2. Toxic Effects in Animals

2.1 General Toxicity

2.1.1 Acute Toxicity

Table 2.1.1 summarizes acute toxicity data for aldrin and dieldrin. The LD_{50}'s vary with the concentration (Barnes and Heath 1964) and the vehicle used (Heath and Vandekar 1964). Organic solvents and vegetable oils increase the toxicity by enhancing the rate of absorption of toxicant into the body (Jager 1970). The toxicity also varies between species, as shown in Table 2.1.2 for dieldrin and its photoisomerization product, photodieldrin.

The main signs of acute aldrin and dieldrin intoxication are increased irritability and tremor, followed by tonic-clonic convulsions, with the central nervous system as the principal site of action. Rats injected with nonlethal doses were found to recover fully and to show no delayed effects (Heath and Vandekar 1964).

2.1.2. Factors Modifying Toxicity

In addition to interspecific differences in susceptibility, the toxicities of aldrin and dieldrin vary according to the mode of administration: highest toxicity via the intravenous route, lower via the oral route, and lowest from dermal application. Studies by Treon and Cleveland (1955) and Heath and Vandekar (1964) indicated that aldrin and dieldrin are slightly more toxic to female than to male rats (see Table 2.1.1). Table 2.1.2 shows this also to be the case for dogs.

TABLE 2.1.1

ACUTE TOXICITY OF ALDRIN AND DIELDRIN IN EXPERIMENTAL ANIMALS

Species	Strain	Sex	Route	Formulation	LD_{50} (mg/kg)	
					Aldrin	Dieldrin
Rat	Wistar	F	Oral	Arachis oil	-	50.8
Rat	Wistar	M	Oral	Arachis oil	-	63.5
Rat	CFE*		Oral	Peanut oil	45.9	38.3
Rat	CFE		Oral	Arom. solv.	18.8	-
Rat	CFE		Oral	40% emulsifiable conc.	56.4	-
Rat	CFE		Oral	40% wettable powder	62.5	-
Rat	CFE		Oral	75% dust conc.	72.2	-
Rat	CFE		Oral	2.5% field strength dust	109.0	-
Rat	CFE		Oral	20% emulsifiable conc.	-	55.9
Rat	CFE		Oral	50% wettable powder	-	52.1
Mouse	-		Oral	-	95	75-100
Mouse	-		Oral	-	44	38
Guinea pig	-		Oral	-	33	49-59
Rabbit	-		Oral	-	50-80	45-50
Dog	-		Oral	-	65-95	56-80
Sheep	-		Oral	-	-	50-75
Rat	CFE		Dermal	40% emulsifiable conc.	194.0	-
Rat	CFE		Dermal	40% wettable powder	274.0	-
Rat	CFE		Dermal	75% dust conc.	269.0	-
Rat	CFE		Dermal	2.5% field dust strength	<100.0	-
Rat	CFE	M	Dermal	20% emulsifiable conc.	-	213.8
Rat	CFE	F	Dermal	20% emulsifiable conc.	-	119.9
Rat	CFE		Dermal	50% wettable powder	-	213.4
Rat	Wistar	F	ip	Glycerol	-	55.9
Rat	Wistar	F	iv	Glycerol	-	8.9
Mouse	-		iv	-	21.5	15.2

*Carworth Farm E strain

Adapted from Jager 1970

TABLE 2.1.2

THE LD_{50}'s OF PHOTODIELDRIN AND DIELDRIN

Species	Approximate LD_{50} (mg/kg)	
	Photodieldrin	Dieldrin
Rat	10	47
Mouse	7	77
Guinea pig	3	24
Dog (M)	140	120
Dog (F)	100	90
Chicken	80	48
Pigeon	90	250

Adapted from FAO/WHO 1971

Another factor modifying the toxicity of aldrin/dieldrin is diet. Heath and Vandekar (1964) found that rats underfed for a prolonged period before dosing were considerably more susceptible to toxic doses of aldrin and dieldrin than those fed normally, probably because of lower storage capacity in adipose tissue and consequently higher dieldrin levels in the blood and in the central nervous system.

2.1.3. Mode of Action

The exact mode of action of aldrin and dieldrin on the central nervous system is still not fully understood. Hathway and Mallinson (1964) re-

ported that the action of dieldrin leads to liberation of ammonia in the brain before convulsions begin and throughout their course. They suggested that dieldrin may inhibit glutamine synthesis in the brain.

Studies conducted on cockroaches suggest that a metabolite, probably trans-6,7-dihydroxydihydroaldrin, may be the active neurotoxic agent, because this metabolite caused an immediate reaction when applied to isolated nerve axons, whereas dieldrin elicited a weaker response which occurred only after a delay (Wang et al 1971).

2.1.4 Effects Observed in Long-Term Feeding Studies

In a study of slightly more than 15 months duration, dogs fed diets containing either aldrin or dieldrin at 1 and 3 ppm survived the entire test period. Increased liver weights were observed in the dogs fed the diets with dieldrin at 1 and 3 ppm and in those given aldrin at 3 ppm. Minor liver cell changes were seen in dogs fed aldrin at 3 ppm, but none were seen in dogs fed dieldrin (Treon and Cleveland 1955).

In a 2-year study conducted by Fitzhugh et al (1964) dogs were fed aldrin or dieldrin in the diet at dosages of 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0 mg/kg. A "no-effect level" of 0.2 mg/kg (equivalent to about 6 ppm in the diet) was established for both compounds. Neither clinical nor histopathologic abnormalities were reported at this dose level. At 0.5 mg/kg, the dogs suffered from convulsions; 4/4 dogs fed aldrin and 1/4 dogs fed dieldrin died during the 2-year exposure period. At higher dose levels, all dogs died within 49 weeks. At autopsy they displayed fatty changes in their livers and renal tubules.

A male and a female dog were exposed to dietary dieldrin at a rate of 0.2 mg/kg/day for 5 years. The only effects observed were increased serum alkaline phosphatase activity in both animals and increased bromosulphthalein clearance in the male. The latter change was considered a sign of stimulation of miscrosomal enzyme activity (Jager 1970).

In a 2-year study, Walker et al (1969) daily gave groups of five male and five female beagles capsules containing 0.05 mg/kg doses of recrystallized dieldrin in olive oil. Control dogs received capsules containing olive oil only. General health, behavior, and body weight were unaffected in each group. Electroencephalographic recordings showed no difference between the control and the high dose group. The results of hematologic studies and urinalyses were similar in all groups. The serum alkaline phosphatase level in the dogs given 0.05 mg/kg was higher after the 18th week than in the controls. At autopsy the only significant finding was an increase in the liver-to-body weight ratio in the high dose group. This increase was not associated with any histologic anomaly.

Treon and Cleveland (1955) fed male and female rats diets containing either aldrin or dieldrin at 2.5, 12.5, and 25 ppm for up to 2 years. A slight increase in the ratio of liver weight to body weight was observed in both males and females, even at the lowest dose level. Liver lesions were recorded for animals in all of the experimental groups.

In a feeding study conducted by Fitzhugh et al (1964), male and female rats received diets containing aldrin or dieldrin at 0.5, 2, 10,

50, 100, and 150 ppm for 2 years. The rats exposed at 50 ppm and above suffered a dose-related reduction in lifespan and a high incidence of nephritis and microscopic liver lesions described as characteristic results of exposure to chlorinated hydrocarbons. Increased liver weight and "minimal" liver cell changes were observed even in the rats fed aldrin or dieldrin at 0.5 ppm. However, 0.5 ppm was considered a "minimal effect level" and was used as the basis for WHO's Acceptable Daily Intake (FAO/WHO 1971). For further evaluation of this experiment see Sections 2.2.1, 2.2.2, and 2.5.

Walker et al (1969) fed recrystallized dieldrin (99% purity) at dietary levels of 0.1, 1.0, or 10 ppm to groups of 25 male and 25 female rats for periods up to 2 years. Forty-five males and 45 females served as controls, but the control diet contained dieldrin at 0.026 ppm. Body weights and food intakes were unaffected by the added dieldrin, but at 10 ppm all the animals became irritable after 8-13 weeks; occasional convulsions occurred in this group during handling. No adverse effect on survival was observed. Liver weights were normal for the first 18 months, but after 2 years increased liver weights and liver-to-body weight ratios were observed in the groups fed 10 ppm. For further evaluation of this experiment see Section 2.5.

A series of long-term feeding studies with recrystallized dieldrin (99% pure) in CFI mice was conducted at Tunstall Laboratory (Walker et al 1972; Thorpe and Walker 1973). The most striking effect was a dose-related increase of liver tumors in all experiments (for details

see Section 2.5). Survival was markedly reduced in mice receiving dieldrin at 10 and 5 ppm and in female mice at 2.5 ppm, but not in males at 2.5 ppm or in either sex at lower concentrations (Figure 2.1.1).

