCI (1978b)
Rat	Osborne-Mendel	50/M,F	29 ppm 65 ppm	80 wk 59 wk	Organochlorine intoxication (tumors, convulsions, hyperexcitability) during the second year.	NCI (1978b)
Rat	Osborne-Mendel	12/M,F	0.5, 1, 2, 10, 50, 100, 150 ppm	2 yr	Decreased survival in rats fed at 50 ppm and above; 42% at 150 ppm dead by the end of one year; female rats died of renal hepatic necrosis; males had higher incidence of chronic nephritis at the 100 and 150 ppm levels.	Reuber (1980)

(continued on the following page)

TABLE 4-4. (continued)

Species	Strain	No./Sex	Dose	Exposure	Effects	Reference
Rat	Albino	40/M	2 mg/kg/day	6 mo	The RNA content and SGOT and alkaline phosphatase (AP) activities increased 4.38, 2.55, and 5.93 times, respectively, while the bilirubin, cholesterol, and total protein contents decreased 80%, 67%, and 30%, respectively. Histologically, hepatic cells increased 40% in size, while the nuclear and nucleolar size increased 12% and 7%, respectively.	Shakoori et al. (1982)
Dog	Beagle	2/M,F	1, 3 ppm	16 mo	1 female at the 3 ppm had vacuolation of the distal renal tubules; no toxic signs at 1 ppm.	Treon and Cleveland (1955)
Dog	Mongrel	2/M,F	0.2, 0.5, 1, 2, 5, 10 mg/kg/day	25 mo	All dogs at the 1, 2, 5, and 10 mg/kg levels died by the 43rd week; weight loss and convulsions were observed at the higher levels; common lesions observed were fatty degeneration of the liver; fat in the renal tubules; reduced erythroid cells.	Fitzhugh et al. (1964)
Dog	Beagle	5/M,F	0.005, 0.05 mg/kg/day	2 yr	In females at 0.05 mg/kg, liver/body wt. ratio and in both sexes serum alkaline phosphate activity were increased.	Walker et al. (1969)

[a]CHIRL = chlorinated hydrocarbon insecticide rodent liver.

No differences were noted in liver/body-weight ratios and liver DNA and RNA of the test animals when compared to those of control animals. No subcellular changes were seen in the hepatocytes. Dose-related increases in microsomal cytochrome P-450 and in the activity of the liver mono-oxygenase enzyme system were observed at the two top dose levels. The concentrations of dieldrin in the subcutaneous fat of the monkeys fed 0.1 ppm were very similar to those measured in humans receiving a similar daily intake of dieldrin per kilogram of body weight. The concentrations in the monkey livers, however, were approximately 200 times higher than in male rats receiving a two-times greater daily intake of dieldrin and were very similar to the concentration in the livers of male mice daily ingesting approximately 50 times more dieldrin per kilogram of body weight (Zavon and Stemmer, 1975; Wright et al., 1978).

4.2.2.4. Teratology and Reproduction Studies--Ball et al. (1953) observed alterations of the estrous cycle of female rats fed a diet containing 20 ppm of aldrin for 10 to 25 weeks.

Treon and Cleveland (1955) studied the effects of aldrin/dieldrin on rat reproduction. Aldrin and dieldrin, at concentrations of 2.5, 12.5, and 25 ppm, were incorporated into the diets of Carworth rats for three generations. Aldrin at the 12.5 ppm level and dieldrin at the 2.5 ppm level reduced the number of pregnancies. This effect was not observed at the other levels for both compounds. Feeding of parent rats during the period of suckling had an effect on the incidence of mortality among the pups. Pup mortality was severe at the 12.5 and 25 ppm levels, and was slight to moderate at the 2.5 ppm level for both aldrin and dieldrin. No differences were observed in the pup weight at weaning between the treated and untreated rats.

Good and Ware (1969) fed male and female CFW Swiss mice with 5 ppm of dieldrin for 120 days, beginning 30 days before mating. The dieldrin-fed mice

produced litters significantly smaller in weight and in number of pups than controls.

Harr et al. (1970) investigated the effect of dieldrin on reproduction in Wistar rats. Dieldrin was fed to male and female rats at concentrations ranging from 0.08 to 40 ppm and monitored for dam survival, conception rate, pup survival, and weaned litter size. These values were normal in rats fed at concentrations of 0.08 and 0.16 ppm. In rats fed 0.31 to 1.25 ppm, a reduction in dam survival and conception rate was noted. In rats fed at concentrations between 2.5 and 10 ppm, the pups died in convulsions (43%) or starved (57%). Deaths of pups due to starvation occurred because both the dams and the pups were too hyperesthetic to permit adequate nursing. Milk curd in the stomach of pups dying when 1 to 3 days old contained dieldrin at concentrations of 2 to 20 ppm. Dam survival, conception rate, pup survival, and weaned litter size were affected in female rats fed dieldrin at 20 to 40 ppm. Most of the pups died in convulsions. The authors reported that the highest dietary level consistent with normal reproduction was 0.24 ppm.

Boucard et al. (1970, as cited in Dix et al., 1977) observed teratogenic effects of dieldrin in rats and mice given a low dose of 2.5 mg/kg and a higher dose of 3.4 mg/kg. These doses were given as a single or repeated dose during gestation. No dose response was demonstrated; however, no statistical analyses were performed, and the incidences of spontaneous abnormalities in these animals were not presented.

Deichmann et al. (1971) reported subnormal reproduction in dogs exposed to aldrin. Eleven (eight females and three males) beagle dogs were dosed with aldrin at 0.15 mg/kg (4 females) and at 0.30 mg/kg (4 females and 3 males) by capsule 5 days per week for a total of 14 months. Following discontinuation of the feeding period, attempts were made to breed all experimental female dogs

with experimental male dogs. The estrous cycle was delayed by 7 to 12 months in the female dogs. Mammary development and milk production were severely depressed. Stillbirth occurred in one female at the 0.15 mg/kg level and in two females at the 0.30 mg/kg level. Two females fed aldrin at the 0.15 mg/kg level did not come into heat during the 8 months following discontinuation of the feeding. No malformations were observed.

Deichmann (1972) presented data on the effects of aldrin and dieldrin when fed to Swiss mice for six generations. Swiss mice were exposed to aldrin at 3, 5, 10, and 25 ppm and to dieldrin at 3, 10, and 25 ppm in their diet for six generations. The indices measured were fertility, gestation, viability, lactation, and survival of the pups. Marked effects on fertility, gestation, viability, lactation, or survival were noted in the parent (first) and second generation, and in their offspring (first and second litters) fed 25 ppm aldrin and dieldrin. Less marked but still significant effects were found in the first and second generations and their offspring that were fed aldrin at 3, 5, and 10 ppm and dieldrin at 3 and 10 ppm.

In a study by Ozoukwa and Sleight (1972), pregnant guinea pigs were given divided doses of dieldrin (up to 120 mg/kg) during gestation. Abortions occurred in guinea pigs after administration of 100 ppm (3 mg/kg) dieldrin in the diet, a dose that was also toxic to the mothers. Dyspnea and convulsions were observed commonly in the dieldrin-treated guinea pigs. Several animals became excitable 2 or more hours after administration of dieldrin, but had CNS depression before death. Mild hepatic fatty metamorphosis and slight vacuolar degeneration in the kidneys were seen in dieldrin-treated guinea pigs. Swelling of the mitochondria occurred in cells of the cerebral cortex and cerebellum after acute dieldrin toxicosis.

Ottolenghi et al. (1974) reported the teratogenic effects of aldrin and

dieldrin in mice and hamsters. Pregnant Syrian golden hamsters and CD-1 mice were given single oral doses of aldrin or dieldrin at one-half the LD_{50} (hamsters at 5, 30, and 50 mg/kg, and mice at 2.5, 15, and 25 mg/kg, respectively). The hamsters were treated orally on days 7, 8, or 9 of gestation, and mice on day 9 of gestation. Both aldrin and dieldrin caused a significant increase in fetal death in hamsters treated on days 7 and 8. Hamsters treated on day 8 also had the highest number of anomalies (i.e., open eyes, webbed feet, cleft palate, and others). A statistically significant ($p < 0.01$ to 0.03) reduction in the fetal weight in hamsters treated on three different days was also observed. In contrast to the hamsters, no significant effects were observed in the weight or survival of fetuses of treated and control mice. However, treated mice showed a significant ($p < 0.01$) increase in the total number of anomalies. Open eye and webbed foot were more frequent after aldrin; cleft palate and webbed foot were more frequent in the dieldrin group. The authors reasoned that both species are susceptible to the teratogenic action of the pesticides; however, the reduced teratogenic effect observed in mice may be due to the lower doses used.

Chernoff et al. (1975) tested dieldrin and the photoproduct, photodieldrin, in CD-1 mice and CD rats. Pregnant mice and rats were given daily oral doses of 1.5, 3, and 6 mg/kg/day of dieldrin and 0.15, 0.30, and 0.60 mg/kg/day of photodieldrin in peanut oil from days 7 to 16 of gestation. In mice at 6 mg/kg, reduced body weight gain and increases in liver-to-body weight ratio were observed. An increase in supernumerary ribs and decreased numbers of caudal ossification centers were noted in the fetuses of mice given 6 mg/kg dieldrin. These findings at the highest dose are a reflection of a fetotoxic response secondary to maternal toxicity. No evidence of teratogenicity without concomitant maternal effects was observed at the given dose levels. The high-

est dose of dieldrin (6 mg/kg) and photodieldrin (0.60 mg/kg) resulted in mortalities (41% and 15%, respectively). Dieldrin was not found to be teratogenic in either the CD rat or the CD-1 mouse at these dose levels. Fetal toxicity at the highest dose was observed. Photodieldrin was not teratogenic or fetotoxic in either the CD rat or CD-1 mouse at these dose levels.

Dix et al. (1977) compared the use of two vehicles (corn oil and DMSO) with various doses of dieldrin in CF-1 mice. The corn oil groups received 1.5 and 4.5 mg/kg/day of dieldrin orally; young (7 weeks) virgin mice were used and the pregnancy rate was very low. With the few mice that survived to termination, the only significant effect was delayed ossification in fetuses of the mice administered the 4 mg/kg level. The DMSO experiments were conducted with older (10 weeks) mice of proven fertility. Fetuses of these mice exhibited a significant ($p < 0.05$) increase in the incidence of delayed ossification and extra ribs. However, the DMSO controls also had a high incidence of delayed ossification (48) and extra ribs (71) compared to the untreated controls, where the number of mice with delayed ossification and extra ribs were 7 and 6, respectively. The authors attributed this to the toxic effects of DMSO. DMSO also produced a reduction in maternal and fetal body weights, whereas the corn oil did not. No differences were observed in the mean litter size, number of resorptions, or fetal death with either vehicle. No dieldrin-related teratogenic effects were observed.

Dix et al. (unpublished results) also reported that no teratogenic effects were observed in the offspring of groups of pregnant banded Dutch rabbits dosed with up to 6 mg/kg/day of dieldrin in carboxyl methylcellulose, from days 6 to 18 of gestation.

4.3. MUTAGENICITY

Evaluation of the published mutagenicity studies on aldrin and dieldrin

have recently (June 1986) been completed by the Office of Pesticides Program (OPP) and presented in reports prepared by Dynamac Corporation. The detailed descriptions and evaluations are appended to this document. Presented below are the overall interpretations of the data taken directly from the OPP aldrin and dieldrin reports, respectively.

4.3.1. Aldrin

The paucity of acceptable studies and the existence of major gaps in the data (point mutation and cytogenetics) severely hinders the development of a definitive genetic toxicology profile for aldrin. However, since aldrin is readily converted both *in vivo* and *in vitro* to dieldrin, the genetic toxicology assessment prepared for dieldrin can be used to infer a genetic activity profile for aldrin.

Although no published bacterial gene mutation assays with sufficient primary data were reviewed, the survey studies with *S. typhimurium* and *E. coli* indicate that aldrin is probably not mutagenic in procaryotes. This finding is supported by the overwhelming conclusion of negative mutagenic effects for dieldrin.

No mammalian gene mutation assays with aldrin were located; the single mammalian cell point mutation assay of dieldrin showed an inconclusive, unconfirmed mutagenic response. A major data gap, therefore, exists for both compounds.

No conclusions could be drawn from the *in vitro* or *in vivo* somatic cytogenetic assays with aldrin; therefore a major data gap remains. However, aldrin was not genotoxic in bacteria, yeast, or rat hepatocytes.

Nonactivated and S9-activated aldrin induced statistically significant increases in UDS in SV-40 transformed (VA-4) human fibroblasts. Dieldrin, also investigated in this study, caused a similar response. However, the validity

of this assay was seriously compromised by numerous technical deficiencies, the observation of strong positive responses by compounds generally accepted as weakly genotoxic or nongenotoxic substances, and the lack of positive controls.

4.3.2. Dieldrin

The published articles reviewed are not considered sufficient to establish a complete genetic toxicology profile for dieldrin. However, certain consistencies emerge which allow us to make reasonable interpretations and to recommend further studies which might generate useful data.

Dieldrin was adequately tested in three acceptable Ames assays with sufficient primary data to conclude that nonactivated and rat or hamster S9-activated dieldrin is not mutagenic in *S. typhimurium*. The results of the seven survey Ames tests with dieldrin support this conclusion. However, based on the inclusive results of a single Ames test, dieldrin, in the presence of mouse S9 activation, is considered presumptively mutagenic in *S. typhimurium*. We caution, however, that this finding has not been confirmed and technical deficiencies preclude acceptance of the results as valid.

The three *E. coli* reverse mutation survey studies with dieldrin further support the conclusion that the chemical is not mutagenic in procaryotes.

In the single published mammalian gene mutation assay, nonactivated dieldrin induced an inconclusive, mutagenic response in Chinese hamster V79 cells. The validity of this assay was seriously compromised because of technical deficiencies, reporting of "positive" responses with compounds that have been previously reported as either weakly mutagenic or not mutagenic, and the lack of a positive control. Although we classified dieldrin 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 completed assay, i.e., three or more noncytotoxic doses, with

and without metabolic activation, and with known mutagens as positive controls.

Although the majority of doses assayed were cytotoxic, dieldrin elicited dose-related clastogenic responses in both cultured human lung and mouse bone marrow cells. Dieldrin, therefore, may be considered weakly clastogenic in mammalian somatic cells.

By contrast, dieldrin administered by gavage or by intraperitoneal injection proved to be nonclastogenic in male germinal cells; HEOD, a major component of dieldrin, was also negative in male germinal cells.

Survey repair studies indicated that dieldrin does not induce primary DNA damage in bacteria. Data from studies with sufficient primary data to draw meaningful conclusions also indicated that dieldrin was not genotoxic in yeast or primary rat and mouse hepatocytes.

However, both S9-activated and nonactivated dieldrin induced a statistically significant increase in unscheduled DNA synthesis in SV-40 transformed human fibroblasts. The validity of this assay is questionable because of numerous technical deficiencies, the reporting of strong "positive" responses by weakly genotoxic or nongenotoxic substances, and the lack of acknowledged positive controls.

It is noteworthy that all assays reporting that dieldrin adversely affects genetic material were either flawed by inadequate study designs (Article Nos. 16, 17, and 26) or showed the greatest activity at cytotoxic doses (Article No. 18).

4.4. CARCINOGENICITY

This section presents the evidence relating to the carcinogenic potential of aldrin or dieldrin based on long-term animal bioassays or results of epidemiologic studies.

4.4.1. Animal Studies

4.4.1.1. Aldrin--

4.4.1.1.1. Studies with Mice. Table 4-5 summarizes the carcinogenicity bioassays of aldrin conducted with mice that are described in this section.

4.4.1.1.1.1. Food and Drug Administration Study (Davis and Fitzhugh, 1962). The authors alluded to a previous 2-year aldrin mouse feeding study that raised the suspicion of tumorigenicity of aldrin although the results were considered inconclusive because the majority of the animals were not available for pathologic examination. No reference was provided and no further information could be found on that study.

In this study, a group of 215 C3HeB/Fe mice were fed a dietary mixture containing 10 ppm aldrin (purity not specified) for a period of up to 2 years. The control group consisted of 217 mice. The number of mice/sex was not given, but they were approximately equally divided by sex. Mice that died during the experiment were necropsied, as were all 2-year survivors. The extent of pathology examination was not clear. Tissues from animals with gross lesions and from some grossly normal mice were preserved. After fixation, the tissues were reexamined and were selected for microscopic study. Slides were prepared from all gross lesions in which neoplasia was suspected, from lungs of mice that had hepatic masses, and also from some animals that had gross pneumonia, intussusceptions, or certain other incidental gross abnormalities. No breakdown as to actual tissues examined was provided.

The average survival of the aldrin-treated groups was approximately 2 months less than controls. Intercurrent diseases, pneumonia, and intestinal parasitism were present and may have influenced the long-term survival rate.

Results, reported for both sexes combined, indicated a statistically significant ($p < 0.001$) increase in the incidence of hepatomas (hepatic cell

adenomas) in the treated animals as compared to controls (Table 4-6). Only one other tumor (lung), was reported in the aldrin group. The incidence of hepatomas, based on necropsied mice, was 23% in the aldrin group and 7% in controls. The hepatic cell adenomas were described as expanding nodules of hepatic parenchymal tissue, usually with altered lobular architecture, and morphologically ranging from very benign lesions to borderline carcinomas. The authors concluded that aldrin had significantly increased the incidence of histologically benign liver tumors. Dr. M. Reuber conducted an independent reevaluation of the liver lesions and considered most of the hepatomas to be liver carcinomas. Drs. Popper, Farber, and Firminger concurred with Reuber's evaluation (Epstein, 1975). The value of this study was compromised by the poor survival rate, lack of detailed pathology, loss of a large percentage of the animals to the study, and failure to treat the results in males and females separately. Despite these inadequacies, the study revealed evidence for hepatocarcinogenicity of aldrin in C_3H mice.

4.4.1.1.1.2. Food and Drug Administration (Davis, 1965). As a follow-up to the previous FDA study, Davis (1965) administered aldrin (purity not specified) at 0 or 10 ppm in the diet, to groups of 100 male and 100 female C_3H mice for 2 years (Table 4-7). The pathology examination was similar to that conducted in the other FDA study. Again, survival in the treated group was reduced compared to the control group, although no breakdown of data by sex and by time of death was given. There was no indication as to the time of tumor detection or deaths in treated versus control groups. The incidence of hepatic hyperplasia and benign hepatomas in the aldrin group was approximately double that of controls, whereas the number of hepatic carcinomas was about the same (Table 4-7). Dr. Reuber also reevaluated the liver lesions from this study and concluded that most of the hepatomas were actually carcinomas. Drs. Popper,

TABLE 4-6. CARCINOGENESIS BIOASSAY OF ALDRIN AND DIELDRIN IN C3HeB/Fe MICE EXPOSED BY DIETARY MIXTURE

Observation	Aldrin (10 ppm)	Dieldrin (10 ppm)	Control
Mice initially on experiment	215	218	217
Average weeks on experiment	51.8	51.4	59.8
Average weeks on experiment for mice with hepatomas	80	77	89
Survivors at 18 months	32 (15%)	33 (15%)	47 (22%)
Survivors at 24 months	2 (1%)	8 (4%)	11 (5%)
Mice discarded at autopsy	64 (30%)	70 (32%)	83 (38%)
Mice potencially available for pathology examination	151	148	134
Number of mice with hepatic cell adenomas	35 (23%)[a]	36 (24%)[a]	9 (7%)

Data not broken down by sex.

[a]Significantly different from controls ($p < 0.001$).

SOURCE: Davis and Fitzhugh, 1962.

TABLE 4-7. SURVIVAL DATA AND LIVER LESIONS IN C3H MICE FOLLOWING TWO-YEAR DIETARY EXPOSURE TO ALDRIN AND DIELDRIN

Observation	Aldrin (10 ppm)	Dieldrin (10 ppm)	Control
Mice initially on experiment	200	200	200
Survivors at 18 months	121 (60%)	117 (58%)	150 (75%)
Survivors at 24 months	31 (15%)	39 (19%)	62 (31%)
Hepatic hyperplasia	72	71	38
Benign hepatoma	65 (32.5%)[a]	69 (34.5%)[a]	27 (13.5%)
Hepatic carcinoma	3 (1.5%)	5 (2.5%)	4 (2%)
Combined hepatoma and hepatic hyperplasia	68 (34%)[a]	74 (37%)[a]	31 (15.5%)

[a]Significantly different from controls by the Fisher Exact Test ($p < 0.05$).

SOURCE: Davis, 1965.

Farber, and Firminger concurred in Reuber's diagnosis (Epstein, 1975). A summary of Reuber's diagnosis is presented in Table 4-8.

Some of the same deficiencies as seen in the 1962 FDA study were also evident in this one; namely, a lack of detailed pathology examination and failure to present data according to sex. However, the survival at 18 or 24 months was acceptable in the controls. The evidence for an oncogenic response (whether benign or malignant) is substantial in male and female C3H mice.

4.4.1.1.1.3. Song and Harville (1964). A total of 55 mice of two strains (C3H and CBA) were administered aldrin in the diet (purity not specified) at 15 ppm for an unspecified time. Ten mice served as controls. A companion study with dieldrin was also conducted. Seven aldrin or dieldrin treated mice had liver tumors by 330 to 375 days. No other details of this study were reported. Little useful information can be obtained from this study due to the lack of details provided.

