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The acute toxicity, hepatic response and neural activity of mirex in rats.

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The acute toxicity, hepatic response and

neural activity of mirex in rats

by

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A Thesis Submitted to the

Graduate Faculty in Partial Fulfillment of

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INTRODUCTION

The use of pesticides in recent years has come under close scrutiny by the public. This concern has stemmed from an increase in the understanding of the deleterious effects of these chemicals on both the environment and nontarget species. DDT was the classical example of an economic poison whose biocidal characteristics ultimately resulted in the banning of the compound. However, many other chemicals have been the subject of intense investigation in recent years, mirex being among them. Although having been aerially sprayed over millions of acres in the south, relatively little is known concerning mirex's effects on nontarget species. In addition, the mechanism of action of mirex on mammals and birds is completely unknown. This research project was designed to add to the existing knowledge of mirex as well as to clarify several conflicts which appear in the literature concerning mirex's toxicity to mammals.

Literature Review

General background

Mirex is a polycyclic chlorocarbon pesticide which has been used in the southeastern United States as the principal ingredient in bait used to control the imported fire ant, _Solenopsis saevissima_ Forel. Mirex, chemically known as 1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1,3,4-metheno-1H-cyclobuta(cd)pentalene, was first prepared in 1946 by Prins. Mirex is a white, odorless, noninflammable, crystalline solid at room temperature. The chemical molecular formula is $C_{10}Cl_{12}$, consisting of 22% carbon and 78% chlorine. It has a molecular weight of 546. Mirex is

insoluble in water but soluble in organic solvents such as hexane, acetone, methyl ethyl ketone, carbon tetrachloride, benzene, dioxane and xylene. The closest chemical related to mirex is Kepone which is also an insecticide and is identical except for the substitution of an oxygen for two chlorine atoms (Markin et al., 1972; Waters et al., 1977). See Figure 1 for the structure of mirex and related pesticides.

The compound was patented in 1955 and was introduced in pesticidal baits in 1959 by Allied Chemical Corporation as GC-1283 (Waters et al., 1977). The actual manufacturing of the pesticide was done by Hooker Chemical Corporation in Niagara, NY. Mirex was developed primarily as a replacement for the highly toxic and less specific insecticides DDT, heptachlor and dieldrin. In addition to mirex's use as a pesticide it was used as a fire retardant under the tradename Dechlorane (Markin et al., 1972). Dechlorane was incorporated into manufactured products which cover an extensive variety of materials, mainly, paints, paper, butylrubber, pyrotechnic mixtures and plastics (Markin et al., 1972). It is possible that mirex could be released into the environment by burning or the weathering of the products into which it was incorporated. The amount of mirex prepared and sold for its fire retardant properties is believed to be several times the amount used for pest control (Markin et al., 1972). Due to possible environmental problems, Dechlorane was discontinued in 1971.

The pesticide mirex, as stated above, was developed primarily to replace endrin and dieldrin in combatting the imported fire ant, Solenopsis saevissima. The ant itself first invaded the southern United States in

mirex$-C_{10}Cl_{12}$

Kepone$-C_{10}Cl_{10}O$

dieldrin$-C_{12}H_8Cl_6O$

DDT$-C_{14}H_9Cl_5$
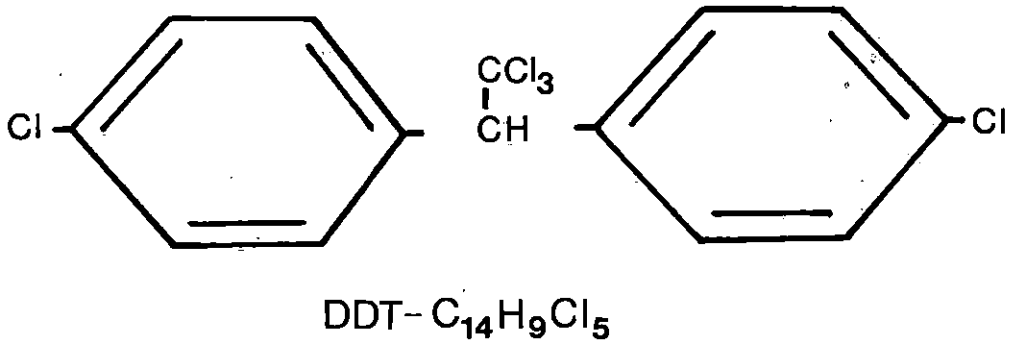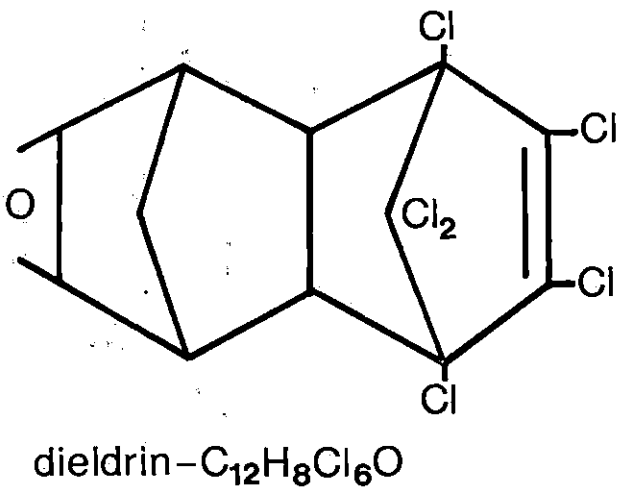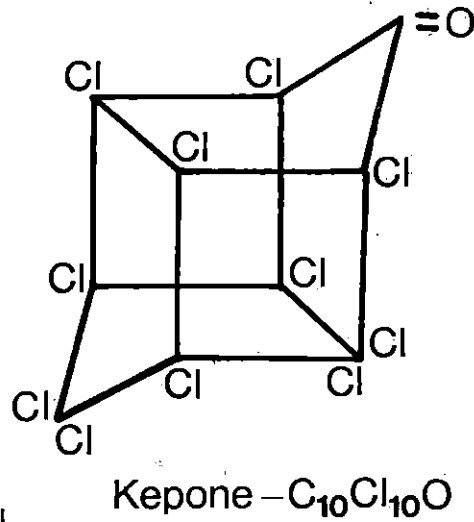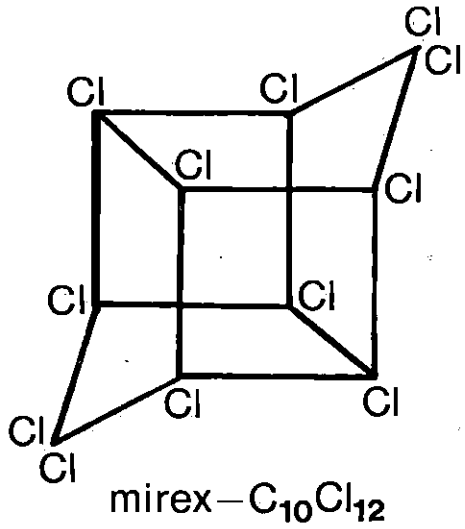
Figure 1. Chemical structures of mirex and kepone and some related chlorinated hydrocarbon pesticides.

1918. Since the ant did not have any natural enemies, the population spread to most states in the southeastern United States by 1950, infesting 126 million acres. Though there is little evidence that the fire ant actually harms plants or wildlife, endrin and dieldrin were used in the early and mid-1950's to combat the growing fire ant population with disastrous effects on nontarget wildlife species such as quail and turkeys (Shapley, 1971). Mirex was used in field trials on the fire ant in 1961 by the USDA. Following the application of mirex to 960 acres near Gulfport, Mississippi 95-99% of the fire ants were eradicated with little or no apparent effect(s) on wildlife. Mirex was then accepted by the USDA as the principal pesticide for fire ant control (Lofgren et al., 1964). Over 269,535 lbs. of technical grade mirex have since been applied to an estimated 70 million acres in the south (Markin et al., 1972).

Mirex was employed for insecticide use in a bait fairly specific for the fire ant. The compound was dissolved in soybean oil and absorbed onto corn cob grits giving a final composition of .15% mirex, 85% grits and approximately 15% soybean oil (Markin et al., 1972; Lofgren et al., 1962). The ants are attracted by the oil and the grits act as the carrier (Lofgren et al., 1963; Lofgren et al., 1964). The bait is applied at 1.25 lbs/acre resulting in 1.7 grams of technical grade mirex per acre. When applied aerially in the recommended manner, mirex will eradicate 98-100% of all fire ants in a treated area in 4-12 weeks (Banks et al., 1971; Lofgren et al., 1963, 1964).

Effects on insects

Mirex has been employed primarily for fire ant control; however, it
has also been used to combat other social insects. The harvester ant in
the west and the leaf cutting ant in Louisiana and Texas have had the most
attention in this regard (Markin et al., 1972). Some mirex has also been
exported to South America for control of umbrella and leaf cutting ants
(Amante, 1968; Markin et al., 1972). In 1971, California used mirex to
control the yellow jacket (Keh et al., 1968; Wagner and Reirson, 1971).
In addition, mirex has been used against a wide variety of other pest
insects with desirable effects, such as scale, mealybug, wasps, termites,
and earwigs (Gross and Spink, 1969; Rai, 1977; Markin et al., 1972).
Mirex has even been used as a systemic control on fleas in rats, kangaroo
rats and cottontail rabbits (Clark and Cole, 1968; Clark et al., 1971).

Tests in the laboratory with desirable social insects such as the
honeybee indicate that mirex will just as effectively kill them (Anderson
and Atkins, 1966). However, under actual field conditions, honeybees are
not affected by mirex application (Glancey et al., 1970).

Mirex has been tried against a wide variety of other insects in addi-
tion to those mentioned above with ineffective results in most cases
(Markin et al., 1972).

Mirex's effectiveness against social insects depends to a large ex-
tent upon its mode of action. The foraging insects ingest mirex from the
oil grit formulation and then are able to distribute the poison throughout
the colony since mirex is a slow acting poison (Echols, 1966). The exact

mechanism of toxicity of mirex in insects is unknown, however, Desiah and Koch (1975) indicated that ATPase activity was not affected.

Environmental impact

There are three important parameters to consider in deciding the suitability of a chemical being applied to the natural environment. These are: its persistence in the environment, its degradation properties, and its residue accumulation in the environment and nontarget organisms. These characteristics of mirex will be considered here.

In 1962 mirex was officially released by the USDA for use as a commercially available pesticide to control the imported fire ant (Markin et al., 1972; Shapley, 1971). Subsequently, mirex was sprayed in vast quantities over 69 million acres in the southeastern United States (Markin et al., 1972). However, it was not until 1968 that the first field studies were made to measure its persistence in the environment. Van Valin et al. (1968) reported in experiments on ponds that mirex, when applied at the recommended rate for control of the fire ant, remained at a relatively constant concentration in plants, soil and water far in excess of 300 days. Baetcke et al. (1972) conducted a study comparing residue levels of DDT and mirex in selected fauna over one year after aerial application of the recommended 1.25 lb/acre of bait. Relatively high levels were found in numerous species. Mirex was measured in 4 out of 5 species of fish and 41 out of 42 species of birds. In addition, mirex residues often exceeded DDT residues. It was concluded that mirex is a highly persistent pesticide.

Studies involving the exposure of mirex to the environment for even longer periods of time also indicate a high persistence. In one case where soil samples were recovered 12 years after treatment with 1 ppm mirex, half of the material was still undegraded. In another, 5 years after an airplane crashed with mirex bait, 640 ppm mirex was present. Both cases represent at least 50% recoveries of mirex. Furthermore, Kepone, a more toxic dechlorinated compound, was found at concentrations of up to 10% of the recovered mirex. This study illustrated mirex's persistence and the fact that it will eventually undergo slow successive dechlorination degradation to a more toxic product, Kepone (Carlson et al., 1976).

This extreme stability of mirex is understandable in view of tests conducted on its degradation characteristics. Exposure to sunlight and UV light showed a slow degradation to chlordecone hydrate, nonochloropentacyclodecan-5-one hydrate and undecachloropentacyclodecane (Ivie et al., 1974a). Holloman et al.(1975) exposed mirex to high temperatures and found hexachlorobenzene as the major product and some hexachlorocyclopentadiene was also found. Baker and Applegate (1974) found that mirex was more persistent than DDT, endrin, chlordane or methylparathion when exposed to 350 μm UV light. Mirex is not degraded apparently by soil bacteria; however, aerobic sludge organisms appear to degrade mirex (Jones and Hodges, 1974; Andrade et al., 1975).

Since mirex is extremely persistent under environmental conditions, widespread distribution of residues has been recorded in both the physical environment and in nontarget organisms as a direct result of fire ant

application. In an extensive study undertaken in Mississippi in 1971 following a single aerial application of mirex to control the fire ant, the residue distribution in the physical environment, terrestrial and aquatic invertebrates and selected vertebrates was monitored by Spence and Markin (1974) in order to determine the characteristics of mirex dispersed in the ecosystem. Mirex appeared to be leached from land sprayed with mirex and this was measured in pond water and sediment as a result of runoff. Residue levels peaked at about one month after spraying and were observed to decline 3-4 months after treatment. Mirex was measured in Bahia grass blades and roots which indicates that mirex can be translocated from the roots upward in the plant.

Invertebrate species showed characteristic residue levels which depended upon feeding habits. Scavenger species such as ants showed the highest levels immediately after treatment indicating that the bait was picked up by these insects directly. Predatory insects showed residue levels that peaked 10 weeks after treatment indicating that mirex passed through the food chain. Herbivorous insects showed the smallest significant levels of mirex. Mirex was detected in 24 out of 25 species measured directly after treatment illustrating the wide dispersal in the environment. After one year, 8 of the 25 species still had detectable levels of mirex (Markin et al., 1974).

The principles illustrated by the invertebrate species were also observed in vertebrates from the same study area (Collins et al., 1974). Carnivorous vertebrates such as loggerhead shrikes and mockingbirds, and predatory fish such as bullheads and sunfish showed residue levels higher

(1-8 ppm) than herbivorous vertebrates such as the bobwhite quail (.01-1.5). These carnivores are high on the food chain and indicate biomagnification as seen with DDT and other related chlorinated hydrocarbon pesticides. Most samples including items in the human food chain such as milk, beef fat, chicken, fish and game animals had detectable levels after one year. Overall, 77% of the samples from the human food chain contained mirex and 88% of all samples taken from 61 species of vertebrates contained mirex. The results of this study indicating the wide dispersal and persistence in nontarget organisms were substantiated by Wolfe and Norment (1973). They found that mirex seemed to be stratified by trophic levels. However, at a lower application rate, .85 g/acre technical mirex, mirex residue levels remained relatively constant in fish for 7 months and did not peak at three months as indicated by the previous investigation. Hollister et al. (1975) and Tagatz et al. (1975) conducted experiments illustrating the extraordinary capacity of various organisms to concentrate mirex. They found that four unicellular marine algae species were able to concentrate mirex 3200-7300-fold from the surrounding growth medium. In an experiment involving mirex leached from bait, minnows had a concentration factor of 40,800, pink and grass shrimp 10,000, and blue crabs 23,000 (Tagatz et al., 1975).

Tissue levels and metabolism of mirex in mammalian and avian systems

Extreme stability and a lipophilic nature are the most notable characteristics of mirex in living systems. Early investigations with mirex indicated that most of the ingested mirex appeared in the feces of rats and monkeys (Mehendale et al., 1972; Weiner et al., 1976). Mehendale

et al. (1972) conducted one of the first investigations on mirex's fate in living systems. Male rats given 6 mg/kg of mirex-^{14}C excreted 55% of the administered dose in the feces within the first 48 hours after administration, reflecting a high amount of unabsorbed mirex. Little was excreted in the urine, 1% of the total dose in 7 days due to the low water solubility of mirex. Most of the mirex uptake was found in adipose tissue: 27% appeared in fat, 3.2% in muscle, 1.75% in the liver, .76% in the small intestine, .23% in the large intestine, .09% in the kidney and .07% in the brain. A large percentage, 34%, of the administered dose remained in the animal. No metabolites were discovered.

The basic findings of this study have been borne out by other investigators. Mirex appears not to undergo any kind of transformation. Only one case appears in the literature of a living system producing a metabolite of mirex. A small amount of metabolite was found in the lower colon of a rhesus monkey by Wiener et al. (1976). This was believed to be due to spontaneous decomposition rather than by way of metabolism or by bacterial decomposition in the gut of the monkey. All other investigators have reported that mirex passed through experimental animals without any kind of biotransformation (Gibson et al., 1972; Ivie et al., 1974a; Khera et al., 1976; Smrek et al., 1977).

In male rats and quail the feces provided the major route of elimination while very little mirex was eliminated in the urine (Gibson et al., 1972; Ivie et al., 1972, 1974). Female rats readily passed mirex through milk to their young and through the placental barrier to fetuses (Gaines and Kimbrough, 1970; Khera et al., 1976). Female quail eliminated 70-85% of

ingested mirex through the eggs (Ivie et al., 1974a,b). Since mirex appeared to concentrate in fat and be eliminated primarily through the feces after oral dosing, the excretion kinetics appear to be biphasic, the first phase lasting 38-48 hours and the long phase lasting in excess of 100 days (Mehendale et al., 1972).

Mirex is highly lipophilic and appears to concentrate in the fat of rats, quail and monkeys (Ivie et al., 1974a,b; Mehendale et al., 1972; Gibson et al., 1972; Weiner et al., 1976). Apparently this tendency for accumulation in fat does not plateau as is the case with DDT and dieldrin (Ivie et al., 1974a). Furthermore, residue accumulations following cessation of treatment dissipate at a much slower rate than either of these pesticides (Ivie et al., 1974a).

Tissues which accumulate mirex at relatively high concentrations usually have a high blood flow and conversely each eliminates mirex residues quickly after the cessation of dosing (Ivie et al., 1974a, 1974b; Gibson et al., 1972). The liver, the kidneys, muscle and the brain are prominent in this respect.