Murphy and Korschgen (1970) conducted 3-year feeding tests on white-tailed deer. Groups of 10 deer and their progeny were given dieldrin at 5 or 25 ppm. No signs of overt intoxication were observed, and 9 or 10 adult deer in each group survived the 3 years. Growth was slower and remained reduced in dieldrin-exposed females that were immature when the study began. Hematologic values and serum protein concentrations were not significantly related to treatment. Liver-to-body weight ratios were significantly larger in deer given dieldrin at 25 ppm, and pituitary glands were smaller and thyroids were larger in deer fed dieldrin.

Male rhesus monkeys were fed dieldrin at 0.1, 0.5, 1.0, 1.75, and 5.0 ppm (0.002-0.07 mg/kg/d) in the diet for about 6 years. No significant liver changes were observed at dietary levels below 1.0 ppm. A dose-related increase in microsomal P450 was found at the higher levels, and increased microsomal enzyme activity was observed at the 1.0 and 1.75 ppm levels, but no changes in subcellular structure were found (Wright 1974, Jager 1970).

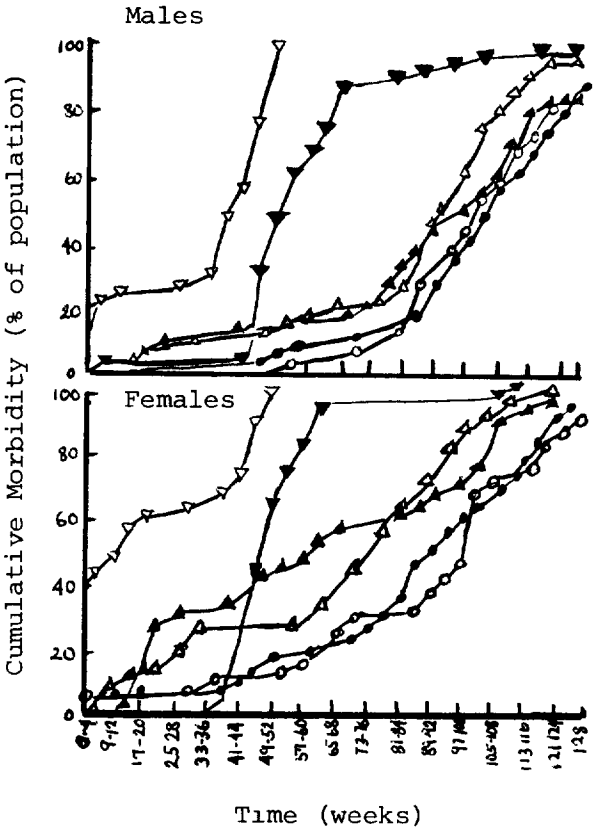
2.2 Organ-Specific Effects

2.2.1 Liver Effects

Histologic changes occur in the livers of rats given repeated doses of certain chlorinated hydrocarbon compounds. These changes have been studied in rats exposed to DDT or to dieldrin and appear to be similar

FIGURE 2.1.1 (Walker et al 1972)

CUMULATIVE MORBIDITY OF MICE FED DIELDRIN
AT LEVELS OF 0 (●), 1.25 (○), 2.5 (▲), 5 (△),
10 (▼), OR 20(▽) PPM



for each compound (USDHEW 1969). The histologic changes in the parenchymal cells of the liver consist of increased deposition of fat, margination of cytoplasmic granules, and hypertrophy of cells. The most characteristic change is the formation of complex lipoid cytoplasmic inclusion bodies termed "lipospheres." In rats fed dieldrin, structural changes in the liver included an increase in the amount of smooth endoplasmic reticulum (SER) associated with induction of microsomal enzymes (Wright 1974). Fitzhugh et al (1964) reported that the severity of these histopathologic changes increased progressively with dosage of both aldrin and dieldrin (see Table 2.2.1).

Kimbrough et al (1971) found morphologic changes, including an increase in SER and atypical mitochondria, in adult male rats fed technical grade dieldrin at 50 or 100 ppm for 8 weeks. Effects were more pronounced in rats fed DDT and dieldrin simultaneously.

In comparative studies, single doses of dieldrin caused a marked proliferation of SER in the livers of rats given 8 mg/kg and in dogs given 2 mg/kg but caused less marked effects in mice given 0.16-7.5 mg/kg. Increases in SER were accompanied by increased microsomal enzyme activity (see Section 2.2.2). In mice, an increase in liver DNA content indicated cell proliferation, whereas rats and dogs showed cellular hypertrophy (Jager 1970; Wright 1974).

Liver lesions induced in mice and rats by aldrin and dieldrin after long-term feeding (15-26 months) have been re-evaluated by Reuber (1974, 1975, 1976). He reported that the lesions spanned all stages of de-

TABLE 2.2.1

CHARACTERISTIC "CHLORINATED INSECTICIDE" CHANGES IN LIVERS OF RATS FED ALDRIN OR DIELDRIN

Dietary Concentration (ppm)	Degree of Liver Change*						Number of Livers Sectioned
	N	T	VS	S	S-M&M	>M	
Control							
None	16	1	0	0	0	0	17
Aldrin							
0.5	15	4	0	0	0	0	19
2	10	8	0	1	0	0	19
10	11	3	7	1	0	0	22
50	0	0	0	6	10	2	18
100	0	0	0	0	5	6	11
150	0	0	0	0	2	7	9
Dieldrin							
0.5	17	4	0	1	0	0	22
2	12	5	5	1	0	0	23
10	7	7	3	1	0	0	18
50	0	0	3	8	6	3	20
100	0	0	1	1	8	8	18
150	0	0	0	1	5	5	11

*N = none, T = trace or minimal, VS = very slight, S = slight, and M = moderate; figures based on microscopic sections

Adapted from Fitzhugh et al 1964

velopment from hypertrophy, through areas or foci of hyperplasia to hyperplastic nodules and well differentiated or moderately well differentiated hepatocellular carcinomas. Reuber (1976) described and illustrated stages in this progressive development. For further details see Section 2.5.

2.2.2 Liver Microsomal Enzymes

Many compounds, including phenobarbital and chlorianted hydrocarbon insecticides such as DDT and dieldrin, stimulate the drug-metabolizing enzyme system of the liver and are frequently associated with liver enlargement (Fouts 1963, Hard and Fouts 1963, Hart et al 1963, Gilbert and Goldberg 1967, Conney et al 1967, Remmer 1967, Kupfer 1967, Rubin et al 1968, Kimbrough et al 1968, Conney 1967, 1969). The increased activity of microsomal enzymes leads to enhanced rates of metabolism of steroid hormones (see Section 2.3.8) and other chemicals, including dieldrin itself (Oberholser et al 1977). Triolo and Coon (1966a,b) reported that a single dose of aldrin and dieldrin at 1 mg/kg body weight administered to rats caused a reduction in the toxicity of parathion, paraoxan, and several other organophosphates administered 4 days later. This effect was attributed to enhance metabolism of the organophosphates.

Rats fed 200 ppm of dieldrin in the diet showed cellular changes observable with electron microscopy within 24 hours. These changes were correlated with an increased activity of certain enzymes, such as those capable of hydroxylating aniline or catalyzing the o-dealkylation of chlorfenvinphos, but the activity of other enzymes such as acid phosphatase or glucose-6-phosphatase did not change (Jager 1970).

In dogs fed dieldrin at 0.05 mg/kg/day for 2 years, cellular changes observable by electron microscopy were similar to those found in rats (Jager 1970, Wright 1974). In the dog, however, unlike in the rat, increased activity of liver and serum alkaline phosphatases was observed at the highest dose level (Walker et al 1969). Street et al (1969) reported a dietary "threshold" level for hepatic enzyme induction by dieldrin of 1 ppm after 2 weeks oral administration to rats, compared with 5 ppm for DDT. Den Tonkelaar and van Esch (1974) found markedly stimulated aminopyrine demethylase activity in male rats fed dieldrin for 2 weeks at 2 ppm, the lowest dietary concentration tested.

2.2.3 Kidney Effects

Fitzhugh et al (1964) observed a marked increase in kidney lesions, diagnosed as slight or moderate nephritis, in rats given aldrin or dieldrin at 50-150 ppm for 2 years (Table 2.2.2). In examining kidney

TABLE 2.2.2

GROSS AND MICROSCOPIC PATHOLOGY IN THE KIDNEYS OF RATS EXPOSED TO ALDRIN OR DIELDRIN FOR 2 YEARS

Dietary Concentration (ppm)	% Survival		No. of Livers Sectioned	Nephritis	
	18 mo	24 mo		S or M	>M
Control					
None	75	50	17	6	1
Aldrin					
0.5	75	50	19	4	2
2	83	50	19	4	3
10	67	42	22	8	1
50	63	25	18	1	2
100	42	17	11	4	5
150	17	4	9	3	2
Dieldrin					
0.5	79	42	22	5	3
2	88	63	23	6	0
10	79	25	18	6	1
50	67	21	20	6	3
100	50	13	18	2	8
150	21	4	11	7	1

*S = slight and M = moderate; figures based on the microscopic sections, except for the inclusion of one markedly damaged kidney, based on gross appearance only, in the 100 ppm aldrin group

Adapted from Fitzhugh et al 1964

sections from this study, Reuber (1974) found that chronic nephritis occurred more commonly in males than females and was very common at high dose levels, particularly of dieldrin; he suggested that the figures in Table 2.2.2 were probably underestimates because of the high mortality after 1 year, when the nephritis was unsually seen. Hemorrhagic and distended urinary bladders were usually associated with severe nephritis and were only seen in males that died. Reuber also found several cases of acute renal necrosis, especially in females, hyperplasia of the renal tubules, and one hyperplastic nodule.