4.4.1.1.1.4. National Cancer Institute (1978a). A bioassay of technical-grade aldrin (95% pure) was conducted using groups of 50 animals of each sex of B6C3F1 mice. The aldrin was administered as a dietary mixture for 80 weeks, with an additional observation for 10 to 13 weeks. Two dose levels were used. Time-weighted average (TWA) doses were 4 or 8 ppm for males and 3 or 6 ppm for females. Matched controls consisted of untreated groups of 20 male mice and 10 female mice.

Pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 92 untreated male and 79 untreated female mice from similar bioassays of other chemicals. All surviving mice were sacrificed at 90 to 93 weeks.

Mean body weights of treated mice were similar to those of controls during the first year of the study, but lower than those of the controls during the

TABLE 4-8. REEVALUATION OF LIVER LESIONS OF THE FDA CARCINOGENESIS BIOASSAYS OF ALDRIN AND DIELDRIN

Observation	Aldrin (10 ppm)	Dieldrin (10 ppm)	Control
Male mice			
Number of mice examined	91	71	73
Average survival (weeks)	86	91	89
Hyperplasia	3%	3%	12%
Hyperplastic nodules	13%	10%	18%
Hepatocellular carcinomas	82%[a]	87%[a]	30%
Female mice			
Number of mice examined	85	71	53
Average survival (weeks)	80	81	93
Hyperplasia	6%	4%	11%
Hyperplastic nodules	8%	8%	13%
Hepatocellular carcinomas	85%[a]	87%[a]	4%

[a]Significantly different from controls by the Fisher Exact Test ($p < 0.05$).

SOURCE: Evaluation by Reuber; as cited in Epstein, 1975.

second year. Hyperexcitability was observed in all treated groups with increasing frequency and severity during the second year. Aldrin produced a dose-related positive trend in the mortality of female mice, but not of male mice.

In male mice, there was a significant dose-related increase in the hepatocellular carcinomas when compared with either matched controls ($p = 0.001$) or pooled controls ($p < 0.01$). The incidence in the low-dose male group was significantly increased when compared to pooled controls ($p < 0.05$), and the incidence in the high-dose male group was significantly increased when compared with matched controls ($p = 0.002$) or pooled controls ($p < 0.001$) (Table 4-9). There was no significant increase ($p < 0.05$) in the female test groups. The authors concluded that aldrin was carcinogenic for the livers of male $B6C3F_1$ mice producing hepatocellular carcinomas.

4.4.1.1.2. Studies with Rats. Table 4-10 summarizes the carcinogenicity bioassays of aldrin conducted with rats that are described in this section.

4.4.1.1.2.1. Kettering Laboratory (Treon and Cleveland, 1955). Aldrin (95% pure) was administered in the diet to groups of 40 Carworth rats of each sex commencing at age 27 to 28 days at concentrations of 2.5, 12.5, or 25 ppm for a period of 2 years. Groups of 40 animals of each sex, which were fed only the powdered diet, served as controls. The mortality of the treated rats was greater than controls with only about one-half surviving at the end of the 2-year period in the 2.5 and 12.5 ppm groups and only about 40% surviving in the high-dose (25 ppm) groups. Weight gain was comparable for all groups. Pathology examination revealed increases in liver weights and the induction of nonspecific, degenerative changes in the hepatic cells typical of lesions produced by chlorinated hydrocarbon compounds. No tumor information was reported by Treon and Cleveland (1955).

TABLE 4-9. INCIDENCES OF HEPATOCELLULAR CARCINOMA IN B6C3F1 MICE EXPOSED TO ALDRIN IN DIETARY MIXTURES

Observation	Male mice	Female mice
Pooled controls	17/92 (18%)[c]	3/78 (4%)
Matched controls	3/20 (15%)[d]	0/10 (0%)
Low dose[a]	16/49 (33%)[e]	5/48 (10%)
High dose[b]	25/45 (56%)[f]	2/43 (5%)

[a]Low Dose = 4 ppm - male mice; 3 ppm - female mice.
[b]High Dose = 8 ppm - male mice; 6 ppm - female mice.
[c]Statistically significant dose-related trend ($p < 0.001$) by Cochran-Armitage test.
[d]Statistically significant dose-related trend ($p = 0.001$) by Cochran-Armitage test.
[e]Statistically significant ($p = 0.048$) when compared to pooled controls by the Fisher Exact Test.
[f]Statistically significant when compared to matched controls ($p = 0.002$) or pooled controls ($p < 0.001$) by the Fisher Exact Test.

SOURCE: NCI, 1978a.

TABLE 4-10. SUMMARY OF DIETARY CARCINOGENESIS BIOASSAYS OF ALDRIN IN RATS

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
>95% pure	Carworth	40 M&F/dose	0 2.5 12.5 25	24 mo	°Increased liver weights °"Chlorinated hydrocarbon" type lesions °No increase in tumors related to aldrin	°High mortality in dose groups °Limitations of study: -small number of animals surviving -inadequate reporting of data °Inadequate test	Treon and Cleveland (1955)
99% pure	Osborne-Mendel	12 M&F/dose	0 0.5 2 10 50 100 150	24 mo	°Significant increase ($p < 0.05$) liver to body weight ratio in males °Higher total tumors in aldrin low-dose groups °No increase in liver tumors °"Chlorinated insecticide" type lesions	°High mortality at 50 ppm and above °Inadequate test	Fitzhugh et al. (1964)
NS	Holtzman	15 (sex not specified)	250	12 mo	°Liver cell necrosis °No tumors reported	°Limitations of study: -too few animals -too short duration -inadequate reporting of data °Inadequate test	Song and Harville (1964)
95% pure	Osborne-Mendel	30 M&F/dose	0 5	25 mo	°No treatment effects on: survival, liver to body-weight ratio, gross pathology, tumor incidences	°Limitations of study: -dose too low -inadequate reporting of data °Inadequate test	Deichmann et al. (1967)

(continued on the following page)

TABLE 4-10. (continued)

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
95% pure	Osborne-Mendel	50 M&F/dose 100 M&F/controls	0 20 30 50	31 mo	•Liver pathology •No treatment-related tumor incidences	•High mortality at 50 ppm •Comparable survival at other doses •Reevaluation by Reuber on sample concluded Deichmann underestimated incidences of malignant tumors. •Only 84% of the animals were microscopically examined •Inadequate test	Deichmann et al. (1970)
NS	Osborne-Mendel	50 F/dose	0 20 50	24+ mo	•No increase in mammary tumors	•Final pathology not reported •Inadequate test	Deichmann (1974)
95% pure	Osborne-Mendel	50 M&F/dose 10 M&F/matched controls 68 M/pooled controls 70 F/pooled controls	0 30 60	Males: 74 wk treatment plus 37-38 wk observation; Females: 80 wk treatment plus 32-33 wk observation; Total = 111-113 wk	•No treatment-related tumors were observed	•Body weight gain of aldrin-treated and rats was less than controls •No significant difference in mortality between treated and control groups. •Adequate test •Suggestive evidence for thyroid follicular cell and adrenal cortex tumor.	NCI (1978a) (Chu et al., 1981) (Griesemer and Cueto, 1980)

NS=Not specified.

Cleveland (1966) reported the tumor incidences observed in the 1955 study. In both male and female rats, the incidences of tumors were less in all treated groups than in the controls. A variety of tumors was seen in several organs of both treated and control groups with no apparent treatment relationship.

This study has limited value as a carcinogenicity test because of the following deficiencies: small number of animals at risk at 2 years and the inadequate reporting of specific animal data as related to survival and associated lesions. However, the dose levels were adequate, as some toxicity and increased mortality were observed in the treated groups.

4.4.1.1.2.2. Food and Drug Administration (Fitzhugh et al., 1964). Aldrin (> 99% purity) was administered to 12 male and 12 female Osborne-Mendel rats in the diet at dose levels of 0, 0.5, 2, 10, 50, 100, or 150 ppm for a period of 2 years. The rats were 3 weeks of age when the aldrin diet was started.

There was no significant effect on growth rate. However, survival was markedly decreased in aldrin groups at 50 ppm or more. Increases in liver to body weight ratio were reported at all dose levels and were statistically increased from controls ($p < 0.05$) at 10 ppm and greater in males and at all dose levels for females. Chronic nephritis, which occurred more commonly in males than females, was reported for high-dose levels.

Only 68% of all animals were examined histologically. Total tumor incidence (all doses combined) in aldrin-treated animals was 36% compared to an incidence in controls of 18%. However, the increase was particularly large among rats at the lower dosage levels. The lower incidence at higher doses may have been related to the decreased survival at the high doses (Table 4-11). There was no predominant tumor type with tumors in various organs including the lungs, breast, and lymphoreticular system. Although no hepatomas or hepatocellular

TABLE 4-11. INCIDENCE OF LIVER LESIONS AND TUMORS AS RELATED TO PERCENT SURVIVAL IN OSBORNE-MENDEL RATS ADMINISTERED ALDRIN IN THE DIET

Dose level (ppm)	Survival percent			Liver lesions	Rats with tumors
	12 mo	18 mo	24 mo		
0	92%	75%	50%	0/17[a]	3/17[b]
0.5	100	75	50	0/19	10/19
2	100	83	50	0/19	7/19
10	96	67	42	0/22	8/22
50	79	63	25	12/18	5/18
100	63	42	17	11/11	5/11
150	33	17	4	9/9	1/9

[a]Incidence of rats with liver lesions of "chlorinated insecticide" type per number microscopically examined.
[b]Incidence of rats with tumors/number microscopically examined.

SOURCE: Fitzhugh et al., 1964.

carcinomas were diagnosed, a high incidence of "chlorinated insecticide" lesions were observed at 50 ppm and above.

4.4.1.1.2.3. Song and Harville (1964). Groups of 15 Holtzman rats of unspecified sex were fed a control diet supplemented with 250 ppm aldrin (purity not specified) for approximately 1 year. Liver cell necrosis and other changes were seen; however, no tumors were found. Two animals from each group were sacrificed "periodically." The test is inadequate as a carcinogenicity bioassay because of its short duration, lack of substantive data, and inadequate number of animals.

4.4.1.1.2.4. Deichmann et al. (1967). Groups of 30 male and 30 female Osborne-Mendel rats were fed a control diet or a diet supplemented with 5 ppm aldrin (95% pure) for a period of 25 months. This level of aldrin had little observable effect as: a) survival at 24 months was 55% in the controls and 65% in the 5 ppm aldrin group, b) there was no increase in liver-to-body weight ratios, c) no difference in gross pathology, and d) no difference in overall or specific tumor incidences. The value of this study is questionable as a test for possible carcinogenicity for the following reasons: a maximum tolerated dose was not achieved and there were no lifetable data or data on mortality from organ-specific tumors.

4.4.1.1.2.5. Deichmann et al. (1970). Groups of 50 male and 50 female Osborne-Mendel rats were administered aldrin (95% pure) in the diet at final concentrations of 20, 30, or 50 ppm, commencing at the age of weaning; dosage for the first 10 weeks was half the final levels. Groups of 100 rats of each sex served as controls. Experiments were terminated at 31 months. Companion dieldrin studies were also conducted at the same time and by the same protocol. There was no difference in body weight between the aldrin dose groups and controls. Survival was comparable in all male groups and in the two low-dose

groups. Mean survival was reduced in the 50 ppm group (13.0 months) as compared to controls (19.5 months).

A moderate but not dose-related increase in the incidence of centrilobular cloudy swelling and liver necrosis was noted in all aldrin-treated male and female rats, but not in controls. There was no evidence for a carcinogenic response in male or female rats fed aldrin at 20, 30, and 50 ppm. The incidence of all tumors is presented in Table 4-12.

While there was no reported increase in tumor incidence, the value of the study was compromised as the result of the lack of pathology data for many animals. The authors reported that all animals were grossly examined. However, only 86% of the aldrin-exposed and 80% of the controls were microscopically examined. In the high-dose female group, only 62% were actually examined microscopically. There is also some concern as to the accuracy of the tumor diagnoses and reporting. Reuber (as cited in Epstein, 1975) conducted an independent reevaluation of most tissues of the 30 ppm dieldrin-exposed rats. Based on this, Reuber claimed that the authors may have underestimated or under-reported the incidence of malignant tumors by approximately threefold. With the reduced survival at the high doses and the insufficient pathology examination this study is not considered an adequate carcinogenicity test.

4.4.1.1.2.6. Deichmann (1974). In a summary that included little experimental detail, Deichmann reported the results of feeding aldrin (purity not specified) at 0, 20, or 50 ppm to groups of 50 female Osborne-Mendel rats and 50 female Sprague-Dawley rats, commencing at weaning. The feeding of aldrin was associated with a dose-related reduced period of survival with the primary cause of death listed as pneumonitis.

This feeding experiment was initiated for the purpose of studying the effect of aldrin on mammary tumors. The authors concluded that there was no

TABLE 4-12. TUMOR INCIDENCES IN OSBORNE-MENDEL RATS FED ALDRIN IN THE DIET

Dose level (ppm)	Males	Females
0	19/75[a] (25%)	60/80 (75%)
20	5/45 (11%)	20/47 (43%)
30	7/46 (15%)	24/44 (55%)
50	4/45 (9%)	11/31 (35%)

[a]Number of rats with tumors/number rats examined histologically.

SOURCE: Deichmann et al., 1970.

indication of an increase in mammary tumor incidence related to the feeding of 20 or 50 ppm aldrin to the two strains of rats. The final pathology diagnoses were not available at the time of the report, so that the distribution of malignant and benign tumors could not be stated.

4.4.1.1.2.7. National Cancer Institute (1978a). Groups of 50 Osborne-Mendel rats of each sex were administered aldrin (95% pure) in the diet at 30 or 60 ppm. The duration of the studies was 111 to 113 weeks with male rats treated for 74 weeks, followed by 37 to 38 weeks of observation; female rats were treated for 80 weeks, followed by 32 to 33 weeks of observation. Matched controls consisted of groups of 10 untreated rats of both sexes. The pooled controls, which were used for statistical evaluation, consisted of the matched-control groups combined with 58 untreated males and 60 untreated females from similar bioassays of other chemicals. A comprehensive pathology examination was performed with only a minor loss of animals to the study.

Mean body weights of treated rats were lower than those of the controls during the second year of the study, with hyperexcitability observed in all treated groups with increasing frequency and severity during the second year. Aldrin produced no significant effect on the mortality of rats of either sex.

There was an increased incidence of follicular-cell adenoma and carcinoma of the thyroid gland in both male (matched controls 3/7, pooled controls 4/48, low dose 14/38, high dose 8/38) and in female rats (matched controls 1/9, pooled controls 3/52, low dose 10/39, high dose 7/46). These incidences were significant in the low-dose but not in the high-dose groups of both males ($p = 0.001$) and females ($p = 0.009$) when compared with pooled controls. However, they were not significant with matched controls. The total number of rats in matched controls was very small. Furthermore, cortical adenoma of the adrenal gland was significant ($p = 0.001$) in aldrin-treated female rats in low-dose (8/45) but

not in high-dose (1/48) groups when compared with pooled controls (0/55). Because these increased incidences were not consistantly significant when compared with matched controls rather than pooled control groups, NCI concluded that the incidences of these tumors were not associated with the treatment. But by present standards these results will be considered equivocal.

4.4.1.1.3. Studies with Other Species.

4.4.1.1.3.1. Treon and Cleveland (1955). Groups of two beagle dogs of both sexes were administered control diets or diets supplemented with 1 or 3 ppm of aldrin (95% pure) and were sacrificed between 15 and 16 months.

In the 3 ppm aldrin group, liver weights of treated animals were increased compared to the controls, and both of the females and one of the males were found to have minor changes in the liver, characterized by local hyaline (droplet) degeneration of the hepatic cells. In the same treatment group, both of the females had vacuolation of the epithelial cells lining the distal renal tubules, and one of the males had slight renal tubular degeneration. In the 1 ppm dieldrin group, one female had vacuolation of the distal renal tubules.

The study is unacceptable as a test of possible carcinogenicity because of the small number of animals tested and the short-term nature of the test relative to the normal lifespan of dogs.

4.4.1.1.3.2. Fitzhugh et al. (1964). A total of 12 mongrel dogs (5 males, 6 females, 1 of unspecified sex), ranging in age from 11 months to 6 years, were fed aldrin at dosages of 0.2 to 10 mg/kg/day, 6 days/week for periods up to 25 months. However, survival at 1, 2, or 5 ppm was very poor (five dogs died by 25 weeks and another at 49 weeks). Except for one dog that died at 4 days, the dogs fed at 0.2 and 0.5 mg/kg/day survived until 24 to 25 months at which time they were sacrificed. Animals fed aldrin at 0.5 mg/kg/day or more showed gross toxic effects, including loss of weight and convulsions,

and they died progressively earlier with increasing dose level. Histologic effects were limited to fatty degenerative changes in the liver and kidneys and bone marrow changes in those dogs fed aldrin at 1 mg/kg/day or more. No gross or microscopic effects were seen in dogs fed aldrin at 0.2 mg/kg/day.

The study is unacceptable as a test of possible carcinogenicity because of the small number of animals tested and the short-term nature of the test relative to the normal lifespan of dogs.

4.4.1.2. Dieldrin--

4.4.1.2.1. Studies with Mice. Table 4-13 summarizes the carcinogenesis bioassays conducted with dieldrin and discussed in this section.

4.4.1.2.1.1. Food and Drug Administration (Davis and Fitzhugh, 1962). The authors alluded to a previous 2-year dieldrin mouse feeding study that raised the suspicion of tumorigenicity of dieldrin in which the results were considered inconclusive because the majority of the animals were not available for pathologic examination. However, no reference was provided and no further information could be found on that study.

In this study, a group of 218 C_3HeB/Fe mice were fed a dietary mixture containing 10 ppm dieldrin (purity not specified) for 2 years. The control group consisted of 217 mice. The number of mice/sex was not given, but they were approximately equally divided by sex. Mice that died during the experiment were necropsied, as were all 2-year survivors. The extent of pathology examination was not clear. Tissues from animals with gross lesions and from some grossly normal mice were preserved. After fixation, the tissues were reexamined and selected for microscopic study. Slides were prepared from all gross lesions in which neoplasia was suspected, from lungs of mice with hepatic lumps and from some animals that had gross pneumonia, intussusceptions, or certain other incidental gross abnormalities. No breakdown as to actual tissues examined was provided.

TABLE 4-13. SUMMARY OF DIETARY CARCINOGENESIS BIOASSAYS OF DIELDRIN IN MICE

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
NS	C3HeB/Fe	215 M/F (treated) 217 M/F (controls)	0 (controls) 10	24 mo	Hepatomas: controls- 9/134 (7%) 10 ppm- 36/148 (24%)[a]	°Reevaluation by Reuber and other pathologists concluded that most liver tumors were hepatocellular carcinomas. °Limitations of study: -poor survival rate -many lost animals -data not tabulated by sex, time of death/tumors °Adequate test	Davis and Fitzhugh (1962) Reuber (as cited in Epstein, 1975)
NS	C3H	100 M&F/dose	0 10	24 mo	Hepatomas: controls-27/200 (13.5%) 10 ppm- 69/200 (34.5%)[b]	°Reevaluation by Reuber and other pathologists concluded that most liver tumors were hepatocellular carcinomas. °Limitations of study: -lack of detailed pathology -data not tabulated by sex, time of death/tumors °Adequate test	Davis (1965) Reuber (as cited in Epstein, 1975)
NS	C3HeB/FeJ CBA/J	55 treated (sex not specified); 10 controls (sex not specified)	0 15	375 days	Liver tumors: 7 treated mice had tumors	°Lack of detail as to sex, time of death, tumors, etc. °Limited value °Inadequate test	Song and Harville (1964)

(continued on the following page)

TABLE 4-13. (continued)

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
Technical Grade	Swiss-Webster	100 M/F/dose	0 3 10	Not clear	Hepatic Nodules: Dose-related 0 ppm-0% 3 ppm-2.5% 10 ppm-48%	°Reevaluation by Reuber and original pathologist. Many nodules reclassified as hepatocellular carcinomas. °Limitations of study: -only 71% histology examination -inadequate reporting of data °Adequate study	MacDonald et al. (1972) Reuber (as cited in Epstein, (1975)
>99% pure	CF-1	300 M/F (ctr) 125 M/F (0.1 ppm) 125 M/F (1.0 ppm) 200 M/F (10 ppm)	0 0.1 1 10	132 wk	Liver Tumors: Dose-related increased in type A, type B, and total liver tumors. Statistically significant ($p < 0.05$) at 10 ppm for both total liver tumors and type B tumors (carcinomas); lung, lymphoid and other tumors in female mice	°Carcinomas mainly in 10 ppm groups °Limitations of study -inadequate reporting of individual deaths and associated tumors °Adequate study	Walker et al. (1972)
>99% pure	CF-1	30 M/F/dose 78 M/F/controls	0 1.25 2.5 5 10 20	128 wk	Liver Tumors: Dose-related, except at 10-20 ppm where early mortality reduced number at risk	°Liver enlargements detected by 36 weeks. °20 ppm-high toxicity early deaths	Walker et al. (1972)

(continued on the following page)

TABLE 4-13. (continued)

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
					(continued)	(continued)	
					5 ppm Males- 87% (controls 12%) Females- 60% (controls 10%) Statistically significant ($p < 0.05$) at 2.5 ppm and above	°<10 ppm- minimal toxicity °No individual data °Adequate study	
>99% pure	CF-1	60 M/F/dose 78 M/F/controls	0 10	128 wk	Liver Tumors: Dose-related, increases in total tumors, type A and type B tumors	°Liver enlargements by 40-50 wk in all dose groups °Adequate study	Walker et al. (1972)
>99% pure	CF-1	29 M/F/dose	0 10	Varied from 2-64 wk	Liver Tumors: Significant increase ($p < 0.05$) with 64 wk of exposure; not significant for 32 or less wk of exposure	°Demonstrates need for long-term exposure to induce effect °Adequate study	Walker et al. (1972)
>99% pure	CF-1	30/M/F/dose 45 M/F/controls	0 10	110 wk	Liver Tumors: Significant increase ($p < 0.01$) in type B tumors of which many metastasized to the lungs Shortened latency period	°Liver enlargements by 50 wk; °Adequate study	Thorpe and Walker (1973)

(continued on the following page)

TABLE 4-13. (continued)

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
95% pure	B6C3F1	50 M&F/dose 20 M (controls) 10 F (controls)	0 2.5 5	80 wk treatment plus 10-13 wk observation	Hepatocellular Carcinomas: Males: HD-16/45 (36%)[a] LD-12/49 (24%)[a] Ctrl (M)-3/18(17%) Ctrl (P)-17/92(18%) Females: HD-3/46 (7%) LD-3/50 (6%) Ctrl (M)-1/18(6%) Ctrl (P)-7/91(8%)	*Dose-related mortality in females. *Adequate test	NCI (1978a)
>99% pure	CF-1	19-82 M/group	0 10	110 wk	Liver Tumors: 10 ppm-48/59 (81%) 0 ppm-6/60 (10%); 39% type B (carcinoma) at 10 ppm and only 1% in controls. Lung metastasia in 14% of 10 ppm animals; 0% for controls. Significant ($p < 0.01$)	*Limitations of study -Only males tested *Adequate study	Tennekes et al. (1981)
>99.9% pure	CF-1	1800 mice used in study. Number varied from 17 to 297 per groups equally divided by sex	0 0.1 1 2.5 5 10 20	Lifetime	Liver Tumors: Dose-related increase in incidences at 10 ppm and greater. Also dose-related reduction in induction time at 0.1 ppm and greater	*No linear relationship between median total dose or median time-to-tumor formation and daily exposure levels.	Tennekes et al. (1982)

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TABLE 4-13. (continued)

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
					(continued)	(continued)	
					in females and 1 in males.	°Authors suggest this indicates enhancement or promoting effect rather than initiation. °Adequate study	
>99% pure	CeH/He B6C3F1 C57/BL/6J	50/50 M 62/76 M 71/69 M (Dose/control groups)	0 10	85 wk exposure plus 47 wk observation (total=132 wk)	Hepatocellular Carcinomas: Significant increase ($p < 0.05$) in all 3 °Also benign liver tumors in B6C3F1 and C57BL/6J mice	°Limitations of test -Only males tested °Adequate study strains.	Meierhenry et al. (1983)

[a]Significantly different from controls ($p < 0.001$).