Toxicology of invertebrates and lower vertebrates

Mirex has been found to be quite effective against various insects; however, it has also proven to be highly toxic to arthropods and moderately toxic to higher organisms such as fish, birds and mammals. As reported earlier, mirex acts on ants primarily as a slow acting poison. After ingestion of the mirex bait there is typically a 72 hour or longer latent period before toxic symptoms are observed in ants, during which time the mirex is distributed throughout the ant colony. Toxic effects are mani-

fested by excitement and loss of social order in the affected ant colonies after 1-4 days (Echols, 1966).

Houseflies have been observed to undergo a similar type of reaction to mirex intoxication. Following exposure, flies were killed in 2-4 days. From 3-5 days were required for a 90% knockdown. Fly strains with a high resistance to cyclodiene pesticides were also tolerant to mirex, suggesting a similar mode of action (Plapp, 1973).

In experiments with the field cricket, a 72 hour latent period was also observed. In addition, increased spontaneous action of the central nerve chord was observed, for mirex as well as dieldrin and DDT. Symptoms of mirex poisoned crickets after the 72 hour latent period were excitability, ataxia, convulsions and finally paralysis which were also characteristic of DDT poisoning. Dieldrin and mirex treated crickets reacted in a similar manner which in one way was different from DDT poisoned crickets. Dieldrin and mirex poisoned crickets did not show DDT-type trains of electrical activity from the central nerve chord, but a prolonged synaptic after-discharge was evident in the early stages of poisoning that was more pronounced for mirex treated animals (MacFarland et al., 1975).

Since mirex is very toxic to insects it is not surprising that it should prove toxic to other arthropods. Mirex residues have been measured to be the second most prevalent pesticide in samples from blue crabs in North Carolina, South Carolina, George and Florida (Markin et al., 1972). Juvenile brown shrimp, grass shrimp, blue crabs and fiddler crabs were observed to be poisoned by mirex bait (Lowe et al., 1971). In blue crabs and crawfish, mirex is believed to be much more hazardous than DDT

(Leffler, 1975; Ludke et al., 1971). Other arthropods are also sensitive to mirex. Crawfish show sensitivity at .1 ppb - 5.0 ppb technical mirex or .3% bait and grass shrimp are sensitive between .01 ppm - 1.0 ppm technical mirex or .15% mirex bait. A characteristic of arthropods is a much higher toxicity in younger animals. Adult crawfish and crabs seem to be unaffected by mirex (McKenzie, 1970). However, larval crawfish, juvenile crabs, larval blue crabs and juvenile shrimp have been shown to be sensitive in the .1 - 10 ppb range (Ludke et al., 1971; Markin et al., 1972).

Fish in general show a much lower toxicity to mirex than insects or arthropods. Fish have been shown to be able to accumulate mirex as a function of concentration of the treatment level directly from the water, or from indirect exposure through the food chain (Markin et al., 1972; Collins et al., 1973). The low toxicity of mirex to fish has been shown by several investigators. Blue gills showed no effect when 30 times the field dose of 1.7 g/acre was applied to aquaria (Van Valin et al., 1968). Earlier it was shown that a 90 hour exposure to 50 mg/1 mirex resulted in no mortality to blue gills, but 20% and 30% of the fish died on exposure to 25 - 50 mg technical mirex/liter using a 50% wettable powder constituting 20,000 - 40,000 times the field dose (Van Valin et al., 1968). Channel catfish, however, showed a 33% decrease in survival from mirex bait treatment of 1.25 lb/acre (Hyde et al., 1974). In experiments with bluegills, goldfish and cutthroat trout, mirex was observed to cause mortality and various pathological alterations in affected fish. Blue gills were observed to show no mortality or pathological changes from either bait

application to ponds or through feeding experiments. Growth rates, however, in the fish exposed to 5 mg/kg/day were observed to be adversely affected.

Goldfish and cutthroat trout seemed to be more susceptible to mirex in that exposure of 1.0 ppm in water resulted in mortality and pathological changes. Death in goldfish was characterized not by typical excited behavior customarily associated with chlorinated hydrocarbon poisoning, but by the fish remaining motionless at the surface, gradually losing equilibrium until death. Gill lesions typified by edematous lamellae were observed in 15-100% of the goldfish by the 24th day after exposure. The kidneys were also altered in that the tubules were markedly dilated. In addition, distended gall bladders were observed to be a symptom of poisoning in these goldfish. Previous experiments with cutthroat trout at 25 ppm resulted in adverse effects on the gills, characterized in a different way: The lamellae in the fish appeared to be fused.

Toxicity of mirex to avian systems

The toxic effects of mirex on birds has been investigated by many experimenters. Most of these studies have been concerned with reproduction effects since reproductive failure of birds exposed to organochlorine substances is perhaps the most deleterious aspect of chronic toxicity of insecticides (Hyde et al., 1973). Adverse effects on avian population are usually mediated through reduced egg production, egg shell thinning, egg breakage, decreased embryonation, decreased hatchability, and reduced fledgling survival (Hyde et al., 1973). Therefore, most researchers have concerned themselves with these parameters.

Early investigations showed that mirex seemed to have no direct effect on adult avian populations. In field pen experiments mirex caused no mortality in quail while a decrease in fertility and hatchability was directly related to rate of exposure (Baker et al., 1972). Pimentel (1971) confirmed this early observation by reporting extremely high LD_{50} and LC_{50} values for avians. The LD_{50} value for the mallard was measured at 2400 mg/kg while LC_{50} values for coturnix quail and pheasant were 10,000 and 1400 to 1600 ppm in feed respectively.

Mirex in low concentrations has been shown to cause few adverse effects on reproduction parameters for birds. Driver et al. (1976) reported that white leghorn laying hens fed .014 ppm mirex for 15 weeks reproduced eggs which had normal fertility and hatchability although the carcass fat from chicks hatched from these eggs had .47 ppm mirex at 1 day of age. Ivie et al. (1974a) examined several reproductive parameters including egg hatchability, chick growth, and survival after feeding mirex at levels up to 30 ppm, he found mirex in egg yolk up to 200 ppm produced no adverse effects on the above measurements. Hyde et al. (1973) found that feeding mirex at 100 ppm to ducks for 25 weeks did result in decreased duckling survival without affecting egg production, shell thickness, shell weight, embryonation or hatchability. Apparently, there is a threshold residue level required for adverse reproductive effects. This was shown by Davidson et al. (1975) and Davidson and Cox (1974) since white leghorn chickens fed for 12 weeks on 160 ppm mirex and quail at 80 ppm showed normal reproduction. Naber and Ware (1965), in contrast, observed decreased hatchability from feeding 600 ppm mirex for 16 weeks to the hen.

Though mirex seems to be deleterious primarily from a reproductive standpoint in birds when administered at high dose levels and for relatively long periods of time, recent studies have shown pathological effects on tissues at lower dose levels. In an investigation into reproductive effects of mirex on chickens and quail, Davidson and Cox (1974) and Davidson et al. (1975) reported, as stated above, the mirex fed at 160 ppm in chickens for 12 weeks resulted in no effects on various reproduction parameters. However, they observed that chickens had lesions in the liver that seemed to be related to mirex residues. In a followup investigation, various characteristics of the liver in quail and chickens were measured in order to detect any change as a result of mirex toxicity. Chickens were fed mirex in the diet for 12 and 16 weeks from 0-160 ppm and Japanese quail were fed 0-80 ppm for 12 weeks. Mirex apparently did not affect the concentration of protein or cytochrome P-450 in hepatic microsomes of chickens or Japanese quail nor did it affect hydroxylation of aniline or demethylation of aminopyrine. Nevertheless, structural changes were apparent in the livers of chickens fed 10-160 ppm mirex. Though chickens appeared healthy, lesions were observed in 11% to 16% of the livers from these groups. The livers were outwardly characterized by congestion, evident reddish streaks on the surfaces and greenish granular lesions. Light microscopic examination revealed mild focal to moderately severe diffuse vacuolar hepatocellular degeneration, mild focal to moderately severe diffuse fatty metamorphosis, irregular focal hepatocellular necrosis, focal coagulation necrosis with fibrosis, multiple foci of telangiectasis, and vacuolar degeneration and focal necrosis of bile duct epithelium. The

most severely affected cells were often those closest to the sinusoids.
Necrosis of parenchymal cells was accompanied by changes in some bile
canaliculi. Proliferation of smooth endoplasmic reticulum was not ob-
served.

Toxicity in mammalian systems

The toxic effects of mirex on mammalian systems has been investigated
numerous times. Generally, mirex has been considered along with DDT,
dieldrin and related compounds to be within the group of chlorinated
hydrocarbon pesticides although its polycyclic structure contains solely
carbon and chlorine (Gaines, 1968).

Early investigations into the acute toxicity of mirex to mammals was
conducted by Allied Chemical Corporation in order to obtain registration for
mirex as a fire ant poison (Markin et al., 1972). The acute oral LC_{50} in
rats was measured by Allied Chemical Corporation to be 312 mg/kg in a corn
oil solution. When fed in an aqueous solution, 6000 mg/kg was needed for
50% mortality (Markin et al., 1972). This would place mirex in the class
of moderately toxic pesticides. Later, in a comprehensive investigation
of the acute toxicity of pesticides, Gaines (1968) calculated the LD_{50} for
mirex at 740 mg/kg for male rats and 600 mg/kg for females when corn oil
was used as the vehicle. Other chlorinated hydrocarbons in the same study
yielded the following results for LD_{50} values in mg/kg: DDT 113, aldrin
39, chlordane 335, dieldrin 48, DDA 740, Kepone 125 and DDE 880. As can
be seen, mirex compares most closely with the breakdown products of DDT
(DDE and DDA) in toxicity to rats. Mirex, like all chlorinated hydro-
carbons tested, was observed to be more toxic in female rats than in males.

Kepone, the most closely related compound from a structural perspective, is roughly 3 to 6 times more toxic than mirex (Gaines, 1968; Naber and Ware, 1965). However, when fed continuously, mirex was found to cause greater mortality in Balb C mice at 7 ppm than kepone at 50 ppm (Ware and Good, 1967). This observation can be partially explained in regard to mirex's proven persistence in the mammalian body (Mehendale et al., 1972). The 90 dose LD_{50} studies have shown that mirex has a very high chronicity factor, 60.8 (one dose LD_{50}/90 dose LD_{50}) when compared with 5.4 for DDT and 12.8 for dieldrin. Thus, these findings indicate that mirex is highly cumulative in effect and is explained by the fact that it is excreted very slowly (Gaines and Kimbrough, 1970; Mehendale et al., 1972).

The toxic effects of mirex on mammalian systems grossly resemble the symptoms which often characterize chlorinated hydrocarbon pesticides. The outward symptoms typically associated with DDT, dieldrin and other related chlorinated hydrocarbons frequently include weight loss, weakness, hair loss, diarrhea, increased liver size, tremor, hyperexcitability, irritability and convulsion (Kendall, 1974a; Gaines, 1960, 1968; Gaines and Kimbrough, 1970). Weight loss as a result of anorexia has been associated particularly with the dieldrin group of compounds (Gaines, 1960). Toxic effects of mirex are generally characterized by weight loss and increased liver size (Abraham et al., 1974; Byard et al., 1975; Davidson et al., 1976; Gaines and Kimbrough, 1970; Baker et al., 1972; Abston and Yarbrough, 1976; Kendall, 1974a). However, other outward observations seem to typify mirex poisoning. Gaines and Kimbrough (1970) noted that rats fed 250 ppm mirex in feed for 16-60 days showed diarrhea, weakness, and

hyperexcitability. Davidson et al. (1976) observed that mirex dosing for 4 weeks at 100 ppm in feed caused weight loss, hair loss and yellowing of the coat. Kendall (1974b) noted diarrhea, hair loss and lethargy. The symptoms of mirex poisoning generally can be seen to be similar to chlorinated hydrocarbon symptoms. However, Gaines and Kimbrough's findings (1970) differ from the other investigators in regard to the excitability of the poisoned animals, which is one of the most characteristic symptoms of chlorinated hydrocarbon toxicity and indicative of CNS activity.

Effects on reproduction

Mirex has been shown to have deleterious effects on reproduction in mice and rats. In an investigation into the effects of mirex, telodrin and DDT on the laboratory mouse, it was concluded that mirex caused the greatest reduction in litter size and fertility (Ware and Good, 1967). Gaines and Kimbrough (1970) measured the effects of mirex on reproduction in Sherman strain rats. They found that when females were fed mirex in the diet it passed the placenta which resulted in fewer live offspring. In addition, fewer young reached weaning. A unique symptom of the young from mirex treated mothers is that 33% developed cataracts. Thus, mirex seems to have a potentially cataractogenic effect. More recently an investigation into the possible teratogenic potential was undertaken (Khera et al., 1976). Low single daily doses of mirex given to female rats on days 6-16 of gestation caused no adverse effects in either parents or offspring. However, at higher dose levels applied under these same conditions maternal toxicity was observed as well as fetal visceral

anomalies. Decreased fetal survival and reduced fetal weight were also observed at the high dose level. Males exposed to mirex showed no effects in a dominant lethal assay (Khera et al., 1976).

Effects on the mammalian liver

One of mirex's most pronounced toxic effects is causing an increase in liver size with a concurrent decrease in body weight (Abraham et al., 1974; Gaines and Kimbrough, 1970; Baker et al., 1972; Byard et al., 1975; Abston and Yarbrough, 1976; Davidson et al., 1976; Fulfs et al., 1977); Robinson and Yarbrough, 1978). It is because of this notable effect on the liver and because the liver manifests the toxicity of other chlorinated hydrocarbon pesticides that extensive research has been conducted on mirex's effect on the liver (Gaines and Kimbrough, 1970). Furthermore, it has been shown that alterations in relative liver weights is a reliable preliminary indication of chemically induced hepatic toxicity (Fulfs et al., 1977).

As stated above many investigations have noted an increase in liver weight to body weight ratio in rats and mice. This increase appears to follow a typical dose response curve with mirex. Robinson and Yarbrough (1978) found maximum liver response in rats from a single 100 ppm dose 4 days after treatment, representing a 100% gain over controls. Even greater increases have been recorded. Byard et al. (1974) recorded a 230% increase in relative liver weight in rats following treatment at 30 ppm for 9 months. This dramatic response on relative liver weight has been demonstrated for many other compounds including chlorinated hydro-

carbon pesticides; however, none has demonstrated as great an effect as mirex (Robinson and Yarbrough, 1978; Gaines and Kimbrough, 1970).

Increases in relative liver weight have been shown to be related to hepatocellular hypertrophy which includes both the increase in size of individual hepatocytes and an increase in the actual number of cells as indicated by the amount of DNA present in the liver (Fulfs et al., 1977; Robinson and Yarbrough, 1978; Kendall, 1974b; Davidson et al., 1976; Byard et al., 1975). Dose dependent protein increases have also been directly related to hypertrophy of the liver and account for most of the increase in dry weight of the liver (Byard et al., 1974; Davidson et al., 1976; Robinson and Yarbrough, 1978).

In addition to increased liver size, various types of lesions have been reported to characterize mirex toxicosis in the liver. Kendall (1974b) reported fibrous white pithy lesions and cellular necrosis. Davidson et al. (1976) observed that 10-100 ppm mirex for 4 weeks disrupted chord cells, caused indistinct hepatocyte borders and necrosis in individual and clusters of hepatocytes in central midzonal areas of the lobule. Fulfs et al. (1977), in addition to observing lesions and cellular necrosis in mice, fed 5, 15, and 30 ppm for 2-18 months, noticed that Kupffer cells phagocytized dead cells.

Numerous structural changes other than an increase in size and appearance have been observed in the liver following mirex treatment in rats, mice and monkeys. Many investigators have observed a proliferation of smooth endoplasmic reticulum as a result of mirex pretreatment (Gaines and Kimbrough, 1970; Baker et al., 1972; Davidson et al., 1976; Fulfs

et al., 1977). This has also been observed for the chlorinated hydro-
carbon pesticides (Gaines and Kimbrough, 1970).

An increase in the number of lipid vesicles or vacuoles has been
noted as a result of mirex treatment, which frequently has been observed
with chlorinated hydrocarbon poisoning (Gaines and Kimbrough, 1970;
Kendall, 1974b; Davidson et al., 1976; Abraham et al., 1974). Bile
canaliculi degenerations and bile stasis are also results of mirex treat-
ment (Gaines and Kimbrough, 1970; Davidson et al., 1976). Hepatomas, neo-
plastic nodules, and hepatocellular carcinomas were measured in rats
treated with 1.00 ppm mirex in the diet over a long period of time (Ulland
et al., 1977; Innes et al., 1969).

Along with structural changes induced in the liver, many biochemical
alterations have been found to result from mirex treatment. In addition
to the proliferation of smooth endoplasmic reticulum, a concurrent in-
crease in cytochrome P-450 and in mixed function oxidase activity has been
reported (Baker et al., 1972; Byard et al., 1974, 1975; Davidson et al.,
1976; Mehendale et al., 1973). In addition, increased respiration has
been shown in the light microsomal fraction (Byard et al., 1974). Byard
et al. (1975) showed that p-chloromethylaniline N-demethylase activity was
stimulated by mirex when fed to mice at 1 ppm for 70 weeks, 15 ppm for 16
weeks and 40 ppm for one week. Parallel increases in 4-biphenyl hydroxyl-
ase activity and cytochrome P-450 were detected. Baker et al. (1972) also
reported that 10 mg/kg of mirex or higher in mice and 25 mg/kg of mirex in
rats increased cytochrome P-450 and O-demethylase activity on the sub-
strate p-nitroanisole. Likewise, increase in p-nitroanisole demethyla-

tion, ethylmorphine demethylation and the activity of UDP-glucuronyl transferase were shown by Mehendale et al. (1973) to occur as a result of treating rats for 5 days at 5-50 mg/kg. Glucose-6-phosphatase activity has been shown to decrease as a response to mirex toxicity (Byard et al., 1975; Fulfs et al., 1977; Abraham et al., 1974). Fulfs et al. (1977) showed that this decrease was dose dependent in mice. Mice fed 5 ppm for 2 months showed no significant decrease while mice fed 30 ppm for two months or 5 ppm for 10 months did show a decrease. In the same investigation Fulfs et al. (1977) showed that acid phosphatase also decreased when the mice received 30 ppm for 2 months. Each of these responses is often used as an indicator of hepatocellular toxicity (Fulfs et al., 1977). Liver toxicity as indicated by loss of potential energy in the form of glycogen has also been shown to be a characteristic of mirex exposure. Gaines and Kimbrough (1970), Kendall (1974b), Davidson et al. (1976) and Abraham et al. (1974) have each reported loss of glycogen in livers from rats and mice fed mirex.