2.2.4 Central Nervous System and Peripheral Motor Effects

As discussed in Section 2.1.3, the effects of aldrin and dieldrin on the central nervous system are not completely understood. Experiments involving long-term dietary exposure have demonstrated a variety of effects, including nonspecific neural lesions, impairment of learning, increased chronaxy, and impairment of muscular performance.

Harr et al (1970b) administered purified dieldrin at concentrations between 0.08 and 40 ppm in the diet to Wistar rats for up to 2 years. They reported nonspecific neural lesions, cranial edema, dieldrin residues in the brain, and convulsions in most exposed rats. No functional effects were observed at dietary concertrations below 2.1 ppm dieldrin, although cranial edema was observed at 0.63 ppm, and cerebral, cerebellar, brain-stem, and vascular lesions were at all dietary levels down to 0.08 ppm. Dieldrin residue levels of 9-11 ppm in the brain were associated with convulsions.

Smith et al (1976) exposed two groups of seven squirrel monkeys to technical dieldrin at two oral dose levels, 0.1 and 0.01 mg/kg/day. Two zero-dose controls were included. After 55 days the higher dose group was shifted to zero exposure and the lower dose group was shifted to exposure at the high dose; controls continued at zero exposure. The new regimens were continued for 54 days. The monkeys were presented with a visual nonspatial successive discrimination reversal task. During the first 55 days, before the change in regimen, control and low-dose monkeys learned the task, whereas high-dose monkeys did not ($P<0.001$). During the subsequent 54 days, the performance of each group remained approximately at the level achieved before the change in regimen. It was concluded that the high dose disrupted learning without affecting retention of the learned task. The authors suggested that this effect could be attributed to disruption of hippocampal activity. The low dose had no effect on task acquisition or retention.

Al-Hachim (1971) reported apparent effects of prenatal exposure to aldrin on the central nervous system of mice. The 38-day-old offspring of mice given aldrin at 2 or 4 mg/kg/day orally for 7 days during the third stage of gestation showed a significant reduction in body weight and a significant increase in electroshock seizure threshold compared to controls.

London and Pallade (1964) exposed rats to aldrin at 3 mg/kg/day in the diet for 6 months and then at 4.5 mg/kg/day for 7 months. They measured chronaxy by applying an electric current to the tails of the

rats and measuring the duration of the voltage pulse required to elicit a withdrawal response. They found that chronaxy was longer in the rats exposed to aldrin than in controls.

Khairy (1960) studied the effects of dieldrin exposure on the muscular performance of rats given diets containing the substance at 25 or 50 ppm for 60 days. The criterion for muscular performance was the time taken to pull a weight along a 250-cm runway. He observed a progressive deterioration of performance related to the amount of dieldrin administered. Jager (1970) reported that the gastrocnemius muscle of rats receiving dieldrin at 50 ppm in the diet for 7 months failed to maintain a tetanus comparable to that of control rats.

Medved' et al (1964) reported that, in cats, feeding of aldrin at 1 mg/kg/day or inhalation of aldrin at 0.1 mg/m^3 for an unspecified period caused marked lowering of conditioned reflexes and of unconditioned orientation reflexes. These reflexes required as much as 6-8 days to return to normal after exposure.

2.2.5 Effects in Other Organs

In 39- to 140-day-old female Wistar rats fed dieldrin at 2.5-10.0 ppm in the diet, Harr et al (1970a) found proliferation of reticuloendothelial components and pancreatic ductal cells. Fibrinoid degeneration, arteritis, endothelial proliferation, and perivascular edema were seen in small to medium sized arteries.

Although no data were found on dieldrin's effects on the mammalian thyroid, Jefferies and French (1972) reported that dieldrin

at 1, 2, and 4 mg/kg/day produced hyperplastic goiters in the thyroids of pigeons. In a visual examination, they observed that the thyroids were significantly enlarged and had small follicles with decreased amounts of colloid, epithelial hyperplasia, and vascular congestion.

2.3 Effects on Reproduction

2.3.1 In Mice

Good and Ware (1969) studied the effects of technical dieldrin on reproduction in 101 pairs of CFW mice, with a similar number of controls. At a dietary level of 5 ppm, dieldrin did not affect maternal mortality, fertility (defined as the percentage of pairs producing young), or fecundity (defined as the number of young per producing pair) but significantly reduced the average size of litters.

Virgo and Bellward (1975 a,b) studied the effects of technical dieldrin at dietary levels of 0, 2.5, 5, 10, 15, 20, and 25 ppm in SWV mice. Virgin and diparous females were studied. Dietary levels of 20 and 25 ppm caused maternal mortality. At levels up to 15 ppm, dieldrin had no effect on the incidence of breeding in parous females, nor did it affect fetal survival, the duration of gestation, or parturition. Levels of 10-15 ppm reduced fertility by 18% and there was a dose-related reduction (maximum 17%) in litter size. The reductions in fertility and litter size resulted from a lesion or lesions preceding implantation. Dieldrin exposure resulted in preweaning losses of entire litters in 100% of litters from dams exposed to dieldrin at 10 ppm or higher, 80% of litters from dams exposed

at 5 ppm, and 47% at 2.5 ppm, versus 31% in controls. Maternal cannibalism and neglect of pups were responsible for deaths in litters of dams fed dieldrin at 15 ppm or more, although it was considered that most of these pups would have died from toxic effects of dieldrin anyway.

In a six-generation study using Swiss mice exposed either to aldrin (3, 5, 10, and 25 ppm) or to dieldrin (3, 10, and 25 ppm), the principal adverse effects were on lactation indices and the viability of pups. Dieldrin at 10 ppm did not affect fertility in the mice in the first mating but decreased it by 31% in the second mating. This dose did not reduce litter size, and reproduction was not substantially affected at 3 ppm. The authors suggested that the adverse effects may have been mediated through hormonal imbalance rather than direct toxicity (Deichmann and Keplinger 1966, Keplinger et al 1970, Deichmann and MacDonald 1971).

2.3.2 In Rats

Results of a three-generation reproduction study in Carworth rats have been reported in summary form only (Treon and Cleveland 1955, Cleveland 1966). Aldrin or dieldrin was fed to rats at dietary levels of 0, 2.5, 12.5, and 25 ppm, and two sets of offspring were obtained from each generation. There was no reported effect on the number of pups per litter nor on the weight of the young rats at weaning. "Initially aldrin in the diet at levels of 12.5 ppm or higher and dieldrin at 2.5 ppm or higher appeared to reduce the

number of pregnancies in these rats. However, this effect tended to diminish to the point of disappearance when the feeding of aldrin at 12.5 ppm or lower was maintained over several generations." Incorporation of aldrin or dieldrin into the diet of parent rats during the period of suckling increased mortality among the offspring, the effect being "slight to moderate" at 2.5 ppm but higher at 12.5 or 25 ppm.

In a factorially designed experiment, Wistar rats weaned at 28 days were placed on diets containing dieldrin at a concentration of 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, or 40 ppm (Harr et al 1970a). Twenty males and 20 females were included in each of the 10 exposure groups and in a control group. The animals were killed at various intervals ranging up to 750 days. Values for dam survival, conception rate, pup survival, and weaned litter size were normal in rats fed dieldrin at 0.08 and 0.16 ppm. In rats fed 0.31-1.25 ppm, there was a slight reduction in the survival of litters and a marked reduction in conception rates (to 73% in the first mating and 33% in the second mating). At dietary levels of 2.5-10 ppm, females survived to breeding age, but nursing pups either died in convulsions or starved. The calculated maximum dietary concentration of dieldrin consistent with normal reproduction was 0.26 ppm in the first breeding and 0.09 ppm in the second breeding. Computed dietary levels of dieldrin associated with various effects are shown in Table 2.3.1.

TABLE 2.3.1

DIETARY LEVELS OF DIELDRIN ASSOCIATED WITH VARIOUS LEVELS OF REPRODUCTIVE PERFORMANCE IN RATS

Measure of Reproductive Performance	Calculated Dietary Levels (ppm)	
	1st Breeding (146-day-old rats)	2nd Breeding (336-day-old rats)
50% dam survival	27.0	16.0
80% dam survival	21.0	9.0
*100% dam survival	10.0	5.0
30% conception rate	13.75	2.88
70% conception rate	2.50	0.24
*90% conception rate	0.36	0.09
3 pups of litter weaned	6.36	2.34
7 pups of litter weaned	1.39	0.50
*9 pups of litter weaned	0.26	0.26

*Normal

Adapted from Harr et al (1970a)

2.3.3 In Dogs

Kitselman (1953) exposed 9 male and 11 female dogs to recrystallized aldrin or dieldrin in food at doses of 0.2, 0.6, and 2.0 mg/kg/day in the diet for up to 1 year. Of the 11 exposed females, 9 became pregnant and came to term, but only 7 pups survived (4/4 from a bitch given dieldrin at 0.2 mg/kg, 2/5 from a bitch given aldrin at 0.2 mg/kg, and 1/5 from a bitch given aldrin at 0.6 mg/kg). The other 32 pups were either stillborn or died within 3 days after birth.

