TOXICOPATHOLOGICAL EFFECTS OF MIREX AND PHOTOMIREX IN CHICK EMBRYOS AND CHICKENS HATCHED FROM EGGS INOCULATED WITH MIREX OR PHOTOMIREX

By

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ABSTRACT

TOXICOPATHOLOGICAL EFFECTS OF MIREX AND PHOTOMIREX IN CHICK EMBRYOS AND CHICKENS HATCHED FROM EGGS INOCULATED WITH MIREX OR PHOTOMIREX

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Eggs were inoculated after 18 hours of incubation with 0.05 or 0.1 ml corn oil or 0.005, 0.05, 0.5, 5 or 25 mg of mirex or photomirex suspended in corn oil. Embryo mortality was recorded and after hatching, 4 of the surviving chickens in each group were killed at the 4th, 5th, 6th and 8th weeks.

Hatchability of chick embryos was lowered in the groups inoculated with 5 or 25 mg of mirex or photomirex. All chicks hatched from eggs inoculated with 25 mg of mirex or photomirex died within 10 days after hatching. Chickens hatched from eggs inoculated with 0.5 or 5 mg of photomirex had ruffled feathers, a pale comb and were weak, but these changes were not seen in chickens hatched from eggs treated with the same doses of mirex. Body weights were less in chickens hatched from eggs inoculated with 0.5 or 5 mg of photomirex by weeks 8 and 5, respectively. The body weight, however, was not affected by mirex at the same dosage levels.

The lymphoid organ weight to body weight ratios as well as the humoral immune response to sheep erythrocytes of chickens hatched from

eggs inoculated with mirex were unaffected. Chickens hatched from eggs inoculated with 0.5 or 5 mg of photomirex had increased bursa weight to body weight ratios at the 5th and 6th weeks, respectively. The chickens which were killed at the 6th week had been given 2 injections of sheep erythrocytes. The spleen weight to body weight ratio in chickens hatched from eggs inoculated with 5 mg of photomirex was increased at the 5th week. The size of the thymus in the chickens hatched from eggs inoculated with 5 mg of photomirex was reduced at the 4th week. The antibody responses to sheep erythrocytes were decreased in chickens hatched from eggs inoculated with 0.5 mg of photomirex. However, there were no histological lesions in the bursas of these chickens. There was depletion of medullary lymphoid cells in bursas of chickens hatched from eggs inoculated with 5 or 25 mg of photomirex.

Mirex or photomirex caused a dose-related increase in the liver weight to body weight ratios of chickens hatched from eggs inoculated with 0.5 or 5 mg of mirex or photomirex. Hepatic lesions were especially prominent in chickens hatched from eggs inoculated with 5 or 25 mg of photomirex. The hepatocytes were swollen, the cytoplasm was vacuolated, and cellular necrosis was evident. The livers of chickens hatched from eggs inoculated with mirex had swollen and vacuolated hepatocytes.

In chickens hatched from eggs inoculated with 5 mg of mirex or photomirex, ultrastructural studies revealed vacuolated hepatocytes and proliferation of smooth endoplasmic reticulum. The mitochondria were swollen and the mitochondrial cristae were disrupted.

There was a delay in spermatogenesis in immature males which had been hatched from eggs inoculated with 0.5 mg of mirex or photomirex

after 18 hours of incubation. Chemical analysis of mirex or photomirex in the tissues indicated that the highest concentration of these chemicals was in the livers, then fat and kidneys, in that order. The concentration was dose related.

DEDICATED

To my parents,

To my husband, Abdel Rahman, and

To my son and daughter, Ayemen and Nuha

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Mirex is a relatively stable hydrocarbon which was introduced as a pesticide in the southern United States in 1960. It was used to control the fire ant (*Solenopsis sp.*). This insect was damaging agricultural land and destroying wildlife as well as harming young livestock. After several years of use, it was discovered that the pesticide accumulated in the tissues of several species of wild animals (Coon and Fleet, 1970) and it was reported in human fat by Suta in 1977.

Mirex is considered to be hepatotoxic (Baker et al., 1972), teratogenic (Khera, 1976) and a tumor inducer in mice (Innes et al., 1969).

Under laboratory conditions mirex undergoes photolytic dechlorination to 8-monohydromirex, commonly known as photomirex (Gibson et al., 1972). Twelve years after its introduction as a pesticide, 20% of the mirex present in the soil was identified as photomirex (Carlson et al., 1976).

Photomirex is hepatotoxic and thyrotoxic to rats (Villeneuve et al., 1979a). It is nonteratogenic in rabbits (Villeneuve et al., 1978).

The persistence of mirex and photomirex in the environment and their lack of biodegradation suggest that both chemicals are now environmental contaminants and may be hazardous to human and animal health.

Mirex has been studied extensively but only a limited amount of research has been done on the toxicity of its photodegradation product,

photomirex. Consequently, there is a need for more research to determine the potential dangers of photomirex.

There are no reports on the toxicity of photomirex in avian species. This suggested that the chicken might serve as a useful experimental animal and chicken embryos were chosen because of their availability, low cost and convenience. The relatively short incubation and developmental period make the chicken embryo a convenient experimental subject for a study of toxic compounds and the young chicks can be observed over a short, rapid growth period.

Both chemicals were used because they are now environmental contaminants and it seemed useful to compare the effects of the 2 products in chickens.

Objectives

The objectives of this experiment were:

1. To determine and compare the toxicity of mirex and photomirex on chick embryos as indicated by embryo mortality.

2. To determine and compare body weight of hatched chicks at the time that they were killed.

3. To compare weights of selected organs compared to controls at the time the chickens were killed.

4. To study selected tissues from hatched chickens for histopathological and ultrastructural changes.

5. To determine the effects of mirex and photomirex on immune competence of hatched chickens.

LITERATURE REVIEW

General Characteristics of Mirex

Mirex has been used as a pesticide for the control of the fire ant, *Solenopsis sp.*, in the southern United States. Trade names of mirex and GC1283 were applied by Allied Chemical Company when the product was used as the active ingredient in baits and dechlorane was the trade name applied by the Hooker Chemical Company when the chemical was used as a fire retardant in polymeric materials (Alley, 1973).

Mirex was first prepared in 1946 (Prin, cited by Alley, 1973) by a reaction of hexachlorocyclopentadiene with aluminum chloride in methylene chloride. It can also be produced by heating kepone with phosphorus pentachloride at 125-150 C, at which time the keto group is replaced by a chlorine atom (Ungnade and McBee, 1958).

The chemical name of mirex is dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalene (Brook, 1974). The chemical structure of mirex (Figure 1) has been determined to be a cage dimer of hexachloropentidine (Griffin and Price, 1964; McBEe et al., 1956) which consists of 10 carbons and 12 chlorine atoms. It is white, crystalline, insoluble in water and is resistant to most common oxidizing and reducing systems as well as strong acids and bases (Dilling et al., 1967; Eaton et al., 1960). Thermally, unlike other chlorinated hydrocarbons, mirex is stable and cannot undergo pyrolysis below 500 C (Holloman et al., 1975). Sunlight plays an important role in

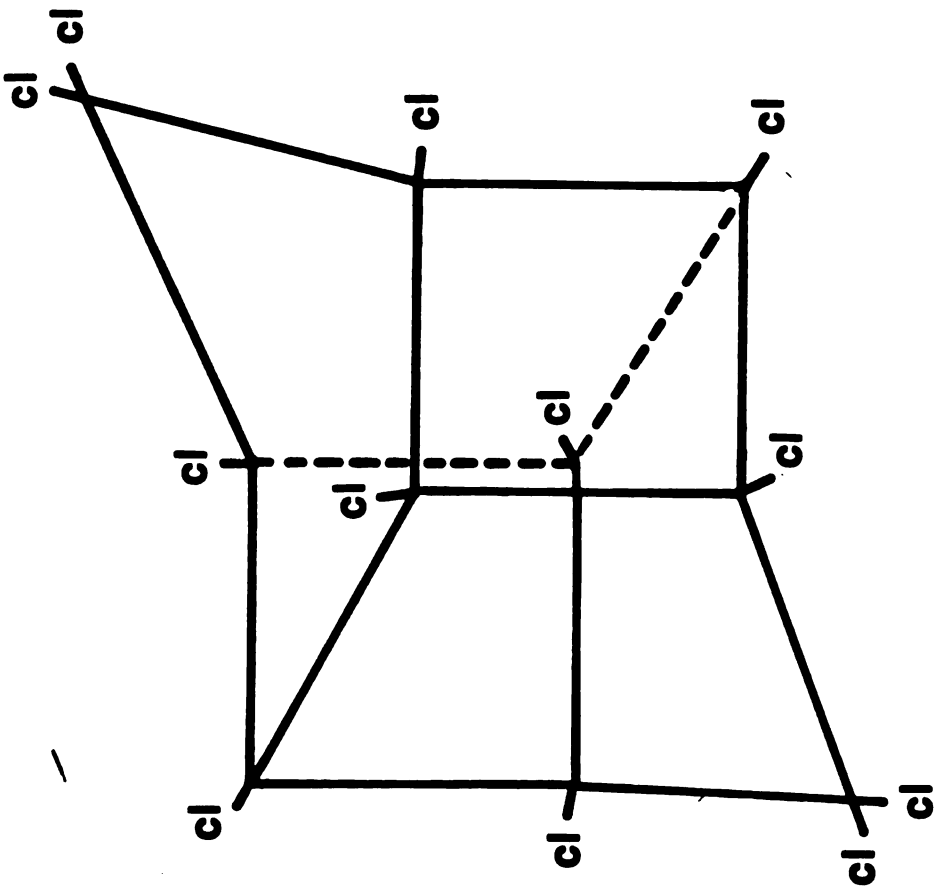

Figure 1. Dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalene

nonbiological degradation of mirex (Gibson et al., 1972). Under anaerobic conditions in soil, sewage sludge organisms slowly metabolize mirex (Andrade and Wheeler, 1974). The main mode of fragmentation of mirex is by dechlorination and cleavage of the pentacyclodecane skeleton (Dilling and Dilling, 1967).

In vitro studies on plant preparations revealed that mirex can be taken up by bean and pea roots (Mehendale et al., 1972). These authors indicated that food crops in the area where mirex was applied can be considered as a source of contamination to human beings and animals.

History of the Fire Ant

The ant, *Solenopsis richertia*, was introduced accidentally in 1918 into the southern United States from South America. The genus and species were identified in 1930 by Creighton (cited by Alley, 1973). The ant is small, dark in color, and belongs to the family Formicidae. The name, fire ant, was given because of the sharp burning sensation which resulted from its sting. The ants cause damage to wildlife, grasses, and newborn livestock (Alley, 1973). They also form mounds which ruin agricultural equipment and pasture land (Coon and Fleet, 1970). In 1963 about 31 million acres had been infested in the southern states.

Eradication Programs

The first organized program for eradication and control of fire ants was initiated by the State of Alabama in 1937. Cyanogas dust was used initially, but in 1949 chlordane replaced cyanogas (Eden and Arant, 1949). In 1957 the United States Department of Agriculture (USDA) joined the eradication program. At that time dieldrin and

heptachlor were used but with limited success. In 1962 mirex was substituted for the other pesticides because of its effectiveness at low concentrations. It was mixed with oil and this combination was used as a poisonous bait for the ants (Coon and Fleet, 1970). The most frequently used formulations were designated 4x, 2x and 1x and they contained 0.3, 0.15, and 0.075% mirex, respectively (Alley, 1973).

Mirex Seen as a Dangerous Contaminant

In 1969, Innes et al. reported that mirex was carcinogenic and by 1970 many reports had appeared indicating that mirex was toxic to other animal life. By that time it was considered to be an environmental contaminant (Coon and Fleet, 1970). Additional alarming information became available when it was learned that mirex was degraded in nature and it and its derivatives might contaminate the environment for many years (Carlson et al., 1976). In the meantime, mirex had been found in human fat but not in human milk (Suta, 1977).

In 1970 the Environmental Protection Agency suspended the interstate shipment of mirex (Alley, 1973), and in 1976 a coordinating committee on scientific and technical assessments of environmental pollutants stopped the use of mirex for control of the fire ant.

In 1979 Matsumura and Madhukar reported that mirex was found in industrial waste products which had drained into White Lake at Muskegon, Michigan, from the Hooker Chemical Company.

Biological Characteristics

Mirex is an aliphophilic compound and accumulates in adipose tissue, kidney, liver, muscle, skin and brain. After continuous feeding of mirex for 16 months to quail, rats and fish, levels of mirex had not

yet reached a plateau in the tissues. However, because mirex accumulates most readily in adipose tissue, levels in this tissue were 200-fold higher than the daily dietary intake (Ivie et al., 1974c).

Mirex is readily absorbed from the intestinal tract (Ivie et al., 1974b). However, it is unaffected by the metabolic processes of animals and is excreted unchanged (Dorough and Ivie, 1974; Gibson et al., 1972). Feces is the most important route for elimination of mirex. Considerable amounts are also excreted through egg yolk (Ivie et al., 1974b). Egg yolks from hens fed 600 mg of mirex/kg of ration for 12 weeks contained 1900 ppm of mirex (Naber and Ware, 1965). These levels of mirex dropped to 500 ppm 3 weeks after mirex was removed from the feed. Traces of mirex have been detected in egg albumen but not in egg shell (Ivie et al., 1974b).

Results of similar studies in hens by Woodham and Bond (1975) indicated that mirex tended to accumulate in the egg yolk and fatty tissues. They also pointed out that the levels of mirex in egg yolk and fatty tissues started to decline as soon as the mirex was removed from the feed. Chicks hatched from eggs of hens fed 30 mg of mirex/kg of ration for 16 months had body fat residues of more than 1000 ppm of mirex. These chicks were never exposed directly to mirex; however, eggs laid by these chicks as adults had a significant amount of mirex in the yolk (Ivie et al., 1974b).