[b]Significantly different from controls ($p < 0.05$).

NS = Not specified; Ctrl (M) = matched controls; Ctrl (P) = pooled controls.

Average survival in the dieldrin-treated group was approximately 2 months less than in controls. Intercurrent diseases, pneumonia, and intestinal parasitism were present and may have influenced the long-term survival rate.

Results, reported for both sexes combined, indicated a statistically significant ($p < 0.001$) increase in the incidence of hepatomas (hepatic cell adenomas) in the treated animals as compared to controls (Table 4-6). The incidence of hepatomas, based on necropsied mice, was 24% in the dieldrin group and 7% in the controls. The hepatic cell adenomas were described as expanding nodules of hepatic parenchymal tissue, usually with altered lobular architecture, and morphologically ranging from very benign appearance to borderline carcinomas. The authors concluded that dieldrin had significantly increased the incidence of histologically benign liver tumors. Dr. M. Reuber conducted an independent reevaluation of the liver lesions, and considered most of the hepatomas to be liver carcinomas. Drs. Popper, Farber, and Firminger concurred with Dr. Reuber's evaluation (Epstein, 1975).

The value of this study was compromised by the poor survival rate, lack of detailed pathology, loss of a large percentage of the animals to the study, and failure to treat the data for males and females separately. Despite these inadequacies, the study revealed evidence for the hepatocarcinogenicity of dieldrin in C_3H mice.

4.4.1.2.1.2. Food and Drug Administration (Davis, 1965). As a follow-up to the previous FDA study, Davis (1965) administered dieldrin (purity not specified) at 0 or 10 ppm in the diet to groups of 100 male and 100 female C_3H mice for 2 years. The pathology examination was similar to that conducted in the other FDA study. Again, survival in the treated group was reduced compared to the control group, although no breakdown of data by sex and by time of death was given. There was no indication as to the time of tumor detection or death

in treated versus control groups. The incidence of benign hepatomas in the dieldrin group was significantly increased ($p < 0.05$) with incidence of 32.5% versus 13.5% in controls. The number of hepatic carcinomas was about the same (Table 4-7).

Dr. Reuber also reevaluated the liver lesions from this study and concluded that most of the hepatomas were actually carcinomas and that the increased incidences were statistically significant ($p < 0.05$) for both males and females. Drs. Popper, Farber, and Firminger checked representative histology and concurred with Reuber's diagnosis (Epstein, 1975). A summary of Reuber's diagnosis is presented in Table 4-8.

Some of the same deficiencies as seen in the 1962 FDA study were also evident in this one; namely, a lack of detailed pathology examination and failure to present data according to sex. However, the survival at 18 or 24 months was acceptable in the controls. The evidence for an oncogenic response (whether benign or malignant) is substantial in male and female C_3H mice.

4.4.1.2.1.3. Song and Harville (1964). A total of 55 mice of two strains (C_3H and CBA) were administered dieldrin (purity not specified) in the diet at 15 ppm for an unspecified time. Ten mice served as controls. A companion study with aldrin was also conducted. Seven dieldrin- or aldrin-treated mice had liver tumors by 330 to 375 days. No other details of this study were reported. Little useful information can be obtained from this study due to the lack of details provided.

4.4.1.2.1.4. MacDonald et al. (1972). This unpublished report was reviewed and evaluated by Epstein (1975). Technical grade dieldrin, dissolved in corn oil, was administered in the diet at 0, 3, or 10 ppm to groups of 100 weanling Swiss-Webster mice of both sexes. The duration of the experiment was unclear. No data were given on the time of death of any animal. Only 71% of

the mice were examined histologically. Decreases in the incidence of spontaneous mammary carcinomas were noted in treated groups. No significant weight loss or changes in liver-to-body weight ratios occurred in mice in the test groups.

The authors claimed that dieldrin is not carcinogenic, but it induced a dose-related increase in nonneoplastic lesions of the liver, such as regenerative hypertrophy and nodulation or regenerative hyperplasia. The incidence of nodulation, for both sexes combined, was 0%, 2.5%, and 48% in the control, 3 ppm, and 10 ppm groups, respectively.

The histopathology was reevaluated by Reuber (cited in Epstein, 1975) and others Deichmann (1973, cited in Epstein, 1975). Anderson, one of the original authors of the study, reversed his original diagnosis of liver lesions from nonneoplastic to neoplastic in approximately half of the only 14 sections originally examined. Of the livers from 10 mice fed 10 ppm dieldrin in which nodulation and nonneoplastic lesions were originally reported, Reuber diagnosed hepatocellular carcinoma in seven instances; these diagnoses were confirmed by Firminger. The results of the histopathologic reevaluation confirm the hepatocarcinogenicity of dieldrin in mice, although revised incidence figures cannot be given, since the reevaluation was based on a relatively small number of sections.

4.4.1.2.1.5. Walker et al. (1972). Walker et al. (1972) conducted two studies on dieldrin carcinogenicity for Shell Research Limited (Tunstall Laboratory). The second study comprised four experiments. Specific-pathogen-free CF_1 mice of both sexes were fed dieldrin (< 99% HEOD) in the diet commencing at 4 weeks of age. The duration varied somewhat with the specific experiment, but all were for at least 2 years. Other mice were fed 4-amino-2,3-dimethylazobenzene and served as positive controls. In all studies, except

one, study No. 2 (Experiment 2.2), mice were fed diets sterilized by ethylene oxide and were maintained under specific-pathogen-free conditions. Initially, the mice were housed five per cage, but after the 6th week, they were placed in individual cages.

Several factors complicate the interpretation of the results of these studies. Statistical analyses of tumor incidence data were not presented. In addition, individual animal data were not reported, thereby restricting the possibility of independent analysis. Some animals were sacrificed when palpable masses became large, even if they were otherwise healthy. This lowered the incidence of tumors, tumor size, and probability of metastases. It is not clear how the incidences of tumors were derived, as these were based on an undefined number of animals.

Liver tumors were classified as Type A or Type B. The Type A tumor appeared as solid cords of closely packed parenchymal cells with a morphology and staining affinity little different from the rest of the normal parenchyma with no fibrous capsule. They were classified as benign tumors. The Type B tumor appeared as areas of papilliform and adenoid growth with some areas of cells proliferating in confluent sheets and often with foci of necrosis. Nuclear abnormalities were often present. The vast majority of mice with Type B tumors also had Type A tumors. In many cases, the two types of lesions were contiguous. Type B tumors were rare in controls.

Study No. 1

In this study, diets containing nominal concentrations of 0, 0.1, 1, and 10 ppm dieldrin (> 99% pure) were fed to groups of 600, 250, 250, and 400 CF-1 mice, respectively, divided equally by sex. The animals were 4 weeks of age at initiation of treatment. The duration of treatment was 132 weeks. The controls and 10 ppm groups were abdominally palpated weekly, commencing at 16

weeks. Once masses were detected, animals were palpated twice weekly and those animals with very big masses were sacrificed. Sections of the brain, heart, lungs, liver, kidneys and testes or ovaries, and of any macroscopic lesions, were prepared for microscopic examination. Palpable liver masses were identified in the 10 ppm groups by 9 months, with one-half having died or been sacrified by 15 months, as compared with 20 to 24 months for the controls. Palpable masses were never detected in the 0.1 or 1 ppm groups, whose lifespans were similar to those of untreated controls. Surviving animals were sacrified from 132 to 140 weeks. The absence of data on the time of death or sacrifice of animals with tumors made accurate determination of survival rates impossible.

An increased incidence of liver tumors was present at all dietary concentrations of dieldrin and this increase was dose related (Table 4-14). Type B tumors were rare in controls, with none seen in females and only 4% in males. While there was an increase in type A tumors in all treated groups, the increase in type B tumors was mainly in the 10 ppm groups.

Dieldrin also induced a significant increase ($p < 0.05$) in the incidence of pulmonary adenomas and carinomas in female mice, and also lymphoid and "other" tumors in female mice. The study was an acceptable test for carcinogenicity with highly positive results in the induction of hepatocellular carcinomas and a slight increase in other tumors, especially in the treated female groups.

Study No. 2

Experiment 2.1--In this experiment, designed to examine the existence of a dose-response relationship between intake of dieldrin and formation of liver tumors, groups of 30 CF-1 mice of each sex received ethylene oxide-sterilized diets containing 1.25, 2.5, 5, 10, or 20 ppm dieldrin (> 99% pure) for 128 weeks. The

TABLE 4-14. INCIDENCES OF TUMORS IN CF-1 MICE ADMINISTERED DIELDRIN IN THE DIET FOR 132 WEEKS

Dose (ppm)	No. of mice	Percent with liver tumors			Percent with lung tumors		Percent with lymphoid tumors	Percent with other tumors
		Total	Type A	Type B	Adenoma	Carcinoma		
					MALES			
0	288	20	16	4	33	8	35	6
0.1	124	26	22	4	38	11	21	3
1	111	31	23	8	38	12	20	5
10	176	94[a]	37[a]	57[a]	18	1	24	2
					FEMALES			
0	297	13	13	0	16	6	40	7
0.1	90	27	23	4	26	13[b]	50[b]	9
1	87	37	31[a]	6	34[a]	14[b]	54[b]	17[b]
10	148	92[a]	37[a]	55[a]	10	0	5	1

[a]Statistically significant ($p > 0.01$).

[b]Statistically significant ($p < 0.05$).

SOURCE: Walker et al., 1972.

control group consisted of 78 animals of both sexes. The number of animals available for examination in the highest dose groups (20 ppm) was much reduced because of early losses from acute toxicity. In these 20 ppm groups, about 25% of the males and nearly 50% of the females died during the first 3 months of the experiment, with the remainder sacrificed by 12 months due to liver enlargements. The liver enlargements were detected after 36 weeks. No signs of dieldrin toxicity were seen in the 10 ppm or lower groups. However, intra-abdominal masses were detected after week 40 in the 10 ppm groups, and most of the mice were sent to necropsy by 16 to 17 months. This was similar to the earlier results at 10 ppm. Palpable masses were detected in the 5 ppm group after week 75 and in the 2.5 ppm animals after week 100, with morbidity of these groups only slightly increased. No liver enlargements could be palpated at 1.25 ppm.

The incidences of liver tumors are presented in Table 4-15. It is noteworthy that a clear, statistically significant ($p < 0.05$) increase in liver tumors of both type A and type B and in total liver tumors occurs from 1.25 to 5 ppm, reaching 87% in males and 60% in females. The decrease at 10 and 20 ppm may be attributable to losses due to toxicity so that fewer mice were actually at risk after 1 year. As the individual data were not presented, a life-table analysis could not be performed. At dose levels of 2.5 ppm and above, a statistically significant ($p < 0.05$) increase in liver tumors was observed. While increased at 1.25 ppm, the increases were not statistically significant ($p < 0.05$).

Experiment 2.2--This study was designed to determine the effect of the method of diet sterilization and dieldrin-induced liver changes. Diets were sterilized with gamma-irradiation or were not sterilized. Groups of 30 male and 30 female CF-1 mice were fed diets of 0 or 10 ppm dieldrin sterilized by gamma-

TABLE 4-15. INCIDENCES OF LIVER TUMORS IN CF-1 MICE ADMINISTERED DIELDRIN IN THE DIET FOR 128 WEEKS

Dose (ppm)	No. of mice	Percentage of mice with liver tumors		
		Total	Type A	Type B
		MALES		
0	78	12[a]	12[b]	0[b]
1.25	30	20	13	7
2.5	30	43[a]	40[a]	3
5	30	87[a]	77[a]	10[a]
10	11	45[a]	36[a]	9
20	17	71[a]	18[a]	53[a]
		FEMALES		
0	78	10[b]	10[b]	0[b]
1.25	30	17	17	0
2.5	28	43[a]	39[a]	4
5	30	60[a]	43[a]	17[a]
10	17	53[a]	41[a]	12[a]
20	21	38[a]	24[a]	14[a]

[a]Statistically different from controls ($p < 0.05$).
[b]Statistically significant dose-related trend by Cochran-Armitage trend test ($p < 0.05$).

SOURCE: Walker et al., 1972.

irradiation for 120 weeks. Groups of 24 [illegible]

Company, the mice were not sacrificed when abdominal masses became large, but only when the animals were moribund (anorexic and clinically affected).

Diets containing 10 ppm dieldrin (> 99% pure) were fed to groups of 30 CF-1 mice of both sexes for 110 weeks. The control group consisted of 45 mice of both sexes. The mice were bred and maintained in individual cages under specific-pathogen-free conditions. Treatment commenced at 4 weeks of age; all survivors were sacrificed after the 110-week exposure. Moribund animals were sacrificed during the study; all animals were grossly examined. The sections of brain, heart, lungs, liver, kidneys, and testes or ovaries, and all macroscopic lesions were processed and microscopically examined. In the mice fed dieldrin, liver enlargement was detected after 50 weeks in both sexes with mortality increased after 22 months. Liver lesions were arbitrarily classified as Type A or Type B as previously described by Walker et al. (1972).

Dieldrin induced a statistically significant increase ($p < 0.01$) in the incidence of liver tumors in both sexes as compared with controls (Table 4-17). Metastases were found only in the lungs of animals with Type B tumors. As in the previous study by Walker et al. (1972), Type B tumors were not detected in female controls and were present in only a small proportion (4%) of male controls.

In contrast to the earlier study of Walker et al. (1972), tumor incidences versus time were presented. Liver tumors appeared much earlier in the treated animals than in controls in that 100% and 50% of all dieldrin-treated males and females, respectively, that died by 17 months had liver tumors; no liver tumors, however, were found in controls at that time.

4.4.1.2.1.7. National Cancer Institute (1978a). A bioassay of technical-grade dieldrin (> 96% purity) for possible carcinogenicity was conducted by

TABLE 4-17. INCIDENCES OF LIVER TUMORS IN CF-1 MICE ADMINISTERED 10 PPM DIELDRIN IN THE DIET FOR 110 WEEKS

Dose (ppm)	No. of mice	Percentage of mice with liver tumors			
		Total	Type A	Type B	Lung metastases
			MALES		
0	45	24	20	4	0
10	30	100[a]	47[a]	53[a]	3
			FEMALES		
0	44	23	23	0	0
10	30	87[a]	40[b]	47[b]	17[a]

[a]Significantly different from controls ($p < 0.01$).
[b]Significantly different from controls ($p < 0.05$).

SOURCE: Thorpe and Walker, 1973.

administered dieldrin at one of two doses for 80 weeks followed by observation for 10 to 13 weeks. Time-weighted average doses were 2.5 or 5 ppm. Matched control groups consisted of 20 untreated male mice and 10 untreated female mice. Pooled controls, used for statistical evaluations, consisted of the matched-control groups and untreated animals from similar bioassays for other chemicals (92 male and 79 female mice). All surviving mice were killed at 90 to 93 weeks. The protocol called for a comprehensive gross and microscopic examination of all animals.

During the second 6-month period, the treated animals showed treatment-related toxicity, mainly neurologic in origin, e.g., hyperexcitability, hyperactivity, and tremors. The toxic effects appeared first in the males and later on in the females. Mean body weights were similar in the dieldrin-exposed and control groups during the first year, but were slightly lower during the second year in the treated groups although not statistically significant. Survival in all groups was excellent with at least 80% of all groups alive at 90 weeks. No significant difference in survival was noted. Based upon achieving a maximum tolerated dose without excess toxicity and increased mortality, the test was considered an acceptable assay for carcinogenicity.

In male mice, there was a significant dose-related increase in hepatocellular carcinomas when compared to pooled controls ($p = 0.02$). The incidence in high-dose males was also significantly increased ($p = 0.025$) when compared with the pooled controls (Table 4-18). No other tumor type was increased in the treated groups. The authors concluded that dieldrin induced a significant increase in the incidence of hepatocellular carcinomas in high-dose male B6C3F1 mice.

4.4.1.2.1.8. Tennekes et al. (1979, 1981). In these studies the incidence of liver tumors was determined in groups of 19 to 82 male CF-1 mice fed control

TABLE 4-18. INCIDENCES OF HEPATOCELLULAR CARCINOMAS IN B6C3F1 MICE EXPOSED TO DIELDRIN IN DIETARY MIXTURE

Study group	Male mice	Female mice
Pooled Controls	17/92 (18%)[a]	7/91 (8%)
Matched Controls	3/18 (7%)	1/18 (6%)
Low Dose (2.5 ppm)	12/50 (24%)	3/50 (6%)
High Dose (5 ppm)	16/45 (36%)[b]	3/46 (2%)

[a]Statistically significant dose-related trend ($p < 0.02$) by Cochran-Armitage test.
[b]Statistically significant when compared to pooled controls ($p = 0.025$) by the Fisher Exact Test.

SOURCE: NCI, 1978a.

or dieldrin-supplemented (10 ppm) diets for 110 weeks and maintained on either of two types of bedding (shredded filter paper or softwood sawdust) and two types of diet (commercial or purified semisynthetic). Animals were bred and reared on the specified diet or bedding. The males, upon weaning, were then housed together at a maximum of five/cage. All animals, during the study, found dead or moribund were necropsied. While all organs were grossly inspected, only the liver and lungs were examined histologically. The mice were weanlings at time of initiation of dieldrin administration. A total of 139 mice were treated with dieldrin with 252 mice fed the control diet. Liver lesions were classified according to the arbitrary method of Walker et al. (1972) as Type A or B. Mice were sacrificed at 15, 52, 65, 92, and 110 weeks.

Dieldrin administration resulted in the relatively early appearance of nodular hepatic lesions, the first being observed in a mouse approximately 43 weeks of age.

Of 59 dieldrin-treated mice killed at 65 weeks of age, 48 (81%) had liver tumors as compared to only 3/60 (5%) in the control groups. Type B tumors were not present in control animals, although they were found in 25% of the animals in the dieldrin groups. Only one animal was found with a hepatocellular carcinoma metastasis to the lung.

The incidence of liver tumors for the total 110 weeks was similar to that seen at 65 weeks in that 81% of treated mice had liver tumors compared to only 10% of the controls. The major difference, however, was in the number of hepatocellular carcinomas (39% vs. 1%) and the number of lung metastases (14% vs. 0%) (Table 4-19). These results were highly significant ($p < 0.01$) and represented a substantial carcinogenic response.

4.4.1.2.1.9. Tennekes et al. (1982). This study was undertaken to investigate the dose-response characteristics of dieldrin-mediated enhancement of

TABLE 4-19. INCIDENCES OF LIVER TUMORS IN CF-1 MICE ADMINISTERED 10 PPM DIELDRIN IN THE DIET FOR 110 WEEKS

Dose (ppm)	No. of mice	Percentage of mice with liver tumors			
		Total	Type A	Type B	Lung metatases
0	252	10	9	1	0
10	139	81[a]	42[a]	39[a]	14

[a]Statistically significant ($p < 0.01$).