Deichmann and MacDonald (1971) and Deichmann et al (1971) exposed beagle dogs by capsule to technical aldrin at 0.15 mg/kg (four females), aldrin at 0.3 mg/kg (four males and three females), or a mixture of aldrin at 0.15 mg/kg plus 6 mg/kg recrystallized p,p'-DDT at 6 mg/kg (four males and four females). Capsules were given 5 days/week for 14 months, and then the dogs were maintained for up to 9 months on uncontaminated diets. Reproduction was severely affected in all three treatment groups, as evidenced by reduced fertility, reduced mammary development, and impaired lactation in the females, and stillbirths and increased mortality of pups. Only 26 pups were raised to weaning in 17 breeding attempts, compared to 78 pups from 18 control females.

2.3.4 In Raccoons

Frederickson (1973) reported on a study of the effects of chronic exposure to dieldrin on reproduction in raccoons. At 2.2 ppm in the diet (wet weight, corresponding to 6 ppm dry weight) most of the females died "under the stress of breeding." At 0.73 ppm in the diet statistically significant adverse effects on the estrous cycle and on the incidence of pregnancy were reported. Fetal death, resorption of embryos, and reduced litter size were also reported. The exposed females produced only 20% of the number of young produced by unexposed females. Seven of 15 exposed females failed to respond normally to the sexual behavior of males. Adverse effects were also noted in males, on the production of sperm, the quality of sperm, and total fertility.

2.3.5 In Rabbits

Wild-trapped cottontail rabbits were confined in 1-acre pens and exposed to granular dieldrin applied at 0.5 or 2.0 lb/acre (Malecki et al 1974). No significant effects were recorded on testis weight, date of breeding, ovulation rates, preimplantation losses, resorption of embryos, or embryonic litter size. No adequate measures of post-natal mortality were obtained. Precise exposure levels were not determined, but food plants contained dieldrin at 0.07-0.25 ppm, and brain residues of dieldrin in the rabbits were in the range 0.11-0.66 ppm.

2.3.6 In Sheep

Thirty-six ewes were fed dieldrin at 0, 1, 5, or 25 ppm during 40 months, which included two gestation periods (FAO/WHO 1971). Reproductive success was apparently normal at exposure concentrations up to 5 ppm. However, at 25 ppm the lambs died shortly after birth.

2.3.7 In Deer

Murphy and Korschgen (1970) studied reproduction in white-tailed deer exposed to dieldrin at dietary levels of 0 (controls), 5, and 25 ppm for 3 years. Ten females (five yearlings, five fawns) were included in each exposure group. No effects on conception rates or on mortality in utero were reported. Fawns from does fed dieldrin at 25 ppm were smaller at birth, and the number of postpartum deaths was increased in the 5 and 25 ppm exposure groups. The fertility of male progeny was not affected. Weight gains of fawns born to does exposed to dieldrin were significantly reduced.

2.3.8 Effects on Steroid Hormones

In a number of experiments dieldrin induced hepatic microsomal enzymes that can enhance the metabolism of steroid hormones such as testosterone and progesterone. Such effects have been reported even at dietary levels as low as 2 ppm (Conney et al 1967, Thomas and Lloyd 1973). Schein and Thomas (1975) showed that administration of dieldrin in five daily doses of 2.5 mg/kg or in one dose of 10 mg/kg increased hepatic microsomal protein and cytochome P450 concentration, increased testosterone hydroxylase activity, and altered the metabolism of testosterone in the liver and prostate of mice. These effects were enhanced by simultaneous or subsequent exposure to parathion (Schein and Thomas 1976). Similar enhancement of dieldrin effects on the metabolism of testosterone by exposure to carbaryl was reported by Schein et al (1976).

Exposure to dieldrin reduced the ability of the prostate gland of rats to assimilate testosterone both in vivo and in vitro (Blend and Schmidt 1971, Thomas et al 1973, 1975). Wakeling et al (1972) reported that dieldrin interfered with the binding of dihydrotestosterone to male sex hormone receptors in the nuclear and cytosol fractions of the rat prostate. Further studies by Wakeling and Visek (1973) suggested that the dieldrin interferes with the in vitro binding of 5-alpha-dihydrotestosterone to its androphilic molecule by a mechanism involving noncompetitive inhibition. Schein and Thomas (1975) showed that dieldrin at 1.25, 2.5, or 5 mg/kg/day for 5 days significantly reduced the total

uptake and subsequent metabolism of androgens in the anterior prostate of the mouse. Dieldrin at concentrations as low as 4 x 10^{-7}M in vitro effectively decreased the formation of dehydrotestosterone in the mouse anterior prostate and of androstanediol in the rat ventral prostate.

In addition to the actions of dieldrin upon the prostate glands of rodents, it apparently produces changes in the levels of serum luteinizing hormone (LH) (Blend and Lehnert 1973). Levels of LH were not affected when the rats were fed dieldrin at 0.7 ppm. At 6.2 ppm, dieldrin produced significant elevations in serum LH. Even in castrated rats, dieldrin (6.2 ppm) caused an increase in the serum levels of this gonadotropin. Dieldrin also caused a slight decrease in the ratio of body weight to pituitary gland weight and a small increase in the ratio of body weight to prostate gland weight. Blend and Lehnert (1973) suggested that the smaller prostate glands observed in the treated (0.7 ppm) rats were possibly caused by an action of dieldrin on hepatic microsomal enzymes that effectively lowered the levels of circulating androgens. These lowered androgen levels in the blood might account for the observed decreases in accessory sex organ weights.

2.4 Teratogenesis

Ottolenghi et al (1974) administered aldrin and dieldrin at approximately one-half the lethal dose to pregnant Syrian golden hamsters and to pregnant CD1 mice. Hamsters given aldrin at 50 mg/kg or dieldrin at 30 mg/kg by oral intubation on day 7, 8, or 9 of gestation showed

a high incidence of fetal death, growth retardation, and congenital abnormalities. The most frequent abnormalities were open eyes, webbed feet, cleft palate, cleft lip, and fused ribs. Dieldrin also induced a smaller number of other defects including exencephaly, platycrania, micrognathia, and ectrodactyly. Hamster pups exposed on day 8 of intrauterine development showed a much higher incidence of abnormalities (22% for aldrin, 33% for dieldrin) than those exposed on day 7 or 9. The same type of defects were induced in mice given aldrin at 25 mg/kg or dieldrin at 15 mg/kg by oral intubation on day 9 of gestation. The percentages were 33% for aldrin and 17% for dieldrin, versus zero in controls.

Chernoff et al (1975) administered dieldrin at doses of 1.5, 3.0, and 6.0 mg/kg to pregnant CD1 mice and CD rats by gastric intubation on days 7-16 of gestation. The highest dose caused maternal death and weight loss in rats but did not cause fetal mortality or anomalies. In mice, all three doses induced an increase in the number of supernumerary ribs in one of two experiments; the increase was statistically significant at 3 and 6 mg/kg. The 6 mg/kg dose also induced a decrease in the number of caudal ossification centers. The authors did not regard these as teratogenic effects. Photodieldrin administered at 0.15, 0.30, and 0.60 mg/kg induced no fetal toxicity or anomalies in rats and mice.

Boucard et al (1970) reported that dieldrin was teratogenic in rats and mice, but the incidence of anomalies was low in all exposed

groups. When data from three dosage regimens (day 6, days 6-14, and days 1-14) are pooled, the incidences in rats were: control, 1/1,336; 2.5 µg/kg/day, 4/655; 3.4 mg/kg/day, 5/617. In mice, the incidences were: control, 2/1,123; 2.5 µg/kg/day, 4/475; 3.4 mg/kg/day, 4/510. These results suggest that dieldrin has a weak teratogenic effect, but the effect was not statistically significant in any one treatment group. The malformations observed in the exposed animals included hydrocephaly (5), hydronephrosis (2), cleft palate (2), and miscellaneous abnormalities (10).

2.5 Carcinogenesis

The carcinogenicity of dieldrin was the principal focus of public hearings held in 1973-74 before the U.S. Environmental Protection Agency (EPA) on the cancellation of registrations of aldrin/dieldrin, and a large volume of expert testimony and exhibits on this subject was introduced into the public record (USEPA 1973-74). The most extensive published review is by Epstein (1975), but it was limited to material introduced by witnesses for parties arguing for cancellation and subsequent to its preparation additional material, including new data, revised diagnoses, and extended statistical analysis, was introduced by witnesses called by Shell Chemical Company (Thorpe 1974, Stevenson 1974, Hunt 1974, Sternberg 1974; see also Stevenson et al 1976).