Mirex in Mammals

Rats rapidly absorbed mirex from the gut. After the administration of a single dose of 6 mg of labeled mirex ^{14}C/kg in corn oil by oral intubation, the rats excreted 55% in the feces within 48 hours and 0.69% in urine after 7 days. Tissues retained approximately 34% of the

total dose of mirex administered. Results of analyses of fat, muscle, liver, kidney, and intestine revealed 27.8%, 3.2%, 1.7%, 0.76%, and 0.23%, respectively, of the total dose remaining 7 days after treatment (Mehendale et al., 1972).

In vitro studies on liver preparations from rats, rabbits and mice incubated with labeled mirex ^{14}C for different lengths of time revealed that the liver was free of any metabolites (Mehendale et al., 1972).

In cattle, mirex was absorbed from the gastrointestinal tract. After giving ^{14}C radiolabeled mirex in gelatin capsules for 28 days to a lactating cow, mirex was detected mainly in feces, milk (0.58 ppm) and adipose tissue (0.21 ppm) but not in the urine (Dorough and Ivie, 1974). Studies by Bond and Woodham (1975) on dairy cows fed daily 0.1 and 1 mg of mirex/kg of ration for 31 weeks resulted in 0.06 and 1.87 ppm of mirex, respectively, in omental fat and 0.08 ppm of mirex in milk. Calves given milk from cows fed daily 1 mg mirex/kg of ration for 20 weeks had mirex residues of 1.67, 0.08, and 0.076 ppm in omental fat, liver and kidney, respectively.

Lofgren et al. (1964) and Hawthorne et al. (1974) reported that no mirex residues were found in the milk of cows grazed in areas treated twice with 1.7 gm of mirex per acre, even at a detection level of 0.3 ppb.

Stein et al. (1976) studied mirex metabolism in monkeys. The metabolites were identified as monohydro- and dihydro-derivatives of mirex (Stein and Pittman, 1977). They reasoned that the presence of these metabolites was due to bacterial action on mirex in the lower gut.

Mirex can cross the placental barrier of rats (Gaines and Kimbrough, 1970; Khera et al., 1976). At the 19th day of gestation, fetuses from dams fed 25 mg of mirex/kg of ration for 78 days contained 0.23 ppm of mirex (Gaines and Kimbrough, 1970).

Mirex has a long environmental half-life (Carlson et al., 1976; Holden, 1976). The rate of dissipation of mirex from the body after being removed from the diet varies with the species. After a single oral dose of 6 mg of mirex/kg body weight, the first half-life time of mirex in rats was 38 hours and the second was in excess of 100 days (Mehendale et al., 1972). In female rats and birds, mirex residues declined by 40% after they were returned to a normal diet for 10 months and 20-30 days, respectively (Ivie et al., 1974c).

Mirex Toxicosis

The degree of mirex toxicity varies among species. It has a high potential for chronic toxicity mainly because it has a tendency to accumulate in the body. However, mirex is considered to be of low acute toxicity. The lowest single oral dose of mirex required to kill adult male and female rats is 400 and 500 mg of mirex/kg body weight, respectively (Gaines and Kimbrough, 1969). The LD_{50} in rats occurs at a level of 600 and 750 mg of mirex/kg body weight for females and males, respectively (Gaines, 1969). In 1970, Gaines and Kimbrough found that the LD_{50} in the same strain of female rats used in the previous experiment was about 365 mg of mirex/kg body weight and the 90 dose LD_{50} was 6 mg of mirex/kg body weight. The chronicity factor which measures the ability of the compound to accumulate was 60.8 and this figure was obtained by dividing the LD_{50} by 90 dose LD_{50}. Mirex, administered in a single oral dose of 80 mg of mirex/kg to 21-day-old rats, resulted in 56% mortality (Lawrence and Kidd, 1978). Studies by Wolfe et al. (1979) in field mice (*Peromyscus polionotus*) indicated that LD_{50} was 17.8 mg of mirex/kg of ration after 105 days. In another study on laboratory mice the LD_{50} occurred after 10 days of feeding at a level

of 330 mg of mirex/kg of ration (Kendall, 1974b), while Ware and Good (1967) reported 100% mortality in mice fed 10 mg of mirex/kg of ration for 60 days, 50 mg of mirex/kg of ration for 15 days, and 250 mg of mirex/kg of ration for 9 days.

The clinical signs reported in rats were lethargy, loss of hair, tremor, diarrhea (Kendall, 1974a; Khera, 1976; Gaines and Kimbrough, 1970), bleeding from the genitalia and severe abdominal swelling (Ivie et al., 1974c). Body weight gain was reduced in female Wistar rats fed 12.5 mg of mirex/kg of ration for 10 days (Khera, 1976). Food consumption was also reduced in rats fed 40 mg and 80 mg of mirex/kg of ration for 4 weeks (Lawrence and Kidd, 1978). In mice 90 mg of mirex/kg of ration in the diet for 7 days reduced body weight gain (Abraham et al., 1974).

Birds were found to be insensitive to acute toxicity of mirex because mirex was excreted rather rapidly (Medley, 1974). Bobwhite quail fed 300 mg of mirex/kg of ration for 10 days were not affected (Kendall, 1974b). Similar results related to body weight change and toxicity were observed when Japanese quail were given 0.3, 3 and 30 mg of mirex/kg of ration for 6 weeks (Ivie et al., 1974b). Studies by Davison et al. (1975) revealed that a concentration up to 160 mg of mirex/kg of ration fed to 24-week-old White Leghorn chickens for 12 weeks did not significantly affect body weight gain. On the other hand, Naber and Ware (1965) reported a loss of body weight in laying hens fed 600 mg of mirex/kg in the ration for 16 weeks but not in the hens fed 300 mg of mirex/kg of ration for 16 weeks. They also reported no change in feed consumption between treated and control birds. Studies by Baetche et al. (1972) indicated that 8 to 81% mortality occurred in

quail, mallards, pheasants, and cowbirds fed diets containing 200 to 500 mg of mirex/kg of ration for 30 to 111 days.

Rhesus monkeys fed a single dose of 1 mg of mirex/kg body weight were not affected (Weiner et al., 1976). One milligram of mirex/kg body weight fed to goats daily for 61 days was not toxic (Smrek et al., 1977). The LD_{50} in dogs was found to exceed 1000 mg of mirex/kg body weight (Larson et al., 1979). No evidence of intoxication was seen in dogs fed a diet containing 4 to 20 mg of mirex/kg of ration for 13 weeks (Larson et al., 1979).

Mirex, when fed to pregnant rats at a rate of 1.5 and 3 mg of mirex/kg body weight daily on days 6 to 15 of gestation, was not teratogenic (Khera, 1976). However, the same author fed 6 and 12.5 mg of mirex/kg of ration to pregnant rats from day 6 through day 15 of pregnancy and found anomalies of the visceral organs, subcutaneous edema, scoliosis, cleft palate, runting, short tail, and fleshy heart with enlarged atria. These anomalies were more prominent in those fed the ration containing 12.5 mg of mirex/kg.

Reproduction

Various effects of mirex on reproduction have been reported in different species. Female rats were fed 25 mg of mirex/kg of ration for 45 days. At that time they were mated and continued on the treated diet through the end of lactation. They produced fewer offspring and the survival rate of offspring was significantly lower than the survival rate of offspring from the controls. Also, the surviving offspring had cataracts. However, pregnant rats fed 5 mg of mirex/kg of ration had normal litters (Gaines and Kimbrough, 1970). Kittens born to queens fed 25 mg of mirex/kg of ration and nursed by untreated foster queens survived to weaning, but there were a few instances of cataracts.

Newborn animals were exposed to mirex by allowing them to nurse foster mothers who had been fed a ration containing 5 mg of mirex/kg for 73 days prior to nursing. This exposure to mirex resulted in an incidence of 38% cataracts in the newborn (Gaines and Kimbrough, 1970). Fuller et al. (1973) gave 45 International Units of pregnant mare's serum to female rats treated with mirex but failed to induce ovulation. They attributed the suppression of ovulation to a depressant effect of mirex on the central nervous system so that it did not release the luteinizing hormone.

Ware and Good (1967) reported that in laboratory mice there is an alteration of fecundity and a reduction in total litter weight as well as the number of offspring born to mothers fed 5 mg of mirex/kg of ration for 30 days.

Reproduction ceased in field mice after they were fed 17.8 mg of mirex/kg of ration for 3 months, but 1.8 mg of mirex/kg of ration only reduced litter size (Wolfe et al., 1979).

Naber and Ware (1965) studied the effect of mirex on egg production and on hatchability of fertile eggs. Mirex fed to laying hens at levels of 300 and 600 mg of mirex/kg of ration for 16 weeks did not affect egg production. Six hundred milligrams of mirex/kg of ration fed to laying hens for 6 and 12 weeks reduced the hatchability of eggs but 300 mg of mirex/kg of ration had no effect. Both levels reduced the survival rate of hatched chickens. Davison et al. (1975) reported that 5, 10, 20, 40, 80, and 160 mg of mirex/kg of ration fed to 24-week-old laying White Leghorn hens for 12 weeks had no effect on egg production, egg weight, shell thickness and calcium content of the shell. Similar results were observed in Japanese quail fed 5, 40, and 80 mg of mirex/kg of feed for 12 weeks (Davison et al., 1975) and 0.3, 3,

or 30 mg of mirex/kg of ration fed for 6 months (Ivie et al., 1974c). In both experiments mirex was recovered from the tissues of the hens and egg yolks. Fertility, hatchability, and survival rate of ducklings were normal from mallard ducks which were fed diets containing 1 or 10 ppm of mirex for one season and of chicks from bobwhite quail which were fed 40 mg of mirex/kg of ration for 2 seasons (Heath and Spann, 1973). Baker (1963) treated quail with various doses of mirex bait. The bait was applied at a rate of 11.2, 112 and 1120 kg/ha. He found a decrease in egg production with increasing levels of mirex, but the hatchability of eggs from the experimental birds was normal. However, Dewitt et al. (1962) found no effect from consumption of mirex bait (1.4 kg/ha) on reproduction or secondary sex characteristics of quail.

Pathology

The liver appears to be a target organ for mirex toxicity because pathologic changes have been observed consistently. Liver weight to body weight ratios were significantly increased in rats fed 25 mg of mirex/kg of ration for 166 days (Gaines and Kimbrough, 1970). However, liver weight to body weight ratios did not change in rats fed 25 mg of mirex/kg body weight for 14 days (Baker et al., 1972), but 100 mg of mirex/kg body weight for 14 days caused a significant increase in relative liver weight. Mehendale et al. (1973) found a significant increase in liver weight to body weight ratios in rats fed 5, 10, 25, and 50 mg of mirex/kg body weight for 5 days. The change in the liver was dose dependent. On the other hand, Byard et al. (1975b) reported that feeding of 60 mg of mirex/kg of diet for one week doubled the liver weight to body weight ratios compared to the control after 2 weeks of feeding. The weight of testes and adrenal glands from rats fed 40 and 80 mg of mirex/kg of ration were increased.

Microscopically, in rats the hepatocytes were enlarged, vacuolated and contained eosinophilic staining inclusions in the cytoplasm (Gaines and Kimbrough, 1970). Intraperitoneal injections of mirex (dosage not given) in rats and mice caused gross focal necrosis on the surface of the liver (Kendall, 1974b). There was fatty infiltration and an increase in collagen in the livers of rats fed 50 mg of mirex/kg of ration for 28 days and then fed a normal feed for 12 days (Singh et al., 1980).

Electron microscopic examination of livers of rats and mice fed mirex revealed proliferation of smooth endoplasmic reticulum (Baker et al., 1972; Gaines and Kimbrough, 1970). Accumulation of ribosomes and dense bodies which may be atypical lysosomal bodies have been reported in rats (Gaines and Kimbrough, 1970). The bile canaliculi were distended, microvilli were reduced in size, and Kupffer cells were disorganized (Singh et al., 1980).

Mirex was considered to be carcinogenic (Innes et al., 1969). Ten milligrams of mirex/kg body weight were intubated daily into 2 strains of 7-day-old mice for 21 days. Then 7 days later, starting at the 28th day, 26 mg of mirex/kg body weight were given in the diet for 70 weeks. They found a 45% incidence of hepatomas in the treated group compared to 4% in the controls. Females were more susceptible than males. In contrast, Ulland et al. (1973) reported an absence of tumors in mice which were given larger doses (dose not given) than those reported by Innes et al. (1969) even after 18 months. Studies in rats fed 50 and 100 mg of mirex/kg of diet for 18 months revealed liver lesions including fatty metamorphosis, megahepatocytes, cystic degeneration, necrosis, nodule formation and carcinomas (Ulland et al., 1977).

Chickens and quail fed 40 to 160 mg mirex/kg of ration for 12 weeks had enlarged livers with necrotic and granular surfaces (Davison et al.,

1976). Microscopically, there was hepatocellular degeneration, fatty changes, focal necrosis and necrosis of bile duct epithelium. Electron microscopy revealed bile canaliculi with thickening of pericanicular ectoplasm, loss of microvilli and formation of myelin figures (Davison et al., 1976).

The induction of hepatic microsomal enzymes has been reported in mice and rats (Gaines and Kimbrough, 1970; Baker et al., 1972; Mehendale et al., 1973). The effect of mirex was more pronounced in rats than mice. Cytochrome P-450 was increased when mirex was given orally or intraperitoneally and a level as low as 1 mg of mirex/kg of body weight given for 14 days to rats resulted in an increase of cytochrome P-450 (BAker et al., 1972). Byard et al. (1975a) reported that mice fed 1 to 90 mg of mirex/kg of ration for 1 to 70 weeks had an increase in total protein in the liver and mixed function oxidases. These increases were related to the dose and the time. Glucose-6 phosphate activity was decreased as mirex levels were increased. The DNA content was increased independently of the dose of mirex and duration of time and reached 150% when compared to the levels in the control animals. These authors attributed the liver enlargement to the stimulation of DNA synthesis.