SOURCE: Tennekes et al., 1981.

liver tumor formation in CF_1 mice. Mice were bred and maintained under specific pathogen-free conditions. A total of 1800 CF_1 mice were administered dieldrin (> 99.9% purity) in the diet beginning at 3 weeks of age and continued for lifetime. Treatment groups are indicated in Table 4-20. The animals were multiply housed, but separated into individual cages after 6 weeks of treatment. Health and behavior were observed daily. Weekly abdominal palpation was started after 16 weeks of treatment; when intra-abdominal masses were detected, the animal was palpated twice weekly and it was sacrificed when the enlargement was considered to be detrimental to health. Liver tumors were classified arbitrarily as Type A or B by the method of Walker et al. (1972).

Liver tumor incidence was calculated on the basis of censored survival data, censorship being imposed on deaths due to other causes, natural or artificial.

Tumor incidence was higher in treated than in control animals and appears to be dose-related through the 10 ppm groups for both sexes (Table 4-20). The reduced response in the 20 ppm groups may be due to compound toxicity, since the authors stress that a significant proportion of both male and female mice exposed to 20 ppm dieldrin died from acute intoxication within the first 13 weeks of treatment.

Dieldrin exposure levels were also associated with a significant reduction of the time-to-tumor development, as compared to controls. The lowest dieldrin exposure level associated with a significant reduction ($p < 0.05$) of median liver tumor induction period was 0.1 ppm in females and 1 ppm in males.

While there was no linear relationship between the median total dose or the median time-to-tumor formation and the daily dieldrin exposure level, the authors suggested that this was not inconsistent with the concept that this compound is devoid of initiating potential, and operates by enhancing the effect

TABLE 4-20. INDUCTION OF LIVER TUMORS IN CF-1 MICE ADMINISTERED DIELDRIN IN THE DIET FOR LIFETIME EXPOSURE

Sex	Expt. no.	Dieldrin (ppm)	No. of mice	Percent animals with liver tumors		Median liver tumor induction period (wk)
				Total	Type B	
Female	1	0	297	12	0	129 (>123)[a]
	2	0	78	10	0	127 (>123)
	1	0.1	120	21	3	119 (>117)[b]
	1	1	117	28	5	116 (113-120)
	2	2.5	28	46	7	106 (89-108)
	2	5	30	60	17	92 (85-99)
	1	10	148	96	60	64 (64-66)
	2	10	17	53	12	50 (>47)
	2	20[c]	21	38	14	48 (>41)
Male	1	0	289	20	4	133 (>130)
	2	0	78	12	0	>128
	1	0.1	124	27	5	130 (121-134)
	1	1	111	32	9	118 (116-122)[b]
	2	2.5	30	47	7	111 (>109)
	2	5	30	87	10	99 (91-101)
	1	10	176	95	60	67 (65-68)
	2	20[c]	17	71	53	41 (39-46)

[a]Numbers in parenthesis indicate 95% confidence intervals.
[b]Lowest dieldrin exposure level associated with a significant reduction of median liver tumor induction period, $p < 0.05$.
[c]In the 20 ppm dieldrin treatment group, 11/21 females and 5/17 males died from acute intoxication within the first 13 weeks of treatment.

SOURCE: Tennekes et al., 1982.

of genetically linked oncogenic factor in CF_1 mouse liver.

4.4.1.2.1.10. Meierhenry et al. (1983). Dieldrin (> 99% pure) was continuously fed in the diet for 85 weeks to 50 C_3H/He, 62 $B6C3F_1$, and 71 C57BL/6J male mice starting at the time of weaning. Fifty C_3H/He, 76 $B6C3F_1$, and 69 C57BL/6J male mice served as controls. The chow had been analyzed and found to be free of a panel of insecticides by Gulf South Research Institute. Eight weeks after the initiation of the experiment and at intervals up to 132 weeks of age, at least three mice in each group were sacrificed and examined. No additional information on animals at risk versus time was provided. Dieldrin (10 ppm) induced a significant increase compared with controls, in the incidence of hepatocellular carcinomas in all three strains of mice (Table 4-21). Hepatocellular carcinomas were defined as liver nodules that contained trabecular areas histologically. The incidence of benign hepatic tumors was also significantly increased in $B6C3F_1$ and C57BL/6J mice.

4.4.1.2.2. Studies with Rats. Table 4-22 summarizes the carcinogenesis bioassays of dieldrin conducted with rats and discussed in this section.

4.4.1.2.2.1. Treon and Cleveland (1955). Dieldrin (> 85% purity) was administered in the diet of 40 Carworth rats of both sexes at concentrations of 2.5, 12.5, or 25 ppm, commencing at age 27 to 28 days and continued for 2 years. Groups of 40 animals of both sexes, which were fed powdered or pelleted diets, served as controls. Dieldrin treatment did not result in an increased mortality or decreased growth rate as compared to controls. No gross pathological or histological data, or any data on tumor incidences were reported. However, Cleveland (1966) reported that many tumors had been seen in the 1955 study. This study is unacceptable as a carcinogenicity test because of the following deficiencies: small number of animals at risk beyond 1 year; no gross pathology or histology data; and no data on tumor incidence even though a

TABLE 4-21. SUMMARY OF TUMORS OBSERVED IN C57BL/6J, C3H/He, AND B6C3F1 MICE TREATED WITH DIELDRIN IN THE DIET AT 10 PPM

Strain	Dietary aldrin (ppm)	Time of observation of first tumor (wk)	No. of mice in treatment group	Percent with hepatocellular carcinoma	Percent with benign hepatic tumors
C57BL/6J	0	56	69	0	14
	10	46	71	30[a]	28[a]
C3H/He	0	37	50	12	18
	10	12	50	38[a]	20
B6C3F1	0	57	76	4	4
	10	49	62	42[a,b]	29[a]

[a]Significantly different from controls ($p < 0.01$).
[b]Two mice had pulmonary metastases.

SOURCE: Meierhenry et al., 1983.

TABLE 4-22. SUMMARY OF DIETARY CARCINOGENESIS BIOASSAYS OF DIELDRIN IN RATS

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
>85% pure	Carworth	40/sex/dose	0 2.5 12.5 25	2 yr	No treatment-related increase in tumors; No increased mortality or decreased growth in dieldrin-treated animals	°Limitations of study -Small numbers alive at 1 year; -No pathology data reported °Inadequate study	Treon and Cleveland (1955) Cleveland (1966)
100% pure	Osborne-Mendel	12/sex/group	0 0.5 2 10 50 100 150	2 yr	Dose-related decrease in mortality > 2 ppm. Dose-related increase in liver to body ratios; Chronic nephritis at high doses, esp. males; No liver tumors, although liver lesions > 50 ppm. Total tumors increased (24% in treated vs. 18% in controls)	°Reuber diagnosed liver cancers at 100 ppm and confirmed multiple-site tumors °Limitations of study -too few animals -high mortality > 2 ppm °Inadequate study	Fitzhugh et al. (1964) Reuber (as cited in Epstein (1975)
Unspecified purity	Holtzman	15/M or F/group	0 250 285	1 yr	No tumor data reported	°Limitations of study -too few animals -too short duration -lack of data reported °Inadequate study	Song and Harville (1964)

(continued on the following page)

TABLE 4-22. (continued)

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
>99% pure	Carworth	25/sex/treated group; 45/sex/ treated/control	0 0.1 1 10	2 yr	No effect on body weight High mortality in all groups Dose-related increase in liver to body-weight ratio No treatment-related increase in tumors Liver pathology at 10 ppm	°Reevaluated by Stevenson-confirmed pathology results °Limitations of study -too few animals at risk > 1 year; -only 58% animals examined -individual animal data not presented °Inadequate study	Walker et al. (1969) Stevenson et al. (1976)
Technical Grade (purity not specified)	Osborne-Mendal	50/sex/dose 100/sex/controls	0 20 30 50	31 mo	No effect on body weight Survival decreased at 50 ppm Liver pathology; no increase in tumors with dieldrin exposure	°Reevaluation by Reuber on sample concluded Deichmann underestimated incidences of malignant tumors °Limitations of study -only 84% of animals microscopically examined -control histology not available °Minimally acceptable study	Deichmann et al. (1970) Reuber (as cited in Epstein, 1975)

(continued on the following page)

TABLE 4-22. (continued)

Substance tested	Strain	Number/Sex	Dose(s) (ppm)	Duration	Effects	Comments	Reference
95% pure	Osborne-Mendel	50/sex/dose 10/sex matched controls 50 males and 60 females-colony controls	0 29 65	80 wk of exposure plus 30-31 wk of observation	Body weights lower in second year; Increase in mortality of treated rats; No treatment-related increase in tumors	°While increased mortality observed in treated groups, it was not significantly different at 18 months °No limitations of study °Adequate study	NCI (1978a)
Technical Grade (purified)	Fischer 344	24/sex	0 2 10 50	104-105 wk	No treatment-related change in body weight or mortality; No increase in tumors related to dieldrin exposure	°Limitations of study -Number of animals small °Adequate study	NCI (1978b)

later report indicated that tumors had been detected.

However, Cleveland (1966) reported the tumor incidence data for these dieldrin feeding studies conducted by Treon and Cleveland (1955). Cleveland reported that the incidences of tumors in male and female rats fed dieldrin for up to 2 years was of the same magnitude as controls. However, the Treon and Cleveland (1955) study is considered unacceptable as a carcinogenicity test for the following reasons: number of animals at risk from late-developing tumors, while not clear, appears to be low; no survival data were presented; data were presented only for all types of tumors combined; and specific histopathology data were not presented.

4.4.1.2.2.2. Food and Drug Administration (Fitzhugh et al., 1964). Dieldrin (100% purity) was administered for 2 years at 0, 0.5, 2, 10, 50, 100, or 150 ppm to groups of 12 male and 12 female Osborne-Mendel rats, starting at 3 weeks of age and continuing for 2 years. By 6 months, there was a dose-related decrease in survival at dieldrin levels > 2 ppm, and the effect was progressively more marked from 6 to 24 months. A significant and dose-related increase in liver-to-body weight ratio was reported, although this did not always occur in both sexes. Chronic nephritis occurred (more commonly in males than in females) at the high-dose levels.

Only 68% of all animals were examined histologically. Sections of liver, kidney, testis, as well as tumors and gross abnormalities were made. Total tumor incidence (all doses combined) in dieldrin-treated animals was 24% compared to an incidence in controls of 18%. According to independent statistical analyses of these data (Fears, as cited in Epstein, 1975), the multiple-site carcinogenic effect is statistically significant (significance level not given) for females and for both sexes combined in low-dose groups. Fitzhugh et al. (1964) reported no hepatomas or hepatocellular carcinomas, although character-

istic "chlorinated insecticide" lesions were said to be generally dose-related, being particularly marked at > 50 ppm.

Tumor histopathology from this experiment plus additional unpublished material from a separate subsequent FDA study was independently reevaluated by Reuber (as cited in Epstein, 1975) and others. The higher incidence of multiple-site tumors in dieldrin-treated animals was confirmed, with statistical significance (significance level not given) at the combined dieldrin 0.5, 2, and 10 ppm levels. In contrast to Fitzhugh et al. (1964), who reported no liver tumors, Reuber found liver cancers in 4/7 females and 1/11 males in the 100 ppm dieldrin group; the incidence is statistically significant ($p = 0.0264$), both sexes combined in relation to controls (no hyperplastic nodules or carcinomas were found in the livers of control animals). Selected histological material was also examined by Drs. Firminger and Farber (Reuber, as cited in Epstein, 1975) who confirmed the above histological findings.

4.4.1.2.2.3. Song and Harville (1964). Groups of 15 Holtzman rats of unspecified sex were fed for approximately 1 year with control diets, diets supplemented with 250 ppm dieldrin (unspecified purity), or diets supplemented with 15 ppm dieldrin, increased by 10 ppm every 2 weeks until 285 ppm was reached. Two animals from each group were sacrificed periodically. This study is unacceptable as a carcinogenicity test because of its short duration, lack of substantive data, and inadequate number of animals.

4.4.1.2.2.4. Walker et al. (1969). Groups of 25 male and 25 female Carworth Farm strain E rats were administered nominal concentrations of 0.1, 1, or 10 ppm dieldrin (> 99% pure) in the diet for 2 years, commencing at 5 weeks of age. Controls consisted of groups or 45 rats of each sex. The pathology protocol included gross examination of all animals dying or sacrificed during the study with microscopic examination of most major organs.

No effect on body weight occurred at any dose level. Mortality was high in all groups, so that groups of only 10 to 16 treated and 10 control rats survived to the end of the test. A treatment-related increase in liver-to-body weight ratio occurred only in females.

Only 58% of all the animals were examined histologically. Organochlorine liver changes were reported only in the 10 ppm group at incidences of 4% (1/23) in males and 26% (6/23) in females. The authors concluded in this study and in their subsequent reevaluation (Stevenson et al., 1976) that no treatment-related increase in tumor incidence occurred in rats. A review of the data as presented supports that conclusion.

However, this study has limitations as a valid test for carcinogenicity for the following reasons: small number of animals at risk for late-developing tumors; high premature mortality in all groups; small percentage of animals examined histologically; and failure to present individual animal data in sufficient detail.

4.4.1.2.2.5. Deichmann et al. (1970). Groups of 50 male and 50 female Osborne-Mendel rats were administered dieldrin (technical grade) in the diet at final concentrations of 20, 30, or 50 ppm, commencing at the age of weaning; dosage for the first 10 weeks was half the above final levels. Groups of 100 rats of both sexes served as controls. The survivors were sacrificed at 31 months.

Feeding dieldrin at the above concentrations had no adverse effect on body weight. Mean survival, however, was reduced in the 50 ppm group (16.6 months) compared to controls (19.5 months). Grossly-observed tumors, lesions and sections of all livers, kidneys, and lungs were to be stained and examined microscopically. However, the authors' tables indicate that sections were examined for only 87% of dieldrin-exposed animals and 81.5% of the controls.

A moderate but not dose-related increase in the incidence of centrilobular cloudy swelling and liver necrosis was noted in all dieldrin-treated male and female rats but not in controls. The authors reported a decrease in tumors in treated animals as compared to controls. Based upon an independent reevaluation of the histopathology for a limited sample of 36 males and 40 females in the 30 ppm dieldrin group, Reuber (as cited by Epstein, 1975) concluded that the original authors underestimated or under-reported the incidence of malignant tumors by approximately threefold. However, the limited scope of this reevaluation, along with the fact that control histology was not available, precluded conclusions on carcinogenicity (Epstein, 1975).

4.4.1.2.2.6. National Cancer Institute (1978a). Groups of 50 Osborne-Mendel rats of both sexes were administered dieldrin (95% pure) at one of two doses in the diet. Low-dose rats were treated for 80 weeks, followed by observation for 30 to 31 weeks. Treatment of high-dose rats was terminated after 59 weeks and followed by 51 to 52 weeks of observation. Time-weighted average doses were 29 or 65 ppm. Matched controls consisted of 10 untreated rats of both sexes. Pooled controls, used for statistical evaluation, consisted of the matched control groups combined with untreated animals (58 males and 60 females) from similar bioassays. All surviving animals were sacrificed at 110 to 111 weeks. A comprehensive pathology examination was performed with only a minor loss of animals to the study.

Mean body weights of treated rats were comparable to controls during the first year but lower than the controls during the second year of the study. Hyperexcitability was observed in all treated groups with increasing frequency during the second year, especially in high-dose animals. There was a marked increase in mortality in treated rats during the first 90 weeks; however, due to a high rate of mortality in controls in the last 20 weeks, survival was not

statistically dose responsive.

In general, there was no predominance of tumors in any particular organ associated with dieldrin-treatment. The only possible exception was an increase in the combined incidence of adrenal cortical adenomas or carcinomas in the low-dose females (6/45) versus the pooled controls (0/55). While this was statistically significant ($p = 0.007$), the authors discounted the finding due to inconsistency in dose-response and variability in other NCI studies.

The authors concluded that none of the tumors occurring in Osborne-Mendel rats treated with dieldrin could clearly be associated with treatment. The study is considered an adequate test for carcinogenicity in rats.

4.4.1.2.2.7. National Cancer Institute (1978b). Groups of 24 Fischer 344 rats of both sexes were administered dieldrin (purified technical grade; purity not indicated) in feed at 2, 10, or 50 ppm for 104 to 105 weeks. Matched controls consisted of groups of 24 untreated rats of both sexes. All surviving rats were killed at 104 to 105 weeks. A comprehensive pathology examination was performed.

Body weights and survival were essentially unaffected by treatment. Adequate numbers of animals were available for meaningful statistical analyses of the incidences of tumors. A variety of neoplasms occurred in control and treated rats, but the incidences were not related to treatment.

The authors concluded that under the conditions of this bioassay, dieldrin was not carcinogenic in Fischer 344 rats. While the numbers of animals was small, the dose levels were sufficiently high without inducing unacceptable mortality or toxicity. It therefore serves as an acceptable bioassay of the carcinogenicity of dieldrin in rats.

4.4.1.2.3. Studies with Other Species.

4.4.1.2.3.1. Cabral et al. (1979). Groups of Syrian golden hamsters were

fed a diet containing 0 (40 animals of both sexes), 20 (34 males, 33 females), 60 (32 males, 34 females), or 180 (41 males, 38 females) ppm of dieldrin (99%) for their lifespan. The age of the animals at initiation of treatment was not clear. The experiment was terminated at 120 weeks of age, when the last survivor was sacrificed. A necropsy was performed on all animals, and the thyroid, lungs, liver, kidneys, spleen, and gonads together with all organs showing macroscopic abnormalities were examined histologically. Mortality was high, especially in the control group for which survival at 70 weeks of age was only 30% in males and 15% in females. Hamsters treated with 180 ppm dieldrin lived longer than those in all other groups (percent survival at 70 weeks of age was 49% for males and 24% for females). Both males and females fed 20 and 180 ppm dieldrin showed a marked retardation of growth. The livers in treated groups showed hepatic cell hypertrophy, and the incidence increased with increasing doses of dieldrin.

No hepatomas were observed in controls, but hepatomas were found in one female and one male in the 180 ppm dieldrin group at 85 and 94 weeks of age, respectively. Although the total tumor incidence in treated animals was not significantly different from that in controls, more animals in the treated groups had more than one tumor than animals in the control groups.

This experiment is unacceptable as a test for possible carcinogenicity for the following reasons: high premature mortality; small numbers of animals at risk from late-developing tumors; and no lifetable data presentation.

4.4.1.2.3.2. Treon and Cleveland (1955). Groups of two beagle dogs of each sex were administered control diets or diets supplemented with 1 or 3 ppm dieldrin for 15 and 16 months and then sacrificed. Liver weights of treated animals were increased, even at 1 ppm. Vacuolization of the distal renal tubules was reported in one female of the 3 ppm dieldrin group. This study is

unacceptable as a test of possible carcinogenicity because of the small number of animals tested and the short-term nature of the study relative to the normal lifespan of dogs.

4.4.1.2.3.3. Fitzhugh et al. (1964). A total of 14 mongrel dogs (seven of each sex), ranging in age from 5 months to 4 years, were fed dieldrin (100% pure) in dosages of 0.2 to 10 mg/kg/day, 6 days/week for periods up to 25 months. Animals fed 0.5 mg/kg/day or more showed gross toxic effects, including loss of weight and convulsions, and they died progressively earlier with increasing dose level. Histological effects were limited to fatty degenerative changes in the liver and kidneys and bone marrow changes in those dogs fed 0.5 mg/kg/day dieldrin or more. No gross or microscopic effects were seen in dogs on dieldrin at 0.2 mg/kg/day. The study is unacceptable as a test of possible carcinogenicity because of the small number of animals tested and the short-term nature of the test relative to the normal lifespan of dogs.

4.4.1.2.3.4. Walker et al. (1969). Groups of five male and five female beagle hounds received, by capsule, daily oral doses of 0.005 or 0.05 mg/kg (equivalent to approximately 0.1 or 1 ppm) dieldrin (> 99% HEOD), commencing at 4 to 7 months of age. The animals were sacrificed after 2 years. At sacrifice, the health and body weights of the treated dogs, as compared to controls, were normal, and there was no observed evidence of gross or microscopic changes in any organs. The study is unacceptable as a test of possible carcinogenicity because of the small number of animals tested and the short-term nature of the test relative to the normal lifespan of dogs.

4.4.1.2.3.5. Wright et al. (1978). Groups of five male rhesus monkeys, all approximately 4 years old, were fed control diets or diets supplemented with technical-grade dieldrin (88.4% purity) at initial concentrations of 0.01, 0.1, 0.5, 1, or 5 ppm. One of the monkeys in the 5 ppm group died after 4 months of

exposure. Dieldrin intoxication was the likely cause of death and, consequently, the dieldrin content of the diet of this group was reduced to 2.5 ppm following a brief period of being fed the control diet. One of the four remaining monkeys in this group died of endocrine failure 6 months from the outset of the experiment. The exposure to dieldrin was not considered to be a contributory factor in its death. However, as a precautionary measure, the concentration of dieldrin in the diet of the three survivors was further reduced to 1.75 ppm at 9 months. Subsequently, one of these animals had its dieldrin intake progressively increased until, at the 23rd month from the outset, it was receiving dieldrin at the initial dietary concentration of 5 ppm. The experiment was terminated after approximately 6 years.

Apart from small changes associated with the hepatocellular endoplasmic reticulum, the overall study revealed no effects that could be attributed to dieldrin. Increases in the activity of the liver microsomal mono-oxygenase system, determined in vitro with a wide range of substrates, provided the most sensitive criterion for an effect; significant increases, to a maximum of threefold, were observed in the 1.75 and 5 ppm treatment groups and these changes were paralleled by increases in cytochrome P-450.

The study is unacceptable as a test of possible carcinogenicity because of the small number of animals tested and the short-term nature of the test relative to the average lifespan of the rhesus monkey of 20 years.