Epstein (1975) concluded that aldrin/dieldrin has been shown to be carcinogenic in the liver in at least five independent experiments

with mice and in other sites, as well as in the liver, in at least one experiment with rats. The reported positive findings in rats were disputed on both pathologic and statistical grounds (Thorpe 1974, Stevenson 1974, Hunt 1974, Sternberg 1974). Dieldrin was also reported to be carcinogenic in the lung and other sites in several experiments with mice (Gross 1974), but this also was disputed on statistical grounds (Stevenson 1974, Hunt 1974). On reviewing the record, the Administrator of EPA concluded that dieldrin has been shown to be carcinogenic in the liver and lung of mice in several experiments and that "there is a strong probability that aldrin/dieldrin is a carcinogen in rats as well as mice" (Train 1974). The following is a brief summary of the experiments and reported findings, including the results of experiments recently reported by the National Cancer Institute (NCI 1977, 1978a,b).

2.5.1 FDA Studies 2 and 3 in C3H Mice

Following an earlier inconclusive experiment, two long-term feeding studies in C3HeB/Fe strain mice were carried out at the U.S. Food and Drug Administration and were reported by Davis and Fitzhugh (1962) and Davis (1965). In Study 2, groups of 215-218 mice, with about equal numbers of males and females, were fed diets containing aldrin or dieldrin at 10 ppm. A similar group of 217 unexposed mice was a control group. Average survival times were 51.6 weeks in exposed mice and 59.8 weeks in the controls. Liver tumors, described as "extending from very benign lesions to borderline carcinomas," were

found in 38/151 (23%) aldrin-exposed, 38/148 (24%) dieldrin-exposed, and 9/134 (7%) control mice. Hepatic tumors developed in mice exposed to aldrin in an average of 80 weeks, in dieldrin-exposed mice in 77 weeks, and in control mice in 89 weeks (Davis and Fitzhugh 1962).

In Study 3, groups of 200 mice, with equal numbers of each sex, were fed diets containing aldrin at 10 ppm, dieldrin at 10 ppm, or no added material. Exposed mice survived less well than controls but showed a markedly increased incidence of liver tumors diagnosed as "benign hepatomas" (Davis 1965: see Table 2.5.1).

TABLE 2.5.1

RESULTS OF FDA STUDY 3 WITH C3H MICE

Dietary Exposure	No. of Survivors			No. of Mice with Lesions* When Killed			No. of Mice with Tumor	
	52 wk	78 wk	104 wk	Hy	H	HC	Benign	Malignant
Control (0)	188	150	64	48	27	4	30	21
Aldrin (10 ppm)	152	121	31	72	65	3	61	9
Dieldrin (10 ppm)	169	117	39	71	69	5	71	9

*Hy = hyperplasia, H = "benign hepatoma," HC = hepatic carcinoma

Adapted by Davis 1965

Histologic material from both studies (excluding dieldrin-exposed mice from Study 3) was examined by Reuber (1974), with the results shown in Table 2.5.2. There were marked and highly statistically significant increases in hepatocellular carinomas in all four treated groups. The carcinomas and other hepatic lesions have been fully described and illustrated by Reuber (1974, 1975, 1976). Carcinomas in controls were generally small and single, in contrast with those in treated animals, which were larger and sometimes multiple. Metastases to the lungs were found in 4% of the males exposed to aldrin and 5% of the females exposed to dieldrin, although no serial sections were made. Hepatic vein thrombosis, causing massive liver necrosis and death, was diagnosed in about 5% of the treated mice. Carcinomas from 7/8 control mice, 9/10 mice exposed to aldrin, and 8/9 dieldrin-exposed mice were successfully transplanted into isologous hosts. The behavior of the transplants correlated well with the degree of malignancy as diagnosed histologically, the more highly malignant tumors growing more rapidly than those judged to be less malignant (Reuber 1974, 1975, 1976; Epstein 1975).

2.5.2 Tunstall Experiment 1 in CF1 Mice

This is the largest-scale experiment on the carcinogenicity of dieldrin. Because of the unusually large numbers of mice involved, the data have been analyzed extensively (Walker et al 1972, Gross 1974, Epstein 1975, Stevenson 1974, Hunt 1974, Thorpe 1974, IARC 1974). In addition to positive controls, the experiment included a total of

1,500 mice segregated into groups containing the following numbers of mice of each sex: 0.01 ppm dieldrin ("control"), 300; 0.1 ppm, 125; 1 ppm, 125; 10 ppm, 200 (Table 2.5.3).

From the 9th month onwards, palpable abdominal masses were detected in mice fed 10 ppm. These mice were killed when the enlargement was considered to be detrimental to their health. Thus, 50% of the mice fed dieldrin at 10 ppm were dead at 15 months and 50% in the other groups were at 20 months (Walker et al 1972).

There was a statistically significant and dose-related increase in liver tumors in dieldrin-exposed mice, in both sexes independently. The increase was significant in female mice even at 0.1 ppm (Table 2.5.3). Liver tumors also appeared earlier in the mice fed dieldrin than in control mice (Gross 1974). The liver tumors were classified into two types, "a" and "b," on the basis of their morphology (Walker et at 1972, Thorpe 1974). Type a tumors were described as nodular growths of solid cords of parenchymal cells, whereas type b tumors were papilliform and adenoid growths with cells proliferating in confluent sheets with necrosis and increased mitoses. Reuber (1974, 1976) pointed out that this morphologic classification does not correspond to the morphologic and biologic behavior of lesions of the liver in mice and rats. Whereas type b tumors were clearly malignant on biologic and morphologic criteria, type a tumors ranged from histologically well-differentiated hepatocellular carcinomas to hyperplastic nodules (Reuber 1974, 1976; Epstein 1975). Lung metastases were observed from 12/138 type b tumors (Walker et al 1972). Successful

TABLE 2.5.2

LIVER TUMORS IN MICE IN FDA STUDIES 2 AND 3

Group (and sex)	No. Examined	Av. Survival (weeks)	% Incidence of Liver Lesions*					
			NH	H	N	SC	LC	TC
Control (M)	73	89	40	12	18	18	12	30
Control (F)	53	93	72	11	13	2	2	4
Aldrin (M)	91	86	1	3	13	21	62	82
Aldrin (F)	85	80	1	6	8	29	55	85
Dieldrin (M)	71	91	0	3	10	17	70	87
Dieldrin (F)	71	81	0	4	8	21	66	87

*NH = no hyperplasia, H = hyperplasia, N = nodules, SC = small carcinomas (less than 5 mm), LC = large carcinomas, TC = total carcinomas

Adapted from Reuber 1974, Epstein 1975

transplantation of tumor tissue to unrelated mice provided further confirmation of malignancy (Thorpe 1974).

In Tunstall experiment 1 the incidences of pulmonary adenomas and pulmonary carcinomas in males and females exposed to dieldrin at 0.1 and 1 ppm were increased above those in controls (Table 2.5.3). The differences were statistically significant in females (Gross 1974, Epstein 1975). Although Stevenson (1974) presented revised data listing more females without tumors, the increased incidence of lung tumors remained statistically significant in both sexes combined (Gross 1974). The increased incidence of lung tumors was also significant in the

TABLE 2.5.3

RESULTS OF TUNSTALL EXPERIMENT 1 IN CF1 MICE

Dose (ppm)	No. of Mice	% with Liver Tumors (Type a/Type b)		% with Lung Metastases	% with Lung Tumors*		% with Lymphoid Tumors	% with Other Tumors
					A	C		
Males								
0	288	20	(16/4)	0.7	33	8	35	6
0.1	124	26	(22/4)	0.8	38	11	21	3
1.0	111	31	(23/8)	0.4	38	12	20	5
10.0	176	94	(37/57)	0.6	18	1	24	2
Females								
0	297	13	(13/0)	0	16	6	40	7
0.1	90	27	(23/4)	0	26	13	50	9
1.0	87	37	(31/6)	1.1	34	14	54	17
10.0	148	92	(37/55)	4.5	10	0	5	1

*A = adenomas, C = carcinomas

Adapted from Walker et al 1972, Epstein 1975

subsample of mice without liver tumors (Gross 1974). These statistical comparisons omitted the mice fed the diet containing dieldrin at 10 ppm, because many died or were killed early in the experiment (Gross 1974). However, a "relative risk" analysis incorporating data on age at death showed a highly significant dose-related increase of lung tumors in both sexes independently (Table 2.5.4, Hunt 1974). There were also statistically significant increases in lymphoid tumors and in "other" tumors in females in the data as originally published (Gross 1974), but the differences were not significant according to the revised data (Hunt 1974).