Photodegradation Products of Mirex

Although mirex is a highly stable chemical, it is degraded slowly by sunlight (Gibson et al., 1972). These workers exposed mirex deposited in silica gel thin layer chromatoplates to sunlight, and Alley et al. (1973) exposed mirex dissolved in hydrocarbon deposited on silica gel surfaces to UV lamp irradiation. Both groups of workers reported production of monohydro- and dihydro-derivative photoproducts of mirex. Ivie et al. (1974a) exposed mirex to sunlight for 28 days and found 90%

of the mirex was unchanged. The photoproduct included monohydro-derivatives (undecachloropentacyclodecane) besides kepone hydrate (nonpentacyclodecane) (Alley et al., 1974; Ivie et al., 1974a). Irradiation of eggs from mallard ducks fed 100 mg of mirex/kg of ration resulted mainly in monohydro- and dihydro-derivatives of mirex photoproducts (Lane et al., 1976). Cripe and Livingston (1977) reported accumulation of mirex photoproducts on bait particles used to control the fire ant. The chlorine atoms in mirex were replaced by hydrogen atoms in the photoproduct derivatives of mirex and the photoproduct was more polar than mirex (Ivie et al., 1974a). The monohydro-derivative of mirex had also been prepared by a reaction of mirex with lithium, water and dry ice (Dilling and Dilling, 1967).

Gibson and co-workers (1972) studied the fate of photodecomposition products of mirex in rats after oral administration. They found that the decomposition products of mirex were similar to mirex in that the decomposition products of mirex were not metabolized by the animal. These products also had a tendency to be stored in fatty tissues with a concentration of 1.1 ppm in fat after 7 days of feeding 0.2 mg/kg of ration. They also noticed a water soluble radiocarbon in the feces, suggesting that the photoproducts were slightly more susceptible to metabolic attack than mirex. Ivie et al. (1974c) administered polar and nonpolar mirex ^{14}C photoproduct orally to rats for 7 days. Eighty percent of the polar compound administered was excreted within 7 days. The excretion was mainly through the feces, but a low level was found in the urine. Similarly, the nonpolar photoproduct was eliminated mainly by way of the feces and only 20% was excreted in 7 days. Tissue analysis indicated that the polar photoproduct was retained mostly by the liver but its concentration declined after treatment was stopped.

The nonpolar photoproduct was retained primarily by fat and the concentration did not decline after treatment was stopped.

MATERIALS AND METHODS

The experimental procedures for the investigation of the characteristics of mirex and photomirex were identical except for the 2 chemicals. It is for this reason that one description will be given for each step with the understanding that whatever was done with one chemical was done with the other.

Eggs

Four hundred eight fertile White Leghorn eggs[a] were candled. The imperfect eggs were discarded and all the remaining eggs were weighed. Only those weighing between 50 and 63 gm were used. The location of each air cell was marked, after which the eggs were stored in a refrigerator until used. The eggs were randomly divided into 9 groups (Table 1). The eggs in the uninoculated group and those in the group in which the air cell was punctured were used as controls for the yolk sac inoculation techniques. Chickens which hatched from the eggs from these 2 groups were discarded at the time they hatched. The chickens hatched from the eggs injected with 0.1 ml corn oil were the control for those hatched from eggs injected with 25 mg of the chemical. Chickens hatched from eggs inoculated with 0.05 ml corn oil were the controls for the 4 remaining experimental groups of chickens.

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[a]From Reichard's Hatchery, St. Louis, Michigan.

Table 1. Experimental design for the study of mirex and photomirex in chickens

Dose	No. of Eggs
Uninoculated	50
Puncture through air cell	50
0.05 ml corn oil	50
0.1 ml corn oil	30
0.005 mg mirex or photomirex	50
0.05 mg mirex or photomirex	60
0.5 mg mirex or photomirex	60
5 mg mirex or photomirex	60
25 mg mirex or photomirex	30
Total	440

Preparation of Dilutions

Both mirex and its photodegradation product (photomirex)[b] were suspended in corn oil. The inoculum was prepared so that the amount of chemical required for each egg was suspended in 0.05 ml of the diluent for the 5, 0.5, 0.05, or 0.005 mg doses that were given. For the 25 mg dose the chemical was suspended in 0.1 ml corn oil. The suspensions of mirex or photomirex were put in a sonicator for 2 hours and then left overnight in a water bath at 37 C.

Method of Injection

Mirex and photomirex were injected into the yolk sac according to the procedure described by McLaughlin et al. (1965). The eggs were left for 2 hours at room temperature prior to incubation. After 18 hours of incubation they were removed from the incubator and the marked area of the air cell was washed with 80% ethyl alcohol. A hole over the center of the air cell was cut through the shell, but care was taken not to puncture the shell membrane. The fine particles of the shell were removed and the area was again wiped with cotton soaked in 80% ethyl alcohol. The eggs were rotated before injection to free the germinal disc and then placed horizontally on a cotton pad. The chemical was injected through a 1 inch 22 gauge hypodermic needle, after which the injection site was sealed with hot paraffin.

The eggs were set with the small ends resting in the incubation trays. They were returned to the incubator and incubated at an optimum temperature of 37 C dry bulb and 30 C wet bulb with 60% humidity. The

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[b]Photomirex was prepared by Dr. M. Zabik, Pesticide Research Center, Michigan State University, East Lansing, Michigan.

eggs were candled on the 5th and 8th days of incubation and just prior to transfer to the hatcher. The number of infertile eggs and dead embryos was recorded. The date of death of the embryos was estimated.

The eggs were transferred to the hatcher on the 19th day. The temperature of the hatcher was maintained at 32 C dry bulb and 36 C wet bulb with a relative humidity of 70%. The hatched chicks were weighed on the hatching day and wing banded.

Autopsy Schedule

The chicks were housed in conventional electrically heated battery brooders with the temperature adjusted to 35 C. They were fed ground commercial chick starter diets[c] and water *ad libitum* until killed.

Four chicks from each group were killed for study at the 4th, 5th, 6th and 8th weeks after hatching. All other chickens were necropsied at other times, but no detailed studies were done on these. The reason for these longer periods was suggested by other published results. These reports indicated that mirex has considerable potential for chronic toxicity since it is not metabolized and is eliminated slowly (Ivie et al., 1974c). It was thought that if such effects do occur they should be present by 8 weeks. Similar information was not known about the effects of photomirex.

Laboratory Procedures

Body and Organ Weights

Chickens were weighed on an electronic Sartorius 3716MP balance[d] prior to killing. The liver, spleen and bursa of Fabricius were cleanly

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[c]Clarksville Elevator, Clarksville, Michigan.

[d]Brinkman Instruments, Inc., Sartorius Balance Division, Cantiague Road, Westbury, New York 11590.

dissected and weighed on an electronic balance.[d] The thymus was scored from 1 (smallest) to 4 (largest) according to the length of the lobules and their sizes, 1 indicating the smallest and 4 indicating the largest.

Blood Samples

The blood was collected from the heart in heparinized tubes. Hemoglobin was determined by the cyanmethemoglobin method, PCV was measured by the microhematocrit method, and red blood cells were counted by an electronic counter.[e] Blood smears were made from each bird and were stained by Wright's stain for the differential leukocyte count.

Serum Electrophoresis

Blood was drawn from the heart and placed in nonheparinized tubes, from which the sera were aspirated and frozen. For serum protein electrophoresis, the pooled sera were applied to cellulose acetate plates[f] and placed on a chamber for 15 minutes at 180 V. The plates were then stained with Ponceau stain,[g] destained by 5% acetic acid, dehydrated with methanol and dried. The plates were then scanned in a densitometer.[h]

Pathologic Techniques

All chickens were necropsied and examined for gross lesions. For histopathologic examination, liver, spleen, bursa of Fabricius, thymus, thyroid, gonad, heart, skin and kidney were fixed in 10% neutral buffered

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[e]Coulter Counter Model ZBl, Coulter Electronic, Hialeah, Florida.

[f]Titan III, Helena Laboratories, Beaumont, Texas.

[g]Helena Laboratories, Beaumont, Texas.

[h]Quick Scan and Quick Quant II, Helena Laboratories, Beaumont, Texas.

formalin. Tissues were processed in an automatic processor,[i] embedded in paraffin and sectioned at 5 to 6 μ. The tissues were stained with hematoxylin-eosin. Frozen sections from the controls and from affected livers were stained by oil red O for lipid visualization.

Transmission Electron Microscopy

Small pieces of liver were fixed in Karnovsky's fixative immediately after necropsy. These thin slices of liver were minced 48 hours later and washed with Zetterqvist solution at a pH of 7.4 (Pease, 1964). The tissues were then post-fixed in 1% osmium in Zetterqvist solution. Tissues were dehydrated in alcohol and transferred to propylene oxide. A mixture of Epon and Araldite was used for embedding.

Semithin sections were made and stained by toluidine blue for rapid scanning. Thin sections were cut by a glass knife on an ultramicrotome.[j] The tissue sections were stained with uranyl acetate and lead citrate and examined under an electron microscope.[k]

Antigenic Stimulation and Antibody Titrations

Four chickens that were given 0, 0.005, 0.05 or 0.5 mg of mirex or photomirex as embryos, for a total of 16 birds, were given injections intravenously of 1 ml of phosphate buffered saline (PBS) containing 2.5 x 10^9 cells of sheep erythrocyte (SE) at the 4th week of age. Sera were collected 7 days after antigen administration and another similar injection of SE was given. Sera were also collected 7 days after the second injection. All the sera were inactivated by heating at 56 C for 30 minutes.

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[i] Autotechnicon, The Technicon Company, Chauncey, New York.

[j] LKB Ultratome III^R, Instrument Group 8800, Sweden.

[k] EM 9S2, Carl Zeiss, Germany.

The hemagglutination test was done on microtiter U plates containing 8 x 12 wells.[l] One drop of phosphate buffered saline was placed in each well with a plastic dispenser[m] that delivered 0.25 ml. From each serum sample, 0.25 ml was added to the first well by plastic dispenser[n] and 2-fold dilutions were made. To each well 0.025 ml of 1% washed SE suspension in PBS was added. A phosphate buffered saline control was done for each serum tested. The plates were then shaken gently and incubated at 37 C for 1 hour and read. They were then shaken again and left overnight in a refrigerator to be read the following day. The endpoint was the highest dilution of serum which resulted in complete agglutination. The titers were expressed as $\log_2$ of the reciprocal of the highest dilution of serum causing complete agglutination of SE.

Preparation of Tissue Samples

Samples from chickens killed from the same group in the same week were pooled for analysis. One gram of the pooled tissues was weighed on a Mettler Analytical Balance.[o] The weighed tissues were rinsed with ether into stainless steel beakers. The tissues were then ground with sand[p] by using a stainless steel rod and dehydrated by adding sodium sulfate.[q] About 15 ml of glass-distilled hexane[r] were added to the

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[l]Cooke Laboratory Products, Alexandria, Virginia.

[m]Helena Laboratories, Beaumont, Texas.

[n]Helena Laboratories, Beaumont, Texas.

[o]Mettler Instrument Corporation, Box 100, Princeton, New Jersey.

[p]J. T. Baker Chemical Company, Phillipsburg, New York.

[q]Mallinckrodt, Inc., Paris, Kentucky.

[r]Burdick and Jackson Laboratories, Inc., Muskegon, Michigan.

beakers and the mixtures were boiled over a heated aluminum plate. The solutions were filtered into a 100 ml volumetric flask. The addition of the same amount of hexane followed by filtration was repeated 3 times. The volume of the filtrate was made up to 100 ml by glass-distilled hexane. A 20 ml portion of the liquid was separated and condensed approximately to 0.5 ml by evaporation.[s]

The samples were eluted in a magnesium silicate column. A 50 ml thistle tube about 200 x 7 mm was used. A small amount of glass wool was placed at its tapered end. About 1.6 gm of Florisil[t] were added to the column, after which a small amount of granular anhydrous sodium sulfate[u] was added. The column was washed with 5 ml of glass-distilled hexane and the washing was discarded. The 0.5 ml condensed sample was poured into the column and was eluted with 13 ml glass-distilled hexane. About 2 ml of the eluate were discarded and the remainder was collected in a 15 ml graduated centrifuge tube. The eluate was evaporated to approximately 0.5 ml. The condensed samples were made up to 2 ml with glass-distilled iso-octane.[v]

Mirex and Photomirex Analysis

The eluted samples were analyzed by gas chromatography. The carrier gas used was nitrogen with a flow rate of 30 ml/min. The column was 6 x 2 mm internal diameter with a temperature adjusted to 240 C for

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[s] N-Evap, Model III, Meyer Organomation Associates, Inc., Shrewsburg, Maryland.

[t] Activated magnesium silicate, 60-100 Mesh, Fisher Scientific Company, Fairlawn, New Jersey.

[u] Mallinckrodt, Inc., Paris, Kentucky.

[v] Burdick and Jackson Laboratories, Inc., Muskegon, Michigan.

detection. The samples were diluted with iso-octate depending on the concentration of the chemical in the sample. Two microliters of each sample were injected into the gas chromatograph.[w] Results were compared to standards containing 0.05 µg of mirex or photomirex/ml. Results were expressed as parts per million (ppm) of the chemical present in the tissue.

Statistical Analysis

Data on hatchability were analyzed by Bonferoni Chi Square (Gill, 1978). The rest of the data were analyzed statistically using the Statistical Package for Social Sciences (SPSS - Northern University) at the Michigan State University Computer Center. Two-way analysis of variance followed by the Dunnett t-test were used.

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[w]CC Model 3700, Varian Instrument Division, Palo Alto, California.

RESULTS

Hatchability

The hatching time of the fertile eggs, in the mirex experiment, was delayed in all the groups. Hatching occurred between the 23rd and 25th days. Some chicks hatched from eggs inoculated with 5 or 25 mg of mirex were too weak to escape from the shell without help.