4.4.1.2.3.6. Zavon and Stemmer (1975). Thirty-one rhesus monkeys were fed dieldrin (purity not indicated) for 3 years at levels of 0.01, 0.1, 0.5, 1, and 5 ppm. Weight and body functions remained normal throughout the 3 years for most animals. No pathology data were reported. This study is not an acceptable bioassay for carcinogenicity due to the small numbers of animals and short duration of the treatment as compared to the average lifespan of the

rhesus monkey.

4.4.2. Epidemiologic Studies

The epidemiologic data for the carcinogenicity of aldrin and dieldrin consists of two studies. The first is a study of a group of pesticide production workers in Holland (Van Raalte, 1977). The cancer cases are presented as case reports. The second is a retrospective cohort mortality study of workers in a pesticide manufacturing plant (Ditraglia et al., 1981). Limitations of both these studies preclude the association of exposure with cancer.

4.4.2.1. Van Raalte (1977)--The author reported his observations on a group of 1000 industrial workers employed at pesticide manufacturing and formulation plants in Pernis, Holland. The number of plants was not reported. Exposure to aldrin and dieldrin began in 1954, with exposure to endrin and telodrin beginning later (no specific information was provided). The results focused on clinical parameters, and the incidence of cancer was discussed only in qualitative terms. Of the 1000 workers, a subset of 166 men with dieldrin exposure was studied in detail. Fifty-one were over 50 years of age. Two cases of cancer were reported. Among men with "comparatively mild exposure," there was a cancer death due to gastric cancer. This case had 5 years of "comparatively mild" exposure. The other case was a lymphosarcoma in a man with 7 years of "very mild exposure." Both of these cases occurred before 1964. The authors stated that no new cases had occurred in the last 11 years, but the exact time period could not be determined. Tumors were not observed in the more heavily exposed workers. No other causes of death were reported that might mask a higher cancer incidence. The authors concluded that there was no excess tumor incidence related to dieldrin exposure. Many study limitations are noted. Exposure was uncertain as to chemicals and concentration levels. Statistical methods were not described, no control group was utilized, and the period of

follow-up after exposure was probably inadequate to detect a carcinogenic effect. Thus, this study is inadequate to determine the carcinogenicity of aldrin and dieldrin.

4.4.2.2. 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 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 Social Security Administration (SSA), state motor vehicle offices, U.S. Postal Mail Corrective Services, and "other" sources.

Four separate cohorts representing four pesticide plants composed the study populations. One plant had manufactured aldrin, dieldrin, and endrin and had done so since 1946. Other pesticides manufactured at this plant included organobromines and organophosphates beginning in 1955 and 1956, respectively. Other chemical products included numerous precursors to the chemicals. The cohort at this plant consisted of 1,155 individuals, representing 24,939 person-years of observation. Ten percent were lost to follow-up. The three remaining plants were not involved in the production of aldrin or dieldrin and, thus, results are not discussed here for these three plants.

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. The U.S. white male age-, calender time-, and cause-specific mortality rates were used to calculate the standardized mortality ratios. Statistical significance between the observed and expected values were tested with the Poisson distribution. Confidence intervals were presented for the SMR estimates, although the method of calculation was not reported by the authors.

Statistically significant ($p < 0.05$) SMR deficits were observed for all causes of death (SMR = 84, observed = 173, 95% CI 72 to 98) and for diseases of the circulatory system (SMR = 77, observed = 69, 95% CI 60 to 97). Statistically significant ($p < 0.01$) SMR increases were observed for nonmalignant respiratory disease (SMR = 212, observed = 22, 95% CI 133 to 320). The SMR for all malignant neoplasms was 82. This was not statistically significant. However, increased SMRs were observed for other specific neoplasms including cancer of the esophagus (SMR = 235), cancer of the rectum (SMR = 242), cancer of the liver (SMR = 225), and cancer of the lymphatic and hematopoietic systems (SMR = 147). None of these was statistically significant.

An analysis by latency was also carried out. Standardized mortality ratios 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. SMRs of 53, 111, and 64 were reported for the three latency time periods, respectively. The individual point estimates were not statistically significant.

This study revealed excess risk of cancer at various tissue sites for all workers at this plant, though none was statistically significant. However, there are limitations to this study. No information on quantitative exposures were available. It was not possible to assess the effects of aldrin or dieldrin independently of the other toxic chemicals at the plant. No attempt was made to exclude or adjust for the effects of sex or race, and no effort was made to control for other confounding variables such as other chemicals produced in the plant, 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, risk may be underestimated for workers involved in the day-to-day manufacture of aldrin and dieldrin. Also, since vital statistics were unknown for 10% of the workers, the risks may be understated. The size of the study population was small in spite of a 25-year or more period of follow-up. Thus, the power of this study to detect a statistically significant result is limited. This study provides insufficient evidence to link aldrin or dieldrin exposure to cancer.

4.5. STRUCTURE-ACTIVITY RELATIONSHIPS

Seven chemicals were identified as important for evaluating the structure-activity of aldrin and dieldrin for carcinogenicity. These chemicals are chlordane, chlorendic acid, endrin, endosulfan, heptachlor, hexachlorocyclopentadiene, and isodrin; their structures are presented in Figure 4-5.

4.5.1. Chlordane

4.5.1.1. Evidence for carcinogenicity - human exposure (IARC, 1979a)--Case reports suggest a relationship between exposure to chlordane or heptachlor (either alone or in combination with other compounds) and blood dyscrasias. Another publication has also suggested an association with acute leukemia; an association between both pre- and postnatal exposure to technical-grade chlordane and the development of neuroblastomas in children was also suggested. These data are inadequate to assess the carcinogenicity of chlordane.

4.5.1.2. Evidence for carcinogenicity - animal studies (IARC, 1979a)--Chlordane (analytical grade) was tested in four experiments in mice and in four experiments in rats by oral administration. It produced hepatocellular carcinomas in mice of both sexes and in four different strains; for rats, one study revealed an increase in hepatocellular adenomas. These data provide sufficient evidence for the carcinogenicity of chlordane.

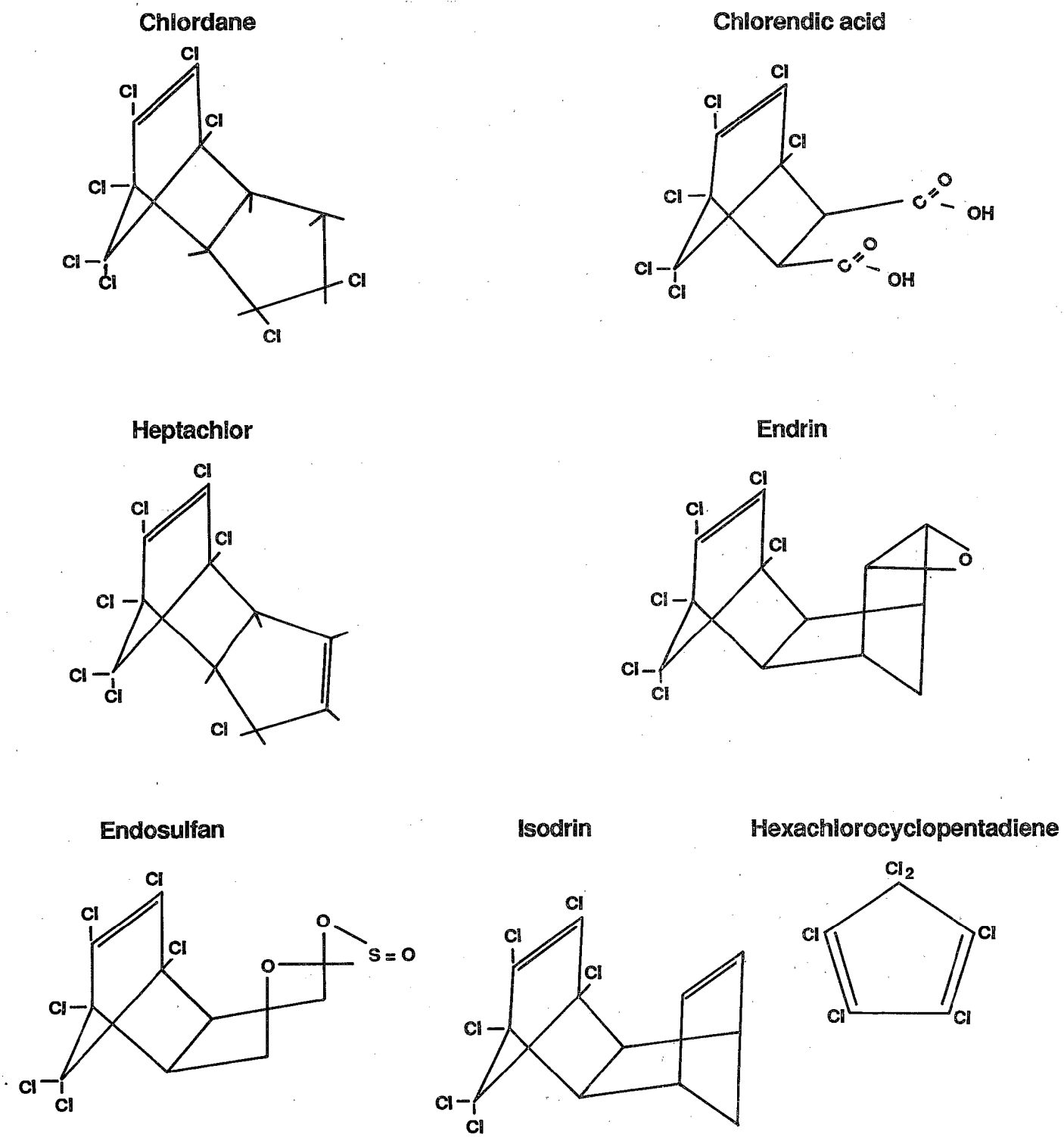

Figure 4-5. Chemicals selected for structure-activity analysis.

4.5.1.3. Evidence for activity in short-term tests (IARC, 1979a)--Chlordane induced gene conversions in yeast but was not shown to be mutagenic in bacteria. It induced mutations in mammalian cells in culture, but was negative in dominant lethal tests in mice. These data are inadequate to assess the carcinogenicity of chlordane.

4.5.2. Chlorendic Acid

4.5.2.1. Evidence for carcinogenicity - human exposure (NTP, 1985b)--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. These data are inadequate to assess the carcinogenicity of chlorendic acid.

4.5.2.2. Evidence for carcinogenicity - animal studies (NTP, 1985b)--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 alveolar/bronchiolar adenomas and preputial gland carcinomas may also have been related to the administration of chlorendic acid. There was also evidence of carcinogenicity 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 $B6C3F_1$ mice as shown by increased incidences of hepatocellular adenomas and of hepatocellular carcinomas. There was no evidence of carcinogenicity in female $B6C3F_1$ mice given chlorendic acid in the diet at concentrations of 620 or 1250 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, 1985b)--Chlorendic acid was not mutagenic in strains TA100, TA98, TA1535, or TA1537 of *S. typhimurium* in the presence or absence of Aroclor 1254-induced male Sprague-

Dawley rats 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% to 5% at doses of 1600 to 1700 mg/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 observed is considered as an indirect mechanism for 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 inadequate to assess the carcinogenicity of chlorendic acid.

4.5.3. Endrin

4.5.3.1. Evidence for carcinogenicity - human exposure (IARC, 1974c)--A study was undertaken in 1968 on 233 workers employed in a factory that had been manufacturing aldrin and dieldrin since 1954 or 1955, endrin since 1957, and dieldrin during 1958 to 1965. 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 left the company were the subject of a subsequent report. The average age at the time of this survey was 47.4 years (range, 29 to 72); the average occupational exposure was 6.6 years (4.0 to 12.3); and the average time since the 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.

4.5.3.2. Evidence for carcinogenicity - animal studies (NTP, 1985b)--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.

4.5.3.3. Evidence for activity in short-term tests (NTP, 1985b)--Endrin did not cause mutations in NTP Salmonella mutagenicity tests. These data are inadequate to assess the carcinogenicity of endrin.

4.5.4. Endosulfan

4.5.4.1. Evidence for carcinogenicity - human exposure (NTP, 1985b)-- 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.

4.5.4.2. Evidence for carcinogenicity - animal studies (NTP, 1985b)--In an NTP 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.

4.5.4.3. Evidence for activity in short-term tests (NTP, 1985b)--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.

4.5.5. Heptachlor

4.5.5.1. Evidence for carcinogenicity - human exposure (IARC, 1979b)--Case reports suggest a relationship between exposure to heptachlor or chlordane (either alone or in combination with other compounds) and blood dyscrasias.

Another publication has also suggested an association with acute leukemia; an association between both pre- and postnatal exposure to technical grade chlordane containing heptachlor and the development of neuroblastomas in children was also suggested. These data are inadequate to assess the carcinogenicity of heptachlor.

4.5.5.2. Evidence for carcinogenicity - animal studies (IARC, 1979b)--Heptachlor containing about 20% chlordane was tested in one experiment in mice and in one in rats by oral administration. Liver carcinomas were observed in mice of both sexes. In rats, the results suggest a carcinogenic effect on the thyroid in females. Heptachlor (97% pure) was also inadequately tested in one experiment in rats by oral administration. A reevaluation of unpublished studies involving the oral administration of heptachlor of unspecified purity to mice and rats of other strains confirms the hepatocarcinogenicity of heptachlor for mice and suggests a carcinogenic effect in female rats. These data provide sufficient evidence for the carcinogenicity of heptachlor.

4.5.5.3. Evidence for activity in short-term tests (IARC, 1979b)--Heptachlor was not mutagenic in *S. typhimurium* or *Drosophila melanogaster* and was negative in dominant lethal tests in mice. These data are inadequate to assess the carcinogenicity of heptachlor.

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 lungs. 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 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 has been scheduled by the 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. Furthermore, HEX did not induce unscheduled DNA synthesis in rat hepatocytes. HEX did not induce *in vitro* cell transformation in 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 on 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 data on the carcinogenicity of isodrin were found.

4.5.7.3. Evidence for activity in short-term tests (HSDB, 1985)--Isodrin is converted to endrin by liver microsomes, and endrin does 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 is used to make this choice. The animal studies are evaluated qualitatively to assure that only properly conducted studies are used. The tumor incidence data are then separated according to organ sites and tumor types. The data sets used in the model are the ones in which the incidence of tumors is statistically significantly higher in at least one test dose level compared to controls or where the tumor incidence rate shows a significant trend with respect to dose level. Both biological and statistical considerations are used to select appropriate data sets.

Because humans may be as sensitive as the most sensitive animal species, potency estimates 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 estimates from the most sensitive sex and strain tested are also reported. This approach is consistent with EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986).

5.1.1. Aldrin

Three data sets are suitable for quantitative risk estimation. These are both male and female C_3H mice in the Davis (1965) study as reevaluated by Reuber and cited in Epstein (1975), and male $B6C3F_1$ mice in the NCI (1978a) bioassay.

5.1.2. Dieldrin

Thirteen data sets are suitable for quantitative risk estimation. These include both male and female C3H mice in the Davis (1965) study as reevaluated by Reuber, male and female CF-1 mice in the Walker et al. (1972) study, male and female CF-1 mice in the Thorpe and Walker (1973) study, male B6C3F1 mice in the NCI (1978a, b) bioassay, male CF-1 mice in the Tennekes et al. (1981) study, and male C57BL/6J, C3H, and B6C3F1 mice in the Meierhenry et al. (1983) study.

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 fit 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 aldrin and dieldrin are described in the previous section.

Although no single mathematical model is recognized as the most appropriate, some mechanisms of the process have become 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. Thus, a model that is linear at low doses is appropriate. The linearized multistage model, which has these properties, is used in this assessment to calculate human risk. This model takes the following form:

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

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

Cancer risks can also be estimated using models such as the probit, logit, Weibull, one-hit, and gamma multi-hit. 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 and pharmacokinetic mechanisms. Hence, animal dosages need to be converted to human equivalent dosages. 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 data to calculate a human equivalent dose. This equation is:

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

where

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

DA = animal dosage (mg/kg/day)

WA = weight of the animal (kg) assumed (0.03 kg for mice, 0.35 kg for rats)

70 = weight of a man (kg).

The surface area correction factor used in this assessment is $(0.03/70)^{1/3}$ = 0.08. 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 5-1 summarizes 16 potency estimates obtained in this way. Tables 5-2 to 5-17 describe the data sets used for these estimates.

5.4.1. Aldrin

Three data sets are suitable for quantitative risk estimation: male and female C_3H mice, and male $B6C3F_1$ mice. The most sensitive sex and strain tested is female C_3H mice. From these the potency can be estimated at 23 per mg/kg/day.

The most sensitive species tested is mice. There are three potency estimates, ranging from 23 down to 12 per mg/kg/day, with a geometric mean of 17 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 17 per mg/kg/day.

These estimates are plausible upper bounds for the increased cancer risk from aldrin, meaning that the true risk is not likely to exceed these estimates and may be lower.

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 aldrin is 6.2×10^3 per mmol/kg/day. This places aldrin in the most potent quartile of suspect carcinogens ranked by the 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

TABLE 5-1. HUMAN POTENCY ESTIMATES BY CHEMICAL

Chemical	Sex, species, strain	Tumor site and type	Potency $(mg/kg/day)^{-1}$	Reference
Aldrin	Male, mice, C3H	Liver, carcinoma	18	Davis (1965), reevaluated by Reuber (cited in Epstein, 1975)
Aldrin	Female, mice, C3H	Liver, carcinoma	23	Davis (1965), reevaluated by Reuber (cited in Epstein, 1975)
Aldrin	Male, mice, B6C3F1	Liver, carcinoma	12	NCI (1978a)
Dieldrin	Male, mice, CF-1	Liver, carcinoma	22	Davis (1965), reevaluated by Reuber (cited in Epstein, 1975)
Dieldrin	Female, mice, CF-1	Liver, carcinoma	25	Davis (1965), reevaluated by Reuber (cited in Epstein, 1975)
Dieldrin	Male, mice, CF-1	Liver, carcinoma	25	Walker et al. (1972)
Dieldrin	Female, mice, CF-1	Liver, carcinoma	28	Walker et al. (1972)
Dieldrin	Male, mice, CF-1	Liver, carcinoma	15	Walker et al. (1972)
Dieldrin	Female, mice CF-1	Liver, carcinoma	7.1	Walker et al. (1972)
Dieldrin	Male, mice CF-1	Liver, carcinoma	55	Thorpe and Walker (1973)
Dieldrin	Female, mice CF-1	Liver, carcinoma	26	Thorpe and Walker (1973)
Dieldrin	Male, mice, B6C3F1	Liver, carcinoma	9.8	NCI (1978a)

(continued on the following page)

TABLE 5-1. (continued)

Chemical	Sex, species, strain	Tumor site and type	Potency $(mg/kg/day)^{-1}$	Reference
Dieldrin	Male, mice CF-1	Liver, carcinoma	18	Tennekes et al. (1981)
Dieldrin	Male, mice C57BL/6J	Liver, carcinoma	7.4	Meierhenry et al. (1983)
Dieldrin	Male, mice C3H/He	Liver, carcinoma	8.5	Meierhenry et al. (1983)
Dieldrin	Male, mice B6C3F1	Liver, carcinoma	11	Meierhenry et al. (1983)

TABLE 5-2. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF ALDRIN FROM TUMOR INCIDENCE IN MICE

Compound: aldrin
Species, strain, sex: mice, C3H, female
Body weight: 0.030 kg (assumed)
Length of experiment: 2 years
Length of exposure: 2 years
Tumor site and type: liver, carcinoma
Route, vehicle: oral, diet
Human potency: 23 per mg/kg/day

Experimental dose (ppm)	Transformed dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Incidence No. responding/ No. examined
0	0	0	2/53
10	1.3	0.104	72/85

SOURCE: Davis, 1965 (evaluated by Reuber; as cited in Epstein, 1975).

TABLE 5-3. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF ALDRIN FROM TUMOR INCIDENCE IN MICE

Compound: aldrin
Species, strain, sex: mice, C3H, male
Body weight: 0.030 kg (assumed)
Length of experiment: 2 years
Length of exposure: 2 years
Tumor site and type: liver, carcinoma
Route, vehicle: oral, diet
Human potency: 18 per mg/kg/day

Experimental dose (ppm)	Transformed dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Incidence No. responding/ No. examined
0	0	0	22/73
10	1.3	0.104	75/91

SOURCE: Davis and Fitzhugh, 1962 (evaluated by Reuber; as cited in Epstein, 1975).

TABLE 5-4. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF ALDRIN FROM TUMOR INCIDENCE IN MICE

Compound: technical grade aldrin
Species, strain, sex: mice, B6C3F1, male
Body weight: 0.030 kg (assumed)
Length of experiment: 93 weeks
Length of exposure: 80 weeks
Tumor site and type: liver, carcinoma
Route, vehicle: oral, diet
Human potency: 12 per mg/kg/day

Experimental dose (ppm)	Transformed dose (mg/kg/day)	Human equivalent dose (mg/kg/day)[a]	Incidence No. responding/ No. examined
0	0	0	17/92
4	0.52	0.04	16/49
8	1.04	0.08	25/45

[a]Human equivalent doses were multiplied by 80/93 to reflect exposure less than the duration of the experiment.

SOURCE: NCI, 1978a.

TABLE 5-5. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mice, C3H, male
Body weight: 0.030 kg (assumed)
Length of experiment: 2 years
Length of exposure: 2 years
Tumor site and type: liver, carcinoma
Route, vehicle: oral, diet
Human potency: 22 per mg/kg/day

Experimental dose (ppm)	Transformed dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Incidence No. responding/ No. examined
0	0	0	22/73
10	1.3	0.104	62/71

SOURCE: Davis, 1965 (evaluated by Reuber; as cited in Epstein, 1975).