Hepatocellular damage as a result of a toxic insult from mirex exposure has been reported, as stated earlier (Davidson et al., 1976; Kendall, 1974a, Fulfs et al., 1977; Gaines and Kimbrough, 1970). Certain soluble hepatic enzymes have often been used as a monitoring technique to determine the extent of the damage from toxic chemicals. These enzymes have been measured directly in the liver and also as a function of their concentration in the blood since ruptured liver cells or liver cells with the type of degeneration of cellular membranes described for mirex often leak measurable hepatic enzymes into the blood.

Thus, several investigators have undertaken this approach for measuring the toxicity of mirex to the liver. Hepatic lactic dehydrogenase and glutamic oxaloacetic transaminase activity of the rat have been shown to be reduced due to mirex exposure (Abston and Yarbrough, 1976). This study showed that the diminished activity was due to reduced enzyme levels and not to enzyme inhibition. Lactic dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), malic dehydrogenase (MDH) and sorbitol dehydrogenase (SDH) activities were measured after mirex exposure of 10-50 ppm in the diets of rats for 4 weeks. Each of these enzymes is a soluble enzyme present in the cytosol of liver cells. The study found that mirex caused a reduction of LDH, MDH, SDH, GOT and GPT in the livers compared to control values. Serum SDH values were shown to be elevated for the first week of exposure and to be concurrent with decreased liver levels of SDH. The authors concluded from this study that the observed reductions in enzyme levels in the liver was due to loss of the enzymes from the soluble portions of hepatocytes and not from mitochondrial portions of the cell since MDH and GOT are involved in the transfer of hydrogen across the mitochondrial membrane. The appearance of SDH in the serum along with the reduction in hepatic SDH is an indication of liver cell damage. The reductions in GPT and glutamic synthetase tissue levels and increases in serum levels of GOT, GPT, and LDH have been reported after single doses of DDT, aldrin, endrin and dieldrin. In addition, similar enzymes losses have been observed with sheep following oral adiministration of CCl_4 and those changes were related to significant hepatocellular damage (Abston and Yarbrough, 1976).

Robinson and Yarbrough (1978), after single oral doses of 50, 100, or 150 mg/kg mirex, reported hypertrophy of livers which is normally associated with the toxic insult of a chemical. However, their findings in regard to the levels of LDH, GPT and GOT along with SLDH, SGPT and SGOT, indicate that mirex did not cause damage to hepatocellular membranes. The authors suggest that chronic and acute exposures of mirex may produce different responses in the liver.

The adverse effect of mirex on both liver function and membrane function has been studied by Mehendale (1976, 1977). He noticed that mirex pretreatment of rats resulted in the suppression of biliary excretion of polychlorinated biphenyl compounds (1-CB;6-CB) in isolated perfused rat livers. In a followup study, Mehendale (1977) proved that mirex also suppressed the biliary excretion of imipramine and sulfobromophthalein. These effects were further shown to be related to neither bile flow nor rate of metabolism since bile flow increased directly with the reduction of excretions. He concluded that mirex somehow affects the transport of metabolites from the liver through the biliary membrane. Thus, the mirex apparently did not alter the actual metabolism of these compounds but did change membrane function.

Behavioral toxicology

Numerous compounds have been shown to affect the central nervous system in animals (Reiter, 1977). Chlorinated hydrocarbon pesticides such as DDT, dieldrin and aldrin have been shown to have CNS effects on mammals which result in hyperexcitability and convulsions (Gaines, 1960). Khairy (1960) noted that dieldrin caused deleterious effects on locomotor activi-

ty in adult rats. Mirex has been shown to have CNS effects in insects
which are similar to both DDT, dieldrin and cyclodienes, depending upon
the species affected (MacFarland et al., 1975; Plapp, 1973; Gaines and
Kimbrough (1970) observed that mirex caused diarrhea, weakness and hyper-
excitability in rats. Kendall (1974a) and Davidson et al. (1976) dif-
fered somewhat in more recent studies. They reported listlessness, yel-
lowing of the coat, diarrhea and weakness but not hyperexcitability from
mirex pretreated rats. Van Valin et al. (1968) also reported that mirex
may act differently since fish died in a manner which was not similar with
poisoning symptoms characteristic of chlorinated hydrocarbons. Apparently
only one paper has been published to date regarding the possible effects
of mirex on the central nervous system in rats. Reiter (19/7) investi-
gated the effects of mirex on the development of locomotor activity in the
rat. Nursing mother rats were intubated daily with mirex on four consecu-
tive days starting at parturition. The Sprague-Dawley rats were dosed
with 0, 2.5 and 10 mg/kg of mirex in corn oil. Acute exploration activity
was measured in residential mazes at 28 and 44 days of age. Mirex treat-
ment caused hyperactivity at 44 days of age. The author concluded that
mirex altered locomotor activity in the developing rat at 44 days of age.
Adults, however, did not show any change in locomotor activity.

Statement of the Problem and Objectives

The review of the literature on mirex and in particular the toxicity
of mirex in mammals reveals that there are important unknowns and con-

flicts present in existing data. Initially, LD_{50} values for mirex vary by more than twofold between investigators. Furthermore, these laboratories used technical grade (98%) rather than a pure form of the compound. Secondly, the mechanism or mode of action of mirex in causing toxicity in mammals is completely unknown. In insects the mechanism of action appears to resemble closely the chlorinated hydrocarbon pesticides in that the CNS is adversely affected which ultimately results in the death of the organism while in mammals this possibility is far from clear. Gaines and Kimbrough (1970) reported hyperexcitability in rats poisoned with mirex. Likewise, Reiter (1977) reported that young rats from mirex treated mothers were hyperactive at 44 days of age. In contrast to these reports, Kendall (1974a) and Davidson et al. (1976) did not report excitability but apparent listlessness in rats.

Research on the adverse effects of mirex on the liver has also introduced an apparent conflict. Abston and Yarbrough (1976) reported that rats treated for 4 weeks at 10-50 ppm mirex showed hepatic damage in that soluble hepatic enzymes were lost from the liver. In addition, concurrent elevation of sorbitol dehydrogenase in the blood was observed. These results are indicative of membrane disruption of the hepatocytes. Robinson and Yarbrough (1978) report, however, that the same enzyme levels did not change as a result of a single oral dose of mirex. They suggested that subacute and chronic exposure may produce different responses in the liver.

In view of these findings in the literature, this investigation was undertaken to study the effects of mirex on Sprague-Dawley rats. The objectives of this research were as follows:

(1) To ascertain the acute toxicity of both technical grade and pure mirex.

(2) To quantify the tissue levels of mirex in rats which died from mirex. These levels do not appear in the literature and may be reflective of the amount necessary to cause death.

(3) To determine the toxic insult of mirex in the liver when mirex was given in a large single dose or in subacute doses.

(4) To ascertain if mirex causes CNS effects as measured by: (a) an effect on locomotor ability; and (b) a change in strychnine and pentylenetetrazol convulsion thresholds.

METHODS AND MATERIALS

Acute Toxicity of Mirex to Rats

Treatment conditions

The housing, care and feeding of the rats were kept constant for all experiments unless stated otherwise. Sprague-Dawley male rats between 12-16 weeks old and weighing approximately 190-220 grams were obtained from Bio Lab Inc. The animals were caged individually in 9.5" x 7" x 7" galvanized metal wire cages. The cages were suspended over cedar chip bedding material with which the animal did not come in contact. The rats were fed ad-libitum with Teklad 4% Mouse and Rat Diet containing 24% crude protein, 4% crude fat and 6% crude fiber. Water access was ad-libitum with inverted rodent water bottles. The temperature of the animal room was maintained at a constant 25°C with constant circulation of air. The lighting was supplied by ceiling lights which were turned on at 7:30 A.M. and turned off at 4:00 P.M. each day.

Dosing for technical and pure grade mirex

This experiment measured the acute toxicity of technical grade (98%) and pure grade (99.95%) mirex. This was accomplished by determining the LD_{50} for technical grade mirex and then comparing the toxicity of the pure grade mirex to the technical grade material.

Food was removed 24 hours prior to dosing. The 50 animals used for the determination of the LD_{50} of technical grade mirex were randomly divided into 5 groups of 10 rats and assigned to one of the dosages of mirex. The dosages employed were 312.5, 500, 800, 1280 and 2048 mg/kg. The toxicity of pure grade mirex 99.95% was compared to the technical

grade mirex with three dose groups. Two dose groups of ten animals received the LD_{50} dose calculated from the technical grade mirex and one group received the LD_{90} dose. These doses were derived from probit vs. log dose plots. The mirex (50 mg/ml) was dissolved in corn oil (Mazola) by heating to 63°C with constant stirring for an hour. The compound was given orally through a stomach tube. A curved 18 gauge needle with a round ball on the tip was passed into the esophagus. The tube did not actually reach the stomach but was extended far enough into the esophagus of the rats to prevent regurgitation or entry into the bronchial passageway.

Rats were not dosed for eight days after arrival in order to allow for equilibration to their new environment. After dosing, the rats were observed closely for the first two days for signs of toxicosis and observed at least 2 times a day thereafter for symptoms. The survivors were kept for at least 10 days or until they appeared healthy.

The LD_{50} was calculated by means of the probit procedure afforded by the Statistical Analysis System. This method for the determination of the LD_{50} value, the probit vs. log dose plot, and the probability vs. log dose plot with 95% fiducial limits, is based on the method of Finney (1971). The method generates the calculated LD_{50} values and the plots mentioned above by computing the maximum likelihood of estimates for the parameters A and $B > 0$ in the probit equation: $y = A + Bx$. This type of analysis estimates the same parameters of dose response data as used by Litchfield and Wilcoxon (1949).

Comparison of the composition of technical grade and pure grade mirex

Since the introduction of mirex as a pesticide, most investigations on animals have used 98% or technical grade mirex. This is understandable since this is the form most commonly used as an insecticide. This investigation was concerned with determining if there were significant differences between chemical compositions of technical grade (98%) and pure grade (99.95%) mirex and was performed in conjunction with the bioassay of the toxicity of these two grades of mirex as described above.

This analysis was done by analyzing one sample of each grade of mirex in a Finnigan Mass Spectrograph.[1] The sequential printout representing particles of progressive molecular weights illustrated the differences in the compounds' compositions.

Tissue Distribution in Rats which Died from Mirex Treatment

The tissue concentration of mirex in rats which died from a single oral dose was measured in this experiment. The extraction of mirex from tissues and quantitation by gas liquid chromatography was the means by which this was accomplished.

Twelve male rats were orally given 573 mg/kg of pure grade mirex in a 5% corn oil solution. Six of the twelve rats died within five days and the rest were kept for at least 10 days or until they appeared healthy. The tissues from those rats which died were removed within several hours

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[1]The mass spectrograph analysis was conducted by Walter Hyde at the Veterinary Diagnostic Laboratory at Iowa State University, College of Veterinary Medicine, Ames, Iowa 50011.

after death. The brains, livers, kidneys, fat samples and quadricept muscle samples were removed, wrapped in aluminum foil and frozen until extraction procedures were carried out.

Extraction procedure for all tissues except fat

The following procedure was used to extract mirex from all tissues except fat. Two grams of tissue were weighed from the original sample and added to 2 grams of Na_2SO_4 in a tissue grinder. The tissue was ground to a homogeneous paste with a glass rod. Approximately 7 ml of methylene chloride certified A.C.S. grade was then added to the tissue grinder and mixed well. This solvent was then filtered off through a small funnel containing glass fiber filter paper topped with 10 grams of Na_2SO_4 into a test tube. This extraction with methylene chloride was repeated two more times. The tissue grinder and funnel were then rinsed well with methylene chloride and combined with the extract and evaporated to dryness with an air jet. A chromatographic macrocolumn, 25 mm outside diameter x 30 cm long fitted with Teflon stopcocks and coarse-fitted glass plates, was then prepared by adding 20 grams of activated Florisil topped with 20 grams of anhydrous, granular sodium sulfate. This column was then positioned over a Kuderna-Danish apparatus fitted with a 10 ml concentration tube. The column was prewet with nanograde petroleum ether (40-50 ml or a sufficient volume to completely cover the sodium sulfate layer). From this point on through the elution procedure the solvent was maintained above the sodium sulfate Florisil interface. The residue from the extract was then re-dissolved with at least 1 ml of petroleum ether and carefully added to the top of the macrocolumn and permitted to percolate through. The tube con-

taining the residue was rinsed at least two more times with petroleum ether and this wash was added to the column. The mirex was eluted from the column with 100 ml of 20% methylene chloride/80% petroleum ether. After the eluting solvent had passed through the column into the Kuderna-Danish assembly, a few grains of sand (washed with petroleum ether) were added to prevent bumping. The solvent was then concentrated to dryness on a steam bath with the use of a Snyder 3-bubble column. The final sample was prepared for gas chromatography by dissolving the residue in 5-10 ml of petroleum ether.

Extraction procedure for fat

Since the fat samples obtained from rats which died from mirex toxicosis were very small, less than 2 grams, the total sample was used for analysis. The sample was weighed and placed in a 250 ml stainless steel beaker. Ten grams of sand, prewashed with petroleum ether, was then added with 10 grams of Na_2SO_4. The tissue was ground to a dry granular mass. Fifty milliliters of petroleum ether were added to the beaker. This was placed on a steam bath and stirred. The supernatant was filtered through a 2" glass funnel with glass fiber filter paper into a pre-tared 250 ml flask. This extraction was repeated two more times. The petroleum ether was evaporated with the steam bath from the 250 ml beaker. The amount of lipid residue was weighed and then dissolved with 2 ml of methylene chloride. Finally, the samples were carried through the Florisil procedure with the macrocolumn and analyzed as described for the other tissues.

Gas chromatographic analysis of mirex

The samples were analyzed for mirex by means of gas chromatography on a Hewlett-Packard 402 gas liquid chromatograph fitted with a ^{63}Ni electron capture detector. The oven was equipped with a glass column 6' long, 1/4" outside diameter and 1/8" inside diameter, filled with a 3% OV-1 chromasorb-P packing. The oven temperature was maintained at 265°C. The injection vaporizer was set at 300°C and the detector block was kept at 300°C. Helium was used for the carrier gas and the flow rate adjusted to 20 ml/min. An argon methane 95%-5% mixture was used as a purge gas at a 60 ml/min flow rate. Using these parameters the instrument was capable of detecting very small (<1 ng) amounts of mirex.

Eluting solvents were nanograde purity and/or checked on the gas chromatograph for contaminants.

The samples were extracted concurrently with a blank sample and a sample spiked with a known amount of mirex (1-10 ppm) in order to determine the efficiency of a given day's extraction procedure.

The amount of mirex in each sample was calculated by determining a standard curve based on the height of the peaks generated by known amounts of mirex. Unknown samples were compared to the standard curve. The following formula depicts the means by which the amount of each sample was determined using the standard curve:

$$\frac{\text{ng of mirex present in sample unknown (determined using standard curve)}}{\text{\#}\mu\text{l of sample unknown injected into gas chromatograph}} \quad \text{X}$$

$$\frac{\text{ml of sample unknown volume after extraction}}{\text{g sample unknown}} = \frac{\text{ng}}{\text{g}}$$

$$= ppb$$

$$\frac{ng}{g} \div 1000 = ppm$$

The standard curve was constructed over a wide range of mirex amounts. Typically, 1 ng, 2 ng, 4 ng, 8 ng, and 10 ng were injected into the gas chromatograph to construct the standard curve. If the injected samples were too concentrated to fall within the limits of the standard curve, they were diluted 10-fold. This permitted a direct comparison of known and unknown amounts of mirex without any extrapolation. All samples were injected two times and their values were averaged and subsequently subjected to statistical analysis by the SAS system. The percent recovery was generally around 90%. Thus, sample values were not multiplied by a correction factor.

Effects of Mirex on the Liver

In order to test the hepatic response of rats to mirex, the following tests were used: (1) hexobarbital sleeping time, (2) histological examination, (3) the measurement of serum levels of soluble hepatic enzymes, and (4) an evaluation of the ability of mirex to induce the microsomal drug metabolizing enzymes.

Rats treated at the acute dose level received one dose of 573 mg/kg of pure grade 99.95% mirex in a 5% corn oil solution through oral intubation as previously described. Hexobarbital sleeping times, serum enzyme levels and histological sections of the liver were taken 48 hours after dosing.

The sub-acute dose protocol involved oral intubation of the rats at a 25 mg/kg/day level for two weeks. The sleeping times, liver sections and serum enzyme samples were taken 24 hours after the last dose of mirex. Control groups for both dose levels received equivalent volumes of corn oil.

Hexobarbital sleeping time

Rats were weighed and given an intraperitoneal injection of a 5% solution of hexobarbital to give 150 mg/kg. The sleeping time was measured from the loss of the righting reflex to the regaining of this reflex.