The effect of mirex on hatchability of the eggs is presented in Table 2. The hatchability of the mirex-inoculated eggs was decreased in a dose-related response (Figure 2). However, the difference was significant only in chicks hatched from eggs inoculated with 5 or 25 mg of mirex compared to those hatched from eggs inoculated with corn oil. The majority of embryos from the eggs inoculated with 25 mg of mirex did not live more than 10 days after inoculation. Only 3 of the 24 fertile eggs inoculated hatched. Thirty-four of the 43 embryos from eggs inoculated with 5 mg of mirex that did not survive died between the 2nd and 14th day of incubation. The remaining 9 of these died during hatching. Embryo mortality from the eggs inoculated with corn oil or 0.005, 0.05 or 0.5 mg of mirex occurred between the 2nd and 19th day of incubation.

Two crippled chicks, characterized by ataxia and spreading of the legs, were found among those hatched from eggs inoculated with 0.5 or 5 mg of mirex (Table 4).

Table 2. The effect on hatchability when mirex was injected into fertile eggs after 18 hours of incubation

Dose	No. eggs inoculated	No. eggs infertile	No. chicks hatched	No. chicks dead	Percent mortality	Percent hatchability	Body weight at hatching (gm)[a]
Uninoculated	50	10	36	4	10	90	39.5±3.5
Punctured through air cell	50	7	30	13	30	70	41.2±7.4
0.05 ml corn oil	50	6	28	16	36	64	38.4±3.3
0.1 ml corn oil	30	6	13	11	46	54	35.3±5.2
0.005 mg mirex	50	3	32	15	32	68	39.1±4.5
0.05 mg mirex	60	8	31	21	40	60	40.1±6.2
0.5 mg mirex	60	5	28	27	49	51	38.1±4.5
5 mg mirex	60	7	10	43	81[b]	19	37.1±3.4
25 mg mirex	30	6	3	21	88[c]	13	34.5±3.4

[a]Values represent mean ± SEM

[b]Different ($p<0.05$) from 0.05 ml corn oil group

[c]Different ($p<0.05$) from 0.1 ml corn oil group

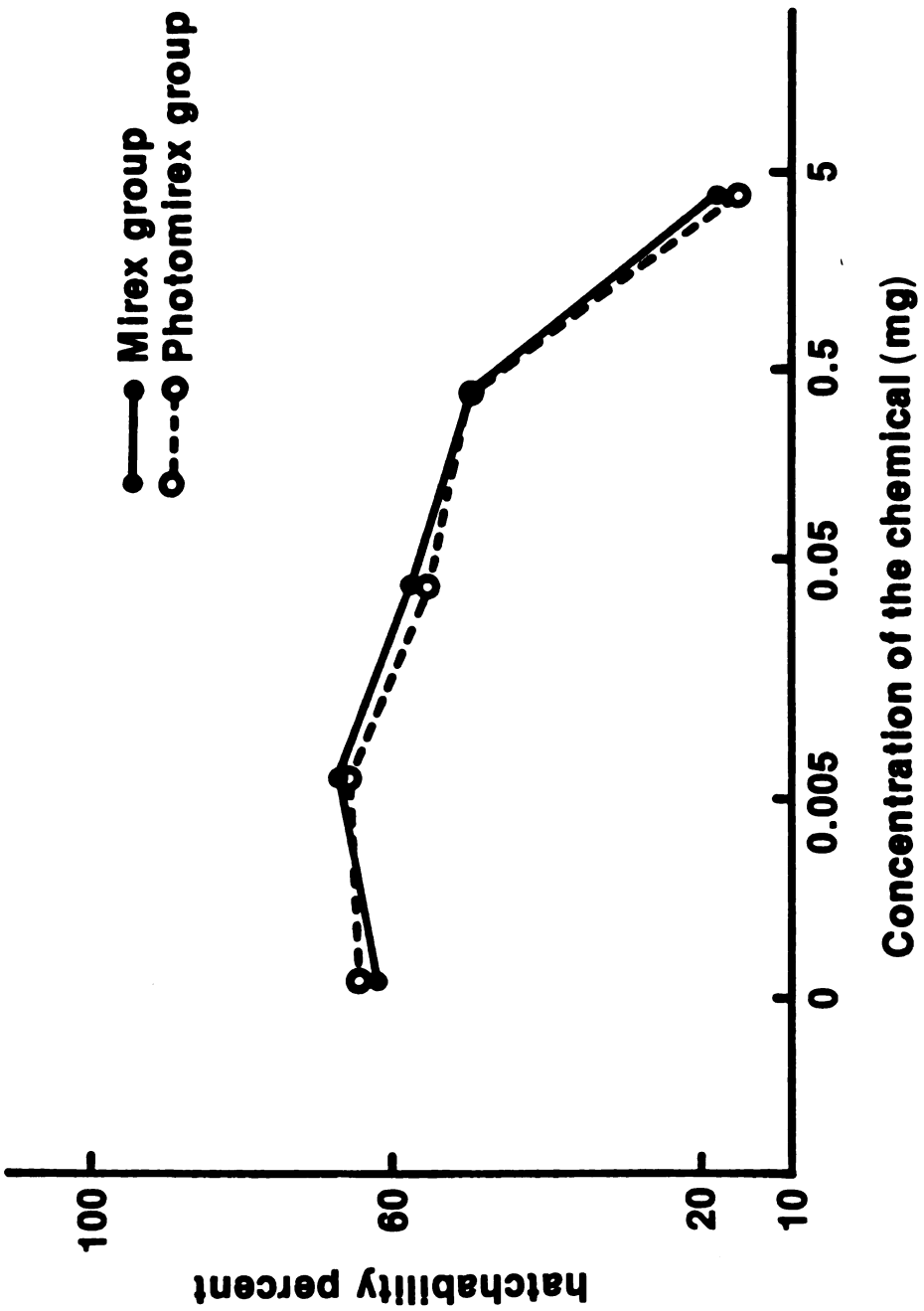

Figure 2. Percent hatchability of chick embryos inoculated with different doses of mirex or photomirex

On the day of hatching, there was no effect on the average body weight of chicks hatched from eggs inoculated with mirex when compared to those of the control group (Table 2).

The hatch time of eggs inoculated with photomirex was increased to 24 days. The 2 chicks hatched from eggs inoculated with 25 mg of photomirex and the majority from the eggs inoculated with 5 mg of photomirex had to be helped out of the shell. The data on hatchability of eggs inoculated with photomirex are presented in Table 3. There was a trend toward an increase in the hatchability as the level of photomirex was decreased (Figure 2). Doses of 5 or 25 mg of photomirex per egg significantly decreased the hatchability when compared to that of the eggs inoculated with corn oil.

Inoculation of 25 mg of photomirex per egg caused embryo mortality within the first 8 days of incubation and only 2 out of 23 fertile eggs inoculated hatched. Ninety percent of the deaths of chick embryos from the eggs inoculated with 5 mg of photomirex occurred within the first 13 days of incubation and 10% at hatching. In the other groups, embryo mortality occurred between the 2nd and 17th days of incubation.

Crippled chicks, similar to those described previously in the mirex experiment, were observed in all groups (Table 4).

The body weights of chicks at the hatching day was not affected by inoculation of 0.005, 0.05, 0.5, or 5 mg of photomirex per egg. However, there was a difference between the body weight of chicks hatched from eggs inoculated with 25 mg of photomirex compared to those hatched from eggs inoculated with 0.1 ml of corn oil.

Table 3. The effect on hatchability when photomirex was injected into fertile eggs after 18 hours of incubation

Dose	No. eggs inoculated	No. eggs infertile	No. chicks hatched	No. chicks dead	Percent mortality	Percent hatchability	Body weight at hatching (gm)[a]
Uninoculated	50	9	37	4	10	90	39.5±3.2
Punctured through air cell	50	8	32	10	24	76	40.1±3.4
0.05 ml corn oil	50	3	31	16	34	66	38.8±2.5
0.1 ml corn oil	30	7	13	10	43	57	37.5±5.5
0.005 mg photomirex	50	5	30	15	33	67	40.4±4.3
0.05 mg photomirex	60	6	31	23	43	57	39.6±2.6
0.5 mg photomirex	60	7	27	26	49	51	38.4±3.4
5 mg photomirex	60	4	10	46	82[b]	18	37.2±2.1
25 mg photomirex	30	7	2	21	91[c]	9	30.2±1.8[c]

[a]Values represent mean ± SEM

[b]Different ($p<0.05$) from 0.05 ml corn oil group

[c]Different ($p<0.05$) from 0.1 ml corn oil group

Table 4. Number of crippled chicks* hatched from eggs inoculated after 18 hours of incubation with different doses of mirex or photomirex

Doses (mg)	Mirex	Photomirex
0.005	0	3
0.05	0	3
0.5	0	2
5	2	2
25	2	1

*Two crippled birds were observed in groups punctured through air cell and in groups given 0.05 ml corn oil. One crippled bird occurred in groups given 0.1 ml corn oil.

Clinical Signs

There were no clinical signs observed in any of the chickens hatched from eggs treated with mirex, although 2 to 4 chickens died from all the groups during the course of the experiment. The 3 chickens hatched from eggs inoculated with 25 mg of mirex died within the first 10 days.

The chickens hatched from eggs inoculated with 5 or 0.5 mg of photomirex were weak and had ruffled feathers and pale combs (Figure 3). They acted normally otherwise. The rest of the chickens from the other groups were clinically normal.

The 2 chickens hatched from eggs inoculated with 25 mg of photomirex were less active and died within the first week after hatching.

Body Weight

The average body weights of chickens hatched from eggs inoculated with 0, 0.005, 0.05, 0.5, or 5 mg of mirex are presented in Table 5. Analysis of variance revealed a significant effect of time ($p<0.001$) and dose ($p<0.005$) but not the interaction between them. The difference in body weight was statistically significant ($p<0.05$) at the 5th and 8th weeks in the chickens hatched from eggs inoculated with 5 or 0.5 mg of photomirex, respectively, as compared to the control.

Liver Weights

The mean liver weights and the ratio of liver weight in mg to body weight in gm of chickens hatched from eggs inoculated with mirex are shown in Table 7. Analysis of variance of the relative liver weights revealed a significant effect by time and dose but not the interaction between them. The relative liver weights were increased as a dose response. However, the difference was more pronounced by the 4th and 5th weeks in chickens hatched from eggs inoculated with 5 mg of mirex

Figure 3. General appearance of a chicken hatched from an egg inoculated with 0.5 mg of photomirex (left) and a control chicken hatched from an egg inoculated with 0.05 ml of corn oil (right).

Table 5. Mean body weight of chickens hatched from eggs inoculated after 18 hours of incubation with different doses of mirex

Doses of mirex (mg)	Body Weights (gm)[a]			
	4th week	5th week	6th week	8th week
0	338.50±71.6	415.85±30.4	700.42±27.5	955.02±46.1
0.005	320.58±65.5	388.35±31.2	673.08±23.8	923.75±41.9
0.05	323.63±76.6	394.53±27.9	657.08±29.8	908.50±44.1
0.5	317.78±81.6	368.65±45.4	632.70±64.8	873.75±99.3
5	263.25±84.0	334.78±22.8	b	b

[a]Values represent means ± SEM, n=4

[b]All chickens had been killed by the end of the 5th week.

Table 6. Mean body weight of chickens hatched from eggs inoculated after 18 hours of incubation with different doses of photomirex

Doses of photomirex (mg)	Body Weights (gm)[a]			
	4th week	5th week	6th week	8th week
0	328.92±47.8	490.58±37.3	708.03±78.1	948.25±142.6
0.005	342.85±61.4	473.08±53.9	647.65±45.3	924.01±143.6
0.05	338.90±63.2	451.70±42.6	643.18±78.1	871.25± 99.6
0.5	316.48±42.5	435.83±28.4	631.58±56.2	810.09± 92.5[c]
5	288.63±40.2	350.63±18.4[c]	b	b

[a]Values represent mean ± SEM, n=4

[b]All chickens had been killed by the end of the 5th week.

[c]Different ($p<0.05$) from control group

Table 7. Mean liver weights (absolute and as a ratio of weights in mg to body weight in gm) from chickens hatched from eggs inoculated after 18 hours with different doses of mirex

Doses of mirex (mg)	Liver Weights (gm)[a]				Ratio of Liver Weight (mg) to Body Weight (gm)[a]			
	4th week	5th week	6th week	8th week	4th week	5th week	6th week	8th week
0	6.94±1.6	9.25±1.3	11.79±1.2	14.78±2.0	20.90±2.9	22.31±2.1	16.84±1.7	15.61±2.8
0.005	6.97±1.3	9.53±1.1	12.08±1.2	15.14±2.2	21.74±2.0	24.02±2.5	17.93±1.3	16.40±2.4
0.05	7.06±1.9	9.40±1.5	12.20±1.0	16.27±1.9	21.81±1.8	23.91±1.4	18.56±1.1	17.92±2.4
0.5	7.70±1.8	9.55±1.2	12.18±1.8	16.60±2.0	24.22±1.4[c]	24.55±1.6[c]	19.21±1.3	19.03±3.3[c]
5	7.39±2.1	9.64±1.6	b	b	27.91±1.9[d]	27.60±2.4[d]	b	b

[a]Values represent mean ± SEM, n=4

[b]All chickens had been killed by the end of the 5th week.

[c]Different ($p<0.05$) from control group

[d]Different ($p<0.01$) from control group

($p<0.01$) and by the 4th, 5th and 8th weeks in those chickens hatched from eggs inoculated with 0.5 mg of mirex ($p<0.05$).

There was no effect on the absolute liver weights of chickens hatched from eggs inoculated with mirex when compared to those of the control chickens.

The mean absolute and relative liver weights from chickens hatched from eggs inoculated with photomirex are shown in Table 8.