TABLE 5-6. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mice, C3H, female
Body weight: 0.030 kg (assumed)
Length of experiment: 2 years
Length of exposure: 2 years
Tumor site and type: liver, carcinoma
Route, vehicle: oral, diet
Human potency: 25 per mg/kg/day

Experimental dose (ppm)	Transformed dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Incidence No. responding/ No. examined
0	0	0	2/53
10	1.3	0.104	62/71

SOURCE: Davis, 1965 (evaluated by Reuber; as cited in Epstein, 1975).

TABLE 5-7. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mice, CF-1, male
Body weight: 0.030 kg (assumed)
Length of experiment: 33 months
Length of exposure: 24 months
Tumor site and type: liver, carcinoma
Route, vehicle: oral, diet
Human potency: 25 per mg/kg/day

Experimental dose (ppm)	Transformed dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Incidence No. responding/ No. examined
0	0	0	58/288
0.1	0.013	0.001	32/124
1	0.13	0.01	34/111
10	1.3	0.104	165/176

SOURCE: Walker et al., 1972.

TABLE 5-8. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mice, CF-1, female
Body weight: 0.030 kg (assumed)
Length of experiment: 33 months
Length of exposure: 24 months
Tumor site and type: liver, carcinoma
Route, vehicle: oral, diet
Human potency: 28 per mg/kg/day

Experimental dose (ppm)	Transformed dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Incidence No. responding/ No. examined
0	0.0013	0.0001	39/297
0.1	0.013	0.001	24/90
1	0.13	0.01	32/87
10	1.3	0.104	136/148

SOURCE: Walker et al., 1972.

TABLE 5-9. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mouse, CF-1, male
Body weight: 0.030 kg (assumed)
Length of experiment: 128 weeks
Length of exposure: 128 weeks
Tumor site and type: liver
Route, vehicle: oral, diet
Human potency: 15 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Tumor incidence
0	0	0	9/78
1.25	0.16	0.012	6/30
2.5	0.33	0.025	13/30
5	0.65	0.049	26/30
10	1.30	0.098	5/11
20	2.60	0.196	12/17

SOURCE: Walker et al., 1972.

TABLE 5-10. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mouse, CF-1, female
Body weight: 0.030 kg (assumed)
Length of experiment: 128 weeks
Length of exposure: 128 weeks
Tumor site and type: liver
Route, vehicle: oral, diet
Human potency: 7.1 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Tumor incidence
0	0	0	8/78
1.25	0.16	0.012	5/30
2.5	0.33	0.025	12/28
5	0.65	0.049	18/30
10	1.30	0.098	9/17
20	2.60	0.196	8/21

SOURCE: Walker et al., 1972.

TABLE 5-11. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mouse, CF-1, male
Body weight: 0.030 kg (assumed)
Length of experiment: 110 weeks
Length of exposure: 110 weeks
Tumor site and type: liver
Route, vehicle: oral, diet
Human potency: 55 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)[a]	Tumor incidence[a]
0	0	0	11/45
10	1.30	0.098	30/30

[a]To calculate human potency, a tumor incidence of 29/30 was used and the dose was adjusted to 29/30 of the above value.

SOURCE: Thorpe and Walker, 1973.

TABLE 5-12. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mouse, CF-1, female
Body weight: 0.030 kg (assumed)
Length of experiment: 110 weeks
Length of exposure: 110 weeks
Tumor site and type: liver
Route, vehicle: oral, diet
Human potency: 26 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Tumor incidence
0	0	0	10/44
10	1.30	0.104	26/30

SOURCE: Thorpe and Walker, 1973.

TABLE 5-13. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: technical-grade dieldrin
Species, strain, sex: mice, B6C3F1, male
Body weight: 0.030 kg (assumed)
Length of experiment: 93 weeks
Length of exposure: 80 weeks
Tumor site and type: liver, carcinoma
Route, vehicle: oral, diet
Human potency: 9.8 per mg/kg/day

Experimental dose (ppm)	Transformed dose (mg/kg/day)	Human equivalent dose (mg/kg/day)[a]	Incidence No. responding/ No. examined
0	0	0	17/92
2.5	0.325	0.026	12/50
5	0.65	0.052	16/45

[a]Human equivalent doses were multiplied by 80/93 to reflect exposure less than the duration of the experiment.

SOURCE: NCI, 1978a.

TABLE 5-14. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: Dieldrin
Species, strain, sex: mouse, CF-1, male
Body weight: 0.030 kg (assumed)
Length of experiment: 110 weeks
Length of exposure: 110 weeks
Tumor site and type: liver
Route, vehicle: oral, diet
Human potency: 18 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)	Tumor incidence
0	0	0	25/252
10	1.30	0.104	113/139

SOURCE: Tennekes et al., 1981.

TABLE 5-15. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mouse, C57BL/6J, male
Body weight: 0.030 kg (assumed)
Length of experiment: 132 weeks
Length of exposure: 85 weeks
Tumor site and type: liver, hepatocellular carcinoma
Route, vehicle: oral, diet
Human potency: 7.4 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)[a]	Tumor incidence
0	0	0	0/69
10	1.30	0.104	21/71

[a]Human equivalent doses were multiplied by 85/132 to reflect exposure less than the duration of the experiment.

SOURCE: Meierhenry et al., 1983.

TABLE 5-16. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mouse, C3H/He, male
Body weight: 0.030 kg (assumed)
Length of experiment: 132 weeks
Length of exposure: 85 weeks
Tumor site and type: liver, hepatocellular carcinoma
Route, vehicle: oral, diet
Human potency: 8.5 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)[a]	Tumor incidence
0	0	0	6/50
10	1.30	0.104	19/50

[a]Human equivalent doses were multiplied by 85/132 to reflect exposure less than the duration of the experiment.

SOURCE: Meierhenry et al., 1983.

TABLE 5-17. CANCER DATA SHEET FOR DERIVATION OF POTENCY OF DIELDRIN FROM TUMOR INCIDENCE IN MICE

Compound: dieldrin
Species, strain, sex: mouse, B6C3F1, male
Body weight: 0.030 kg (assumed)
Length of experiment: 132 weeks
Length of exposure: 85 weeks
Tumor site and type: liver, hepatocellular carcinoma
Route, vehicle: oral, diet
Human potency: 11 per mg/kg/day

Experimental dose (ppm)	Average daily dose (mg/kg/day)	Human equivalent dose (mg/kg/day)[a]	Tumor incidence
0	0	0	3/76
10	1.30	0.104	26/62

[a]Human equivalent doses were multiplied by 85/132 to reflect exposure less than the duration of the experiment.

SOURCE: Meierhenry et al., 1983.

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/day, an air concentration of 1 $\mu g/m^3$ is equivalent to a dose of:

$$(1\ \mu g/m^3)\ (10^{-3}\ mg/\mu g)\ (20\ m^3/day)\ /\ (70\ kg) = 2.89 \times 10^{-4}\ mg/kg/day.$$

Multiplying this dose by the potencies calculated above gives unit risks of 4.9×10^{-3} per $\mu g/m^3$ and 6.6×10^{-3} per $\mu g/m^3$ for potencies of 17 and 23, respectively.

The unit risk in water, which is the potency expressed in terms of $\mu g/L$ drinking water concentration, 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/day, a water concentration of 1 ug/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 4.9×10^{-4} per $\mu g/L$ and 6.6×10^{-4} per $\mu g/L$ for potencies of 17 and 23, respectively.

5.4.2. Dieldrin

Thirteen data sets are suitable for quantitative risk estimation. The most sensitive sex and strain tested is male CF_1 mice in the Thorpe and Walker (1973) study. From these data the potency can be estimated at 55 per mg/kg/day.

The most sensitive species tested is mice. There are 13 potency estimates, ranging from 55 down to 7 per mg/kg/day, with a geometric mean of 16 per mg/kg/day. Therefore the potency for the general population is estimated at 16 per mg/kg/day.

These estimates are plausible upper bounds for the increased cancer risk from dieldrin, meaning that the true risk is not likely to exceed these estimates and may be lower.

The molecular potency index for dieldrin is 6.1×10^3 per mmol/kg/day. This places dieldrin in the most potent quartile of suspect carcinogens ranked by the CAG.

The potency estimates yield unit risks in air of 4.6×10^{-3} per $\mu g/m^3$ and 1.6×10^{-2} per $\mu g/m^3$. The unit risks in water are 4.6×10^{-4} per $\mu g/L$ and 1.6×10^{-3} per $\mu g/L$.

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APPENDIX A

EPA: 68-02-4225
DYNAMAC No. 1-77-A4
June 25, 1986

DATA EVALUATION RECORD

TERMITICIDES--ALDRIN
Mutagenicity--Multiple Genetic Toxicology Studies

STUDY IDENTIFICATION: Mutagenicity--Multiple genetic toxicology studies.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 6-25-86

1. CHEMICAL: Aldrin.

2. TEST MATERIAL: N/A.

3. STUDY/ACTION TYPE: Mutagenicity-multiple genetic toxicology studies

4. STUDY IDENTIFICATION: N/A.

5. REVIEWED BY:

Brenda Worthy, M.T.
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Date: 7-3-86

I. INTRODUCTION

The purpose of this document is to summarize from published articles the review findings of *in vitro* and *in vivo* genetic toxicology assays conducted with aldrin. Findings which were evaluated as part of a screening assay and reported limited primary data are presented in Table 1. Although survey findings had insufficient data to evaluate assay acceptability, they offer useful information in the overall genetic toxicology review of aldrin.

Table 2 contains the review findings of published primary data with sufficient information to make an assessment of their acceptability.

The results of published articles reviewed on aldrin are discussed according to genetic effect categories; the data included in the survey studies consisted of 7 gene mutation assays (Category 1), 1 chromosomal aberration assay (Category 2), and 2 assays that assessed other mutagenic mechanisms (Category 3); the data from primary studies consisted of 3 chromosomal aberration assays (Category 2) and 5 assays in Category 3.

II. SURVEY STUDIES:

A. Summary:

1. Gene Mutation (Category 1):

Aldrin was reported negative (Article No. 1) using the plate incorporation method with five strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, and TA100) in the presence or absence of rat S9 activation, at doses up to 5000 µg/ml. Negative results were reported for the concentration agar gradient assay (Article No. 2) using *S. typhimurium* G46, C3076, D3052, TA1535, TA1537, TA1538, TA98, and TA100 with or without rat S9 activation, over a 1-10,000 fold concentration range. Nonactivated aldrin was also negative for reverse mutation (omitted from list of positive compounds) at 20 µg/disc in *S. typhimurium* strains TA1535, TA1536, TA1537, and TA1538 (Article No. 3).

Aldrin was investigated in four survey *Escherichia coli* tryptophan reversions assays. Nonactivated and S9-activated aldrin, assayed up to 5000 µg/plate, did not increase tryptophan reversion in *E. coli* WP2 *hcr* (Article No. 1). Qualitative negative results were reported over a 1-10,000-fold nonactivated and S9-activated concentration range of aldrin in the concentration agar gradient test with *E. coli* WP2 and WP2 *uvrA* (Article No. 2). In two nonactivated spot tests (Article Nos 3 and 4), 20 µg and 1 mg/disc aldrin were negative in *E. coli* WP2 and WP2 *hcr* or *E. coli* WP2, respectively.

TABLE 1. SUMMARY OF MUTAGENICITY TESTING OF ALDRIN

Article/Authors/Study Identification	Genetic Endpoint	Indicator Organism	Purity	Application	Dose Range	Response	Conclusion	Comment
1 Further mutagenicity studies on pesticides in bacterial reversion assay systems/Moriya, M., Ohta, T., Watanabe, K., Miyazawa, T., Kato, K., Shirasu, Y./Mutat. Res. 116: 185-216, 1983.	Gene Mutation	S. typhimurium TA100, TA98, TA1535, TA1537, and TA1538 E. coli WP2 hcr	Not reported	Salmonella Microsome Plate Assay (Ames Test) and E. coli WP2 hcr tryptophan reversion assay	Tested up to 5000µg/plate unless cytotoxic; +/- S9 activation	Negative	Negative	Preliminary screening assay (228 pesticides)
2 Chemically induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds/Probst, G. S., McMahon, R. E., Hill, L. E., Thompson, C. Z., Epp, J. K., Neal, S. B./Environ. Mutagen. 3: 11-22, 1981.	Gene Mutation	S. typhimurium G46, C3076, D3052, TA1535, TA1537, TA1538, TA98, and TA100 E. coli WP2 and WP2 uvrA⁻	Not reported	Concentration agar gradient plate; +/- S9 activation	1-10,000 fold conc. range	Negative not included in tables showing positive responses	Negative	Preliminary screening assay for 218 compounds of various chemical classes
3 Mutagenicity screening of pesticides in the microbial system/Shirasu, Y., Moriya, M., Kato, K., Furuhashi, A., Kada, T./Mutat. Res. 40: 19-30, 1976.	Gene Mutation	S. typhimurium TA1535, TA1536, TA1537, TA1538 E. coli WP2 and WP2 hcr	Not reported	Disc Test Cells were exposed for 2 days at 37 C -S9 activation	20 µg/disc	Negative by omission from list of positive results	Negative	Preliminary screening assay of 166 pesticides
	DNA damage	B. subtilis H17(Rec⁺) and M45 (Rec⁻)		Disc Test Cells were exposed for 24 hr at 37 C -S9 activaton	20 µg/disc	Negative by omission from list of positive results	Negative	Preliminary screening assay of 166 pesticides

TABLE 1. SUMMARY OF MUTAGENICITY TESTING OF ALDRIN (Continued)

Article/Authors/Study Identification	Genetic Endpoint	Indicator Organism	Purity	Application	Dose Range	Response	Conclusion	Comment
4 Mutagenicity of Dichlorvos/ Ashwood-Smith, M. J., Trevino, J., Ring, R./Nature 240: 418-420, 1972.	Gene Mutation	E coli WP2	Not reported	Disc Test Cells were exposed for 48 hr at 37 C -S9 activation	10% of active ingredient ~ 1mg	Negative	Negative	Preliminary screening assay of 15 pesticides
5 Mutagenicity testing of herbicides, fungicides and insecticides. I. Chromosome aberrations in Vicia faba/Njagi, G. D. E., Gopalan, H. N. B./Cytologia 46: 169-172, 1981.	Chromosomal aberrations	Root tip cells of Vicia faba	40%	The roots of intact seedlings were treated for ~ 5 hours. Cells were collected every 30 minutes during treatment	10, 50, 100, 1000, 5000, and 10,000 ppm	Positive for: mitotic inhibition, pynosis, premature chromosome condensation, plasmolysis. Reported to be dose related	Results suggest severe cytotoxicity; but no assessment for chromosomal aberrations	Results are from anaphase cells. This study was intended as a screening assay of 12 pesticides
6 In vitro breakage of plasmid DNA by mutagens and pesticides/ Griffin, III, D. E., Hill, W. E./Mutat. Res. 52: 161-169, 1978.	DNA damage	E. coli colicinogenic plasmid E1 (CoLE1)	Not reported	[Me^{3}H] thymine labelled cells were exposed for an unspecified time, layered on a alkaline sucrose gradient on which fraction analysis was made to assess DNA strand breakage. -S9 activation	1 mg/mL	Negative	Negative	Preliminary screening assay for development of a new procedure

TABLE 2. SUMMARY OF MUTAGENICITY TESTING OF ALDRIN

Article/Authors/Study Identification	Genetic Endpoint	Indicator Organism	Purity	Application	Dose Range	Response	Conclusion	Comment
7 The comparative cytogenetic effects of aldrin and phosphamidon/ Georgian, L./Mutat. Res. 31: 103-108, 1975.	Chromosomal aberration	Human peripheral blood	Not reported	Cells cultured for 50 hrs were exposed for 22 hrs.	19.125, 38.25, 76.5 µg/mL	Positive at lowest dose; however, dose was cytotoxic (19.125) µg/mL All cells died at higher doses	Inconclusive evidence for a positive response	Aberrations observed near limit of cell survival; gaps included in % aberrant cells; no. of aberrations/ cell slightly lower than Mitomycin C; slides not coded
	Chromosomal aberration	Rat and mouse bone marrow		5 rats and 5 mice received one ip dose; sacrificed 24 hr after dosing	9.56, 19.125, 38.25, 76.50 µg/g	100 and 50% mortality at 76.5 and 38.25 µg/g; positive at 38.25 and 19.125 µg/mL Negative at 9.56 µg/mL	Inconclusive evidence for a positive response	Aberrations observed, near limit of animal survival. Gaps included in % aberrants. No positive control; slides not coded. Comments on both studies: 1, limited information on methodology (i.e., staining procedures) 2, presumed reactive dose was comparable for in vivo and in vitro studies 3, positive effects noted near limit of cell and animal survival.

TABLE 2. SUMMARY OF MUTAGENICITY TESTING OF ALDRIN (Continued)

Article/Authors/Study Identification	Genetic Endpoint	Indicator Organism	Purity	Application	Dose Range	Response	Conclusion	Comment
8 Mutagenicity studies involving aldrin, endosulfan, dimethoate, phosphamidon, carbaryl, and ceresan/Usha Rani, M. V., Reddi, O. S., Reddy, P. P./Bull. Environ. Contam. Toxicol. 25:277-282, 1980.	Chromosomal aberrations (micronucleated polychromatic erythrocytes)	Swiss male mice bone marrow	Not reported	Six mice received two equal oral doses separated by a 24 hr interval	13 mg/kg	Negative	Unacceptable	Only one dose assayed; no evidence of a cytotoxic or toxic effects; only one sampling interval; no positive control assayed.
9 Detection of chemical mutagens by the dominant lethal assay in the mouse/Epstein, S. S., Arnold, E., Andrea, J., Bass, W., Bishop, Y./ Toxicol. Appl. Pharmacol. 23: 288-325, 1972.	Chromosomal breaks in male germinal Dominant lethal assay	Mouse spermatogenesis cycle (8 week mating sequence)	Not reported	A single ip dose (7 males low dose 9 males high dose, 1 male mated to 3 females/week)	8 and 40 mg/kg	Negative 5/9 or 56% of males died at 40 mg/kg; At week 5: 1.2 increase in early deaths/ female in high dose	Negative (Acceptable)	Increase was not significant, and no other increases were noted throughout study; assayed up to a toxic dose
				Five consecutive daily administrations by gavage (10 males/ group; 1 male mated to 3 females/ week)	0.5 and 1 mg/kg/ day	Negative: 20% males in 1.0 mg/ kg group and 10% in 0.5 mg/kg group died; At wk 2 in high dose 1.0 early deaths/ pregnancy At wk 8: decrease in % pregnancy at both doses; 1.5 increase in early deaths per pregnancy and 100% pregnant females with early death observed in 2 females at the low dose only	Results are uninterpretable	As reported by authors, increases were not significant and these variances occurred late in the study (wk 8) and only in the low dose; however, they did not rule out dose reversal

TABLE 2. SUMMARY OF MUTAGENICITY TESTING OF ALDRIN (Continued)

Article/Authors/Study Identification	Genetic Endpoint	Indicator Organism	Purity	Application	Dose Range	Response	Conclusion	Comment
10 Chemically induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds/Probst, G. S., McMahon, R. E., Hill, L. E., Thompson, C. Z., Epp, J. K., Neal, S. B./Environ. Mutagen. 3: 11-22, 1981.	DNA repair UDS	Primary rat hepatocytes; Unscheduled DNA Synthesis (UDS)	Not reported	Cells were exposed for 5 and 18-20 hr with tritiated thymidine	8 doses ranging from 0.5 to 1,000 nmoles/mL	Negative	Negative (Acceptable)	The conc. scored was 100 nmoles/mL, the highest noncytotoxic dose.
11 Effect of pesticides on scheduled and unscheduled DNA synthesis of rat thymocytes and human lymphocytes/Rocchi, P., Perocco, P., Alberghini, W., Fini, A., Prodi, G./Arch. Toxicol. 45: 101-108, 1980.	Scheduled DNA Synthesis (SDS)	Rat thymocytes	95%	Preliminary Study: Cells were exposed for 4 hrs with tritiated thymidine	10, 100, and 1000 µg/mL	Positive inhibition of SDS 10, 35, and 96% at 10, 100, and 1000 µg/mL, respectively	N/A	Range-finding study to determine that dose which inhibited synthesis by 50%; selected dose for UDS assay with human lymphocytes was 100 µg/mL
	Unscheduled DNA Synthesis (UDS)	Human lymphocytes		UDS Assay Cells were exposed for 4 hr with hydroxyurea and tritiated thymidine	100µg/mL	Weak positive; slight increase in UDS	Inconclusive evidence for a genotoxic response	Multiple doses should have been assayed
		Human lymphocytes		Cells were irradiated with UV light, exposed for 4 hr with hydroxyurea and tritiated thymidine to measure the effects of the chemical on DNA repair after UV damage	100µg/mL	Weak interference with DNA repair	N/A	Correlation between interference with DNA repair and genotoxicity is not clear.