2.5.3 Tunstall Experimental Series 2 in CF1 Mice

The Tunstall experimental series 2 (Walker et al 1972) comprised six independent tests, each with its own controls but involving smaller numbers of animals (10-33 mice per treated group) than experiment 1. Study 2.1 was a dose-response experiment in which mice were exposed to dieldrin at dietary concentrations of 1.25, 2.5, 5, 10, and 20 ppm. Study 2.2 involved three groups exposed to dieldrin at 10 ppm, two of which were fed diets sterilized with gamma rays or ethylene oxide. Study 2.3 compared tumor incidences in groups of mice fed DDT at 50 ppm, DDT at 100 ppm, and a mixture of dieldrin at 5 ppm and DDT at 50 ppm. Study 2.4 compared tumor incidences in mice exposed to dieldrin, beginning early in life, for 2, 4, 8, 16, 32, and 64 weeks.

The incidence of liver tumors was significantly increased in both males and females independently in each of the six experiments (Tables

TABLE 2.5.4

SUMMARY OF CHI-SQUARE VALUES FROM RELATIVE RISK ANALYSIS OF TUMOR INCIDENCE IN TUNSTALL EXPERIMENTS

Tumors	Experiment					
	1 (df=3)	2.1 (df=5)	2.2a (df=1)	2.2b (df=1)	2.2c (df=1)	4 (df=1)
Male Mice						
Liver, type a	210*	90.2*	18.4*	9.4*	9.0*	3.1*
Liver, type b	535*	93.5*	6.2*	6.0*	5.1*	29.7*
Liver, total	725*	143*	28.3*	19.5*	16.1*	37.3*
Lung, benign	31.8*	4.89	3.8*	0.1	0.7	0.1
Lung, malignant	6.83	-	--	-	-	0.0
Lung, total	30.2*	3.03	3.8*	0.1	0.8	0.0
Female Mice						
Liver, type a	261*	113*	11.0*	22.3*	18.2*	0.3
Liver, type b	547*	45.1*	9.8*	2.1	7.1*	66.0*
Liver, total	807*	165*	24.5*	26.9*	28.5*	56.5*
Lung, benign	27.5*	5.37	1.8	-	4.6*	1.7
Lung, malignant	2.82	-	-	-	-	0.0
Lung, total	21.2*	6.38	1.1	1.0	4.6*	0.9

*Statistically significant (P less than 0.05); df = degrees of freedom

Note: Data for experiment 3 not included, because the statistical analysis included data on other chemicals

Adapted from Hunt 1974

2.5.4 and 2.5.5, Walker et al 1972, Epstein 1975). In study 2.1, the increase was uniformly dose-related in both sexes when allowance was made for age at death (Hunt 1974). In study 2.3, there was evidence for synergistic action of dieldrin and DDT (Table 2.5.5). In study 2.4, liver tumor incidence was increased even in animals exposed for only 8, 4, or 2 weeks (Table 2.5.6). In addition, the age-adjusted incidence of

lung tumors was increased in 10 of 12 groups exposed to dieldrin, the increase being statistically significant in males in Study 2.2a and in females in Studies 2.2c and 3 (Table 2.5.4). The age-adjusted incidence of other tumors was statistically significantly increased in females in Study 2.4 (Hunt 1974).

TABLE 2.5.5

LIVER LESIONS* IN MICE IN TUNSTALL EXPERIMENT 2.3

Dietary Exposure	No. and Sex of Mice Examined	% Incidence of Liver Lesions**					
		NH	H	N	SC	LC	TC
Control	45 M	62	29	9	0	0	0
Control	32 F	47	44	9	0	0	0
DDT at 50 ppm	31 M	31	34	28	6	0	6
DDT at 50 ppm	31 F	32	16	35	13	3	16
DDT at 50 ppm and Dieldrin at 5 ppm	33 M	3	18	21	15	42	58
DDT at 50 ppm and Dieldrin at 5 ppm	31 F	0	0	6	29	65	94

* Diagnosed by Reuber (1974)

** NH = no hyperplasia, H = hyperplasia, N = nodules, SC = small carcinomas (less than 5 mm), LC = large carcinomas, TC = total carcinomas

Adapted from Epstein 1975

2.5.4 Tunstall Experiment 3 in CF1 Mice

In Tunstall experiment 3, CF1 mice were exposed, beginning at 4 weeks, to dieldrin in the diet at one concentration, 10 ppm. Relatively few mice were used. Mice were not killed when abdominal masses became large, but were if they became moribund. Mice surviving to 110 weeks were killed then (Thorpe and Walker 1973).

TABLE 2.5.6

INCIDENCE OF LIVER TUMORS IN MICE EXPOSED TO DIELDRIN FOR DURATIONS OF 2-64 WEEKS

Duration of Feeding (weeks)	No. of Mice		No. of Mice with Liver Tumors (Type a/Type b)	
	M	F	M	F
0	18	16	2/0	1/0
2	13	9	2/0	2/0
4	10	12	0/1	3/1
8	10	12	3/1	4/0
16	11	8	4/0	3/0
32	10	10	4/0	4/0
64	13	9	6/7	6/2

Adapted from Walker et al 1972, Epstein 1975

The incidence of liver tumors was increased significantly in treated mice of both sexes (Table 2.5.7), and the tumors occurred significantly earlier in treated mice than in controls (Thorpe and Walker 1973, Epstein 1975). The incidence of pulmonary metastases was much higher than in Studies 1 and 2 (Table 2.5.7), presumably because the animals were not killed prematurely (Epstein 1975). The age-adjusted incidence of lung tumors was increased in females, but not significantly (Hunt 1974).

2.5.5 Tunstall Experiment 4 in Three Strains of Mice

In Tunstall experiment 4, the effects of exposure to dieldrin at 10 ppm were compared in CF1 mice, in LACG mice, and in hybrids of the two strains (Stevenson 1974, Thorpe 1974, Hunt 1974). Forty mice of each sex were used in each treatment group, with groups of 60 controls.

The age-adjusted incidence of liver tumors was highly significantly increased in all six treated groups (Table 2.5.4, Hunt 1974). The incidence of lung tumors was also increased in all six treated groups, although not significantly so in any one considered alone (Hunt 1974). However, the incidence of other tumors was significantly increased in treated female CF1 mice (Hunt 1974).

TABLE 2.5.7

INCIDENCE OF LIVER TUMORS IN MICE IN TUNSTALL EXPERIMENT 3

Group	No. of Mice	% with Liver Tumors (Type a/Type b)		% with Lung Metastases
Males				
Control	45	24	(20/4)	0
10 ppm	30	100	(47/53)	3
Females				
Control	44	23	(23/0)	0
10 ppm	30	87	(40/47)	17

Adapted from Thorpe and Walker 1973

2.5.6 University of Miami Study with Swiss-Webster Mice

Four hundred Swiss-Webster mice were exposed to dieldrin at 3 or 10 ppm in the diet, with appropriate controls (MacDonald et al 1972). The death rate was high because of fighting and amyloidosis, and the mean lifespan of the males was only 12-13 months. There was a marked increase in liver lesions in the treated groups. The lesions were originally reported as "nodulation or restorative hyperplasia," but a

number of these diagnoses were later amended to hepatocellular carcinomas (MacDonald et al 1973 addendum). Diagnoses as carcinomas were confirmed on representative slides by Reuber (1974) and other pathologists, as reviewed by Epstein (1975).

2.5.7 NCI Study with B6C3F1 Mice

The National Cancer Institute recently reported the results of a bioassay of technical aldrin and technical dieldrin for carcinogenicity in B6C3F1 mice (NCI 1978a). Groups of 50 mice of each sex were fed diets containing aldrin or dieldrin for 80 weeks, then observed for 10-13 weeks. The time-weighted average dietary concentrations of aldrin were 4 and 8 ppm for males and 3 and 6 ppm for females; dieldrin was fed at 2.5 and 5 ppm. Untreated matched controls were groups of 20 untreated male and 10 female mice. Pooled control groups, used for statistical evaluation, consisted of the matched controls combined with 92 untreated male and 79 untreated female mice from similar bioassays of other chemicals. All surviving mice were killed at 90-93 weeks.

The mice fed aldrin or dieldrin and the control mice had similar mean body weights. Hyperexcitability was observed in all treated groups with increasing frequency during the 2nd year of the study. Female mice fed aldrin showed a dose-related increase in mortality.

There was a significant dose-related increase in the incidence of hepatocellular carcinomas in male mice fed either chemical (matched controls 3/20, pooled controls 17/92, low-dose aldrin 16/49, high-dose aldrin 25/45, low-dose dieldrin 12/50, high-dose dieldrin 16/45). The incidence of hepatocellular carcinomas in females was higher in

all the exposed groups than in controls, but the differences were not statistically significant. No other tumors appeared at a significantly higher frequency in the exposed groups than in the controls.

2.5.8 NCI Study of B6C3F1 Mice Exposed to Photodieldrin

The National Cancer Institute has also reported the results of a bioassay of photodieldrin (recrystallized and without detectable residual dieldrin) for carcinogenicity in B6C3F1 mice (NCI 1977). Groups of 50 mice of each sex were fed diets containing photodieldrin at concentrations of 0.32 and 0.64 ppm for 80 weeks, then observed for 10-13 weeks. Matched controls were 10 untreated mice of each sex at each dose; pooled controls groups consisted of 60 untreated mice of each sex.