Analysis of the relative liver weights revealed a significant effect of time ($p<0.001$), dose ($p<0.001$) and the interaction between them ($p<0.05$). There was a significant increase in the relative liver weights by the 4th and 5th weeks in chickens hatched from eggs inoculated with 5 mg of photomirex ($p<0.01$) and in the chickens at the 5th, 6th and 8th weeks ($p<0.05$) hatched from eggs inoculated with 0.5 mg of photomirex. Apparently photomirex had no effect on the absolute liver weights as compared to the liver weights from control chickens.

Bursa Weights

The mean bursa weight and the bursa weight in mg to body weight in gm of chickens hatched from eggs inoculated with mirex are presented in Table 9. Analysis of variance of the relative bursa weights revealed a significant effect of time ($p<0.001$) but neither of dose nor the interaction between them. Apparently, mirex had no effect on the absolute and relative weights of the bursa compared to those of the controls.

The bursa weights and the ratio of bursa weight in mg to body weight in gm of chickens hatched from eggs inoculated with photomirex are shown in Table 10. Analysis of variance applied to the relative bursa weights revealed a significant effect of time but of neither dose

Table 8. Mean liver weights (absolute and as a ratio of weights in mg to body weight in gm) from chickens hatched from eggs inoculated after 18 hours with different doses of photomirex

Doses of photomirex (mg)	Liver Weights (gm)[a]				Ratio of Liver Weight (mg) to Body Weight (gm)[a]			
	4th week	5th week	6th week	8th week	4th week	5th week	6th week	8th week
0	7.24±1.3	10.06±1.2	12.65±1.3	15.36±1.8	22.80±1.3	20.54±1.8	17.89±1.9	17.14±2.2
0.005	7.47±1.2	10.06±1.3	11.05±1.2	17.63±1.4	21.87±1.6	21.25±1.7	17.03±1.8	18.87±1.9
0.05	7.56±1.6	10.09±1.9	11.66±2.3	17.00±2.3	22.84±1.5	22.42±1.4	18.01±1.6	19.42±1.6
0.5	7.48±1.0	10.12±1.8	13.01±1.5	17.52±2.6	23.68±1.8	23.30±1.6[c]	20.70±1.7[c]	21.74±2.1[c]
5	7.88±1.4	10.46±1.5	b	b	27.12±2.7[d]	28.35±2.9[d]	b	b

[a]Values represent mean ± SEM, n=4

[b]All chickens had been killed by the end of the 5th week.

[c]Different ($p<0.05$) from control group

[d]Different ($p<0.01$) from control group

Table 9. Mean bursa weights (absolute and as a ratio of weights in mg to body weight in gm) from chickens hatched from eggs inoculated after 18 hours of incubation with different doses of mirex

Doses of mirex (mg)	Bursa Weights (gm)[a]				Ratio of Bursa Weight (mg) to Body Weight (gm)[a]			
	4th week	5th week	6th week	8th week	4th week	5th week	6th week	8th week
0	1.72±0.4	2.76±0.7	3.40±0.4	2.07±0.5	5.08±0.5	6.65±0.5	4.85±0.4	2.15±0.6
0.005	1.53±0.9	2.15±0.3	2.83±0.6	2.40±0.7	4.84±0.8	5.54±0.9	4.22±1.0	2.59±0.7
0.05	1.46±0.3	2.45±0.3	3.22±0.4	2.72±0.6	4.76±1.0	6.20±0.5	4.89±0.6	2.99±0.6
0.5	1.89±1.0	2.25±0.5	3.21±0.7	2.17±0.9	5.91±2.6	6.07±0.5	5.09±1.1	2.51±0.4
5	1.75±0.3	2.26±0.6	b	b	5.39±2.4	6.52±1.9	b	b

[a]Values represent mean ± SEM, n=4

[b]All chickens had been killed by the end of the 5th week.

Table 10. Mean bursa weights (absolute and as a ratio of weights in mg to body weight in gm) from chickens hatched from eggs inoculated after 18 hours of incubation with different doses of photomirex

Doses of photomirex (mg)	Bursa Weights (gm)[a]				Ratio of Bursa Weight (mg) to Body Weight (gm)[a]			
	4th week	5th week	6th week	8th week	4th week	5th week	6th week	8th week
0	1.58±0.5	1.98±0.4	2.97±0.8	2.30±1.0	4.88±1.4	4.01±0.6	4.42±1.5	3.02±0.6
0.005	1.51±0.7	2.15±0.3	3.01±1.1	3.10±1.4	5.16±0.9	4.59±0.9	5.00±1.3	4.31±1.1
0.05	1.87±0.5	2.16±0.4	2.15±0.8	2.04±0.4	5.64±1.5	4.80±0.7	3.86±1.2	3.59±0.8
0.5	1.60±0.5	1.83±0.5	3.83±0.7	2.36±0.8	4.94±1.1	4.25±1.2	6.04±1.1[c]	4.11±0.5
5	1.95±0.8	2.02±0.3	b	b	5.25±1.9	5.76±0.5[c]	b	b

[a]Values represent mean ± SEM, n=4

[b]All chickens had been killed by the end of the 5th week.

[c]Different ($p<0.05$) from control group

nor the interaction between them. At the 5th and 6th weeks of age the ratios were significantly higher in the chickens hatched from eggs inoculated with 5 or 0.5 mg of photomirex, respectively. The photomirex did not affect the absolute bursa weights.

Spleen Weights

The mean spleen weight and the ratio of spleen weight in mg to body weight in gm of chickens hatched from eggs inoculated with mirex are presented in Table 11. Mirex did not affect the absolute and the relative weights of the spleens as compared to the spleens of the controls.

The mean spleen weight and the ratio of spleen weight in mg to body weight in gm of chickens hatched from eggs inoculated with photomirex are shown in Table 12. The spleen to body weight ratio in the chickens hatched from eggs inoculated with 5 mg of photomirex was larger at the 5th week than that in the control chickens. There was no effect observed on the absolute spleen weights from chickens hatched from eggs inoculated with photomirex compared to the spleens of the control chickens.

Thymus Scoring

The effect of mirex or photomirex on the thymus is presented in Table 13. Mirex had no effect on the thymus size at all levels tested. Photomirex significantly decreased the size of the thymus at the 4th week in chickens hatched from eggs inoculated with 5 mg photomirex. All other levels of photomirex did not cause any difference in thymus size compared to the thymuses from control chickens.

Table 11. Mean spleen weights (absolute and as a ratio of weights in mg to body weight in gm) from chickens hatched from eggs inoculated after 18 hours of incubation with different doses of mirex

Doses of mirex (mg)	Spleen Weights (gm)[a]				Ratio of Spleen Weight (mg) to Body Weight (gm)[a]			
	4th week	5th week	6th week	8th week	4th week	5th week	6th week	8th week
0	0.54±0.2	0.86±0.1	0.97±0.1	1.69±0.3	1.75±0.7	2.12±0.3	1.39±0.2	1.77±0.3
0.005	0.59±0.1	0.81±0.2	1.05±0.3	1.90±0.5	1.84±0.1	2.08±0.4	1.61±0.4	2.06±0.6
0.05	0.60±0.2	0.77±0.1	1.01±0.4	1.57±0.3	1.87±0.3	1.98±0.4	1.53±0.6	1.74±0.4
0.5	0.60±0.3	0.73±0.3	0.93±0.3	1.59±0.2	1.92±0.4	1.94±0.7	1.46±0.3	1.91±0.2
5	0.47±0.1	0.69±0.1	b	b	1.81±0.3	1.82±0.2	b	b

[a]Values represent mean ± SEM, n=4

[b]All chickens had been killed by the end of the 5th week.

Table 12. Mean spleen weights (absolute and as a ratio of weights in mg to body weight in gm) from chickens hatched from eggs inoculated after 18 hours of incubation with different doses of photomirex

Doses of photomirex (mg)	Spleen Weights (gm)[a]				Ratio of Spleen Weight (mg) to Body Weight (gm)[a]			
	4th week	5th week	6th week	8th week	4th week	5th week	6th week	8th week
0	0.54±0.2	1.03±0.1	1.80±0.6	1.78±0.5	1.65±0.5	2.10±0.2	2.50±1.2	1.86±0.3
0.005	0.54±0.1	0.96±0.1	1.64±0.7	1.96±0.9	1.64±0.5	2.06±0.2	2.56±0.6	2.05±0.6
0.05	0.64±0.4	0.90±0.2	1.53±0.3	1.63±0.2	1.87±0.3	1.76±0.3	2.37±0.2	1.91±0.4
0.5	0.55±0.1	0.90±0.2	1.70±0.4	1.48±0.6	1.75±0.3	2.05±0.4	2.72±0.8	1.85±0.7
5	0.53±0.3	0.82±0.5	b	b	1.77±0.8	2.34±0.6[c]	b	b

[a]Values represent mean ± SEM, n=4

[b]All chickens had been killed by the end of the 5th week.

[c]Different ($p<0.05$) from control group

Table 13. Thymus scoring of chickens hatched from eggs inoculated after 18 hours of incubation with different doses of mirex or photomirex

Doses of the chemical (mg)	Thymus Scoring*							
	Mirex[a]				Photomirex[a]			
	4th week	5th week	6th week	8th week	4th week	5th week	6th week	8th week
0	3.3±0.2	3.7±0.4	2.9±0.7	3.2±0.4	3.8±0.5	3.5±0.6	3.1±0.6	3.1±0.3
0.005	3.0±0.6	3.3±0.7	3.2±0.8	2.8±0.2	2.8±0.6	3.0±0.8	3.3±0.7	3.1±0.2
0.05	2.8±0.8	3.0±0.9	3.5±0.4	3.0±0.4	2.8±0.5	2.9±0.9	2.5±1.0	3.0±0.4
0.5	3.4±0.4	2.9±0.6	3.0±0.2	2.8±0.9	3.0±0.4	2.8±0.9	2.4±0.6	2.6±1.1
5	2.5±0.6	2.9±0.3	b	b	2.5±0.7[c]	2.5±0.6	b	b

*A score of 1 indicates the smallest; a score of 4 indicates the largest.

[a]Values represent mean ± SEM, n=4

[b]All chickens had been killed by the end of the 5th week.

[c]Different ($p<0.05$) from control group

Hematologic Findings

The hematologic values of chickens hatched from eggs inoculated with mirex are summarized in Table 14. Hemoglobin concentration and packed cell volume (PCV) were not affected by treatment. The number of red blood cells (RBC) was in the normal range. The differential leukocyte counts (Appendix, Table A1) were not affected by mirex. The mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) values are shown in the Appendix (Table A3).

Table 15 summarizes the hematologic profile of chickens hatched from eggs inoculated with photomirex. Photomirex did not affect the RBC counts and PCV. However, the hemoglobin concentration in chickens hatched from eggs inoculated with 0.5 or 5 mg of photomirex was decreased at the 6th and 4th weeks, respectively. There were no changes in the differential leukocyte counts of chickens hatched from eggs inoculated with photomirex (Appendix, Table A2). The MCV, MCH and MCHC values are shown in the Appendix (Table A4).

Serum Electrophoresis

The concentrations of albumin, globulins and the albumin to globulin ration (A/G) in serum of chickens hatched from eggs inoculated with mirex are shown in Table 16. Mirex did not cause changes in these values.

The concentrations of albumin, globulins and A/G ratios of chickens hatched from eggs inoculated with photomirex are shown in Table 17. There was a significant increase in the amount of albumin and this explains the increase in A/G ratios in the serum of chickens hatched from eggs inoculated with 5 mg or 0.5 mg photomirex at weeks 5 and 6, respectively.

Table 14. The hematological values from chickens hatched from eggs inoculated after 18 hours of incubation with different doses of mirex

Doses of mirex (mg)	4th week			5th week			6th week			8th week		
	RBC[a] mm^3	Hb[a] gm/dl	PCV[a] %	RBC mm^3	Hb gm/dl	PCV %	RBC mm^3	Hb gm/dl	PCV %	RBC mm^3	Hb gm/dl	PCV %
0	2.16 ±0.5	11.32 ±0.1	31.22 ±4.2	2.32 ±0.1	11.50 ±0.8	31.10 ±2.6	2.45 ±0.2	12.52 ±2.1	31.54 ±2.2	2.36 ±0.3	11.41 ±2.5	29.01 ±2.9
0.005	2.32 ±0.3	10.81 ±0.4	32.42 ±3.1	2.01 ±0.7	10.42 ±1.0	28.91 ±1.9	2.18 ±0.4	11.81 ±2.4	29.49 ±1.9	2.39 ±0.3	12.40 ±3.0	30.15 ±2.3
0.05	2.36 ±0.1	10.80 ±0.4	34.02 ±2.1	2.30 ±0.5	11.54 ±1.5	30.12 ±2.7	2.22 ±0.5	11.12 ±3.5	28.51 ±3.4	2.58 ±0.4	13.04 ±2.1	33.24 ±3.3
0.5	2.12 ±0.1	10.44 ±0.3	29.30 ±5.1	2.18 ±0.7	10.82 ±1.3	29.29 ±4.5	2.19 ±0.3	11.40 ±4.1	28.00 ±3.2	2.05 ±0.5	10.40 ±3.2	27.06 ±5.4
5	1.94 ±0.6	10.00 ±0.3	28.12 ±4.2	2.04 ±0.5	9.11 ±1.2	29.52 ±3.1	b	b	b	b	b	b

[a]Values represent mean ± SEM, n=4

[b]All chickens had been killed by the end of the 5th week.