TABLE 2. SUMMARY OF MUTAGENICITY TESTING OF ALDRIN (Continued)

Article/Authors/Study Identification	Genetic Endpoint	Indicator Organism	Purity	Application	Dose Range	Response	Conclusion	Comment
12 Pesticide induced DNA damage and its repair in cultured human cells/ Ahmed, F. E., Hart, R. W., Lewis, N. J./Mutat. Res. 42: 161-174, 1977.	DNA repair (UDS)	SV-40 transformed human cells (VA-4)	Not reported	UDS Assay Cells were treated with tritiated thymidine +/- S9 activation and exposed for 1, 3, 5, 8 and 12 hours	1, 10, 100 and 1000 μM	Positive at all doses +/- S9 activation (qualitative results)	Inconclusive; presumptive positive	The use of a transformed cell line is not recommended The reporting of qualitative results prohibits an accurate assessment of the data; assay is also technically flawed.
13 Evaluation of the alkaline elution/ rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential Sina, J. F., Bean, C. L., Dysart, G. R., Taylor, V. I., Bradley, M. O./Mutat. Res. 113: 357-391, 1983.	DNA damage (DNA strand breakage)	Rat hepatocytes	Not reported	Cells were exposed for 3 hours.	0.03, 0.3 and 3mM	Reported positive at cytotoxic doses	Inconclusive evidence for a positive response	Noncarcinogens that gave results similar to aldrin (+ at cytotoxic doses) were classified as "false positives" by the authors
14 Cytogenic effect of some chlororganic insecticides on mouse bone-marrow cell nuclei/Markaryan, D. S./Genetika 2(1): 132-137, 1966.	In vivo spindle apparatus inhibition	Anaphase and early telophase in male mouse bone marrow cells	Not reported	Single ip administration to 3 male mice; bone marrow harvested 21 hr. posttreatment	0.002 mg/g	Positive; increase in nuclear disturbances; marked increase in chromosome and chromatid bridges and fragments	Unacceptable; technically deficient	Data are uninterpretable due to staining protocol (probably high incidence of artifacts); unable to determine if increases in nuclear disturbances include severe cytotoxic signs observed at 4% of LD_{50} (50 mg/kg)

2. Chromosomal Aberrations (Category 2):

Meristematic root cells of *Vicia faba* were treated with 8 doses of aldrin ranging from 10 to 10,000 ppm (Article No. 5). Root samples were collected at varying times up to 310 minutes, stained, and scored for chromosomal aberrations and cytological effects. The results from anaphase cells collected 120 minutes following exposure to 100 ppm showed increased mitotic inhibition, formation of pynotic nuclei, premature chromosome condensation, and plasmolysis. The authors stated that the cytotoxic effects were dose and time related; however, there was no apparent increase in chromosomal aberrations. We assess that aldrin was severely cytotoxic but not clastogenic in this study.

Other Mutagenic Mechanisms (Category 3):

Nonactivated aldrin, at 20 µg/disc, did not cause preferential inhibition of *Bacillus subtilis* repair-deficient M45, compared to the repair-competent H17 strain (Article No. 3).

One mg/mL aldrin did not increase the number of plasmid breaks in *E. coli* CoL E1 when compared to the control (Article No. 6).

B. Conclusion - Survey Data:

There was insufficient data presented in the survey studies to draw unequivocal conclusions about the genotoxic responses to aldrin. However, the results suggest that aldrin did not cause gene mutations or DNA damage in bacteria, or induce chromosomal aberrations in plants.

III. STUDIES WITH PRIMARY DATA:

1. Gene Mutation (Category 1):

No studies were submitted for this category; therefore, a significant data gap exists.

2. Chromosomal Aberrations (Category 2):

a. Mammalian Cells: Georgian (Article No. 7) exposed synchronized human peripheral blood cells to 19.125, 38.25 and 76.5 µg/mL aldrin. No cells survived the 22-hour treatment with 38.25 and 76.5 µg/mL. At the lowest dose assayed, which was reported as cytotoxic, there was a 20% increase in chromosomal aberrations per cell as compared to the negative control. No cells survived the two higher concentrations, and a mammalian metabolic activation system was not employed in this study.

Our reviewers noted, however, that gaps were included in the scored aberrations, and that only 84 cells were counted for the 19.125 µg/mL dose group, whereas 273 cells each were scored for the negative and positive (Mitomycin C, 1 µg/mL) controls, respectively. The authors stated that the increase in aberrations occurred near the limit of cell survival, and that the observed chromosomal lesions are probably not perpetuated in other abnormal cells. We assess that the inclusion of gaps in the total aberration counts and the increase in aberrant cells near the limit of cell survival are insufficient evidence for a clastogenic response. The study is, therefore, inconclusive for a positive response.

Georgian (Article No. 7), also administered single ip injections of 9.56, 19.125, 38.25, and 76.50 ug/g aldrin to five rats and mice (sex not specified) per dose; bone marrow cells were collected 24 hours later. All mice and rats receiving 76.50 µg/g and 50% of the 38.25 µg/g dose groups died. Although increased chromosomal aberrations were reported in the 38.25 µg/g group, only 26 cells were scored for surviving rats and 30 for surviving mice. Approximately 7- and 3-fold increases in chromosomal aberrations per cell were reported for rats and mice, respectively, exposed to 19.125 µg/g aldrin. The results for the lowest dose (9.56 µg/g) were not reported; however, the authors indicated that no chromosomal aberrations were found. Hence, there were no quantitative data available to be evaluated at the lowest dose. As noted for the <u>in vitro</u> cytogenetic assay performed by Georgian, gaps were included in the total number of aberrations. The author formed the same conclusions about chromosomal aberrations near the limit of survival. We assess that these data are deficient in some details, and are insufficient for concluding that aldrin is clastogenic in rodent bone marrow cells. The study is, therefore, inconclusive for a positive response.

Usha et al. (Article No. 8) performed a micronucleus assay in mouse bone marrow using aldrin. Six male mice received two equal oral administrations of aldrin (13 mg/kg total) separated by 24-hour intervals. Six hours after the second administration, animals were sacrificed by cervical dislocation and bone marrow cells were stained. Two thousand polychromatic erythrocytes (PCE) were scored for micronuclei and the ratio of PCE to normochromatic erythrocytes (NCE) was calculated. No toxic signs were reported and the ratio of PCE:NCE was comparable to the control. No significant increase in the frequency of micronuclei was observed. We assess that the assay was technically flawed because only one dose was evaluated, no evidence of a cytotoxic or clinically toxic effect was observed, at the single sampling interval, and a positive control was not evaluated. We concluded that the test material was not adequately investigated; therefore, the study is unacceptable.

b. Germinal Cells: Epstein et al. (Article No. 9) investigated the clastogenic effects of 172 chemicals in mouse dominant lethal assays. Aldrin was administered to 7 or 9 male mice using a single ip dose of 8 or 40 mg/kg, respectively; in a second experiment, 10 male mice/group received an oral daily dose of 0.5 or 1 mg/kg/day for 5 consecutive days. The concurrent negative control group consisted of 10 males. For both experiments, male mice were mated 1 male to 3 females for 8 weeks. In the acute assay 56% (5/9) of the males died at the highest dose (40 mg/kg). A nonsignificant increase ($p > 0.05$) in early deaths/female was recorded at week 5; no other increases in measured parameters were noted; therefore, the study is acceptable.

In the subacute assay, 10% (1/10) and 20% (2/10) of the males died in the 0.5 and 1.0 mg/kg dose groups, respectively. The following nonsignificant changes were observed: at week 2, an increase in early deaths/pregnancy at the high dose; at week 8, a decrease in percent pregnancy (both doses) and increases in early fetal deaths and percent pregnant females with early deaths. The authors stated that since the changes in dominant lethal parameters were not significant and were generally noted at the low dose, the results did not suggest a positive effect; they did not, however, rule out the possibility of dose reversal. We concluded this assay cannot be properly interpreted.

c. Conclusion for Chromosomal Aberrations: No conclusion can be drawn from these *in vitro* and *in vivo* somatic cytogenetic assays with aldrin; therefore, a major data gap exists.

Based on the dominant lethal studies (Article No. 9) aldrin was not clastogenic in the acute assay; however, the results of the subacute assay cannot be properly interpreted.

3. Other Mutagenic Mechanisms (Category 3):

a. DNA Repair/Damage: Probst et al. (Article No. 10) tested 8 aldrin doses ranging from 0.5 to 1000 nM/mL in an *in vitro* unscheduled DNA-synthesis (UDS) assay using primary rat hepatocytes. Aldrin did not cause an increase in UDS at the highest noncytotoxic dose (100 nM/mL). Although 100 nM/mL was the only dose scored in this article, the study was properly controlled, and is acceptable.

Rocchi et al. (Article No. 11) tested aldrin's action on semiconservative DNA synthesis (SDS) to determine the 50% inhibition dose with rat thymocytes. They assayed aldrin for its potential to induce unscheduled DNA synthesis (UDS) in human lymphocytes in the presence of hydroxyurea and

tritiated thymidine without metabolic activation. In addition, they tested aldrin's ability to interfere with human lymphocyte repair capability following damage exerted by ultraviolet (UV) rays.

Based on the results of the inhibition of thymidine uptake assay, aldrin caused a 50% inhibition in rat thymocytes at a dose of 100 μg/mL; this dose was used in the UDS assay with human cells. Induced UDS repair effects were assessed after calculations were made to eliminate the effect of SDS inhibition. Aldrin induced an increase in UDS over the control. Similarly, with human cells damaged by UV irradiation and dosed with aldrin (100 μg/mL), the chemical slightly interfered with UDS repair. We assess that since the observed effects were noted at only one dose, the evidence for a genotoxic response is inconclusive. Therefore, the assay should be repeated with multiple doses in the presence and absence of metabolic activation.

Ahmed et al. (Article No. 12) evaluated aldrin at doses of 1, 10, 100 and 1000 μM with or without S9 activation in a UDS assay with SV-40 human (simian virus) transformed cells (VA-4). The authors reported only qualitative positive responses at all nonactivated and S9-activated doses following the 8-hour treatment. We assess that the reporting of qualitative results prohibits an accurate assessment of any dose-related response. In addition, the cells used in this assay were not grown under reduced serum or arginine deprivation conditions in conjunction with hydroxyurea to insure blockage of S-phase synthesis. Several agents which are generally considered nongenotoxic or weakly genotoxic (e.g., Diquat, 2,4-D fluid, and carbaryl) induced strong responses in this study. We discussed the findings of this assay with Dr. Gregory Probst,[1] Lilly Research Labs. He concurred with our assessment and noted that cells which may have escaped hydroxyurea treatment could easily be misinterpreted as undergoing UDS synthesis, thereby increasing the chances of a false positive.

In addition, the use of a transformed cell line to assess primary DNA damage does not conform with recommended[2] procedures, which use non-transformed diploid human cells.

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[1] Probst, G. S., Lilly Research Labs., Greenfield, IN, personal communications 4/86.

[2] Mitchell, A. D., Casciano, D. A., Meltz, M. L., Robinson, D. E., San, R., H. C., Williams, G. M., and 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.

We classify aldrin as an inconclusive presumptive genotoxin for SV-40 transformed human cells. We recommend confirmation of these data in a rigorously controlled experiment that conforms to the currently recommended protocols for UDS assays with human cell lines.

Sina et al. (Article No. 13) evaluated the DNA-damaging activity of 91 chemicals, which included aldrin, in a DNA-strand breakage assay using primarily rat hepatocytes. Cells were exposed to three aldrin doses of 0.03, 0.3, and 3 mM for 3 hours. Cytotoxicity was measured either by glutamate-oxaloacetate transaminase release or by trypan blue exclusion. DNA strand breaks were analyzed by alkaline elution, and the DNA content was determined fluorimetrically.

In contrast to the authors' criteria for a positive response, (3-fold increase in elution rates at <30% toxicity), aldrin was listed as positive. The authors reported a dose-related increase in single strand breaks (SSB) at the two highest doses; however, no activity was seen at the low dose. Based on the authors' statement that trypan blue exclusion proved to be a more reliable indicator of cell viability and a >30% reduction in cell viability was indicative of a biologically significant cytotoxic effect, we concluded from the data that the two active doses were cytotoxic (4 and 62% viability at 3 and 0.3 mM, respectively). Interestingly, several nonmutagens/noncarcinogens (cupric sulfate, phenanthrene, toluene, and 2-nitrobiphenyl) elicited responses similar to aldrin but were classified as "false positives." The authors stated that "Each of these compounds, however, caused SSB only when cytotoxicity was greater than 30%. DNA damage produced by these compounds may, therefore, be secondary to cell lysis."

We assess that the results for aldrin did not provide convincing evidence of a genotoxic effect.

b. Inhibition of Spindle Apparatus: Markaryan (Article No. 14) studied the effects of aldrin on the bone marrow of male mice. He reported that anaphase and early telophase harvested cells in mice injected ip with 0.002 mg/g showed increased frequencies of "nuclear disturbances," total chromosome rearrangements (chromosome and chromatid bridges and fragments) and "adhesions." We discussed the findings of this study with Dr. James Ivett,[3] and concur with his recommendation that the study is unacceptable for the following reasons:

1. The method used to prepare anaphase and telophase plates (slices of bone marrow stained with acetocarmine) tends

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[3] Ivett, J. L., Hazleton Biotechnologies, Vienna, VA (personal communications, 4/86.

to increase the number of artifacts, and lends itself to a planer effect (causes chromosomes to be excluded from a field being viewed); this makes the distinction between true chromosomal rearrangements and artifact difficult.

2. The number of animals investigated (three male mice) is probably not an inadequate sample population.

3. The inclusion of cytotoxic effects (vacuolation, karyopycnosis, and karyolysis) in the total percent nuclear disturbances suggests that the assayed dose, which was approximately 4% of the LD_{50}, was severely cytotoxic.

4. The slides were not reported as being coded.

We concluded, therefore, that the results could not be interpreted, and that these findings should be excluded from the genetic assessment of aldrin.

c. Conclusions for Other Genetic Mechanisms:

Only one published article (No. 10) in the category provided convincing evidence that aldrin is not genotoxic; this was the UDS assay using primary rat hepatocytes.

Based on the inconclusive and unconfirmed findings of a UDS assay with transformed human cells, aldrin is listed, with reservations, as presumptively genotoxic in human cells. We caution, however, that these findings are suspect and should be confirmed. The additional UDS assay with human lymphocytes or the DNA-strand break assay with primary rat hepatocytes were equivocal and provided insufficient evidence of a genotoxic response.

IV. OVERALL INTERPRETATION OF STUDY RESULTS AND RECOMMENDATIONS

1. Interpretation

The paucity of acceptable studies and the existence of major gaps in the data (point mutation and cytogenetics) severely hinders the development of a definitive genetic toxicology profile for aldrin. However, since aldrin in readily converted both *in vivo* and *in vitro* to dieldrin, the genetic toxicology assessment prepared for dieldrin can be used to infer a genetic activity profile for aldrin.

Although no published bacterial gene mutation assays with sufficient primary data were reviewed, the survey studies with *S. typhimurium* and *E. coli* indicate that aldrin is probably not mutagenic in procaryotes. This finding is supported by the overwhelming conclusion of negative mutagenic effects for dieldrin.

No mammalian gene mutation assays with aldrin were located; the single mammalian cell point mutation assay of dieldrin showed an inconclusive, unconfirmed mutagenic response. A major data gap, therefore, exists for both compounds.

No conclusions could be drawn from the in vitro or in vivo somatic cytogenetic assays with aldrin; therefore a major data gap remains. However, aldrin was not clastogenic in an acute dominant lethal assay in mouse germinal cells.

In agreement with both survey and acceptable DNA damage/repair assays with dieldrin, aldrin was not genotoxic in bacteria, yeast, or rat hepatocytes.

Nonactivated and S9-activated aldrin induced statistically significant increases in UDS in SV-40 transformed (VA-4) human fibroblasts. Dieldrin, also investigated in this study, caused a similar response. However, the validity of this assay was seriously compromised by numerous technical deficiencies, the observation of strong positive responses by compounds generally accepted as weakly genotoxic or nongenotoxic substances, and the lack of positive controls.

2. Recommendations

Although no persuasive evidence of adverse genetic effects was uncovered in the review of the available genetic toxicology assay with aldrin, adequate tests are needed to assess the following:

1. In vitro and in vivo gene mutation assays.

2. In vitro and in vivo chromosomal aberration assays.

3. In vitro UDS assays with SV-40 transformed (VA-4) cells and non-transformed human cells.

In conclusion, we assess that the most useful information will come from experiments on promotion activity, since interference with cellular communication has been shown for dieldrin and other related chlorinated hydrocarbons such as heptachlor and chlordane. We therefore recommend these additional tests be performed with aldrin:

1. In vitro metabolic cooperation and/or transformation with promotion assay.

2. In vivo promoter activity assays.

APPENDIX B

EPA: 68-02-4225
TASK: 077-A3
June 23, 1986

DATA EVALUATION RECORD

TERMITICIDES--DIELDRIN

Mutagenicity--Multiple Genetic Toxicology Studies

STUDY IDENTIFICATION: Mutagenicity--Multiple genetic toxicology studies.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 6-23-86

1. CHEMICAL: Dieldrin.

2. TEST MATERIAL: N/A.

3. STUDY/ACTION TYPE: Mutagenicity--multiple genetic toxicology studies.

4. STUDY IDENTIFICATION: N/A.

5. REVIEWED BY:

Brenda Worthy, M.T.
Principal Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 6-23-86

Nancy E. McCarroll, B.S.
Independent Reviewer
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6. APPROVED BY:

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Irving Mauer, Ph.D.
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Date: 06-30-86

Jane E. Harris, Ph.D.
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Signature: Marvin C (for JEH)
Date: 7-3-86

I. INTRODUCTION:

The purpose of this document is to summarize the review findings from published articles of *in vitro* and *in vivo* genetic toxicology assays conducted with dieldrin. Articles that evaluated dieldrin as part of a screening assay and reported limited primary data are presented in Table 1. Although survey articles had insufficient data to evaluate assay acceptability, they offer useful information in the overall genetic toxicology review of dieldrin.

Table 2 contains the review findings of published articles with sufficient data to make an assessment of assay acceptability. The results of all published articles reviewed on dieldrin are discussed based on categories of genetic effect according to EPA Guidelines: survey data from 8 gene mutation assays (Category 1) and 6 assays that assessed other mutagenic mechanisms (Category 3); primary data from 5 gene mutation assays (Category 1), 5 chromosomal aberration assays (Category 2), and 8 assays classified as other mechanisms (Category 3).

II. SURVEY STUDIES (Table 1):

A. Summary:

1. Gene Mutation (Category 1):

Dieldrin was reported negative in four survey articles (Article Nos. 1, 2, 3, and 5) which reported plate incorporation assays with strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, or TA100) in the presence or absence of rat S9 activation; doses ranged from 4 to 5000 µg/mL, depending on solubility or toxicity. Similarly, dieldrin was negative in two spot tests (Article No. 4 and 7) with *S. typhimurium* strains TA98 and TA100 or TA1535, TA1536, TA1537, and TA1538 without S9 activation, at doses of 0.05 and

1 mg/disc (Article No. 4) and at 20 µg/disc (Article No. 7). The chemical was also reported negative over a 1-10,000-fold concentration range using a concentration agar gradient method (Article No. 6) with S. typhimurium strains G46, D3052, C3076, TA1535, TA1537, TA1538, TA98, and TA100 with and without rat S9 activation.

Dieldrin was negative when assayed with Escherichia coli strains WP2, WP2uvrA and/or WP2hcr (Article Nos. 3, 6, 7, and 8). The test chemical was assayed at levels up to 5000 µg/mL +/- S9 activation in a plate incorporation assay (Article No. 3); over a 1-10,000 fold dose range in the concentration agar gradient test +/-S9 activation (Article No. 6), and using the nonactivated spot test at 20 µg/disc and 1 mg/disc (Article Nos. 7 and 8).

2. Other Mutagenic Mechanisms (Category 3):

Dieldrin, assayed at levels up to 1000 µg/mL with or without S9 activation, did not cause preferential inhibition of E. coli DNA repair-deficient WP67 or CM871 in a micro-suspension assay (Article No. 5). Similarly, nonactivated 20 µg/disc dieldrin was not genotoxic in Bacillus subtilis DNA repair-deficient M45 (Article No. 7). Dieldrin (0.1 mg/mL) was assayed with E. coli cells which carry plasmid E1 (Article No. 9) to assess breakage in Col E1 plasmid DNA. The chemical did not increase the number of plasmid breaks relative to the negative control.

Dieldrin's ability to cause DNA damage in Chinese hamster V79 cells was assessed using the alkaline elution method (Article No. 10). It was assayed at nine doses ranging from 0.001 to 10 mM with S9 activation, and reported as negative.

The potential for dieldrin to affect DNA synthesis in testicular cells of male mice was examined in Article No. 11. Mice were orally administered a single dose of 50 mg/kg dieldrin. The authors reported that the chemical inhibited DNA synthesis as assessed by a decrease in uptake over the control in tritiated thymidine by testicular cells. In another article inhibition of DNA synthesis by dieldrin was also assessed in human HeLa cells but only in the presence of S9 activation (Article No. 12). The authors reported that dieldrin inhibited HeLa cell DNA-synthesis at 4×10^{-4} M, and that after the chemical was removed, the rate of DNA synthesis continued to decline. The authors suggested that the continued depression of DNA synthesis after removal of the chemical can be used to distinguish between DNA-damaging chemicals and those that inhibit DNA synthesis but do not cause DNA damage. Although we agree that the assay may distinguish DNA inhibition from DNA repair synthesis, the lack of quantitative data for example on UDS does not permit us to assess the validity of this assumption.

B. Conclusion:

The data presented in these survey articles were insufficient to form unequivocal conclusions. However, the data collectively suggest that dieldrin is not mutagenic nor does it cause DNA damage in bacteria. Although dieldrin was shown to inhibit DNA synthesis in mammalian cells, the distinction between cellular toxicity and direct damage to DNA was not clearly established.