Histological examination of the liver

Following exsanguination, the livers were quickly removed from the rats, weighed, cut into pieces, and placed in a 4 % buffered neutral formalin solution. The solution contained 10 ml of 37-40% formaldehyde in 90 ml of distilled water with 42 grams sodium phosphate monobasic and 6.52 grams sodium phosphate dibasic (anhydrous). After at least one week of fixation, a cross section of the central lobe of each of the livers was removed and subjected to a 12 hour dehydration embedding process in an Auto Technicon.[1] The steps of dehydration involved a sequential one hour exposure to each of the following 12 baths: 70% alcohol, 70% alcohol, 95% alcohol, 95% alcohol, absolute alcohol, absolute alcohol, xylene, xylene, paraffin, paraffin, paraffin, and para-plast. The embedded section was then cut into cross sections with a microtome and subjected to a standard

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[1]The slide preparation was carried out in the histology laboratory in the Department of Veterinary Anatomy, Pharmacology and Physiology, College of Veterinary Medicine, Iowa State University, Ames, Iowa 50011.

staining technique with hematoxylin and eosin dyes. The staining procedure also involved consecutive stages. The liver cross sections were placed on a slide and then immersed in baths for specific time periods. The following baths were used for the hydrating, staining and dehydration steps: xylene - 5 min., xylene - 5 min., absolute alcohol - 2 min., absolute alcohol - 2 min., 95% alcohol - 2 min., 70% alcohol - 2 min., distilled water - 2 min., Harris hematoxylin - 8 min., tap water - 2 min., 99 ml 70% alcohol with 1 ml HCl - 1 sec., tap water - 5 min., lithium carbonate - 1 min., tap water - 5 min., distilled water - 2 min., eosin - 2 min., 95% alcohol - 1 min., 95% alcohol - 1 min., absolute alcohol - 2 min., absolute alcohol - 2 min., xylene - 2 min., xylene - 2 min., xylene - 2 min. Following these stages, the coverslip was applied and the slides evaluated with a light microscope.

Serum enzyme levels

Following the determination of the hexobarbital sleeping times, rats were decapitated for exsanguination. Whole blood samples were collected in 10 ml centrifuge tubes and allowed to clot at room temperature, 25°C. The tubes were then centrifuged at approximately 5000 rpm for 15 min. and the serum pipetted into disposable plastic 5 ml test tubes. All hemolyzed samples were discarded. The samples were then quickly analyzed with a Rotochem IIA Centrifigal Analyzer.[1] All samples were obtained from single animals. Serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic

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[1]This analysis was conducted by the Pathology Laboratory at Iowa State University, College of Veterinary Medicine, Ames, Iowa 50011.

transaminase and alkaline phosphatase values were measured in Internation-
al Units/Liter. Serum albumin levels are expressed in grams/deciliter.

Serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic
transaminase values were measured using Smith Kline French (SKF) reagents
and the oxidation of $NADH_2$ to NAD. This method for the quantification of
serum glutamic-oxaloacetic transaminase (SGOT) values was modeled after
the original method developed by Karmen (1955). However, the refinements
introduced by Henry et al. (1960) and Amador et al. (1967) have been in-
corporated to improve accuracy. Figure 2 depicts the reactions for meas-
urement of SGOT. Since the activity of SGOT is proportional to the ap-
pearance of oxaloacetate, the oxidation of $NADH_2$ is proportional to SGOT
activity. Thus SGOT can be quantified by measuring the decrease in ab-
sorbance at 340 nm caused by the formation of NAD from $NADH_2$.

The quantification of serum glutamic-pyruvic transaminase (SGPT) in-
volved the same basic principle as for SGOT. This series of reactions is
shown in Figure 3. Henry et al. (1960) developed this system for SGPT
activity measurement. The SGPT activity can be seen to be proportional to
the oxidation of $NADH_2$. Thus SGPT activity is once again quantified
through the decrease in absorbance induced by the appearance of NAD at
340 nm.

The alkaline phosphatase determination was carried out by the method
of Wilkinson et al. (1969) which used SKF reagents and is based on the
conversion of p-nitrophenylphosphate to p-nitrophenol (see Figure 4).
This product has a characteristic yellow color which thus allows the
activity of alkaline phosphatase to be measured as a function of the

COOH
|
CH$_2$
|
H-C-NH$_2$
|
COOH

\+

COOH
|
CH$_2$
|
CH$_2$
|
C = 0
|
COOH

SGOT →

COOH
|
CH$_2$
|
C = 0
|
COOH

\+

COOH
|
CH$_2$
|
CH$_2$
|
HCNH$_2$
|
COOH

aspartate α-ketoglutarate   oxaloacetate   glutamate

COOH
|
CH$_2$
|
C = 0
|
COOH

\+ NADH$_2$ MDH →

COOH
|
CH$_2$
|
H-C-OH
|
COOH

\+ NAD

oxaloacetate   malic acid
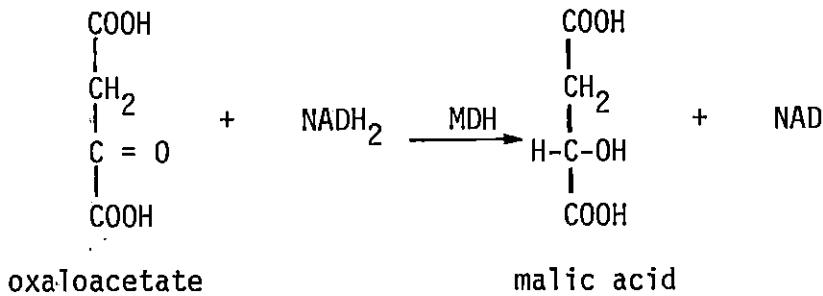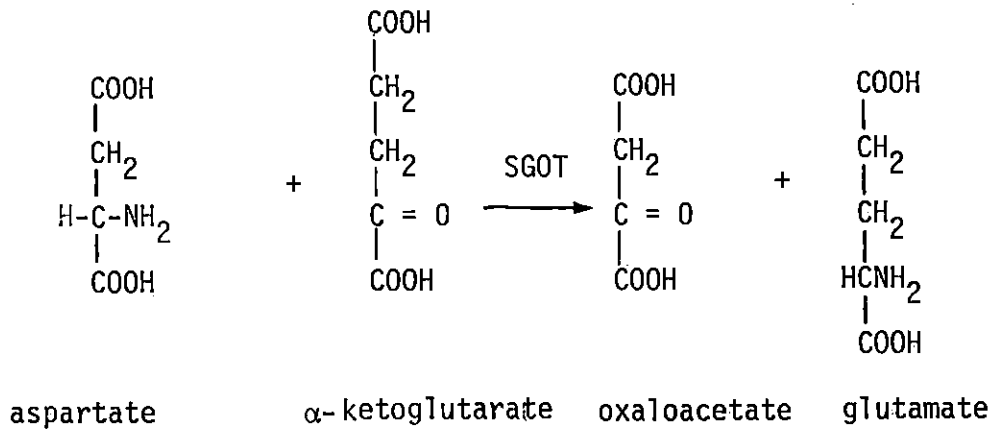
SGOT = serum glutaric-oxaloacetic transaminase

MDH = malic dehydrogenase

NAD = nicotinamide adenine dinucleotide

NADH$_2$ = reduced NAD

Figure 2. The chemical reactions used to quantify the activity of
glutaric oxaloacetic transaminase present in the serum samples.

COOH   COOH
|
CH_2 CH_3 CH_2 CH_3
|
CH_2 + $H-C-NH_2$ SGPT CH_2 +   C = 0
|
C = 0   COOH $H-C-NH_2$ COOH
|
COOH   COOH

α-ketoglutarate   alanine   glutamate   pyruvate

CH_3 CH_3
|
C = 0   + $NADH_2$ LDH   H-C-OH   +   NAD
|
COOH   COOH
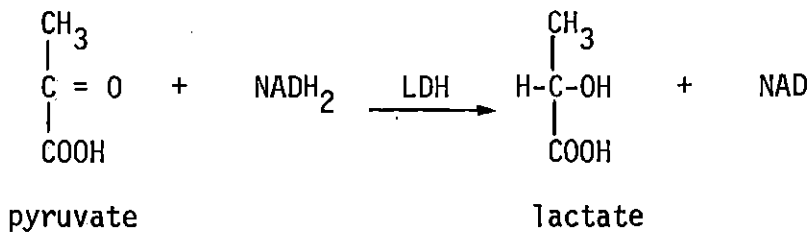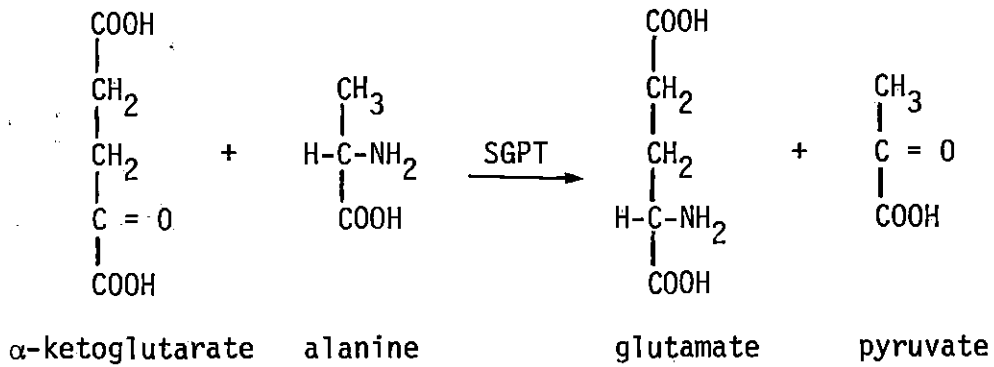
pyruvate   lactate

SGPT = serum glutamic-pyruvic transaminase

LDH = lactate dehydrogenase

NAD = nicotinamide adenine dinucleotide

$NADH_2$ = reduced NAD

Figure 3. The chemical reactions used to quantify the amount of glutamic pyruvic transaminase activity present in the serum samples.

$$O_2N-\langle\!\!\!\bigcirc\!\!\!\rangle-OPO_3^{2-} + H_2O + OH^- \longrightarrow O_2N-\langle\!\!\!\bigcirc\!\!\!\rangle-O^- + HPO_4^{2-}$$

p-nitrophenylphosphate   p-nitrophenol
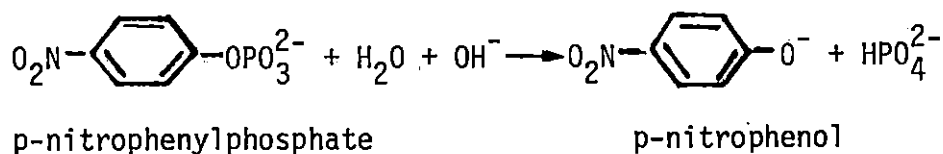
Figure 4. The chemical reaction used to monitor the activity of alkaline phosphatase in serum.

increase in absorbance caused by the product. The absorption in this case was measured with a spectrophotometer at 405 nm wavelength.

Serum albumin levels were measured with Pierce bromcresol green albumin reagent.

Measurement of microsomal activity

The liver microsomal activity of rats pretreated with mirex was investigated in this experiment by monitoring the conversion of p-nitroanisole to p-nitrophenol (see Figure 5). In order to achieve this, four groups of 10 rats were used. The experimental mirex group was treated four times with 50 mg/kg/day of pure grade mirex (P.O.) in a 5% corn oil solution. The control group received an equivalent volume of corn oil. The third group received 60 mg/kg/day of phenobarbital intraperitoneally for four days. Since phenobarbital is a known microsomal enzyme inducer, this group would serve as a reference to which mirex could be compared. The fourth group, which was the control for the phenobarbital group, received an equivalent volume of saline intraperitoneally for four days.

$$CH_3O-\langle\rangle-NO_2 \xrightarrow[O_2]{NADPH} HO-\langle\rangle-NO_2$$

microsomes

p-nitroanisole   p-nitrophenol
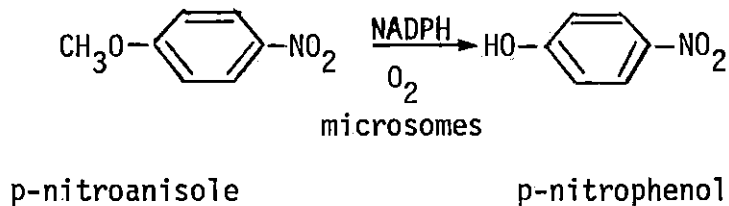
Figure 5. The chemical reaction used to quantify the activity of the microsomes.

Four days after the last dose, 96 hours from the end of administration, the rats were weighed and killed by a blow to the head. The livers were removed, weighed, and quickly wrapped in aluminum foil and placed on ice. The livers were cut into small pieces and placed in 50 ml centrifuge tubes containing 0.1 M phosphate buffer (pH 7.4). A 25% homogenate was prepared with the aid of a Sonifier Cell Disrupter. The nuclei and mitochondria were removed by centrifugation at 9000 x g for 15 minutes. The super-natant portion containing the microsomes, and the soluble enzymes were kept cool with ice and refrigeration during homogenation and centrifuga-tion respectively.

Next, 1 ml of supernatant was combined with .1 ml of 100 mmoles/L p-nitroanisole and 2 ml of incubation reagent in a clean centrifuge tube. The incubation reagent contained: phosphate buffer pH 7.4 (100 mM/L), nicotinamide (20 mM), glucose-6-phosphate (4 mM), NAD (0.4 mM), and $MgCl_2$ (10 mM). The tubes were mixed well and then placed in a 37°C bath for 60 minutes. After incubation, .5 ml of 20% trichloroacetic acid was added and the contents mixed. The tubes were then centrifuged 5 minutes at 3000 x g. The trichloroacetic acid extract was transferred to another

tube to which 1.0 ml of 1.0 N sodium hydroxide was added. These tubes
were agitated and allowed to stand for 10 minutes at which time they were
centrifuged for 5 minutes at 3000 x g. Samples were then read on a Bauch
and Lomb Spectrophotometer at 410 millimicrons and compared with a stand-
ard curve constructed from known concentrations of p-nitrophenol.

The protein content of the homogenate was determined by the method of
Lowry et al. (1951).

Effect of Mirex on the Nervous System

Locomotor ability

The locomotor ability of rats pretreated with mirex was measured in
this experiment. To accomplish this a simple rotorod device such as that
described by Watzman et al. (1964) and Watzman and Barry (1968) was used.
A 4' wooden 1 3/4" diameter rod was covered with 1/2" foam insulation.
The outside diameter measured 2 1/4". Compartments for the rats were con-
structed using cardboard disks surrounding the bar at 9" intervals. This
permitted 5 rats to run at one time. A D.C. motor with a gear reduction
unit was attached to one end of this bar thus allowing the bar's speed to
be regulated to 5, 10, 15, 20, 25, 28, 30, 35, and 40 rpm. The bar was
situated at 24" from the floor (see Figure 6). Uncooperative rats
were trained initially by permitting them to fall into a bucket of
water. The locomotor performance was measured by allowing the rats to run
for 30 seconds at each consecutive speed until they fell from the bar.
The speed at which they fell or clung to the bar to keep from falling was
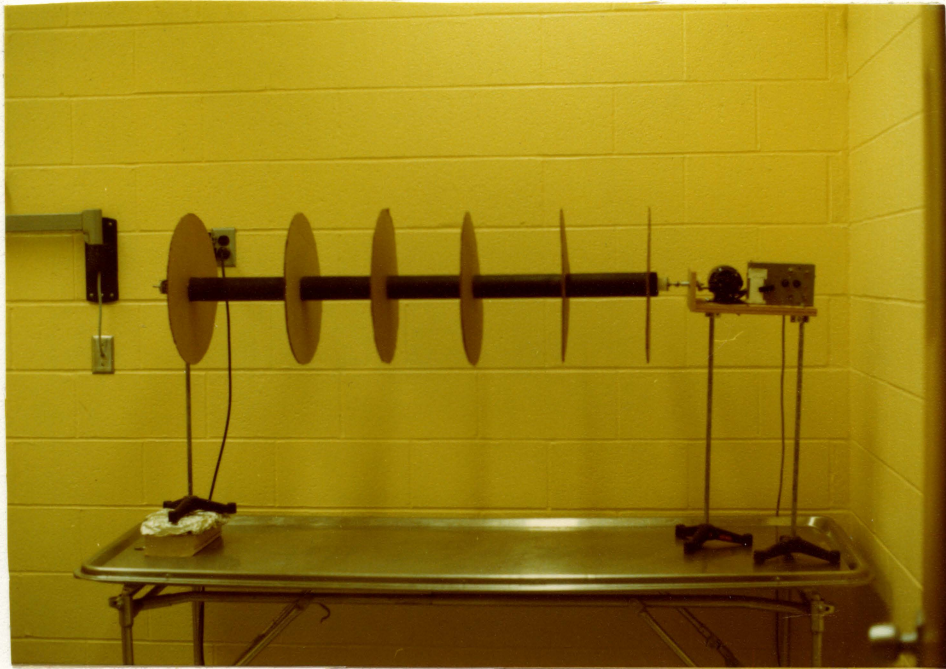taken as the endpoint in each trial. The forty rats used in this experi-

Figure 6. The rotobar device used to test the locomotor ability of the rats.

ment were given 5 running trials, one per day, to establish a level of performance for each rat before administration of mirex. After the rats were trained, one group of twenty rats was given 50 mg/kg/day of pure grade mirex in a 5% solution through oral intubation for four consecutive days. The control group received the equivalent amount of corn oil during this dosing period. None of the rats were subjected to running trials during the dosing days. At 24, 48 and 72 hours after the last dose, running trials were carried out for all rats.

The data were evaluated by means of the analysis of variance procedures by SAS.