Table 15. The hematological values from chickens hatched from eggs inoculated after 18 hours of incubation with different doses of photomirex

Doses of photomirex (gm)	4th week			5th week			6th week		
	RBC[a] mm^3	Hb[a] gm/dl	PCV[a] %	RBC mm^3	Hb gm/dl	PCV %	RBC mm^3	Hb gm/dl	PCV %
0	2.24 ±0.1	12.11 ±2.1	30.53 ±3.1	2.58 ±0.9	11.30 ±1.9	31.11 ±2.5	2.63 ±0.4	12.61 ±1.2	32.42 ±3.6
0.005	2.26 ±0.3	11.22 ±1.2	29.10 ±2.9	2.49 ±0.3	11.11 ±2.1	31.42 ±2.9	2.66 ±0.5	13.04 ±1.6	32.01 ±2.2
0.05	2.20 ±0.2	11.00 ±1.9	28.56 ±2.7	2.43 ±0.1	11.60 ±1.0	30.09 ±3.1	2.49 ±0.6	12.02 ±1.9	30.21 ±4.2
0.5	2.30 ±0.1	10.0 ±1.2	29.20 ±3.1	2.32 ±0.8	10.31 ±0.9	29.17 ±4.1	2.53 ±0.9	10.20 ±0.9[c]	30.91 ±3.4
5	2.19 ±0.4	8.11 ±2.3[c]	27.50 ±2.6	2.47 ±0.5	10.02 ±0.9	29.45 ±3.6	b	b	b

[a]Values represent mean ± SEM, n=4

[b]All chickens had been killed by the end of the 5th week.

[c]Different ($p<0.05$) from control group

Table 16. The concentration of albumin (Alb), globulin (Glob) and albumin/globulin ratio (A/G) in serum of chickens hatched from eggs inoculated after 18 hours of incubation with different doses of mirex

Doses of mirex (mg)	Values[a]								
	4th week			5th week			6th week		
	Alb(g/dl)	Glob(g/dl)	A/G	Alb(g/dl)	Glob(g/dl)	A/G	Alb(g/dl)	Glob(g/dl)	A/G
0	1.04	1.35	0.77	1.25	1.57	0.79	1.45	1.71	0.85
0.005	1.14	1.44	0.79	1.30	1.60	0.81	1.34	1.71	0.78
0.05	0.96	1.34	0.71	1.23	1.55	0.79	1.46	1.67	0.87
0.5	0.94	1.20	0.78	1.36	1.64	0.82	1.33	1.60	0.83
5	0.99	1.23	0.80	1.15	1.47	0.78	b	b	b

[a]Values are from pooled sera from 4 chickens.

[b]All chickens had been killed by the end of the 5th week.

Table 17. The concentration of albumin (Alb), globulin (Glob) and albumin/globulin ratio (A/G) in serum of chickens hatched from eggs inoculated after 18 hours of incubation with different doses of photomirex

Doses of photomirex (mg)	Values[a]								
	4th week			5th week			6th week		
	Alb(g/dl)	Glob(g/dl)	A/G	Alb(g/dl)	Glob(g/dl)	A/G	Alb(g/dl)	Glob(g/dl)	A/G
0	1.03	1.37	0.75	1.04	1.46	0.71	1.09	1.71	0.6
0.005	1.02	1.28	0.80	1.15	1.45	0.79	1.03	1.60	0.6
0.05	1.06	1.34	0.79	1.02	1.52	0.71	1.06	1.64	0.6
0.5	0.99	1.21	0.82	1.01	1.40	0.72	1.41[c]	1.59	0.8
5	0.97	1.33	0.73	1.43[c]	1.37	1.04	b	b	bb

[a]Values are from pooled sera from 4 chickens.

[b]All chickens had been killed by the end of the 5th week.

[c]Different ($p<0.05$) from control group

Antibody Response

Evaluations of the humoral immune responses are shown in Table 18 for the chickens hatched from eggs inoculated with mirex or photomirex. The primary and secondary responses to sheep erythrocytes were significantly decreased ($p<0.05$) in chickens hatched from eggs inoculated with 0.5 mg photomirex. However, there was a trend toward a reduction in the humoral response in chickens hatched from eggs inoculated with the same level of mirex. There were no differences in the antibody response in chickens hatched from eggs inoculated with other concentrations of mirex or photomirex compared to the antibody response in the controls.

Mirex and Photomirex Analyses

The concentrations of mirex in the liver, kidney and fat (pooled samples) of chickens hatched from eggs inoculated with mirex are presented in Table 19. The concentration of mirex was highest in the liver and lowest in the kidney, in decreasing order. In general, the concentration of mirex in the tissues was dose related and decreased with time.

The concentration of mirex on the 10th day of age in chickens hatched from eggs inoculated with 25 mg of mirex was 158 and 101 ppm in the liver and fat, respectively.

The concentrations of photomirex in the liver, kidney, and fat (samples pooled) of chickens hatched from eggs inoculated with photomirex are shown in Table 20. The concentration of photomirex in the tissues was proportional to the dosage of photomirex injected into the eggs. The liver contained the highest concentration of photomirex. Body fat and kidney were next highest, in that order. Photomirex was present in the liver at a higher concentration in chickens hatched from eggs inoculated with 0.5 mg of photomirex up to the 8th week when compared to livers from other treated chickens.

Table 18. Humoral antibody response of chickens at the 5th and 6th weeks of age which were hatched from eggs inoculated after 18 hours of incubation with different doses of mirex or photomirex

Doses of the chemical (mg)	Mirex Antibody to SE* (hemagglutination test)[a]		Photomirex Antibody to SE* (hemagglutination test)[a]	
	Primary response	Secondary response	Primary response	Secondary response
0	7.2±0.9	8.0±1.2	5.0±0.6	6.5±0.3
0.005	6.7±1.1	7.9±0.8	4.3±0.4	6.0±0.4
0.05	7.4±2.3	7.6±0.9	4.7±0.45	6.7±2.3
0.5	6.0±0.5	7.5±2.4	3.8±0.5[b]	4.2±1.1[b]

*Sheep erythrocytes

[a]Values represent mean $\log_2$ ± SEM, n=4

[b]Different ($p<0.05$) from control group

Table 19. The concentration of mirex in the tissues of chickens hatched from eggs inoculated after 18 hours of incubation with different doses of mirex

Doses of mirex (mg)	Concentrations of Mirex in the Tissues (ppm)[a]											
	4th week			5th week			6th week			8th week		
	Liver	Kidney	Fat	Liver	Kidney	Fat	Liver	Kidney	Fat	Liver	Kidney	Fat
0	0.00	0.00	0.01	0.00	ND	0.01	0.00	ND	ND	0.00	ND	ND
0.005	0.03	0.01	0.01	0.04	0.02	0.02	0.03	ND	0.01	0.02	ND	ND
0.05	0.41	0.03	0.04	0.17	0.03	0.03	0.17	ND	0.14	0.11	ND	0.03
0.5	4.00	0.05	0.69	1.56	0.05	0.07	0.58	ND	0.07	0.34	ND	0.07
5	38.2	11.14	19.53	21.42	ND	9.28	b	b	b	b	b	b

[a]Pooled samples from 4 chickens. The values were expressed on a whole organ weight.

[b]All chickens had been killed by the end of the 5th week.

ND = not done

Table 20. The concentration of photomirex in the tissues of chickens hatched from eggs inoculated after 18 hours of incubation with different doses of photomirex

Doses of photomirex (mg)	Concentrations of Photomirex in the Tissues (ppm)[a]											
	4th week			5th week			6th week			+ 8th week		
	Liver	Kidney	Fat	Liver	Kidney	Fat	Liver	Kidney	Fat	Liver	Kidney	Fat
0	0.00	0.00	0.00	0.00	ND	0.02	0.00	ND	ND	0.00	ND	ND
0.005	0.04	0.01	0.02	0.04	0.01	0.02	0.03	ND	0.01	0.02	ND	0.01
0.05	0.34	0.05	0.12	0.33	0.06	0.07	0.26	ND	0.06	0.22	ND	0.06
0.5	6.79	0.06	0.80	2.70	ND	0.43	1.61	ND	0.21	1.39	ND	0.07
5	47.35	12.76	30.94	41.20	ND	15.81	b	b	b	b	b	b

[a]Pooled samples from 4 chickens. The values were expressed on a whole organ weight.

[b]All chickens had been killed by the end of 5th week.

ND = not done

In chickens hatched from eggs inoculated with 25 mg of photomirex, the chemical was present at a concentration of 172 and 125 ppm in the liver and fat, respectively, on the 7th day after hatching.

Gross Lesions

Gross lesions were found only at the 4th week in the chickens hatched from eggs inoculated with 5 mg of mirex. The livers of these chickens were friable and had small areas of hemorrhage on the surface.

The principal gross lesions in chickens hatched from eggs inoculated with photomirex were also in the liver. The livers of the chickens hatched from eggs inoculated with 25 mg of photomirex were friable and had areas of necrosis throughout. The spleens were hemorrhagic and were larger in size in chickens which died at 7 days after hatching when compared to their controls of the same age.

Chickens hatched from eggs inoculated with 5 mg of photomirex had pinpoint areas of necrosis in the livers (Figure 4). Gross lesions were not seen in the chickens hatched from eggs inoculated with 0.005, 0.05 or 0.5 mg of photomirex.

Histopathology

Liver

Microscopically there were no lesions seen in the livers of chickens hatched from eggs inoculated with 0.05 ml of corn oil (Figure 5).

Livers from chickens hatched from eggs inoculated with 0.005 or 0.05 mg of mirex had small vacuoles in the cytoplasm but other basic structures were normal. In the chickens hatched from eggs inoculated with 0.5 mg of mirex, the liver changes included swollen hepatocytes and slight fatty changes (Figure 6), as demonstrated by oil red O stain.

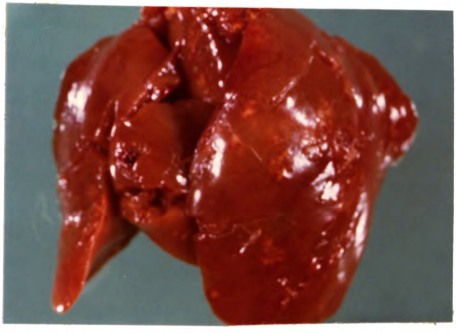

Figure 4. Gross appearance of liver from 5-week-old chicken hatched from an egg inoculated with 5 mg of photomirex. Notice the areas of focal necrosis.

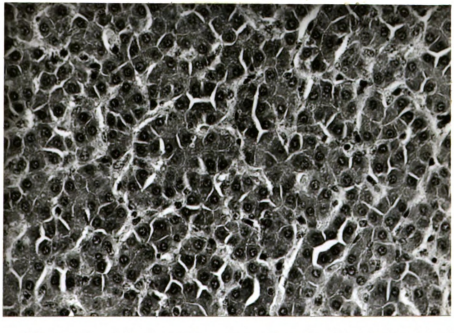

Figure 5. Liver section from 5-week-old chicken hatched from an egg inoculated with 0.05 ml corn oil. Notice normal appearance of hepatocytes. H&E stain; X170.

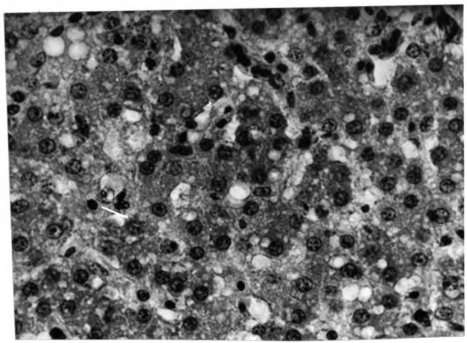

Figure 6. Liver section from 5-week-old chicken hatched from an egg inoculated with 0.5 mg of mirex. Notice vacuolation and swelling of hepatocytes. H&E stain; X425.

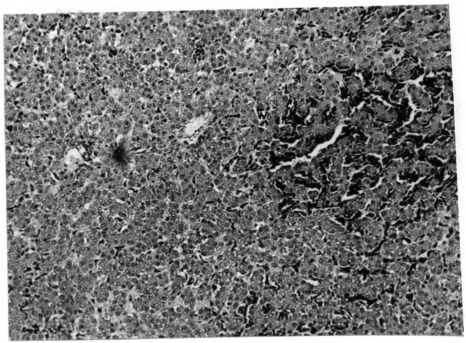

Figure 7. Liver section from 5-week-old chicken hatched from an egg inoculated with 5 mg of mirex. Notice the pale foamy cytoplasm and hemorrhage. H&E stain; X42.5.

The changes were more marked in the livers of chickens hatched from eggs inoculated with 5 mg of mirex. The hepatocytes were swollen, and there was more cytoplasmic vacuolation (Figure 7). The vacuoles were caused by fatty changes, as indicated by the positive oil red O stain.

The hepatic lesions of chickens hatched from eggs inoculated with 0.05 or 0.005 mg of photomirex were similar to those lesions described for mirex. The livers from chickens hatched from eggs inoculated with 0.5 mg of photomirex had more fatty metamorphosis than those from chickens hatched from the same level of mirex (Figure 8). The hepatic lesions of chickens hatched from eggs inoculated with 5 or 25 mg of photomirex were more extensive than those seen in chickens hatched from eggs given the same level of mirex. There were areas of necrosis (Figures 9 and 10). The necrosis tended to be in the midzonal areas. A few mitotic figures were present. The sinusoids were narrowed by the swollen hepatocytes and vacuolation of cytoplasm was seen. There were a few foci of lymphocytes in the livers of both treated and control chickens.

Testes

The testicles of chickens hatched from eggs inoculated with 0.05 ml of corn oil were normal. The seminiferous tubules were uniform and lined by tall columnar cells. Spermatogenic activity was present (Figure 11). In the chickens hatched from eggs inoculated with 0.5 mg mirex there was delay in the process of spermatogenesis. The seminiferous tubules were immature with large vacuolated areas (Figure 12). There were no changes in the testes of the chickens hatched from eggs inoculated with 0.05 or 0.005 mg of mirex compared to the controls. Changes observed in the testes from chickens hatched from eggs inoculated with

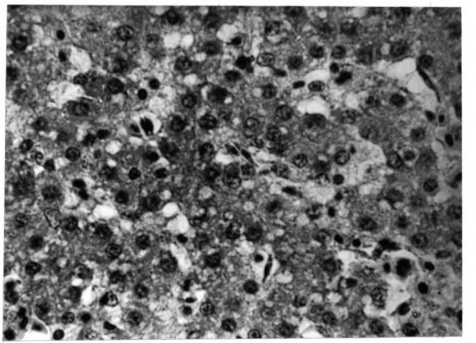

Figure 8. Liver section from 8-week-old chicken hatched from an egg inoculated with 0.5 mg of photomirex. Notice vacuolation and swelling of hepatocytes. H&E stain; X425.