Convulsions and hyperactivity were noted in exposed male mice, but body weights and mortality were unaffected by exposure to photodieldrin. Exposed mice and controls showed no statistically significant differences in tumor incidence.

2.5.9 FDA Experiment 1 and 2 with Osborne-Mendel Rats

In the FDA experiment 1, initiated at the U.S. Food and Drug Administration (FDA) in 1952 but not published until 1964, 12 groups of 12 male and 12 female Osborne-Mendel rats were fed diets containing aldrin or dieldrin at 6 concentrations, from 0.5 to 150 ppm (Fitzhugh et al 1964). Exposure began at 3 weeks and continued until mice surviving after 2 years were killed. Twelve rats of each sex were controls. Only 68% (227/336) of the animals, including only 17/24

controls, were examined histologically. The many small groups of animals makes the experiment difficult to analyze with statistical rigor.

The exposed rats could be classified into two groups (Table 2.5.8). Those exposed to aldrin or dieldrin at high dietary concentrations (50-150 ppm) showed a dose-related decrease in survival, acute renal necrosis, chronic nephritis, and a high incidence of liver lesions (Fitzhugh et al 1964, Reuber 1974, Epstein 1975). Rats exposed to aldrin or dieldrin at lower concentrations (0.5-10 ppm) survived well and had a low incidence of liver and kidney lesions but a higher incidence than controls of tumors in other organs, primarily the lymphatic system and mammary glands (Fitzhugh et al 1964, Reuber 1974, Epstein 1975, Thorpe 1974). The increase in tumor incidence in the rats exposed at low concentrations is statistically significant if groups are pooled (Gross 1974, Hunt 1974). The increase in liver lesions is also statistically significant (Gross 1974, Epstein 1975), but the pathologic diagnoses have been disputed vigorously. Reuber (1974) diagnosed 18 hepatocellular carcinomas in exposed animals, whereas other pathologists diagnosed most of the lesions as hyperplastic nodules or even milder lesions (Thorpe 1974, Sternberg 1974). In the terminology of the Liver Cancer Workshop (Squire and Levitt 1975) most of the lesions would be classified as neoplastic nodules.

In a second experiment conducted at FDA in 1963, 43 Osborne-Mendel rats were exposed to dieldrin at 1 ppm for 2 years. There were 39 controls. The results were similar to those for the groups at the lower concentrations

TABLE 2.5.8

GROSS AND MICROSCOPIC PATHOLOGY OF SOME RATS IN FDA EXPERIMENT 1

Dietary Exposure (ppm)	Number Sectioned	Nephritis*				Urinary Bladder**		No. of Rats with Tumors***						
		0	<S	M&S	>M	Dis	Hem	A	B	C	D	E	F	Total
Control (0)	17	5	5	6	1	0	0	1	1	0	0	0	1	3
Aldrin														
0.5	19	3	10	4	2	0	0	5	3	2	3	0	1	10
2	19	4	8	4	3	0	0	2	3	2	0	0	0	7
10	22	3	10	8	1	0	0	2	3	0	0	2	2	8
50	18	8	7	1	2	2	1	2	3	0	0	0	0	5
100	11	1	2	4	5	3	2	4	0	0	1	0	0	5
150	9	1	3	3	2	4	2	1	0	0	0	0	0	1
Dieldrin														
0.5	22	5	9	5	3	1	0	4	1	1	1	0	2	8
2	23	9	8	6	0	1	0	2	4	0	0	1	2	8
10	18	5	6	6	1	1	0	2	0	1	0	1	0	4
50	20	5	6	6	3	0	0	1	2	0	0	0	1	4
100	18	5	3	2	8	2	1	0	0	0	1	0	2	3
150	11	1	2	7	1	2	2	0	0	0	0	0	0	0

*S = slight, M = moderate; based on microscopic sections except one markedly damaged kidney based on gross appearance only in the 100 ppm aldrin group

**Dis = distended, Hem = hemorrhagic

***A = pulmonary lymphosarcoma, B = fibroadenoma of breast, C = carcinoma of breast, D = lymphoid except lung, E = fibrosarcoma, F = other

Adapted from Fitzhugh et al 1964

in experiment 1, in that there was a statistically significant increase in lung and other tumors in males and a small incidence of liver lesions of disputed significance (Reuber 1974, Epstein 1975, Thorpe 1974).

2.5.10 Tunstall Experiment with CFE Rats

Groups of 25 male and 25 female CFE strain rats were fed diets containing recrystallized dieldrin added at concentrations of 0.1, 1, and 10 ppm. Exposure began at 5 weeks of age and continued for 2 years. A control group of 45 animals of each sex was fed a diet containing dieldrin at 0.026 ppm. The results of the experiment as originally reported (Walker et al 1969) are summarized in Table 2.5.9. Subsequently, revised data have been reported (Stevenson et al 1976). There was an increase in tumor incidence, primarily in the thyroid and mammary glands, in the females exposed at 0.1 and 1 ppm. This increase was of marginal statistical significance in the pooled groups (Gross 1974, Epstein 1975). With the revised data the difference was statistically significant in a conventional analysis but not in an actuarial analysis (Stevenson et al 1975). There was no increase in tumor incidence in rats of either sex fed diets containing dieldrin at 10 ppm.

2.5.11 NCI Experiment with Aldrin and Dieldrin in Osborne-Mendel Rats

The National Cancer Institute recently reported the results of a bioassay of technical aldrin and technical dieldrin for carcinogenicity in Osborne-Mendel rats (NCI 1978a). Groups of 50 rats of each sex were fed diets containing aldrin or dieldrin at one of two concentrations for 59-80 weeks and were then observed for an additional 30-52 weeks. The dietary concentrations of aldrin were 30 and 60 ppm, and the time-

TABLE 2.5.9

TUMORS IN RATS FED DIELDRIN FOR 2 YEARS

Dietary Concentration (ppm)	No. of Rats	Tumors				No. of Rats with Tumors	% Tumor Incidence
		Thyroid	Pituitary	Mammary	Other		
Males							
0.026	43	3	2	1	6	12	28
0.1	23	2	2	-	2	6	26
1.0	23	2	1	-	3	5	22
10.0	23	4	2	-	2	8	35
Females							
0.026	43	3	2	13	3	19	44
0.1	23	6	1	11	2	15	65
1.0	23	4	1	10	4	14	61
10.0	23	3	2	8	-	12	52

Adapted from Walker et al 1969

weighted average dietary concentrations of dieldrin were 29 and 65 ppm. Matched control groups were 10 untreated rats of each sex; pooled controls, 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. All surviving rats were killed at 111-113 weeks.

Hyperexcitability was observed in all exposed groups with increasing frequency and severity during the 2nd year. Rats fed dieldrin had a higher death rate than controls during the first 90 weeks of the experiment. During the 2nd year, the mean body weights of rats fed either aldrin or dieldrin were lower than those of the controls. Rats fed aldrin had an increased incidence of thyroid tumors, including follicular-cell adenoma and carcinoma. In males, the incidences were 4/48 for pooled controls, 14/38 at the low dose, and 8/38 at the high dose; in females, they were 3/52 for pooled controls, 10/39 at the low dose, and 7/46 at the high dose. The increases were statistically significant in the males (P=0.001) and the females (P=0.009) at the low dose but not in either high dose group. There was also an increased incidence of adrenal cortical adenomas in exposed females (pooled controls 0/55, low dose 8/45, high dose 1/48), which was also statistically significant in the rats at the low dose (P=0.001) but not in those at the high dose. In the males, a significant increase in pancreatic islet tumors occurred at the low dose (P=0.043) but not at the high dose.

Female rats fed dieldrin had an increased incidence of adrenal cortical tumors (adenoma or carcinoma). The incidences were 0/55 in

pooled controls, 6/45 at the low dose, and 2/40 at the high dose. As in rats fed aldrin, this increase was statistically significant in the rats at the low dose (P=0.007) but not in those at the high dose. There was also a statistically significant (P=0.030) dose-related increase in incidence of thyroid tumors in females.

2.5.12 NCI Experiment with Dieldrin in Fischer Rats

The National Cancer Institute also reported the results of a bioassay of recrystallized dieldrin for carcinogenicity in Fisher 344 rats (NCI 1978b). Groups of 24 rats of each sex were fed diets containing dieldrin at concentrations of 2, 10, and 50 ppm for 104-105 weeks. Matched control groups consisted of 24 untreated rats of each sex. All surviving rats were killed at 104-105 weeks.

The body weights of the exposed rats were essentially unaffected, but hyperexcitability, tremors, and coma were observed beginning in the 76th week in males at the high dose and in the 80th week in females at the high dose. Survival was not adversely affected by exposure.

There was no statistically significant differences in the tumor incidences in exposed rats and the controls; however, the thyroid and adrenal glands of the exposed rats were not examined histopathologically in this study.