Strychnine and pentylenetetrazol convulsion thresholds

The principle involved in this experiment is to determine if mirex pretreatment influences the convulsion thresholds of rats treated with either pentylenetetrazol or strychnine. This method has been used to study the effects of chlorinated hydrocarbon pesticides, such as dieldrin, on the central nervous system (Natoff and Reiff (1967).

The rats in the locomotor experiment were used in this experiment. Twenty-four hours after the last running trial, which was 96 hours after the last mirex treatment, rats were divided into 4 groups of 10 animals. Ten rats which had been pretreated with mirex at 50 mg/kg/day for four consecutive days, and ten control rats which had received an equivalent amount of corn oil were injected intraperitoneally with a sufficient volume of a 2% pentylenetetrazol solution to give 64 mg/kg. The same procedure was also carried out with two more groups, a mirex pretreated and a control group, using a .05% solution of strychnine to give a dose of

3.2 mg/kg. The time from the moment of injection until the onset of convulsions was measured by a stopwatch for each rat.

The results from this experiment were analyzed with the Statistical Analysis System.

List of Chemicals, Reagents, Materials and their Manufacturers

Chemicals

1. Mirex

Courtesy of Joseph Ford
National Monitoring Residue Analysis Lab
APHIS PIQ
Box 3209
Gulfport, Miss. 39503

2. Mazola corn oil

Best Foods
Englewood Cliffs, N.J. 07632

3. Trichloroacetic acid
Na2SO4
Methylene chloride
Florisil
NaOH
Petroleum ether

Fisher Scientific Company
Chemical Manufacturing Division
Fair Lawn, N.J. 07410

4. He gas
O2 gas
Alcohol
Petroleum ether
Xylene
Formalin

Chemistry Stores
Iowa State University
Ames, Iowa 50011

5. Hexobarbital
Phenobarbital
Nicotinamide
Glucose-6-phosphate
NADPH
MgCl2

Sigma Chemical Company
P.O. Box 14505
St. Louis, Mo. 63178

6. p-nitrophenol

Mallinckrodt Chemical Works
St. Louis, Mo. 63178

7. p-nitroanisole

Eastman Kodak Company
Rochester 3, N.Y.

8. Argon-methane (95-5%) gas — Matheson Gas Company
East Rutherford, N.J.   07073

9. Para-plast — Sherwood Medical Supply
St. Louis, Mo.   63103

Reagents

1. SKF reagents — Smith Kline French Company
15005 Gordon Street
Philadelphia, Pa.   19101

2. Pierce reagents
(Bromcresol green albumin
reagent) — Pierce Chemical Company
Box 117
Rockford, Ill.   61105

3. Harris hematoxylin solution — Chemistry Stores
Iowa State University
Ames, Iowa   50011

Materials

1. Sprague-Dawley rats — Bio Lab, Inc.
5228 Centerville Road
St. Paul, Minn.   55110

2. Teklad 4% Mouse/Rat Diet — Teklad Standard Lab Diet
P.O. Box 75
Winfield, Iowa   52699

3. Statistical Analysis System — Barr, Goodnight, Sall and Helwig
SAS Institute. Inc.
P.O. Box 10066
Raleigh, N.C.   27605

4. Hewlett-Packard 402 Gas
Chromatograph — Hewlett-Packard Company
Avondale Division
Route 41
Avondale, Pa.   19311

5. Autotechnician IIA — The Technicon Company
Chauncey, N.Y.

6. Kuderna-Danish Assembly
Snyder bubble columns
Concentrator tubes
500 ml flasks — Kontes Glass Company
Vineland, N.J.

7. Rotochem IIA Analyzer — American Instruments
Silver Springs, Md.

8. Finnigan Spectometer

    Finnigan Instruments
    845 West Maude Ave.
    Sunnyvale, California 94086

9. Sonifier Cell Disrupter

    Heat Systems Company
    Melville, L.I., N.Y.

PRESENTATION OF RESULTS

Acute Toxicity of Mirex to Rats

Following examination of the literature and preliminary experiments, the dose range (312.5 mg/kg to 2048 mg/kg) was selected to determine the LD_{50} for technical grade mirex. The results of this experiment are summarized in Table 1. The group dosed with 312.5 mg/kg technical grade

Table 1. The acute toxicity of technical grade mirex on male rats

Dose (mg/kg)	Number of rats surviving	Number of rats which died	% mortality	Time until death in days (No. of rats which died on each day)
312.5	9	1	10	4 (1)
500.0	6	4	40	3,4 (3,1)
800.0	2	8	80	2,3,4 (1,5,2)
1280.0	1	9	90	2,3,4 (1,6,2)
2048.0	0	10	100	2,3 (1,9)

mirex had only one death which occurred on the fourth day. The group receiving the highest dose showed 100% mortality with rats dying on either the second day (1 rat) or third day post-treatment (9 rats). At the higher dose the rats died at an earlier time. However, the mechanism(s) by which mirex produces death take a considerable period of time when compared to chlorinated hydrocarbon pesticides. Therefore, had the LD_{50} been calculated on the basis of 24 hour mortality, mirex would be evaluated as a much less toxic pesticide than it really is.

Two graphical representations of the data were generated using the method developed by Finney (1971). The graph of probability vs. log (dose) appears in Figure 7. This graph illustrates the standard s-shaped dose-response relationship of the data. A graph of probit vs. log dose gave the expected linear relationship as shown in Figure 8.

The graph in which the probit versus log dose is plotted indicates an LD_{50} of 573.2 mg/kg for technical grade mirex. The 95% fiducial limits for the LD_{50} for technical grade mirex occur within the dose range of 427.7 mg/kg to 733.4 mg/kg.

Utilizing the information gained from the above results, the toxicity of pure grade mirex was determined for comparison purposes. Two groups received the LD_{50} dose and one group received the LD_{90} dose as derived from the dose-response relationship for technical grade mirex. The results of this experiment are summarized in Table 2.

Table 2. The toxicity of pure grade mirex to rats

Group	Dose (mg/kg)	Number of rats surviving	Number of rats which died	% mortality	Time until death in days (No. of rats which died on each day)
1	573	2	8	80	3,4 (5,3)
2	573	6	6	50	3,4 (2,4)
3	1100	0	10	100	3,4 (4,6)

Figure 7. A graph of the probability versus the log of the dose, a dose response curve, for technical grade mirex.

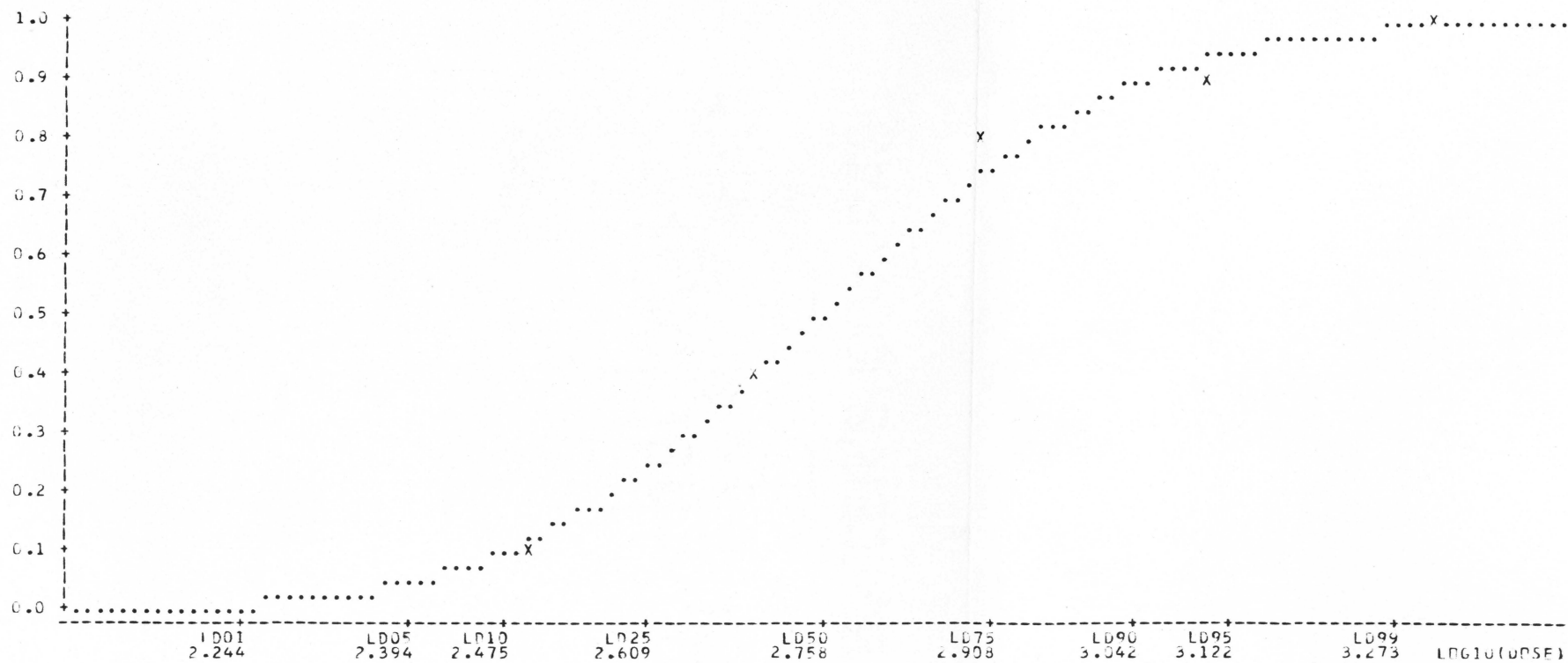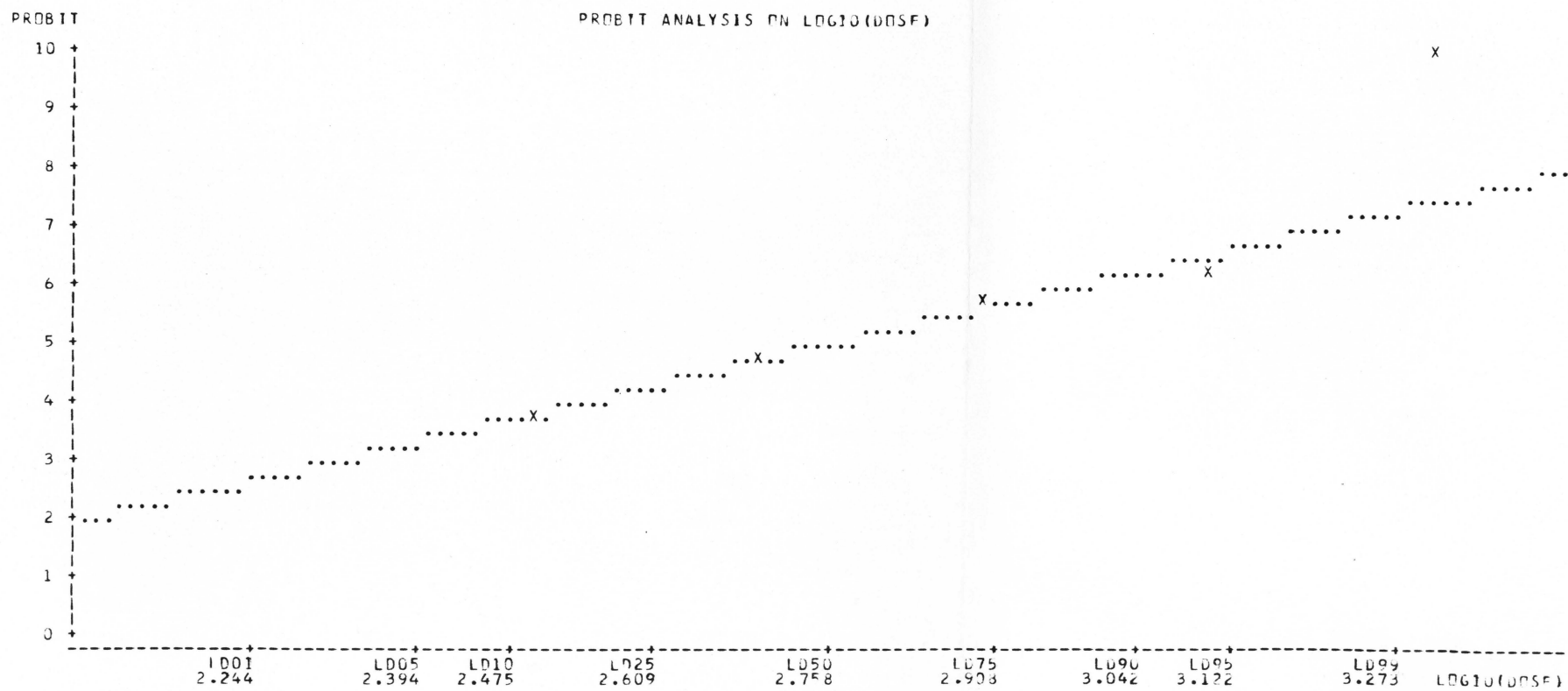PROBABILITY   PROBIT ANALYSIS ON LOG10(DOSE)

Figure 8. A probit transformation plot of percent mortality versus log
(dose) for technical grade mirex. A chi-square value of .8975
indicates that the data are accurately portrayed by the fit
computed through the probit procedure.

PROBIT ANALYSIS ON LOG10(DOSE)

The data indicate that pure grade mirex causes mortality well within the 95% confidence intervals derived from the technical grade mirex study. Thus, any difference in the toxicity of the two grades of purity are sufficiently small as to be nondetectable with the accuracy afforded with groups of ten rats per dose group.

Symptoms of toxicity

The rats poisoned with mirex showed characteristic symptoms of toxicity. The most common symptoms included: yellowing of the fur, diarrhea, and listlessness. Rats exhibiting listless behavior appeared weak and ataxic. None of the rats showed the excitable behavior as described by Gaines and Kimbrough (1970). However, when the rats were handled, they appeared to be sensitive to abdominal pressure. Some rats which received mirex showed marked eye secretions which pasted their eyes shut. In addition, a few rats seemed to have blood in their feces.

Mass spectrometer analysis

The differences in the two grades of mirex indicate that small amounts of contaminants are present. These appear to be low molecular weight nonchlorinated aliphatic compounds. The graphical representation of the composition of the two grades of material appear in Figures 9 and 10. It is not known whether these compounds influence the toxicity of mirex at this time; however, the toxicity of the two grades of mirex to rats appears to be the same.

Figure 9. Mass spectrophotometer recording of technical grade mirex. ↓ = not present in pure mires.
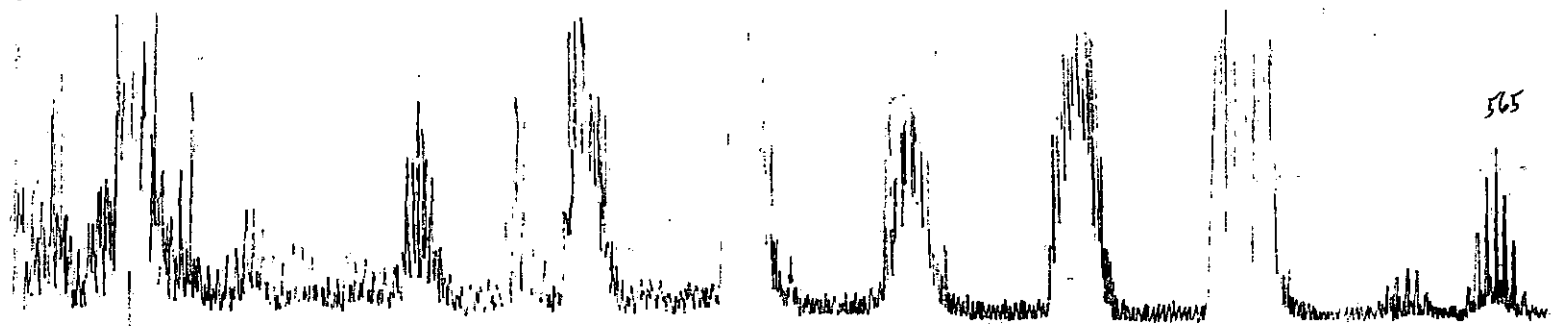
↓ = not present in Pure mixes

565

57

300   500

O f R

Figure 10. Mass spectrophotometer recording of pure grade mirex. X indicates background noise. Bkg = background.