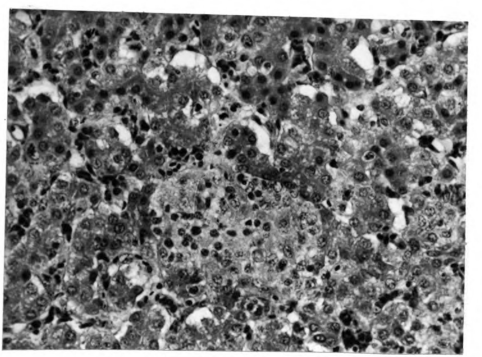

Figure 9. Liver section from 5-week-old chicken hatched from an egg inoculated with 5 mg of photomirex. Notice vacuolation and necrosis of hepatocytes. H&E stain; X170.

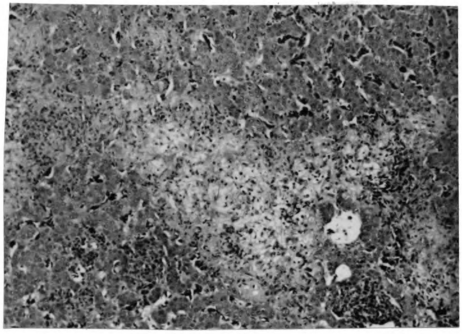

Figure 10. Liver section from 7-day-old chicken hatched from an egg inoculated with 25 mg of photomirex. Notice necrosis of hepatocytes. H&E stain; X42.5.

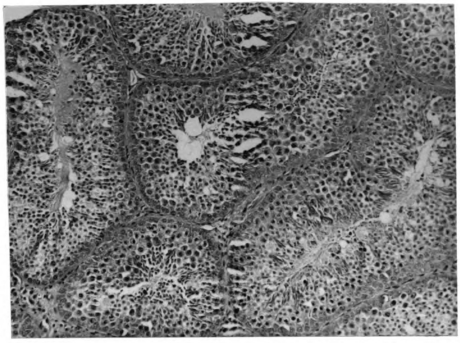

Figure 11. Section of a testis from 8-week-old chicken hatched from an egg inoculated with 0.05 ml corn oil. Notice the active process of spermatogenesis. H&E stain; X68.

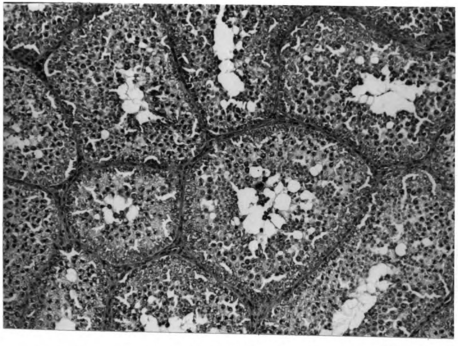

Figure 12. Section of a testis from 8-week-old chicken hatched from an egg inoculated with 0.5 mg of mirex. Notice the delayed process of spermatogenesis and vacuolation. H&E stain; X68.

photomirex were similar but more prominent than those seen in chickens hatched from mirex-treated eggs (Figure 13).

Other Organs

The spleens of the 2 chickens hatched from eggs inoculated with 25 mg of photomirex were hemorrhagic with slight depletion of lymphocytes and there was depletion of lymphoid cells from the medulla of the bursa of Fabricius. However, in chickens hatched from eggs inoculated with 5 mg of photomirex there was only slight depletion of medullary lymphoid cells of the bursa.

No histologic changes were observed in other organs.

Transmission Electron Microscopy

Electron micrographs of hepatic cells from control chickens were normal. The mitochondria were round to elongated and distributed throughout the cytoplasm. The mitochondrial cristae were well developed (Figure 14).

The electron microscopic changes were generally proportional to the dose. Hepatocytes of chickens hatched from eggs inoculated with 5 mg mirex had cytoplasmic vacuolation, swelling of mitochondria, and disruption of mitochondrial cristae and hyperplasia of smooth endoplasmic reticulum (Figure 15). Similar lesions were observed in chickens hatched from eggs inoculated with 5 mg of photomirex (Figure 16). Hepatocytes from chickens hatched from eggs inoculated with 0.5 mg of mirex or photomirex had slightly swollen mitochondria and cytoplasmic vacuolation (Figure 17). Chickens hatched from eggs inoculated with 0.005 or 0.05 mg of mirex or photomirex had no ultrastructural changes.

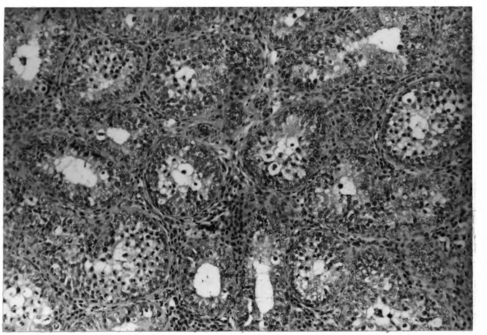

Figure 13. Section of a testis from 8-week-old chicken hatched from an egg inoculated with 0.5 mg of photomirex. Notice the delayed process of spermatogenesis and the vacuolation similar to that shown in Figure 12. H&E stain; X68.

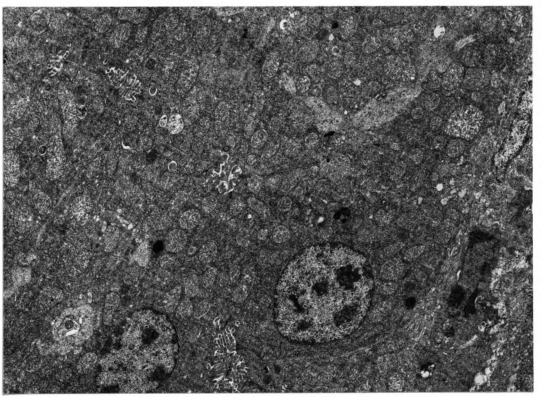

Figure 14. Electron micrograph of liver cell of control chicken. Notice the mitochondria with well developed cristae were distributed throughout the cytoplasm. Uranyl acetate and lead citrate staining; X5880.

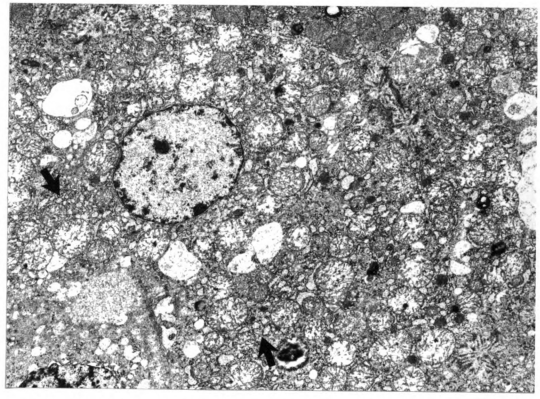

Figure 15. Electron micrograph of liver cell from chicken hatched from an egg inoculated with 5 mg of mirex. Notice the swollen mitochondria with disruption of mitochondrial cristae. The smooth endoplasmic reticulum (arrow) was increased. Vacuolation was present. Uranyl acetate and lead citrate staining; X5880.

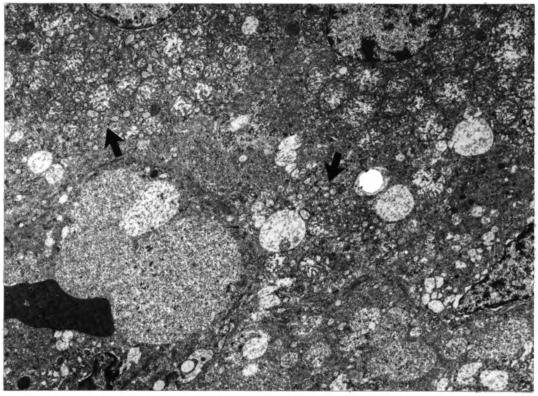

Figure 16. Electron micrograph of liver cell from chicken hatched from an egg inoculated with 5 mg of photomirex. Notice the size of mitochondria, vacuolation and increased smooth endoplasmic reticulum (arrow). Uranyl acetate and lead citrate staining; X5880.

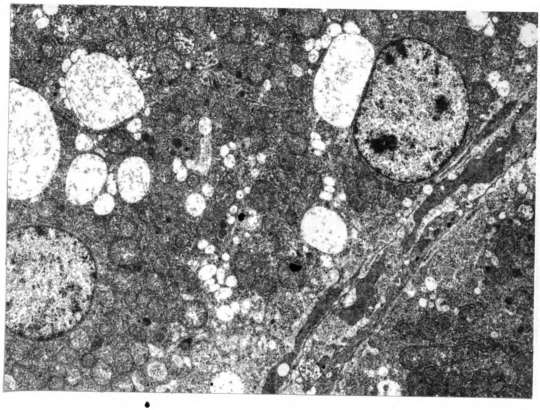

Figure 17. Electron micrograph of liver cell from chicken hatched from an egg inoculated with 0.5 mg of photomirex. Notice the swollen mitochondria and vacuolation. Uranyl acetate and lead citrate staining; X5880.

DISCUSSION

Hatchability

In the present study, 5 or 25 mg of photomirex injected into chick embryos caused high mortality during the early stage of development. This may be due to the toxic effect of the chemical which was in direct contact with the embryos. However, even the few chicks hatched from eggs inoculated with the high dose of photomirex failed to survive, which could be attributed to the delayed toxic effect. A delayed toxic effect could be a plausible explanation, since 30% of the egg yolk remains at the time of hatching and is absorbed during the first week.

The effects of mirex and those of photomirex were similar. These findings suggest that toxicity of photomirex to chick embryos was similar to the parent chemical, mirex. The possible delayed effect of mirex and photomirex described above was supported by a report which stated that hatchability of eggs laid by hens fed 300 mg of mirex/kg of ration was not affected but the survival rate of hatched chicks was reduced (Naber and Ware, 1965). The level of mirex in these eggs was 668 ppm (33.4 mg/egg). However, the same authors reported reduction in hatchability of eggs laid by hens fed 600 mg of mirex/kg of ration in which the level of mirex in the egg yolk was 1864 ppm (93.2 mg/egg). Heath and Spann (1973) also reported no effect on hatchability of eggs laid by mallard ducks fed 10 mg of mirex/kg of ration or bobwhite quail fed 40 mg of mirex/kg of feed.

The hatching time of chick embryos in the mirex and photomirex experiments was prolonged and the embryos required more time to develop to a stage normally attained at 20 days. This might be attributed to the interruptions associated with handling during the incubation period, such as injections and candling. However, mercuric chloride, another toxic chemical, has been reported to prolong the hatching time of embryos to 35 days (McLaughlin et al., 1965).

Mirex was found to have a teratogenic effect in rats (Khera, 1976). Villeneuve and co-workers (1978) reported that photomirex was not teratogenic in rabbits. In the present investigation, photomirex as well as mirex had no teratogenic effect on chicks.

Clinical Signs and Body Weights

Weakness, ruffled feathers, and pale color were seen in chickens hatched from eggs inoculated with 0.5 or 5 mg of photomirex. However, these signs were not observed in chickens hatched from eggs inoculated with mirex. Villeneuve et al. (1979a) reported cyanosis in the hind limbs, irritability, and hypoactivity in rats fed 125 mg of photomirex/kg of ration. Similar results were reported by Gaines and Kimbrough (1970) in rats given mirex in the feed. The insensitivity of chickens to mirex was also reported by Davison et al. (1975).

Body weight was reduced in chickens hatched from eggs inoculated with 0.5 or 5 mg of photomirex by weeks 8 and 5, respectively. Mirex had no significant effect on body weight, although there was a trend towards reduction in body weight at the higher dose level. Unfortunately, the feed consumption was not measured. The reduction in body weight could be associated with reduction of feed consumption. Both treated and control groups were fed standard chick diet *ad libitum*.

In poultry, mirex administered in the feed had no effect on body weight (Ivie et al., 1974c; Davison et al., 1975) but decreased the body weight gain in rats (Khera, 1976) and mice (Abraham et al., 1974). Photomirex also reduced body weight gain in rats fed 5 mg of photomirex/kg of ration for 27 days (Villeneuve et al., 1979a).

Laboratory Results

Villeneuve and co-workers (1979b) reported a normochromic macrocytic anemia in rats fed photomirex in the feed. Larson et al. (1979) reported an elevated hematocrit and WBC count in dogs fed 100 mg mirex/kg of ration. However, none of the hematological values was affected in the chickens hatched from eggs inoculated with mirex in the present investigation. On the other hand, there was a reduction of Hb concentration in chickens hatched from eggs inoculated with 0.5 or 5 mg of photomirex on the 4th and 6th weeks, respectively. This may have been due to a disturbance in Hb synthesis caused by photomirex or a disturbance in iron metabolism.

The serum albumin concentration was increased in chickens hatched from eggs inoculated with 0.5 or 5 mg of photomirex at the 6th and 5th weeks, respectively. These effects were not observed in chickens hatched from eggs inoculated with mirex. This increase may be due to an increase of protein metabolism by the liver. Baker et al. (1975) reported a similar increase in total proteins in rats which they attributed to the increased synthesis of DNA by the liver.

There were no published reports on the effects of photomirex on immunological functions of inoculated animals. Humoral immunity was reduced in chickens hatched from eggs inoculated with 0.5 mg of photomirex, but mirex had no effect. This reduction in the immunity may

be due to functional changes of B-cells, since no changes were observed histologically in the bursas of these chickens. Further investigation may be needed in this regard. No attempt was made to test for cellular immune functions in this study.