III. STUDIES WITH PRIMARY DATA (Table 2):

1. Gene Mutation (Category 1):

a. Bacteria: In an acceptable study, Haworth et al. (Article No. 13) assayed dieldrin using a 20-minute preincubation

plate incorporation method with S. typhimurium strains TA1535, TA1537, TA98, and TA100 at dose levels of 33, 100, 333, 1000, and 3333 µg/plate with and without rat or hamster S9 activation. The chemical precipitated at the three highest doses. Dieldrin did not cause an increase in the number of histidine revertants in any of the tester strains, with or without rat or hamster S9 activation. The positive controls demonstrated the sensitivity of the assay to detect a mutagenic response.

In Article No. 14, Glatt et al. assayed dieldrin with S. typhimurium strains TA98, TA100 (+/- rat S9 activation), TA1535, and TA1537 (-S9 activation) at six doses ranging from 10 to 3000 µg/plate. In addition, strain TA98 was dosed with rat S9-activated dieldrin in the presence or absence of the epoxide hydrolase inhibitor and glutathione depletor 1,1,1-trichloropropene 2,3-oxide (TCPO). Exposure was for 3 days at 37°C using the plate incorporation method. Precipitation was observed at the three highest doses (300, 1000, and 3000 µg/plate). Dieldrin was negative in this study with or without S9 activation whether TCPO was present or absent. The positive controls demonstrated the sensitivity of the test to detect a mutagenic response. We assess that the study was well designed, properly controlled, and that dieldrin was assayed up to the limit of solubility; the study is acceptable.

Marshall et al. (Article No. 15) assayed the chemical using S. typhimurium strains TA1535, TA1536, TA1537, and TA1538 at doses from 50 to 1000 µg/plate, with or without rat S9 activation; cytotoxic levels were reported as >2500 µg/plate -S9 and >1000 µg/plate +S9, but these data were not included in the article. The authors reported that dieldrin did not induce a mutagenic response. Although the more sensitive strains, TA98 and TA100 were not evaluated, we assess that the study was properly controlled and is acceptable.

Majumdar et al. (Article No. 16) tested dieldrin with S. typhimurium strains TA1535, TA98, and TA100 using the plate incorporation method. Cells were exposed to 1, 25, and 50 µg/mL with or without S9 activation and scored at 72 hours. Cytotoxicity (decrease in the number of revertants) was reported at the highest dose when compared to the two lower doses. Dieldrin caused an increase in the number of revertants in strains TA98 and TA100 either in the presence or absence of mouse S9 activation, but only with S9 activation in strain TA1535. The highest number of revertants was induced at 25 µg/mL; however, 1 µg/mL also induced a mutagenic response that was twofold or greater than the solvent controls. We assess, however, that a repeat assay should be performed because positive controls were not included; the background frequency of the TA98 solvent controls was lower and the nonactivated solvent control for TA1535 was higher than those published by Ames et al.[1] Additionally the authors stated that the reduction in revertants at 50 µg/mL may have been due to cytotoxicity. It is noteworthy that the results of 10 other Ames assays with dieldrin, reviewed here, showed that the test material was noncytotoxic at doses up to 1000 µg/plate. The study is, therefore, considered inconclusive, and dieldrin is classified as a presumptive mutagen under mouse S9-activated conditions.

b. Mammalian Cells: Ahmed et al. (Article No. 17) assayed dieldrin for mutation at the ouabain locus in Chinese hamster lung (V79) cells. Based on a preliminary cytotoxicity test with dieldrin at doses ≥100 µM, a dose of 10 µM was chosen for the mutation assay. Nonactivated dieldrin caused

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[1] Ames, B. N., McCann, J., Yamasaki, E. "Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutat. Res. 31(1975): 347-364.

an increase in the number of revertants with 77.8% survival. 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:

1. Only a single dose of dieldrin was evaluated for mutagenicity.

2. The presumptive mutagenic response was not confirmed in a repeat assay at the concentration range expected to give dose-response data.

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

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

Although we assess that the study provides inconclusive evidence for a positive response, nonactivated dieldrin is classified as an unconfirmed presumptive mutagen in Chinese hamster lung cells.

c. Conclusion for Gene Mutation (Category 1): Dieldrin was tested in 4 different studies using the *S. typhimurium* plate incorporation assay. It was reported that dieldrin did not induce a mutagenic response with or without rat S9 activation in three acceptable studies (Article Nos. 13, 14, and 15). However, in Article No. 16, it was reported that the number of revertants increased with or without mouse S9 activation.

The weight of evidence from both survey (Table 1) and primary data (Table 2) indicates that dieldrin is not mutagenic in bacteria in the presence or absence of rat S9. However, an inconclusive presumptive positive response with or without mouse S9 activation was observed in a single assay. Hence, we assess that it is unlikely that dieldrin causes gene mutations in bacteria, unless a validated repeat assay using mouse S9 can be submitted for review.

The presumptive mutagenicity of a single nonactivated dose of dieldrin in Chinese hamster lung cells was not confirmed. Hence the evidence that dieldrin causes gene mutations in mammalian cells is inconclusive; the results of this study should be confirmed in valid mammalian gene mutation assays.

2. Chromosomal Aberrations (Category 2):

a. Somatic Cells: Majumdar et al. (Article No. 18) exposed cultured human lung cells (WI-38) to nonactivated dieldrin at 1, 10, and 30 μg/mL. Dieldrin induced significant ($p = <0.01$) dose-related increases in the number of chromosomal aberrations (chromatid breaks, fragments, chromosome interchanges, and rings) at all doses. The authors also reported a decrease in the mitotic index from 8.0 to 1.5% with increasing doses of dieldrin compared to control (11.0) and the highest dose tested resulted in a cell degeneration of 50% at 24 hrs. We assess that the dose-related response indicates a clastogenic effect. However, the assay should also be performed with S9 activation; therefore the study is inconclusive.

The same investigators administered single ip doses of 1, 30, or 50 mg/kg of dieldrin to STS mice (sex not specified). Two hours prior to the 24-hour sacrifice and bone marrow harvest, the mice received an ip injection of colcemid. The chemical caused an increase in chromosomal aberrations (chromatid

breaks, fragments, chromosome interchanges, and rings). They reported decreases in the mitotic index from 6.0 to 2.8% with increasing doses of dieldrin compared to controls (10.5). Although this assay was performed with only one cell harvest interval and without a positive control we assess that the dose-related response indicates a clastogenic effect; therefore the study is acceptable.

Dean et al. (Article No. 19) administered single oral doses of 30 or 60 mg/kg HEOD to male and female Chinese hamsters (oral LD_{50}, 120 mg/kg). At 1.5 hours prior to the 8- and 24-hour sacrifices, the mice received an ip dose of colcemid. The authors reported that the test chemical did not cause an increase in the number of chromosomal aberrations at the 8- or 24-hour intervals. However, since no clinical toxicity or cytotoxic effects were reported, and no positive control was used, this study is unacceptable.

The same investigators monitored the lymphocytes of workers in a dieldrin manufacturing plant. They screened 17 presumably non-exposed controls, 9 former and 12 current plant workers, and reported no differences in chromosomal aberrations. Levels of dieldrin and/or other manufacturing components were not reported.

b. Germinal Cells: To assess the chromosomal effects of HEOD on male germinal cells, Dean et al. (Article No. 19) performed two separate dominant lethal tests. In the first experiment, 8 male mice were administered single oral doses of HEOD at 12.5 or 25 mg/kg; the concurrent negative control group consisted of 16 animals. Each male was mated to 3 females per week for 8 weeks. The authors reported that there were no differences in the number of early fetal deaths between the dosed and the control groups; however, the pregnancy rate was low for all groups, i.e., 65.1% in the controls and 65.9

and 64.3% in the dosed groups. Fetal implantations were statistically decreased at week 1 in the high-dose group and at week 3 in the low-dose group. In addition, the authors indicated that housing conditions may have been "substandard" (although these conditions were not detailed); therefore, a second experiment was performed. Male mice were dosed once orally with 12.5, 25, or 50 mg/kg of HEOD and each male was mated to 3 females per week for 5 weeks. The authors reported that clinical toxicity was observed in the mid and high doses. Under the presumably "improved" housing conditions, the pregnancy rate for the dosed groups was ≥80.2% compared to the controls (76.6%). There was no reduction in the number of fetal implantations or increases in the number of early fetal deaths compared to controls over the 5-week mating period.

We assess that HEOD at the doses tested (12.5, 25, and 50 mg/kg) did not cause a clastogenic response in male germinal cells over a 5-week period. Although it is recommended that the dominant lethal assay be performed over an 8-week period, we assess that the study is acceptable for all stages of the spermatogenic cycle, except differentiating spermatogonia and stem cells.

Epstein et al. (Article No. 20) evaluated the chromosomal effects of dieldrin among 172 other chemicals tested on mouse germinal cells in dominant lethal assays. Dieldrin was administered to male mice as a single ip dose at 5.2 or 26 mg/kg in one experiment, and in a second experiment male mice received oral daily doses of 2 or 3 mg/kg/day for 5 consecutive days. In both experiments male mice were mated, 1 male to 3 females per week for 8 weeks. The authors reported, in both experiments, that dieldrin did not cause significant differences in the number of fetal implantations or total fetal deaths when compared to the control groups.

We assessed that at the doses tested, dieldrin did not cause any clastogenic effects in mouse germinal cells. Although no cytotoxic responses were reported, the test chemical was assayed at approximately the LD_{50} which was 30 mg/kg; 1/9 deaths occurred at 26 mg/kg, the highest dose tested. The study is acceptable.

c. Conclusion for Chromosomal Aberrations: In single unconfirmed tests dieldrin was reported to induce chromosomal aberrations in human lung cell cultures (WI-38) and mouse bone marrow cells. HEOD (99%) did not cause chromosomal aberrations in hamster bone marrow; however, there was no evidence of cytotoxicity; therefore, the study was not acceptable. Neither dieldrin nor HEOD induced chromosomal breaks in mouse germinal cells in two dominant lethal studies. Based on this evidence dieldrin may be considered presumptively clastogenic in somatic mammalian cells, requiring confirmation in adequately controlled repeat experiments.

3. Other Mutagenic Mechanism (Category 3):

a. DNA Damage/Repair: Dean et al. (Article No. 19) examined HEOD in six independent host-mediated assays in which *Saccharomyces cerevisiae* strain D4 was scored for mitotic gene conversion. In assays 1-3 male mice were dosed once orally at 25 and 50 mg/kg and inoculated ip with yeast cells. In assays 4-6, male mice were dosed orally at 5 or 10 mg/kg/day for 5 consecutive days, and on day 5 mice were inoculated ip with yeast cells. All animals were sacrificed 5 hours after inoculation. No toxic or cytotoxic effects were observed in the acute phase of the host mediated assays; however, in a concurrent dominant lethal assay, evidence of chemical intoxication was observed following the acute administration of 50 mg/kg HEOD. In the subacute assays animals receiving the chemical at 10 mg/kg/day x 5

were lethargic throughout the dosing intervals. The chemical did not cause an increase in the numbers of S. cerevisiae adenine or tryptophan convertants relative to the controls at any dose level in either experiment. We assess, therefore, that HEOD was evaluated at the appropriate doses in properly controlled host mediated assays; the study is acceptable.

Probst et al. (Article No. 21) tested dieldrin in an unscheduled DNA-synthesis (UDS) assay with primary rat hepatocytes at 8 doses ranging from 0.5 to 1000 nM/mL. Dieldrin did not cause an increase in UDS at the highest noncytotoxic dose (500 nM/ml) scored. The study was properly controlled, and is acceptable.

In another UDS assay, Klauning et al. (Article No. 22) assessed the DNA damaging potential of dieldrin in mouse hepatocytes at concentrations of 10^{-4}, 10^{-5}, and 10^{-6} M. No increase in net grains/nucleus was reported. Phenobarbital pretreated mouse hepatocytes exposed to comparable doses of dieldrin also showed no increases in net grains/nucleus. The study was properly controlled and is acceptable.

Rocchi et al. (Article No. 23) tested dieldrin's action on semiconservative DNA synthesis (SDS) to determine the 50% inhibition dose with rat thymocytes. They assayed aldrin for its potential to induce unscheduled DNA synthesis (UDS) in human lymphocytes in the presence of hydroxyurea and tritiated thymidine without metabolic activation. In addition, they tested aldrin's ability to interfere with human lymphocyte repair capability following damage exerted by ultraviolet (UV) rays.

Based on the results of the inhibition of thymidine uptake assay, dieldrin caused a 50% inhibition in rat thymocytes at a dose of 100 μg/mL; the dose was used in the UDS assay

with human cells. Induced UDS repair effects were assessed after calculations were made to eliminate the effect of SDS inhibition. Dieldrin induced an increase in UDS over the control. Similarly, with human cells damaged by UV irradiation and dosed with dieldrin (100 μg/mL), the chemical slightly interfered with UDS repair. We assess that since the observed effects were noted at only one dose, the evidence for a genotoxic response is inconclusive; therefore, the assay should be repeated with multiple doses in the presence and absence of metabolic activation.

Ahmed et al. (Article No. 24) evaluated S9 activated and nonactivated dieldrin at doses of 1, 10, and 100 μM in a UDS assay using SV-40 (virus-transformed) human cells (VA-4). The authors reported a significant ($p=<0.05$) net increase in UDS at 1 μM, (12.5±1.8 grains/nucleus) versus the control (3.5±0.9 grains/nucleus). However, the results for the mid- and high-doses were reported only as qualitative (+) positive responses with or without S9 activation after 8 hours of exposure. The lack of primary data for the mid- and high-dose prohibits an assessment of any dose-related response.

The cells used in this assay were not grown under reduced serum or arginine deprivation conditions in conjunction with hydroxyurea to insure blockage of S-phase synthesis. Several agents which are generally considered nongenotoxic or weakly genotoxic (e.g., Diquat 2,4-D fluid, and carbaryl) induced strong responses in this study. We discussed the findings of this assay with Dr. Gregory Probst,[1] Lilly Research Labs.

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[1] Probst, G. S., Lilly Research Labs., Greenfield, IN, personal communications 4/86.

He concurred with our assessment and noted that cells which may have escaped hydroxyurea treatment could easily be misinterpreted as undergoing UDS synthesis, thereby increasing the chances of a false positive.

In addition the use of a transformed cell line to assess primary DNA damage does not conform with recommended[2] procedures, which use non-transformed diploid human cells.

We classify dieldrin as an inconclusive presumptive genotoxin in SV-40 transformed human cells. We recommend confirmation of these data in a rigorously controlled experiment that conforms to the currently recommended protocols for UDS assays with human cell lines.

Ahmed et al. (Article No. 24) also assessed dieldrin's potential to cause DNA strand breakage in VA-4 cells using photolysis of BrdU at a wavelength of 313 nm light. The authors reported that the test material increased the number of strand breaks/10^8 Daltons at the 100 μM dose. Since the control data were not reported, and the use of a transformed cell line was employed, we assess the results to be inconclusive evidence for a positive response.

b. Inhibition of Spindle Apparatus:

Markaryan (Article No. 25) studied the effects of dieldrin on bone marrow cells of male mice. He reported that anaphase and early telophase harvested cells in mice injected ip with

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

0.0012 mg/g dieldrin had increased frequencies of "nuclear disturbance," total chromosome rearrangements (chromosome and chromatid bridges, and fragments), and "adhesions." We discussed the findings of this study with Dr. James Ivett,[3] and concur with his recommendation that the study is unacceptable for the following reasons:

1. The method used to prepare anaphase and telophase plates (slices of bone marrow stained with acetocarmine) may cause an increase in the number of artifacts, and lends itself to a planer effect (complete chromosomal displays are not visible in all viewing fields). This makes the distinction between true chromosomal rearrangements and artifact difficult.

2. The number of animals investigated (three male mice) do not represent a sufficient sample population.

3. The inclusion of cytotoxic effects (vacuolation, karyopycnosis, and karyolysis) in the total percent nuclear disturbances suggests that the assay dose (approximately 4% of the LD_{50}) was severely cytotoxic.

4. The slides were not reported as being coded.

We concluded, therefore, that the results are unacceptable because they cannot be interpreted, and that these findings should not be included in the genetic assessment of dieldrin.

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[3] Ivett, J. L., Hazleton Biotechnologies, Vienna, VA (personal communications), 4/86.

c. Conclusion for Other Mutagenic Mechanisms (Category 3):

Published articles that evaluated the potential of dieldrin to interact with and damage DNA provided evidence that the chemical appears not to be genotoxic in yeast, or in rat or mouse primary hepatocyte cultures. Based on the inconclusive and unconfirmed findings of a UDS assay with transformed human cells, dieldrin is listed, with reservations, as presumptively genotoxic in human cells. We caution, however, that these findings are suspect and should be confirmed. The results of the spindle mechanisms/inhibition assay performed with dieldrin could not be interpreted.

4. Epigenetic Effects:

Wade et al. (Article No. 26) reported a new technique to measure cell-to-cell communication, which they termed, "gap fluorescence redistribution after photobleaching" (FRAP). To support the ability of this method to measure interference with gap junction communication, the investigators included a known tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA), a nontumorigenic phorbol ester, 4-phorbol-12,13-didecanoate (4PD), and the pesticide, dieldrin.

Cultured human teratocarcinoma cells were labeled with 6-carboxy-fluorescein diacetate and exposed to 7 μg/mL of dieldrin (a reported noncytotoxic dose). Pairs of contacting cells were selected and one of each pair was photobleached (dye was photochemically destroyed) by an argon ion laser beam (ACAS 470 laser/imaging). Transfer or redistribution of the dye from the unbleached cell to the bleached cell was measured over time using a series of image scans.

Dieldrin blocked the fluorescence recovery of photobleached cells. Fifteen minutes after bleaching the fluorescence intensity of dieldrin-treated photobleached cells was ≈1000 units, compared to the control (≈14,000) units. It was reported; however, data were not included in article that the tumor promoter, TPA, also blocked fluorescence recovery in exposed cell cultures, and the authors stated that inhibition of transport by TPA may be related to its tumorigenicity. In contrast, the nontumorigenic phorbol ester, 4PD, did not block transport; again however, the 4PD data were not included.

Although this is a new technique that has not been fully validated, we assess these preliminary results indicate that dieldrin can inhibit junctional communication in cultured mammalian cells.

IV. OVERALL INTERPRETATION OF RESULTS AND RECOMMENDATIONS:

1. Interpretation:

The published articles reviewed are not considered sufficient to establish a complete genetic toxicology profile for dieldrin. However, certain consistencies emerge which allow us to make reasonable interpretations and to recommend further studies which might generate useful data.

Dieldrin was adequately tested in three acceptable Ames assays with sufficient primary data to conclude that nonactivated and rat or hamster S9-activated dieldrin is not mutagenic in *S. typhimurium*. The results of the seven survey Ames tests with dieldrin support this conclusion. However, based on the inconclusive results of a single Ames test, dieldrin, in the presence of mouse S9 activation, is considered presumptively mutagenic in *Salmonella*. We caution, however, that this finding

has not been confirmed and technical deficiencies preclude acceptance of the results as valid.

The three E. coli reverse mutation survey studies with dieldrin further support the conclusion that the chemical is not mutagenic in procaryotes.

In the single published mammalian gene mutation assay, non-activated dieldrin induced an inconclusive, mutagenic response in Chinese hamster V79 cells. The validity of this assay was seriously compromised because of technical deficiencies, reporting of "positive" responses with compounds that have been previously reported as either weakly mutagenic or not mutagenic, and the lack of a positive control. Although we classified dieldrin 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 mutagens as positive controls.

Although the majority of doses assayed were cytotoxic, dieldrin elicited dose-related clastogenic responses in both cultured human lung and mouse bone marrow cells. Dieldrin, therefore, may be considered weakly clastogenic in mammalian somatic cells.

By contrast, dieldrin administered by gavage or ip proved to be nonclastogenic in male germinal cells; HEOD, a major component of dieldrin, was also negative in male germinal cells.

Survey repair studies indicated that dieldrin does not induce primary DNA damage in bacteria. Data from studies with sufficient primary data to draw meaningful conclusions also indicated that dieldrin was not genotoxic in yeast or primary rat and mouse hepatocytes.

However, both S9-activated and nonactivated dieldrin induced a statistically significant increase in UDS in SV-40 transformed human fibroblasts. The validity of this assay is questionable because of numerous technical deficiencies, the reporting of strong "positive" responses by weakly genotoxic or nongenotoxic substances, and the lack of acknowledged positive controls.

It is noteworthy that all assays reporting that dieldrin adversely affects genetic material were either flawed by inadequate study designs (Article Nos. 16, 17, and 26) or showed the greatest activity at cytotoxic doses (Article No. 18).

In a promising new technique, Wade et al. tested dieldrin against the known tumor promoter TPA to demonstrate the effectiveness of the FRAP technology to detect interference with cellular communication. We assess that their results suggest that dieldrin may act by an epigenetic mechanism possessing promotional activity.

2. Recommendations:

Although the evidence from the majority of available published articles with procaryotic gene mutation assays shows that dieldrin does not induce gene mutations, its mutagenic potential for *S. typhimurium* with or without mouse S9-activation has not been fully investigated. Likewise, mutations of Chinese hamster V79 cells to ouabain resistance with or without mouse S9 should be validated. A reassessment of UDS using human diploid cell lines in a well controlled experiment would also be useful in completing information which, at present, constitutes a data gap.

We assess that the most useful information, however, will come from experiments on promotion activity. Wade et al. demonstrated that dieldrin may interfere with intercellular communication.

The following tests are recommended to confirm the potential promotional activity of the test material:

1. *In vitro* transformation with promotional assay

2. *In vivo* promoting activity assay

3. *In vitro* UDS assays with SV-40 (Simian virus) transformed cells (VA-4) and non-transformed human cells.

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