X - background

59
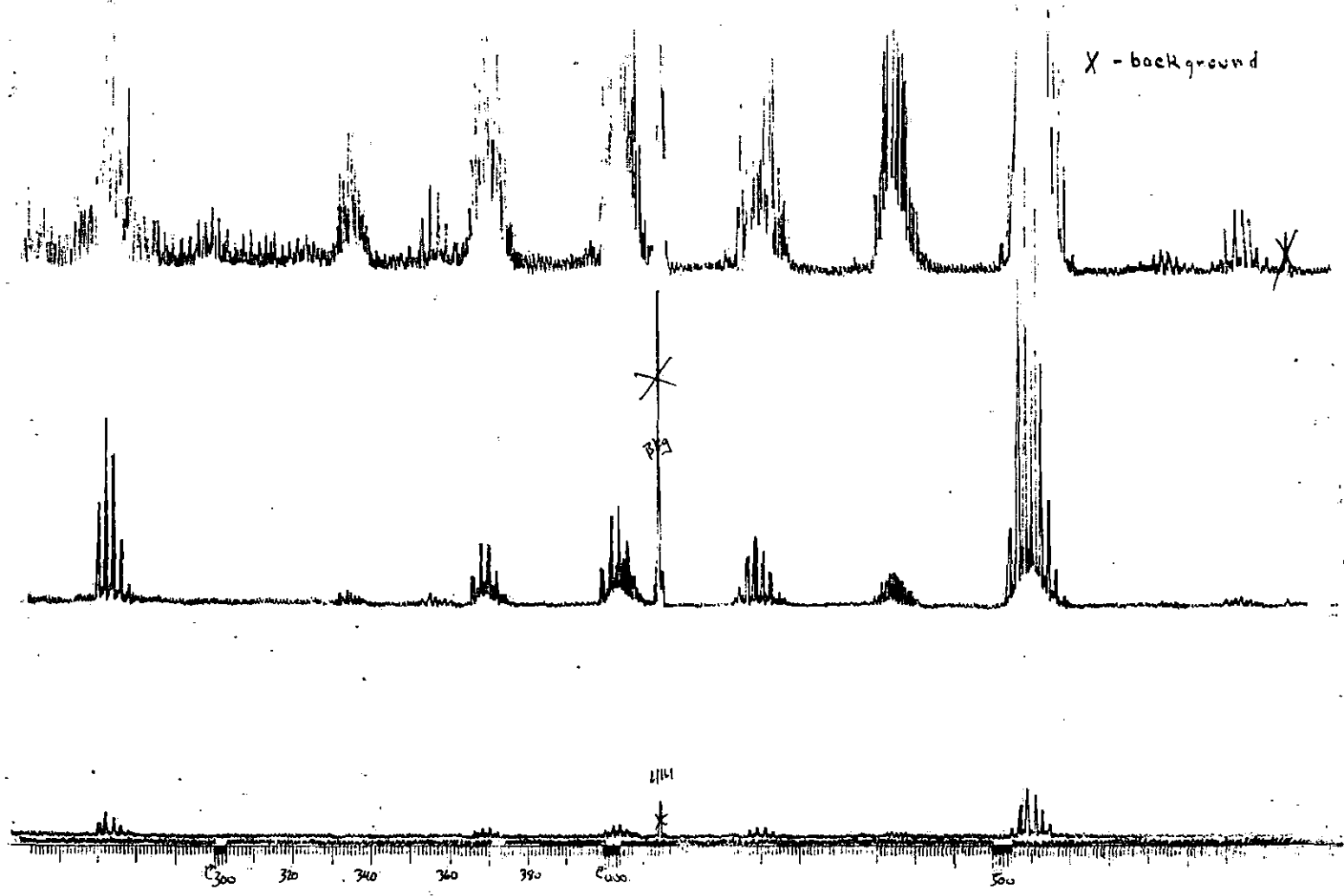
Tissue Distribution in Rats which Died from Mirex Treatment

This experiment measured the residue levels of mirex in rats which died from mirex (see Table 3). The residue levels were determined by gas chromatographic procedures.

Table 3. The tissue levels of mirex in rats which died after receiving 573 mg/kg of mirex

Rat #	Day on which death occurred after treatment	Amount of mirex (ppm[a])				
		Liver	Brain	Kidney	Muscle	Fat
4	5	33.0	26.8	88.0	27.9	.b
6	3	112.4	33.2	66.8	30.7	195.0
8	3	151.1	30.9	66.0	33.1	531.0
10	4	109.5	31.1	84.6	38.1	682.0
11	4	257.5	45.5	71.8	27.5	745.0
12	5	50.7	46.9	109.0	40.2	.b
Mean±S.E.[c]		119.0 ±32.3	35.7 ±3.4	81.0 ±6.7	32.9 ±2.2	538.3 ±122.9

[a]Parts per million by weight.

[b]The residue levels in the fat of these rats could not be determined since the amount of fat present on the carcass was negligible.

[c]The standard error of the mean is abbreviated with S.E.

These data illustrate the trends apparent regarding the sequestration of mirex in tissues. Fat contained the highest concentration of mirex. A typical gas chromatographic recording for the determination of mirex is shown in Figure 11.

Figure 11.   An example of a gas chromatograph recording for mirex at
             different amounts. The chart speed was .25 inches/minute.
             The range in amounts of mirex injected was from .7-4 ng,
             illustrating the high sensitivity of the gas chromato-
             graph with the ^{63}Ni detector.

↑ .7 ng mirex   ↑ 1 ng mirex   ↑ 2 ng mirex   ↑← 4 ng mirex
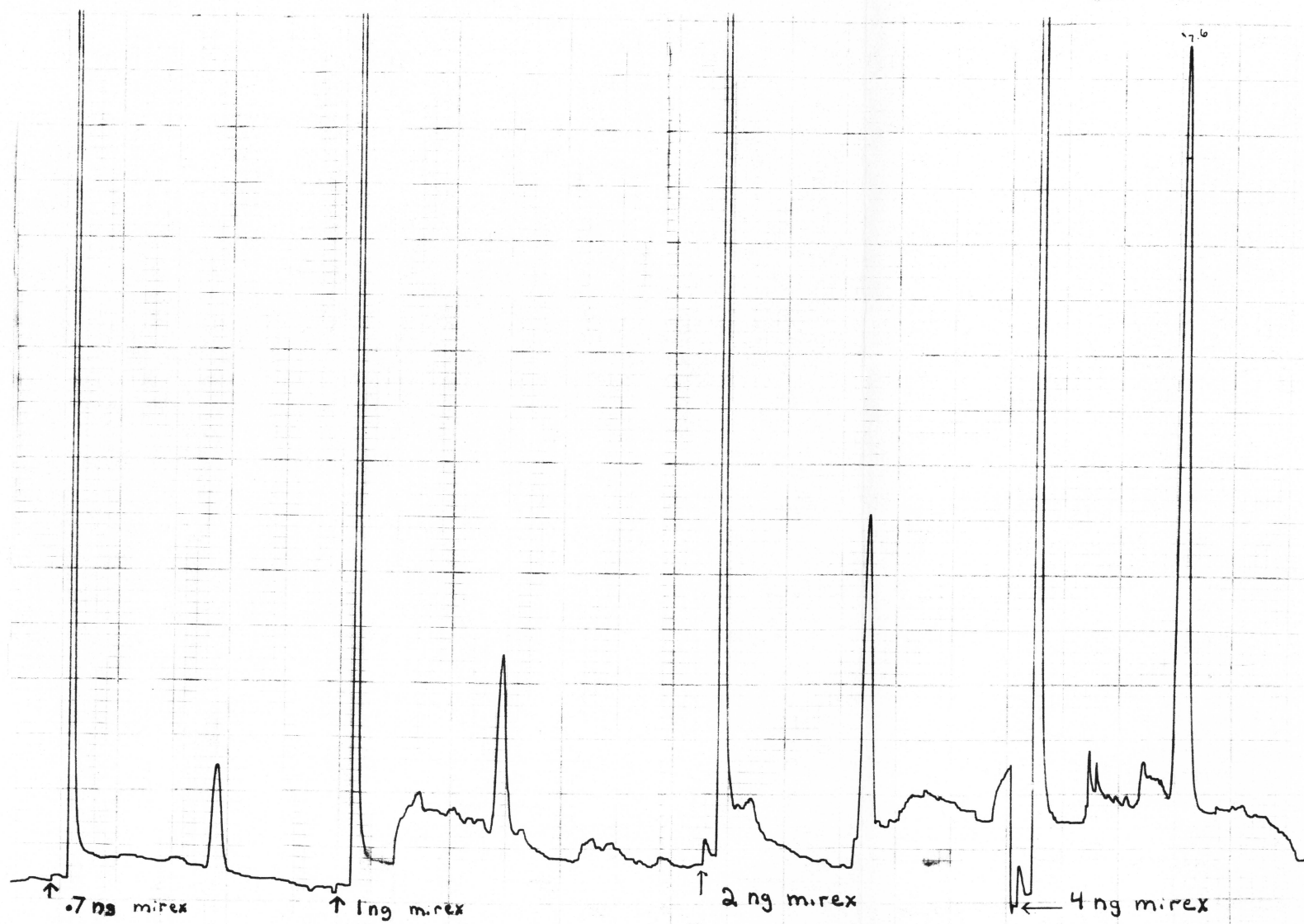
Confirmation of the extraction and analysis procedure for mirex was accomplished by comparing tissue sample residue levels with two other laboratories, the Iowa State University Veterinary Diagnostic Lab and the University of Iowa Pesticide Analysis Laboratory, Institute of Agricultural Medicine. A homogenized tissue sample was used for this purpose. The tissue was divided into nine samples, three being measured by each laboratory. The average values in parts per million were ; .10-I.S.U., .45-U.I., .50-Lasley.

Hepatic Toxicity of Mirex in Rats

The effects of mirex on the liver were investigated in this experiment using four standard techniques: (1) hexobarbital sleeping time; (2) changes in the circulating levels of soluble hepatic enzymes and albumin; (3) histological examination; and (4) effect on drug metabolism.

Rats receiving the acute dose of mirex (573 mg/kg in a single oral dose using a 5% corn oil solution) and animals in the sub-acute regime (25 mg/kg/day for 2 weeks using the same mirex solution) were compared to controls which received an equivalent volume of corn oil. Table 4 summarizes the results of the acute dose experiment. The measurements on the acute dose group were taken 48 hours after the single oral dose of mirex. The sub-acute group was tested 24 hours after the last dose. Table 5 summarizes the results of the sub-acute dose experiment.

The most pronounced effects on the rats receiving an acute dose of mirex can be seen in Table 4 to be a decrease in body weight which was not associated with any change in liver weight. The control rats on the

Table 4. The effects of an acute dose of mirex (573 mg/kg) on the rat[a]

Measurement	N	Control Mean with standard error	N	Mirex treated Mean with standard error	Change due to mirex	F-value	Level of significance
Body weight (g)[b]	10	304.6±12.6	10	258.7±10.7			
Body weight change	10	21.1±6.3	10	-19.5±8.4	Decrease	14.96	99%
Liver weight	10	13.1±.4	10	13.6±.4			
Liver weight (as percent of body weight)	10	4.4±.2	10	5.3±.2	Increase	8.33	99%
Serum glutamic-oxaloacetic transaminase (SGOT) IU/L[c]	10	154.5±12.3	4	230.0±26.0	Increase	11.01	98%
Serum glutamic-pyruvic trans-aminase (SGPT) IU/L	10	37.3±3.6	7	138.9±21.5	Increase	30.97	98%
Alkaline phosphatase IU/L	10	194.8±15.6	8	132.9±14.5	Decrease	8.05	98%

[a]All values were obtained 48 hours after the administration of mirex.

[b]Grams.

[c]International Units/Liter.

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Table 4. (Continued)

| Measurement. | Control | | Mirex treated | | Change due to mirex | F-value | Level of significance |
	N	Mean with standard error	N	Mean with standard error			
Serum albumin g/DL[d]	10	4.0±.1	8	4.3±.1	Increase	2.33	N.S.[e]
Hexobarbital sleeping time (minutes)	9	28.8±1.6	9	37.7±3.7	Increase	4.84	95%

[d]Grams/deciliter.

[e]N.S. = not significant.

Table 5. The effects of sub-acute administration of mirex (25 mg/kg/day - 2 weeks) on the rat[a]

Measurement	Control		Mirex treated		Change due to mirex	F-value	Level of significance
	N	Mean with standard error	N	Mean with standard error			
Body weight (g)[b]	10	279.1±5.0	10	259.6±7.6			
Liver weight	10	11.0±0.4	10	18.7±0.5	Increase	168.45	99%
Liver weight as % of body weight	10	4.1±0.1	10	7.2±0.2	Increase	168.45	99%
Serum glutamic-oxaloacetic trans-aminase (SGOT) IU/L[c]	9	151.7±5.6	8	124.2±7.7	Decrease	8.62	99%
Serum glutamic-pyruvic trans-aminase (SGPT) IU/L	9	29.4±2.0	8	31.0±3.5	Increase	.16	N.S.[d]
Alkaline phosphatase IU/L	0	181.8±9.8	8	131.4±12.7	Decrease	10.11	99%

[a]Values were obtained 24 hours after the last dose of mirex.

[b]Grams.

[c]International Units/Liter.

[d]N.S. = not significant.

Table 5. (Continued)

Measurement	N	Control Mean with standard error	N	Mirex treated Mean with standard error	Change due to mirex	F-value	Level of significance
Serum albumin g/DL[e]	9	3.5±0.1	8	4.0±0.1	Increase	11.71	99%
Hexobarbital sleeping time (minutes)	9	21.4±2.5	8	30.2±2.4	Increase	6.55	98%

[e]Grams/deciliter.

average increased their body weights by 21.1 grams while the mirex-treated rats lost 19.5 grams. Although the livers increased as a percentage of the body weight, from 4.4% in controls to 5.3% in mirex-treated rats, the actual livers did not change in weight. Thus the increase as a percentage of body weight can be attributed almost entirely to the loss of body weight and not to hypertrophy of the liver over this short time period.

These observations after the acute insult of mirex contrasted with the findings of the sub-acute test. The rats receiving 25 mg/kg/day for 2 weeks did not lose weight as a result of mirex dosage. The average weights for the control and mirex-treated rats can be seen to be roughly equal. The livers, however, showed a marked hypertrophy in the mirex group. Control rats' livers weighed 11.0 grams on the average while mirex-treated rats had livers averaging 18.7 grams. These weights represent 4.1% of the body weight in the control animals and 7.2% in the mirex treated rats.

The measurement of hepatic function in each group, using hexobarbital sleeping time as an index of drug metabolism, indicated that sleeping times were increased. The sleeping times after acute mirex dosage increased from 28.8 minutes as an average in the control group to 37.7 minutes for the mirex-treated group. This would be indicative of a decrease in the ability of the liver to hydroxylate the hexobarbital which would result in an increased sleeping time. A surprising finding was that the rats which received mirex at 25 mg/kg/day for 2 weeks and which had hypertrophied livers had increased sleeping times. This would again sug-

gest that the liver was not able to metabolize hexobarbital faster than the control rats.

This implied damage to the liver is substantiated by the levels of the circulating soluble hepatic enzymes SGOT, SGPT but not alkaline phosphatase. SGOT and SGPT values can be seen in Table 4 to be dramatically raised in the acute mirex-treated rats indicating loss from the liver due to damage from mirex. Alkaline phosphatase levels, however, were lower in rats treated with mirex than in control rats.

The study with mirex treatment for 2 weeks at 25 mg/kg/day showed that rats receiving mirex had lower levels of circulating SGOT and alkaline phosphatase than control rats while SGPT values in control and mirex-treated rats appear to be the same. Control rats from both studies appear to have the same levels of serum SGOT, SGPT and alkaline phosphatase enzymes.

Albumin levels were the same for controls and rats which received 573 mg/kg of mirex. However, rats which received 25 mg/kg/day had significantly higher levels of circulating albumin than control rats.

Cross sections of the rat livers showed marked effects of mirex in both the acute and sub-acute experiments. Livers from rats receiving the acute dose (573 mg/kg) were characterized by extensive vacuolation in the lobule associated with fatty infiltration. The vacuolar distribution was primarily associated with the portal areas. However, midzonal portions of the lobules were significantly vacuolated. In addition, these livers showed significant portal cellular degeneration and individual cell death

throughout the lobule. See Figures 12 and 13 for photographs of liver cross sections from control and experimental rats.

The livers from rats which received the sub-acute dose regime appeared to have a basically different response to mirex. These sections were characterized by a general hepatocellular hypertrophy. In addition, these hepatocytes seemed to lack the vacuolation, and thus fatty change, found in the livers of rats which received mirex acutely. Death of individual cells was found to be distributed throughout the lobule in these livers (see Figures 14 and 15).

Measurement of Microsomal Activity

The microsomal induction test involved the measurement of the activity of the microsomal enzymes as a result of mirex and phenobarbital pretreatment. The induction of the microsomal enzymes was measured by monitoring their activity with a standard reaction. This was accomplished by monitoring the conversion of p-nitroanisole to p-nitrophenol. The rats in this experiment were treated on 4 consecutive days with 50 mg/kg mirex (P.O.) or 60 mg/kg phenobarbital, IP. Saline and corn oil were used as the controls for the experimental groups. The measurements were taken 96 hours after the last dose. The results from this experiment are depicted in Tables 6 and 7.

From Table 6 it can be observed that mirex did not produce a significant change in body weight yet it produced a significant hypertrophy of the liver. These changes observed in the mirex-treated group were not observed in the phenobarbital group (Table 7).
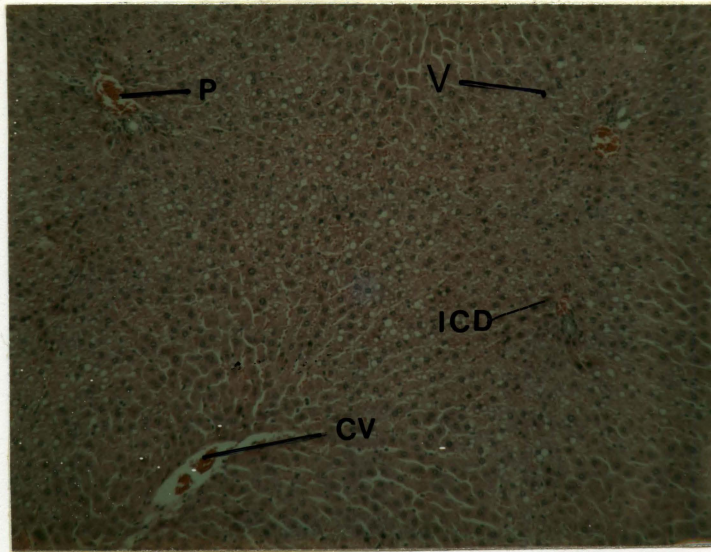
Figure 12. Photograph showing a histological liver cross section from a
rat treated with 573 mg/kg mirex. Magnification 100X. Note
portal and midzonal vacuolation (V) and individual cell death
(ICD), portal system (P), central vein (CV).

Figure 13. Photograph showing a histological liver cross section from a
rat receiving corn oil at equivalent volume to experimental
group. Magnification 100X. Note (P), (CV).