Pathological Changes

Liver weight to body weight ratios were increased in chickens hatched from eggs inoculated with 0.5 or 5 mg of mirex or photomirex. The increases in liver weight ratios were directly proportional to the concentration of the chemicals. These findings were in agreement with earlier studies with mirex in chickens and quail (Davison et al., 1976), rats (Gaines and Kimbrough, 1970) and mice (Byard et al., 1975). Photomirex was also found to increase the liver weight ratios in rats (Villeneuve et al., 1978, 1979a). Several explanations for the cause of enlargement of the liver have been postulated. Such increases were related to proliferation of the smooth endoplasmic reticulum and an increase in mixed function oxidases in rats fed mirex in the ration (Gaines and Kimbrough, 1970; Baker et al., 1972). Byard et al. (1975) attributed the increase to the stimulation of DNA synthesis which led to cellular growth of the liver. In the present study the increase in liver weight may have been due to an increase in smooth endoplasmic reticulum in the cells.

The histological changes observed in the livers of chickens that were hatched from eggs inoculated with 5 or 25 mg of photomirex included vacuolation, swelling of hepatocytes and necrosis. These changes were similar to those reported in rats fed 0.2 mg/kg of ration for 13 weeks (Villeneuve et al., 1979b). Mirex caused similar but milder changes, but there was no necrosis. In addition to vacuolation and hepatic cell

swelling, which were reported here, Davison et al. (1976) described focal hepatic and bile duct epithelium necrosis in chickens and quail fed mirex in their rations. The difference may be due to the difference in age and method of administration of the chemical.

The electron microscopic observations in the hepatocytes from chickens hatched from eggs inoculated with 5 mg of photomirex included vacuolation, swelling of mitochondria and disruption of mitochondrial cristae and increased smooth endoplasmic reticulum. These changes have been reported in rats (Singh et al., 1980). The changes seen in the livers of chickens hatched from eggs inoculated with mirex were similar but less severe. Similar changes have been reported by other investigators in rats (Gaines and Kimbrough, 1970; Baker et al., 1972). In chickens fed mirex in the ration, changes in bile canaliculi and formation of myelin figures were reported (Davison et al., 1976), but these changes were not observed in the chickens in this investigation. In rats, reduction in glycogen, appearance of atypical lysosomal bodies and an increase in smooth endoplasmic reticulum were reported (Gaines and Kimbrough, 1970; Baker et al., 1972). These responses may be related to the difference in tolerance to the toxic substance in different species and in different breeds within the species and to differences in method and age at the time of administration of the chemical.

In general, the liver was the organ most sensitive to the toxic effects of mirex or photomirex.

There were no reports regarding the effect of mirex or photomirex on the histology of lymphoid organs in chickens. There was no effect on the weights of the bursas of Fabricius of chickens hatched from eggs inoculated with mirex or photomirex. However, the unexpected increase in the bursa weight to body weight ratios in the chickens hatched from

eggs inoculated with 0.5 or 5 mg of photomirex at the 5th and 6th weeks, respectively, could be due to reduction in growth rate or stimulation by SE. The numerical method of scoring the thymus was chosen arbitrarily and seemed convenient for the purpose. Admittedly, the scores were arrived at subjectively. There is no ready explanation for the smaller size of the thymuses at the 4th week in chickens hatched from eggs inoculated with 5 mg of photomirex, but maybe involution had started prematurely in the 4 chickens. However, this is only speculation, because thymic involution generally does not start until the 17th week. The apparent increase of the spleen weight of chickens hatched from eggs inoculated with 5 mg of photomirex most likely is due to reduction in growth rate. Histologically, chickens hatched from eggs inoculated with 25 mg photomirex had lymphoid depletion in the spleen and the medulla of the bursa. The spleen and thymus were not altered histologically with other levels of either mirex or photomirex.

In the present studies there was a delay in spermatogenesis in the chicks hatched from eggs inoculated with mirex or photomirex. Villeneuve and co-workers (1979b) reported delayed spermatogenesis, degeneration of tubular epithelium and reduction in interstitial cells in rats fed photomirex in the ration. Female rats treated with mirex failed to ovulate (Fuller et al., 1973). These authors suggested there was inhibition of release of luteinizing hormone. The changes observed in the chickens in this investigation could be either related to hormonal changes or to retarded growth rate.

The thyroid glands of chickens hatched from eggs inoculated with mirex or photomirex were normal. However, Villeneuve et al. (1979a) reported histological changes in the thyroid glands in rats.

Chemical Analysis

Analysis revealed mirex and photomirex in the livers, fat and kidneys of chickens hatched from eggs inoculated with the chemicals. The concentrations of mirex or photomirex in these tissues was dose dependent. The livers retained more mirex or photomirex than fat and kidneys. The concentrations of photomirex retained by the tissues were higher than mirex. Other workers reported that mirex accumulates and persists mainly in fat as well as fat-containing tissue (Ivie et al., 1974c; Gibson et al., 1972). Villeneuve et al. (1979a) recovered photomirex mainly from fat and livers of rats fed the chemical in the ration. The reason for higher levels of photomirex in the livers of chickens in this study compared to fat levels is not known.

Ivie et al. (1974c) and Medley (1974) reported a rapid disappearance of mirex from birds after mirex was removed from the feed. Interestingly, hens hatched from mirex contaminated eggs laid eggs which contained mirex, even though the hens themselves had not been exposed to mirex (Ivie et al., 1974c). In our study the presence of mirex or photomirex in the chickens' tissues was detected up to the 8th week after hatching. The level of mirex or photomirex in the tissues decreased with time. The presence of these chemicals in these tissues and the reduction of their concentrations by time reflect their lipophilic character as well as slow excretion and their tendency not to be metabolized.

The use of mirex as a pesticide was discontinued because of the danger of environmental contamination and because it was found in the tissues of some species of wild animals in areas where it had been used. If these species of wild animals were eaten by people, this would pose a threat to human health. In this investigation mirex and photomirex

were found in chicken tissue up to the 8th week of age. This, too, would pose a threat to human health through the food chain. Therefore, the results of this research lend support to the decision to discontinue the use of mirex for control of the fire ant.

SUMMARY AND CONCLUSIONS

This experiment was conducted to determine and compare the toxic effects of mirex and photomirex in chickens. Four hundred eighty eggs were randomly allotted to 9 groups. The eggs in groups 1 and 2 were left as uninoculated controls or controls that were punctured through the air cell. Four chickens hatched from eggs inoculated with 0.05 ml corn oil, 0.005, 0.05 or 0.5 mg of mirex or photomirex were killed at 4, 5, 6 and 8 weeks after hatching. Chickens hatched from eggs inoculated with 5 mg of mirex or photomirex were killed at the 4th and 5th weeks. There were no more living birds from these groups after the 5th week. However, no chickens hatched from eggs inoculated with 25 mg of either mirex or photomirex survived to the 4th week.

In general, there were no clinical signs in chickens hatched from eggs inoculated with mirex. However, photomirex caused ruffling of feathers, paleness of combs and weakness. The body weight was reduced in chickens hatched from eggs inoculated with 0.5 or 5 mg of photomirex at the 8th and 5th weeks, respectively.

The liver weight to body weight ratio was incresaed in a dose-related manner in the chickens hatched from eggs inoculated with 0.5 or 5 mg of mirex or photomirex. The histologic and electron microscopic lesions in the liver were more severe in chickens hatched from eggs inoculated with photomirex than those hatched from eggs inoculated with mirex.

In general, the lymphoid organs were not affected in chickens hatched from eggs inoculated with mirex. However, there was depletion of medullary cells in bursas of chickens hatched from eggs inoculated with 5 or 25 mg of photomirex. The humoral antibody responses to sheep erythrocytes were decreased in chickens hatched from eggs inoculated with 0.5 mg of photomirex.

Hematological and serum electrophoresis values were not affected by mirex. However, a reduction in hemoglobin concentration, at weeks 6 and 4, and an increase in serum albumin, at weeks 6 and 5, were found in chickens hatched from eggs inoculated with 0.5 or 5 mg of photomirex, respectively, compared to their controls.

There was a delay in spermatogenesis in immature males which had been hatched from eggs inoculated with 0.5 mg of mirex or photomirex after 18 hours of incubation.

The results of this investigation indicated that, in general, photomirex induced the same toxic effects as the original chemical, mirex.

APPENDIX

Table A1. The average relative differential leukocyte counts of chickens hatched from eggs inoculated after 18 hours of incubation with different doses of mirex

Doses of mirex (mg)	Differential Count (%)[a]				
	Heterophils	Lymphocytes	Eosinophils	Basophils	Monocytes
			4th Week		
0	20.9±5.2	69.4± 7.4	2.9±1.1	3.1±0.5	3.7±0.3
0.005	21.6±3.4	70.2± 5.6	2.2±0.9	2.9±0.4	3.1±0.5
0.05	23.5±2.1	70.3± 9.2	2.1±0.7	2.0±0.2	4.1±0.4
0.5	21.4±3.5	68.2± 4.1	3.2±1.4	2.8±0.5	4.4±0.6
5	20.1±4.2	59.7± 6.4	2.8±0.8	3.5±0.1	3.9±0.2
			5th Week		
0	22.2±7.1	68.4±10.3	2.4±0.9	3.3±1.1	3.7±0.9
0.005	22.8±5.4	66.2± 6.4	2.3±0.8	3.8±0.9	4.9±0.7
0.05	20.9±2.6	69.6± 7.2	1.9±0.4	4.1±0.7	3.5±1.0
0.5	21.5±4.6	67.0± 9.6	2.2±0.3	3.9±0.5	4.4±0.8
5	23.1±7.8	69.9±11.4	1.8±0.5	2.1±0.3	3.1±0.7
			6th Week		
0	23.1±4.1	73.4± 9.1	0.9±0.3	0.9±0.1	1.7±0.4
0.005	23.2±6.2	73.1±11.1	1.1±0.5	1.2±0.4	2.4±0.2
0.05	25.1±4.6	70.9± 8.4	0.8±0.5	1.1±0.5	2.1±0.4
0.5	24.2±8.1	71.3±10.8	0.9±0.7	1.0±0.7	2.6±0.6

[a]Values are expressed as means ± SEM, n=4

Table A2. The average relative differential leukocyte counts of chickens hatched from eggs inoculated after 18 hours of incubation with different doses of photomirex

Doses of photo-mirex (mg)	Differential Count (%)[a]				
	Heterophils	Lymphocytes	Eosinophils	Basophils	Monocytes
			4th Week		
0	18.9±4.2	71.2± 8.4	3.1±0.3	1.6±0.5	5.2±1.1
0.005	21.2±6.5	70.1± 7.2	1.9±0.4	2.6±0.3	4.2±0.9
0.05	20.4±3.9	69.9± 6.6	2.8±0.3	3.2±0.5	3.7±0.7
0.5	20.1±4.1	68.5± 6.2	2.2±0.2	3.1±0.4	6.1±0.8
5	22.2±7.1	67.7± 5.4	1.8±0.1	2.8±0.3	5.5±0.7
			5th Week		
0	21.1±6.1	70.8± 9.2	1.9±0.3	2.1±0.2	4.1±0.9
0.005	19.5±7.2	72.3± 7.1	2.5±0.5	3.0±0.8	2.7±1.6
0.05	20.9±3.4	70.4± 8.0	2.3±0.3	1.9±0.3	4.5±0.8
0.5	20.1±6.1	71.4± 8.4	2.2±0.1	2.9±0.5	3.5±0.5
5	22.1±4.5	69.4± 6.1	2.0±0.1	2.7±0.4	3.8±0.4
			6th Week		
0	19.9±6.1	69.5±10.2	1.8±0.4	2.4±0.3	6.4±1.2
0.005	22.2±7.4	66.2± 9.1	2.2±0.3	3.0±0.7	5.6±1.1
0.05	20.9±8.3	67.7±11.2	2.6±0.3	2.2±0.4	6.6±1.5
0.5	20.6±6.6	69.2± 8.1	3.0±0.6	2.3±0.5	4.9±0.9

[a]Values are expressed as means ± SEM

Table A3. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) from chickens hatched from eggs inoculated after 18 hours of incubation with different doses of mirex

Doses of mirex (mg)	4th Week			5th Week			6th Week			8th Week		
	MCV μ^3	MCH μ_{UG}	MCHC %	MCV μ^3	MCH μ_{UG}	MCHC %	MCV μ^3	MCH μ_{UG}	MCHC %	MCV μ^3	MCH μ_{UG}	MCHC %
0	144.4	52.3	36.6	134.1	49.6	37.0	126.5	51.0	39.6	122.9	48.3	39.3
0.005	137.9	46.6	33.8	143.8	51.7	36.0	135.3	54.1	40.0	125.5	51.9	41.3
0.05	144.1	45.8	31.8	130.9	50.0	38.2	128.4	50.1	39.0	127.9	50.4	39.4
0.5	136.8	49.1	35.9	134.4	49.5	36.9	127.9	52.1	40.7	131.7	50.7	38.5
5	144.3	51.5	35.7	144.6	44.6	30.8	---	---	---	---	---	---

Table A4. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) from chickens hatched from eggs inoculated after 18 hours of incubation with different doses of photomirex

Doses of photomirex (mg)	4th Week			5th Week			6th Week		
	MCV μ^3	MCH μ_{UG}	MCHC %	MCV μ^3	MCH μ_{UG}	MCHC %	MCV μ^3	MCH μ_{UG}	MCHC %
0	136.2	54.0	39.7	120.5	43.8	36.3	123.2	47.9	39.1
0.005	128.2	49.6	38.5	126.1	44.2	35.0	121.7	52.9	40.6
0.05	129.5	50.0	38.6	123.9	47.7	38.5	121.3	48.2	39.7
0.5	126.1	43.5	34.5	125.9	44.4	35.4	122.1	40.3	33.0
5	125.5	37.0	29.5	119.4	40.5	33.9	---	---	---

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