2.5.13 NCI Experiment with Osborne-Mendel Rats Exposed to Photodieldrin

The National Cancer Institute also reported the results of a bioassay of photodieldrin for carcinogenicity in Osborne-Mendel rats (NCI 1977). The photodieldrin was recrystallized and contained no detectable residual dieldrin. Groups of 50 rats of each sex were fed

diets containing photodieldrin for 80 weeks and then observed for an additional 31-32 weeks. Male rats received photodieldrin at 5 and 10 ppm; the time-weighted average dietary concentrations for females were 3.4 and 7.5 ppm. Matched control groups were 10 untreated rats of each sex; pooled control groups, used for statistical evaluation, consisted of the matched controls combined with 65 untreated rats of each sex from other similarly conducted bioassays.

Convulsions and hyperactivity were noted in exposed rats of both sexes, but mortality and body weights were unaffected. The incidence of tumors of several types (mammary tumors and thyroid tumors in females and multiple-site hemangiomas in males) was higher in exposed rats than in controls. However, these increases were only marginally statistically significant and were considered not clearly associated with exposure to photodieldrin (NCI 1977).

2.5.14 Other Experiments

Several other long-term feeding experiments in mammals have been reported but are of little value as carcinogenicity tests because of defects in methodology or reporting (Epstein 1975, IARC 1974). Song and Harville (1964) reported neoplastic effects in mice and rats exposed to aldrin and dieldrin at high doses for short periods, but did not provide details. Treon and Cleveland (1955) and Cleveland (1966) referred to tests with aldrin and dieldrin in rats, but the data published were somewhat conflicting and were insufficient for evaluation. Deichmann et al (1967) reported a study with aldrin in rats exposed at 5 ppm. Deichmann et al (1970) fed rats aldrin and

dieldrin at 20, 30, and 50 ppm in the diet and reported a significant reduction in tumor incidence, but the lifespan in the exposed groups was markedly reduced and the data given were insufficent for estimating relative risks. Epstein (1975) also reviewed three studies with dogs exposed to aldrin and dieldrin for up to 2 years and one study with rhesus monkeys exposed to dieldrin for up to 6 years, but these studies are too short in relation to the lifespan of the animals to be acceptable carcinogenicity tests (IARC 1974).

2.6 Mutagenesis and Related Cytotoxic Effects

Bidwell et al (1975) conducted a comprehensive evaluation of the mutagenic potential of dieldrin, but the results have been reported only in abstract form. They used direct bacterial tests with and without microsomal activation, a host-mediated assay, analyses of blood and urine for active metabolites, a micronuclei test, metaphase analysis, a dominant lethal assay, and a heritable translocation test. In most of the tests with mammals, dieldrin was administered by gavage on a "sub-acute basis" at 0.08, 0.8, and 8 mg/kg in corn oil. The authors' overall evaluation of the data from mice was that dieldrin was negative for mutagenicity in all tests. Dieldrin did not increase the number of mutants in five tests with Salmonella, including excision repair-deficient mutants and frame-shift and base-analogue detection strains.

Dean et al (1975) reported the results of three tests of the mutagenic potential of recrystallized dieldrin. In a dominant lethal assay with mice, dieldrin gave marginally positive results in one

experiment, in that the number of fetal implantations in female mice mated with males given dieldrin at 12.5 or 25 mg/kg was significantly reduced in the 1st-3rd weeks after dosing, although the number of early fetal deaths was not increased. These results were not duplicated in a second experiment, in which the number of fetal implantations was increased in all three treatment groups. The test system seems to have been insensitive, because a positive control substance (cyclophosphamide) produced small effects even at 100 mg/kg. Dieldrin at 30 or 60 mg/kg caused a nonsignificant decrease in polyploidy in bone marrow cells from Chinese hamsters and no increase in chromatid gaps. In a host-mediated assay, dieldrin induced no changes in the rate of mitotic gene conversion in Saccharomyces cerevisiae (strain D4) when it was administered to mice in single doses of 25 and 50 mg/kg or repeated doses of 0.2, 5, and 10 mg/kg/day. However, the positive control (ethyl methanesulfonate of 400 mg/kg) produced only a slight increase in the rate of gene conversion.

McCann et al (1975) and McCann and Ames (1976) reported that dieldrin gave negative results for mutagenicity in the reversion bioassay with Salmonella typhimurium strains TA 1535, TA 1536, TA 98, and TA 100, both with and without activation by rat liver microsomal preparations (S-9). Van Dijck and van de Voorde (1976) similarly reported that aldrin and dieldrin were negative in this bioassay with activation by mouse liver microsomes. Marshall et al (1976) reported that dieldrin was not mutagenic in the S typhimurium bioassay, with or without rat liver microsomal homogenates. They used four strains of

S typhimurium: TA 1535, TA 1536, TA 1537, and TA 1538. They did not use the more sensitive strains, TA 98 and TA 100. Shirasu et al (1976) also reported that aldrin and dieldrin were negative for mutagenicity in the four strains of S typhimurium used by Marshall et al and in two tryptophaneless strains of E coli, but they did not use microsomal activation. They also reported that aldrin and dieldrin were negative for mutagenicity in recombination assays with Bacillus subtilis strains H17 Rec^+ and M45 Rec^-.

Swenberg et al (1976) found negative results for dieldrin in an in vitro alkaline elution assay for DNA damage in Chinese hamster (V79) cell culture with rat liver microsomal activation. Both McCann and Ames (1976) and Swenberg et al (1976) interpreted the results with dieldrin as "false negatives," because the systems used in their experiments otherwise usually give positive results with carcinogens.

In contrast to the negative results summarized above, a number of investigators have reported positive results for aldrin and dieldrin in other bioassay systems. Majumdar et al (1976) reported that recrystallized dieldrin caused chromosome damage in bone marrow cells of mice in vivo and in human embryonic lung cells in vitro. Single intraperitoneal injections of dieldrin at 1, 30, and 50 mg/kg into STS mice caused pronounced mitotic inhibition and produced twofold to sixfold increases in chromosome abnormalities, primarily breaks and fragments, in bone marrow cells; these changes were statistically significant even at the lowest dose. At 1, 10, and 30 µg/ml, dieldrin caused similar effects in human lung cell cultures (WI-38) in vitro. The chemical also produced chromosomal interchanges

and rings. Cytotoxic studies using the WI-38 cell line revealed dose-response and time-response reactions to dieldrin.

Ahmed (1975) and Ahmed et al (1977) reported that aldrin and dieldrin, with rat liver microsomal activation, induced unscheduled DNA repair in human fibroblasts (VA-4) transformed by the SV-40 virus. The kinetics of dieldrin-induced damage and repair were studied through incorporation of bromodeoxyuridine into the damaged regions. Dieldrin also increased the mutation frequency in vitro of spontaneously transformed Chinese hamster cells (V79) to ouabain-resistant mutants.

Georgian (1975) found that aldrin induced chromosome aberrations in human lymphocyte cultures in vitro and in bone marrow cells of rats and mice exposed in vivo. Dose-response relationships were observed in both bioassays. In the lymphocyte bioassay, aldrin showed a narrow range of doses causing chromosomal changes, between 19 and 38 µg/ml, close to the cytotoxic concentrations. In the in vivo rodent bioassays the minimal dose inducing chromosomal aberrations was 19 mg/kg (single dose ip). No effects were observed at 9.6 mg/kg.

Markaryan (1966) reported that dieldrin administered to mice caused mitotic inhibition and a variety of chromosomal aberrations, including significant increases in the incidence of breaks, fragments, chromosome and chromatid bridges, and stickiness, but not of translocations or dicentrics. Guerzoni et al (1976) detected mutagenic activity on S cerevisiae by aldrin at 5 and 50 ppm.

Bunch and Low (1973) fed technical dieldrin at dietary concentrations of 4, 10, and 30 ppm to mallard ducks for 60 days and examined

bone marrow cell cultures from the ducks' offspring. No significant increase in chromosomal aberrations was observed, but dieldrin at 30 ppm caused a significant reduction in the rate of mitosis. Duck lymphocyte cultures exposed to dieldrin at 100 ppm showed a significant increase in the incidence of chromosomal aberrations, including gaps and breaks. Mitotic indices were significantly reduced at all dieldrin concentrations down to 0.1 ppm.

Walker et al (1977) reported that recrystallized dieldrin markedly inhibited incorporation of amino acid precursors of DNA, RNA, and protein into Ehrlich ascites tumor cells in vitro. Effects on incorporation of thymidine and uridine were marked at concentrations as low as 10^{-6}M (0.4 ppm). Daily injections of dieldrin at 1.5 mg/kg for 5 days into mice inhibited the growth of Ehrlich ascites tumor cells in vivo. Chung et al (1967) had earlier reported inconsistent effects of dieldrin on the synthesis of DNA, RNA, and protein in HeLa cells. Sheinman and Yannai (1974) reported that dieldrin, at concentrations as low as 25 µg/ml, was toxic to rat fetal liver primary culture cells and human kidney cell line B in vitro. Observed morphologic changes included granulation and shrinkage of the cytoplasm, formation of long and narrow cytoplasmic projections, and the appearance of giant cells.