Figure 14. Photograph showing a histological liver cross section from a
            rat receiving 25 mg/kg mirex for 2 weeks. Magnification 100X.
            Note: hypertrophy, individual cell death (ICD), portal system
            (P), central vein (CV).
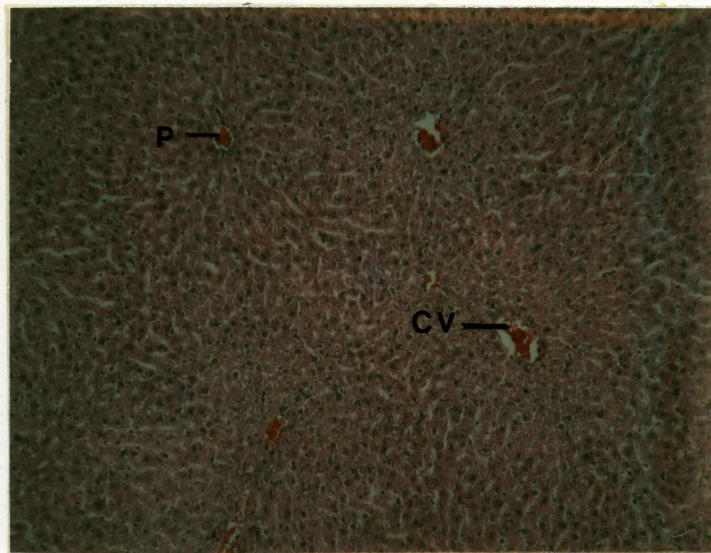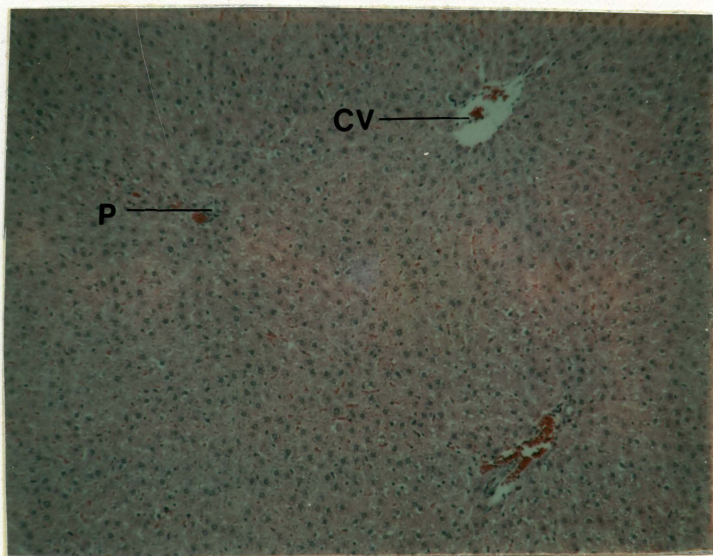

Figure 15. Photograph showing a histological liver cross section from a
            rat receiving corn oil for 2 weeks at equivalent volumes to
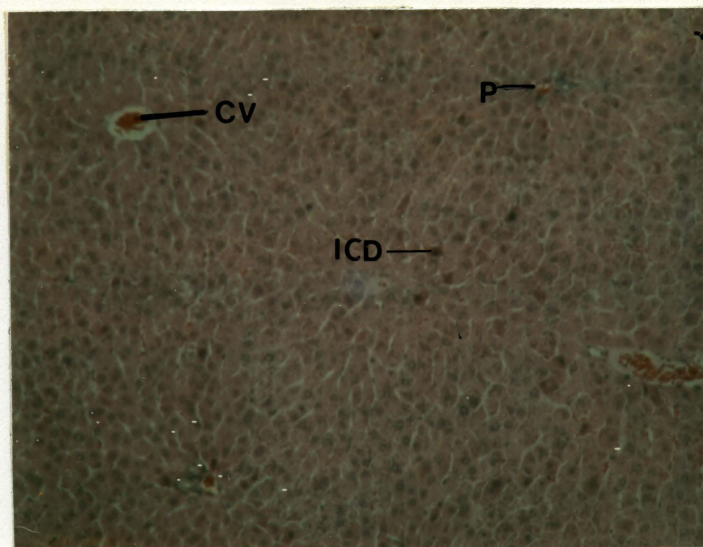   the experimental group. Magnification 100X. Note: (P),
            (CV).

Table 6. Evidence for induction of microsomal enzymes by mirex[a]

Measurement	Corn oil control		Mirex experimental		Change due to mirex	F-value	PR > F
	N	Mean with S.E.[b]	N	Mean with S.E.[b]			
Body weight (g)[c]	10	260.3±6.8	10	250.4±10.8	No change	.79	.3786
Liver weight (g)	10	11.4±0.5	10	17.9±0.6	Increase	65.20	.0001
Liver weight as % of body weight	10	4.4±0.1	10	7.2±0.2	Increase	161.75	.0001
Protein concentration[d]	10	2.1±0.1	10	2.0±0.1	No change	.19	.6682
umoles of p-nitrophenol formed/liter/hour	10	11.3±1.0	10	24.6±0.8	Increase	87.53	.0001
mmoles of p-nitrophenol formed/kg.protein/hour	10	5.5±.5	10	12.2±0.5	Increase	87.23	.0001

[a]Measurements were taken 96 hours after administration of mirex (50 mg/kg/day - 4 days). The control group received just corn oil.

[b]S.E. = standard error of mean.

[c]Grams.

[d]Protein concentration as grams/liter.

Table 7. Evidence for induction of microsomal enzymes by phenobarbital[a]

| Measurement | Saline control | | Phenobarbital experimental | | Change due to mirex | F-value | PR > F |
	N	Mean with S.E.[b]	N	Mean with S.E.[b]			
Body weight (g)[c]	10	265.5±8.3	10	263.8±4.0	No change	.02	.8792
Liver weight (g)	10	11.0±0.8	10	11.8±0.3	No change	.95	.3359
Liver weight as % of body weight	10	4.1±0.2	10	4.5±0.1	No change	2.75	.1058
Protein concentration[d]	10	2.4±0.1	10	2.2±0.1	No change	1.92	.1748
umoles p-nitrophenol formed/liter/hour	10	16.3±1.2	9	19.8±1.1	Increase	5.86	.0209
mmoles of p-nitrophenol formed/kg.protein/hour	10	6.9±0.6	9	9.1±0.5	Increase	9.27	.0044

[a]Measurements were taken 96 hours after administration of phenobarbital (60 mg/kg/day - 4 days). The control group received just saline.

[b]S.E. = standard error of the mean.

[c]Grams.

[d]Protein concentration as grams/liter.

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The protein concentration was not different from control in either mirex or phenobarbital-treated groups. This is to be expected since the 25% homogenates were made on a weight-volume basis.

The rate of p-nitrophenol formed was increased in both the phenobarbital and mirex-treated groups. Mirex caused a greater increase than that observed in the phenobarbital group. Thus mirex and phenobarbital were both observed to induce the microsomal system, but under the conditions employed, mirex was the better inducer.

Effects of Mirex on the Nervous System

Two tests were used in this investigation in order to ascertain whether or not mirex had any measurable effects on the nervous system in rats. Initially, rats were dosed orally for four days with 50 mg/kg/day mirex in a 5% corn oil solution. After this treatment the rats were tested for locomotor impairment with a rotating bar device. Following this test the rats were tested for differences in the time until onset of convulsion following pentylenetetrazol or strychnine injection.

Locomotor ability

The locomotor performance of the rats, measured by their ability to accomplish the running task, showed little difference between the control group receiving corn oil and the mirex pretreated group. Table 8 illustrates these findings for the locomotor performance test. The average speeds at which the rats fell from the bar before treatment were 19.6 rpm and 18.7 rpm for the mirex and control groups respectively, indicating about the same locomotor ability in the two groups prior to treatment.

Table 8. The results of the rotobar test[a]

Group	Time	Number of observations	Number of rats	rpm causing rats to fall mean	Change	PR > F
Mirex treated	Before treatment	100	20	19.6		
Mirex treated	After treatment	60	20	22.0	Increase	.0118
Control	Before treatment	100	20	18.7		
Control	After treatment	60	20	18.5	Decrease	.3318

[a]Measurements were taken 24 hours after treating rats with mirex (50 mg/kg/day - 4 days). The control group received just corn oil. Analysis of variance of the overall performance between groups indicated that there was no difference in locomotor ability between controls and mirex-treated rats (PR > F = .1439.

Locomotor trials after mirex treatment indicate a slight increase in the group's locomotor ability. The analysis of variance for these changes, however, indicated that there was no significant difference in locomotor ability between the control group and the mirex-treated group (probability of a greater F-value = .1439).

Effect of mirex pretreatment on strychnine and pentylenetetrazol induced convulsions

The effects of mirex treatment on the time it takes for rats to convulse after pentylenetetrazol or strychnine administration are illustrated in Tables 9 and 10. Rats were treated with 50 mg/kg for four days and then used for this test 96 hours post-treatment. Rats which received a

Table 9. The effect of mirex pretreatment on pentylenetetrazol induced
         convulsions[a]

Treatment	Rat #	Time to convulse (minutes)	Mean±standard error (minutes)
	1	2.30	
	3	1.45	
	4	1.58	
Mirex	5	1.96	1.68±.13[*]
(50 mg/kg-4 days)	6	1.87	
	7	1.85	
	8	0.96	
	9	2.16	
	10	1.28	
	41	1.45	
	21	0.75	
	22	0.80	
	23	0.98	
	24	0.93	
Control	25	1.13	1.00±.10[*]
(corn oil)	26	0.96	
	27	1.73	
	28	1.30	
	29	0.78	
	30	0.71	

[a]The measurements were taken 96 hours after the last dose of mirex
(50 mg/kg/day - 4 days). The control group had been dosed with just corn
oil. Pentylenetetrazol was given intraperitoneally (64 mg/kg), and the
time until the onset of convulsions was measured with a stopwatch.

[*]The F-value for a difference between the means = 17.05. Thus a
highly significant difference at PR > F = 0.0006 was generated.

Table 10. The effects of mirex pretreatment on strychnine induced con-
           vulsions[a]

Treatment	Rat #	Time to convulse (minutes)	Mean±standard error (minutes)
	11	D.N.C.[b]	
	12	"	
	13	"	
	14	"	
Mirex	15	"	
(50 mg/kg-4 days)	16	5.17	
	17	D.N.C.	
	18	"	
	19	"	
	20	"	
	31	5.75	
	32	3.83	
	33	4.00	
	34	5.83	
	35	5.17	
Control	36	6.92	5.68±.46[*]
(corn oil)	37	5.75	
	38	6.83	
	39	4.25	
	40	8.50	

[a]The measurements were taken 96 hours after the last dose of mirex
(50 mg/kg/day - 4 days). The control group had been dosed with just corn
oil. Strychnine was given intraperitoneally (3.2 mg/kg), and the time
until the onset of convulsions measured with a stopwatch.

[b]D.N.C. = these rats did not convulse after strychnine injection.

[*]A highly significant difference existed between the mirex and control
group. F-value = 9.84, PR > F = .0001.

convulsant dose of pentylenetetrazol after mirex pretreatment convulsed in an average of 1.68±.13 minutes while control rats convulsed in 1.00±.10 minutes. This difference was significant at the 99% level.

A more striking effect was observed with rats given strychnine after mirex pretreatment. These rats were dosed with 2 times the convulsant dose of strychnine, 3.2 mg/kg, I.P. but only one of ten rats convulsed, and in this rat the strychnine was injected directly into the liver. All ten of the control rats convulsed requiring an average of 5.68±.46 minutes to onset.

DISCUSSION

Acute Toxicity of Mirex to Rats

The toxicity of mirex in adult male rats as measured in this experiment indicates that mirex can be considered to be a moderately toxic pesticide. The oral LD_{50} value for technical grade mirex was evaluated to be 573 mg/kg. Since the pure grade mirex did not differ significantly in lethality or composition from the technical grade, the LD_{50} value for it would probably be applicable. Our value for the oral LD_{50} is somewhat higher than the values of 306 mg/kg reported by Martin and Worthing (1974) and of 312 mg/kg reported by Allied Chemical Company (Markin et al., 1972), but Gaines (1968) reported a value of 740 mg/kg. However, the rats used by Gaines were of a different strain and were not fasted before dosing which may account for a slightly higher tolerance to the pesticide.

The symptoms observed in rats suffering from mirex intoxication were different from the symptoms most commonly associated with chlorinated hydrocarbon poisoning. Gaines (1960, 1968) noted that symptoms of chlorinated hydrocarbon poisoning were characterized by tremor, hyperexcitability, irritability and convulsions. The rats observed in this experiment though showing weight loss and diarrhea which are common symptoms of chlorinated hydrocarbon poisoning never exhibited excitability or convulsions. The most notable symptoms of toxicity were listlessness and a general deterioration of health. Another observation, although difficult to quantify, was that the rats seemed to resist handling after mirex administration. These results conflict with the symptoms used by Gaines and

Kimbrough (1970) to characterize mirex intoxication in rats. Gaines reported that hyperexcitability was one sign of poisoning in rats treated with mirex. Our results are more consistent with the observations made by Kendall (1974a) and Davidson et al. (1976) who reported that mirex caused listlessness.

Thus, the differences observed in the symptoms of intoxication between mirex and other chlorinated hydrocarbon pesticides would imply that mirex is not acting on the central nervous system as a stimulant-type neuropoison but has an altogether different mechanism of action in eliciting toxicity in rats.

Distribution of Mirex in Rats which Died from Mirex Treatment

The distribution and concentration of mirex in tissues substantiate the lipophilic nature and stability in living mammalian systems. After oral administration of 573 mg/kg mirex in corn oil, the rats which died from the mirex treatment showed high residue levels. The highest was found in fat, followed by liver, kidney, brain and muscle respectively. The brain and muscle samples showed amounts of mirex which were almost equal (35.7 ppm and 32.9 ppm). The residue levels of chlorinated hydrocarbon pesticides in the brains of animals which died from intoxication with these compounds have been considered to be a good indicator of acute poisoning (Stickel et al., 1966). Thus since mirex is considered to be much less toxic than DDT in regard to an acute insult, it is surprising that the brain levels of mirex in rats which died from mirex intoxication were similar to levels observed for DDT by other researchers (Stickel

et al., 1966). They measured a range in DDT levels in rats dying from DDT to be between 35-52 ppm.

The lipophilic nature of mirex reflected by residue levels found in the tissue compartments in this experiment are consistent with the results of other investigators. Mehendale et al. (1972) found that male rats which received a single oral dose of mirex (6.0 mg/kg in corn oil) sequestered most of the mirex in fat followed by kidney, liver, brain and muscle on a weight basis. Brain and muscle concentrations were equal in this experiment while the kidneys had a higher concentration than the liver. Gibson et al. (1972), however, found that more mirex appeared in the liver than in the kidney 48 hours after exposure to a single oral dose of mirex which is consistent with the result observed in the present study. Though these researchers used relatively low doses of mirex, higher doses as used by Khera et al. (1976) reflect the same trend in tissue levels as found in our experiment. After dosing male rats for ten consecutive days with 12.5 mg/kg they found the following residue levels: 2810.0 + 51 ppm in fat, 110.6 + 16.0 ppm in the liver and 15.3 +-1.7 ppm in the brain.

Although we did not specifically study mirex metabolism, gas chromatographic analysis of the various tissues did not reveal any peaks which would suggest the presence of metabolites. This finding is consistent with other researchers in that they did not find any metabolites of mirex in mammalian systems (Khera et al., 1976; Mehendale et al., 1972; Gibson et al., 1972).

The variability observed in the tissue levels indicates that the fat compartment showed the greatest fluctuations in the amounts of mirex present. This result for fat is explicable when one considers the extremely small amounts of fat on the carcasses of the dead rats which made the calculation inherently less accurate. Possibly, the fat had been metabolized to maintain energy requirements in the dying animals. Fat mobilization as a result of pesticide poisoning has been observed in birds dying from DDT (Stickel et al., 1966).

Hepatic Toxicity of Mirex in Rats

The primary purpose of this experiment was to determine the effects of mirex on the liver from acute and sub-acute administration. In addition, the effects of an intermediate dose regime were monitored in order to evaluate the state of the liver under conditions identical to those found in the experiment assessing mirex's effect on the nervous system.

The methods used in this experiment have been found to be reliable indicators for determining the effects of noxious chemicals on the liver by other researchers. The alteration in relative liver weights has proven to be an effective preliminary indicator of hepatic toxicity (Fulfs et al., 1977). Thus, many researchers have utilized this effect for the measurement of mirex toxicity on the liver. Mirex has been found by many researchers to cause dramatic increases in relative liver weight of rats (Fulfs et al., 1977; Gaines and Kimbrough, 1970; Byard et al., 1974; Davidson et al., 1976; Robinson and Yarbrough, 1978). This increase has been related to cellular hypertrophy with concurrent increases in the

protein levels (Byard et al., 1974; Davidson et al., 1976). In the present investigation, the acute dose of 573 mg/kg caused a significant increase in the liver weight to body weight ratio. However, no actual change occurred in the liver weights themselves. Thus, the increase in relative liver weight can be attributed to a relatively severe decrease in body weight as a result of mirex intoxication. The sub-acute dose group, however, involving doses of 25 mg/kg/day for 2 weeks, showed a highly significant increase in both liver weight and relative liver weight. This hypertrophy of the liver was associated with a marked increase in the size of the liver cells as revealed by histologic examination. The liver sections from the acute group were not hypertrophied, probably due to the brief time of exposure. However, rats treated for 4 days at 50 mg/kg showed a highly significant increase in both liver weights and liver weight/body weight ratios. In addition, these livers showed the same protein concentration as control livers which accompanies the type of hypertrophy shown by the sub-acute dose regime. Hypertrophy of the liver as observed for these two groups of rats has been suggested to be a compensatory mechanism resulting from the impairment of liver function, caused by the toxic agents (Robinson and Yarbrough, 1978).

Serum hepatic enzyme levels were consequently employed as a means to enable the detection of functional liver damage by mirex treatment. These have traditionally been good indicators of hepatocellular tissue damage (Dinman et al., 1973; Paget, 1970). The elevation in the circulating levels of soluble hepatic enzymes after treatment with known hepatotoxic chemicals such as CCl_4 have been related to necrosis, permeability changes

and an increase in the hepatic levels of the enzymes (Plaa, 1975; Dinman et al., 1963; Dinman and Bernstein, 1968; Ideo et al., 1971). The enzymes monitored in this experiment after acute and sub-acute mirex administration were alkaline phosphatase , glutamic-oxaloacetic transaminase and glutamic pyruvic-transaminase. The findings following acute intoxication with mirex indicate that liver damage occurred by either necrosis or changes in cellular permeability, since the SGOT and SGPT levels were significantly higher than control values. The alkaline phosphatase activity was lower than control levels. This discrepancy can be attributed to a different time course for this enzyme. Ideo et al. (1971) found peak levels for alkaline phosphatase at 24 hours and levels lower than control 48 hours after treatment with CCl_4. The deleterious effects suggested by the changes in the enzyme levels were confirmed by histopathology examination. The rats which had been dosed with 573 mg/kg mirex showed death of individual hepatocytes and a significant increase of vacuoles. However, the membrane degeneration as described by Davidson et al. (1976) was not observed. These enzyme level elevations conflict with the results observed by Robinson and Yarbrough (1978). After acute administration of 50, 100, and 150 ppm mirex in the diet, Robinson and Yarbrough (1978) did not observe any change in serum levels of soluble hepatic enzymes. This finding could be attributed to the lower doses of mirex which were employed. The insult may not have been enough to elicit necrosis or permeability changes. The findings of Abston and Yarbrough (1976) were consistent with the present study. They observed increases in the circulating hepatic enzyme levels in rats; however, they employed 10-50 ppm mirex (in feed)

for four weeks which exposed the animals to amounts of mirex comparable to this study.

The rats subjected to the sub-acute administration of mirex in the present study showed SGOT and alkaline phosphatase serum levels which were lower than control values and SGPT levels which were not different from control values. These results may indicate that the livers had compensated enough by hypertrophy, or that the hepatic enzymes had leaked into the serum to an extent that they exhausted the hepatic supply. Abston and Yarbrough (1976) noted that losses of enzymes to the serum were concurrent with lower hepatic enzyme levels. This may have been the case for the sub-acute dose group since individual cell death was evident in sections of the livers in mirex-treated rats.

The impairment of the liver as suggested by the elevated serum enzyme levels was further characterized with a functional test, namely, hexobarbital sleeping time. The metabolic transformation of barbiturate anesthetics has often been used as a means to determine the functional state of the liver. Furthermore, serum enzyme elevations used in conjunction with barbiturate anesthetics have shown that a positive relationship exists between these separate measurements and hepatocellular injury (Klaassen and Plaa, 1966; Balazs and Grice, 1963).

Chlorinated hydrocarbons such as DDT have been shown to be potent microsomal enzyme inducers (Hart and Fouts, 1963). Thus, when these chemicals have been given to mammals prior to treatment with hexobarbital or pentobarbital, there has been a significant decrease in barbiturate sleeping times due to the induction of the microsomal enzymes. In con-

trast, organophosphate pesticides have been shown to have an opposite effect, that of lengthening barbiturate sleeping time (Hart and Fouts, 1963).

In the present study, the hexobarbital sleeping times were increased as has been reported for the organophosphate pesticides. This result seems to be in conflict with the known capacity of mirex to induce the drug metabolizing microsomal enzymes. Numerous researchers have reported that mirex is a potent inducer of the smooth endoplasmic reticulum, i.e., the cytochrome P-450 (Baker et al., 1972; Byard et al., 1974; Mehendale et al., 1973; Davidson et al., 1975). Thus, it would seem that mirex pretreatment would hasten hexobarbital metabolism. However, the results from the serum enzyme assay and the tissue damage observed in the histological sections support the notion that liver damage occurred and thus partially explains the increased hexobarbital sleeping time. The positive relationship between cellular disfunction or cell death and increased barbiturate sleeping time has been observed by other researchers. Balazs and Grice (1963) noted a positive relationship between liver necrosis and increased pentobarbital sleeping times and increased serum SGPT levels in the rat, a result consistent with this present study. Thus, the increased hexobarbital sleeping time after acute and sub-acute mirex administration indicates hepatocellular disfunction in these rats. This effect may be dose related since the test of the liver function after treating rats for four days with 50 mg/kg mirex showed that microsomal activity was increased. These rats had hypertrophied livers which were apparently not adversely affected

by mirex to the extent that their function was impaired, as was the case with rats treated with the acute or sub-acute dose regimes.

Though rats receiving sub-acute and acute doses of mirex may exhibit a similar type of response to mirex insult which is manifested in the serum hepatic enzyme responses and hexobarbital sleeping time, extensive morphological differences were observed in the livers of these rats. It has been shown by other researchers that mirex produces proliferation of smooth endoplasmic reticulum, lipid vacuoles, hepatocellular enlargement and necrosis of clusters and individual hepatocytes (Gaines and Kimbrough, 1970; Davidson et al., 1976; Kendall, 1974b; Robinson and Yarbrough, 1978; Fulfs et al., 1977). Examination of livers from the present experiment revealed individual cellular death distributed throughout the lobule and extensive portal and midzonal vacuolation and fatty change associated with acute insult of mirex similar to that observed by Kendall (1974b). The hypertrophy observed by Robinson and Yarbrough (1978) was not observed from the single oral dose due to the fact that the rats were sacrificed 48 hours after treatment. The rats undergoing sub-acute treatment showed significant hypertrophy and individual cell death, however, vacuolation as observed in the group receiving the acute dose of mirex was not present. This difference in vacuolation and hypertrophy may indicate, as Robinson and Yarbrough (1978) suggested, that there is a difference in the response of the liver cell to acute and sub-acute mirex insult. However, this supposition conflicts with the responses of the soluble hepatic enzymes to acute and sub-acute mirex treatment.

The Neural Activity of Mirex in Rats

Chlorinated hydrocarbon pesticides have been shown to have effects on the central nervous system by many researchers. The typical symptoms of poisoning include irritability, hyperexcitability to outside stimuli, disturbed equilibrium, tremor and tonic and clonic convulsions (Murphy, 1975; Gaines, 1968; Al-Hachim and Fink, 1967). Al-Hachim (1971) and Al-Hachim and Fink (1968) have shown that treatment of mice with pesticides such as aldrin and DDT have offspring with lowered audiogenic and electro-shock convulsion thresholds, indicating biochemical or physiological brain damage from these compounds. Dieldrin has been shown to alter EEG recordings in humans weeks or months after exposure (Murphy, 1975). In addition, locomotor activity has been shown to be affected by dieldrin and DDT as a consequence of central nervous system affects (Khairy, 1960).

As a consequence of the symptoms of chlorinated hydrocarbon toxicity, much attention and research has been focused on the elucidation of the mechanism for the action of these compounds on the central nervous system. Most research has been conducted on DDT since it is the best known and was the most widely used pesticide. DDT has been shown to exert its primary action upon the sensory and motor nerve fibers (Murphy, 1975). The mechanism of action has been shown in recent studies to be related to the ion flux of the neural membrane. DDT is believed to alter the flow of potassium and sodium ions across the membrane in nerve axons by combining with a component of the membrane to create a complex which alters the normal functioning of the membrane (Hayes, 1975; Murphy, 1975).

The cyclodiene pesticides have been shown to act in a different way. Rats poisoned with dieldrin have been observed to release gamma butyrobetaine and related compounds from the mitochondria. It is believed that these substances are responsible for the effects of endrin since injection of betaine esters intracranially caused violent convulsion and death in rats (Murphy, 1976).

This present experiment was designed to detect whether or not mirex caused any effects on the central nervous system which could be measurable with a simple locomotor task or with changes in the convulsion thresholds for strychnine or pentylenetetrazol. The rotobar device has been well-documented as a test for psychotropic compounds (Kinnard and Watzman, 1966; Watzman et al., 1964; Dunham and Miya, 1957). In addition, strychnine and pentylenetetrazol have been used to detect neural effects of dieldrin treatment in mice (Natoff and Reiff, 1967).

Locomotor Effects of Mirex

The results for the rotobar test clearly indicate that mirex did not impair the rats' ability to perform the locomotor task. The performance of the control and mirex-treated animals was shown not to be different (PR > F = .1439). The change in locomotor ability after the treatment with mirex indicates that the mirex-treated rats slightly but significantly improved in their ability to remain on the rotating bar. The control rats, however, showed no significant change in locomotor performance. Though the mirex-treated group was measured to have a slight but statistically significant increase in locomotor ability, this observation may

not be biologically significant since the net increase was only a fraction of the 5 rpm interval used to denote a performance change.

Thus, the type of deleterious locomotor performance change as reported for DDT and other chlorinated hydrocarbon pesticides did not occur in this experiment. Though a slight increase in locomotor ability was observed for the mirex-treated group, caution must be exercised in interpreting this result since the increase in performance was so small. However, an increase in locomotor behavior was observed by Reiter (1977) in offspring from mirex-treated mothers and the results observed in this experiment may be due to a similar CNS effect. Other experiments with the locomotor ability of rats treated with mirex would be needed to substantiate any effects of mirex causing the enhancement of locomotor performance in rats.

Convulsion Thresholds for Strychnine and Pentylenetetrazol

The convulsive effects of chlorinated hydrocarbon pesticides on rats and mice have been one of the characteristic symptoms of toxicity. This effect has lead to a considerable amount of research on the mechanism of action. One observation has been that DDT and related hydrocarbon pesticides seem to augment an animal's response to external stimuli which would indicate that sensory pathways are involved (Murphy, 1975). As a consequence of these effects, electroshock, audiogenic and chemical seizure thresholds have been used to detect effects of these pesticides on the central nervous system in developing rodents and in adults (Al-Hachim and Fink, 1967, 1968; Al-Hachim, 1971; Natoff and Reiff, 1967). Strychnine and

pentylenetetrazol have been used to differentiate between the probable sites of action of dieldrin. Acute oral administration of dieldrin (15, 30 and 60 mg/kg) to mice significantly increased their sensitivity to pentylenetetrazol in the central nervous system, but not strychnine. Dieldrin is believed to facilitate the transmission of impulses from the higher motor centers and the cerebrospinal axis while strychnine is believed to act on sensory modalities and motor pathways (Natoff and Reiff, 1967).

This experiment was thus designed to determine if mirex, which has been classified as a chlorinated hydrocarbon pesticide and compared to dieldrin, would exert any action on the central nervous system which would alter the convulsion threshold for pentylenetetrazol or strychnine, as has been shown with dieldrin.

The results found in the present experiment in which rats were injected with pentylenetetrazol or strychnine subsequent to mirex treatment were not expected. The group of rats receiving pentylenetetrazol after mirex treatment convulsed in 1.68 + .13 minutes while the control group convulsed in 1.00 + .10 minutes. Thus there was a highly significant net increase in the time it took for the rats to convulse. The effect after strychnine injection was even more striking. The control group convulsed in 5.68 + .46 minutes while only one rat in the mirex pretreated group convulsed. The experimental rats, although showing exaggerated responses to external stimuli which seemed to bring them to the brink of convulsion, never did convulse. This finding is surprising in light of the fact that the rats receiving strychnine received 3.2 mg/kg, twice the reported

convulsant dose (Barnes and Eltherington, 1973). The single rat which did convulse was incorrectly injected into the liver rather than the abdominal cavity.

Two possible explanations exist for these findings. Either mirex was acting as a depressant which would antagonize the effects of strychnine and pentylenetetrazol in the central nervous system or the liver microsomes, which have been shown to be induced after 4 days of mirex treatment at 50 mg/kg/day, were metabolizing the convulsant compounds at a rate which would prolong or prevent the compound from reaching a threshold concentration in the blood. The evidence for a depressant effect would be consistent with the lengthening of the hexobarbital sleeping time at acute and chronic doses. In addition, the listless behavior observed at the acute dose could be an extension of this type of mirex activity. However, it is important to note that the doses of mirex given before hexobarbital sleeping time measurements were different from those observed in this experiment. Furthermore, the lengthening in these sleeping times may have been a result of the disfunction of the liver as indicated by histology and the elevated soluble hepatic enzymes. The state of the liver after doses equivalent to that used in this experiment were measured in the test for microsomal activity after mirex treatment. It was found that the liver microsomes were significantly induced after mirex treatment and were capable of metabolizing p-nitroanisole at a significantly higher rate than controls or phenobarbital-treated rats. Furthermore, the increase in smooth endoplasmic reticulum, mixed function oxidases, and microsomal activity of the livers after mirex pretreatment has been well-documented

by other researchers (Gaines and Kimbrough, 1970; Baker et al., 1972; Mehendale et al., 1973; Byard et al., 1974; Davidson et al., 1976; Fulfs et al., 1977). In addition, strychnine and pentylenetetrazole have been shown to be metabolized by the microsomal systems of the liver (Howes and Hunter, 1966; Adamson and Fouts, 1959; Ko, 1969; Ko and Hosein (1970); Vohland et al., 1974). Strychnine is metabolized by microsomal activity to a hydroxylated product which is blocked by the known microsomal in- hibitor SKF-525A (Adamson and Fouts, 1959). Pentylenetetrazol has also been shown to be metabolized to 6-hydroxy-pentylenetetrazol and 8-hydroxy- pentylenetetrazol by the microsomes and these reactions can also be blocked by SKF-525A (Vohland et al., 1974; Rowles et al., 1971; Ko, 1969; Ko and Hosein, 1970).

The rate at which the microsomes can metabolize a compound like strychnine also supports the supposition that these two convulsant com- pounds may have been metabolized to the extent that (1) greater time was required for the blood concentration of pentylenetetrazol to reach convul- sant threshold levels and (2) the concentration needed for strychnine to induce convulsions was never achieved. Howes and Hunter (1966) showed that pretreatment of rats with other compounds which stimulate the microsomes of the liver enabled the microsomes from these livers to metabolize strychnine at a significantly greater rate. Pretreatment of rats with several compounds was able to induce the catabolism of strychnine in the .58-.64 umoles/gram liver/hour range. Since the rats in the present ex- periment weighed approximately 250 grams and would have had hypertrophied livers weighing approximately 18 grams as shown in the microsomal induc-

tion experiment, it is feasible that their livers could metabolize enough strychnine to keep the concentration below convulsant levels. A 250 gram rat would receive .8 mg of strychnine at the 3.2 mg/kg dose level. This is equal to 2.4 umoles of strychnine. If the rats were capable of metabolizing strychnine at rates equivalent to that observed in the study by Howes and Hunter (1966), they would be capable of metabolizing about 11.52 umoles in an hour. However, since the control group convulsed in 5.68 minutes, this would equal only .094 hours. Thus, 1.049 umoles of strychnine could be metabolized in 5.68 minutes if the rats' livers were performing on a comparable level as in the Howes and Hunter (1966) experiment. Since 2.4 umoles were originally administered, just 1.35 umoles would be left and not detoxified. This is equal to .45 mg or 1.8 mg/kg of strychnine left in the rat which is just in excess of the convulsant dose. Thus, the supposition that the activity of the microsomes in the liver, as induced by mirex, could account for the prolongation of the time it takes for the rats to convulse from pentylenetetrazol or strychnine seems more tenable than a possible CNS depressant effect of mirex on the rats. To answer this question would undoubtedly require further experimentation such as monitoring the convulsant concentrations in the blood after mirex pretreatment.

Several other inconclusive results have arisen during the course of this investigation. The apparent discrepancy between the hexobarbital sleeping time prolongation and mirex's proven ability to induce drug metabolizing enzymes could be resolved by measuring hexobarbital sleeping

times under treatment conditions in which mirex has been shown to cause a significant increase in microsomal activity, i.e., 50 mg/kg/day for four days.

SUMMARY

This research dealt with the acute toxicity, hepatic response, and the effects on the nervous system of mirex in adult male rats. The acute toxicity for technical grade mirex (98%) was assessed with an LD_{50} test. This value was determined to be 573 mg/kg for oral exposure. Pure grade mirex (99.95%) was found not to differ significantly in either chemical composition or lethality to rats. The distributions of mirex in rats which died from mirex treatment indicates that mirex is highly stable since no metabolites were observed. In addition, mirex was observed to be highly lipophilic with the highest residue levels appearing in the fat (538.3 + 122.9 ppm), followed in decreasing order by the liver, kidney, brain and muscle.

The hepatic responses to acute, sub-acute, and an intermediate dose regime were determined. After acute administration (573 mg/kg), mirex was observed to cause hepatotoxicity as manifested by elevated serum enzyme levels (SGOT, SGPT), a prolonged hexobarbital sleeping time, and the infiltration of fat vacuoles in the liver. The sub-acute dose regime (25 mg/kg/day - 2 weeks) was also observed to cause toxic insult in that both the liver and liver cells were hypertrophied and the hexobarbital sleeping time was prolonged. The serum enzyme levels, however, were not elevated. The intermediate dose regime (50 mg/kg/day - 4 days) showed that marked hypertrophy of the liver resulted from mirex treatment which was associated with a significant induction of the microsomal enzyme activity and a concurrent increase in protein.

Possible effects of mirex on the nervous system in rats were tested using locomotor and convulsion threshold techniques. Mirex was observed not to cause any type of locomotor impairment. The times until the onset of pentylenetetrazol and strychnine induced convulsions were significantly lengthened. These effects could be due to a CNS depressant effect of mirex. However, we feel that the "anticonvulsant effect" could be due to the liver's ability to metabolize these compounds at an enhanced rate.

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