Fundamental Principles of Thermodynamics

By: R.M. Thwaites

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Title: A PHARMACOLOGIC AND TOXICOLOGIC COMPARISON OF TWO ISOMERIC CHLORINATED HYDROCARBON INSECTICIDES: ENDRIN AND DIE LD RIN

Abstract approved: ____________________________

The lethal potency, intensity of action, tissue distribution, and cardiovascular toxicity of two isomeric chlorinated cyclodiene insecticides, endrin and dieldrin, have been compared intravenously in mice and dogs. Endrin has 5 times the lethal potency of dieldrin in mice, measured as the median lethal dose (LD50) plus 95% confidence limits. At equimolar doses, endrin produces greater intensity of action than dieldrin, measured as the reciprocal of the median lethal times (LT50), plus 95% confidence limits. The dose-response curves of endrin and dieldrin (dose vs. 100/LT50) are roughly parallel over a 15 to 40 mg/kg dose range. Dieldrin produces greater intensity of action than endrin, when the compounds are administered in equilethal doses.

The lethal potency of endrin or dieldrin is relatively unaltered by changes in ambient temperature or social aggregation, but is
decreased by induced hypothermia. The LT50s, resulting from administration of an LD90 of either compound, are prolonged by decreases in ambient temperature below 23°C, indicating a decrease in the intensity of action of each compound. Increases in ambient temperature above 23°C, or aggregating animals at any ambient temperature, increases the intensity of action of endrin, but not that of dieldrin.

The seizure patterns of endrin and dieldrin, induced in mice by an intravenous LD90, are very similar, differ only in the duration of the various components of the pattern, and resemble the seizure pattern induced by an equilethal intravenous dose of picrotoxin. The intensity of action and lethal potency of dieldrin are decreased to a greater extent than those of endrin, by pre-treatment of mice with tranquilizers, barbiturates, anticonvulsants, and general depressants.

The relative order of magnitude of tissue concentrations of endrin or dieldrin 300 seconds after i.v. administration of an LD90 to mice is liver > brain = fat > blood. The ratios of dose to a particular tissue concentration at equilibrium are equal for endrin and dieldrin. Dieldrin equilibrates sooner with brain, and endrin equilibrates sooner with liver, blood and fat. The rate of accumulation of either insecticide into the brain does not correlate with the onset or duration of the seizure pattern.
At an equimolar dose, endrin and dieldrin attain equal concentrations in the brain.

A lethal dose of endrin (5 mg/kg) administered to dogs produces a period of pronounced hypertension, followed by a period of terminal hypotension. During one or both of these periods, oscillations in blood pressure occur which are recurring and independent of respiration and tonic convulsions. Immobilization of the animals with d-tubocurarine HCl plus artificial respiration prevents convulsions and the development of the hypotensive phase. Such a preparation facilitates and sustains endrin-induced oscillations in blood pressure. Bilateral adrenalectomy prevents the hypertensive phase, but does not eliminate oscillations. In the spinal-ectomized animal, endrin produces a slow decrease in blood pressure. Endrin antagonizes the actions of norepinephrine and tyramine on the heart and peripheral vasculature. Endrin does not alter the contractile performance of isolated rat trabecular muscle.

Dieldrin, at three times the dose of endrin, does not produce hyper- or hypotension, or recurrent oscillations in blood pressure, nor does it antagonize the effects of injected catecholamines, at the dose used.

These findings have indicated that differences in toxicity between endrin and dieldrin are not based on differential distribution
of these compounds into various tissues. The differences are most likely based on the nature of interaction between the insecticides and active sites in the central nervous system. Since endrin and dieldrin respond in a similar fashion to a variety of experimental conditions, produce identical patterns of convulsive activity, and have a parallel relationship between their respective dose-response curves, it is probable that these two insecticides combine with the same active sites in the central nervous system. Differences that exist between the two compounds indicate that endrin has greater intrinsic activity and/or affinity for the active site than dieldrin. It is further suggested that endrin may possess alpha-adrenergic blocking properties, and that dieldrin is much less potent in this respect or does not possess these properties at all.
A Pharmacologic and Toxicologic Comparison of Two Isomeric Chlorinated Hydrocarbon Insecticides: Endrin and Dieldrin

by

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A PHARMACOLOGIC AND TOXICOLOGIC COMPARISON
OF TWO ISOMERIC CHLORINATED HYDROCARBON
INSECTICIDES: ENDRIN AND DIELDRIN

I. GENERAL INTRODUCTION

The chlorinated hydrocarbon insecticides represent a group of chemically stable, but highly toxic compounds. For convenience, they are divided into three categories: the DDT group, the lindane group, and the aldrin-toxaphene group (Soloway, 1965). The latter group is comprised of derivatives of cyclodienes. Endrin and dieldrin are the most potent of the cyclodienes, and, as such, they represent the two most toxic chlorinated hydrocarbon insecticides.

Dieldrin was developed in 1948 and endrin in 1951 for use in the protection and preservation of crops (Sowell, Lawrence, and Coleman, 1968). Since that time, these compounds as well as other chlorinated hydrocarbon insecticides have accumulated in virtually all ecosystems. This has posed a potential threat to man and his environment, and consequently abundant research has been undertaken to elucidate the pharmacologic and toxicologic properties of these compounds.

Structurally, endrin (the epoxide of isodrin) and dieldrin (the epoxide of aldrin) are stereoisomers containing a napthalene nucleus with a methylene bridge across each of the two six-membered rings. As seen in Figure 1.1, in the dieldrin molecule
Figure 1-1. The structures of endrin and dieldrin
the bridges are trans with respect to each other, while in endrin they are in the cis orientation. This singular difference in orientation of the methylene bridges imparts to each of the compounds a distinct and different topography (Soloway, 1965). Both compounds are white crystalline solids with melting points between 175-176° C. They are stable to mild acid and alkalai, insoluble in water, sparingly soluble in alcohols, and moderately soluble in acetone and aromatic solvents. The vapor pressure of both is between $1.7-2.0 \times 10^{-7}$ mm Hg at 25° C. Upon exposure to light or strong acids, endrin and dieldrin undergo chemical rearrangement or decomposition. Dieldrin may give rise to diols while endrin tends to be converted to an aldehyde or ketone. Endrin is particularly sensitive to heat, and above 200° C it undergoes thermal decomposition.

Scientific research on chlorinated hydrocarbon insecticides can be divided into two categories. The first of these is research on the occurrence and distribution of the compounds in ecosystems. The second deals with problems of acute and chronic toxicity. The first category, is replete with documentation (Jegier, 1969; Lichtenstein, 1969; Robinson, 1969; Winnett and Reed, 1968; Zavon, Hine, and Parker, 1965; Robinson and Hunter, 1966; Duggan, 1969), but not particularly germane to the subject matter of this thesis. It should be noted that research in this
area has dealt more with dieldrin than endrin, perhaps because
dieldrin is stored in fat to a greater degree than endrin (Kunze
and Laug, 1953; Claborn, Radeleff, and Bushland, 1960;
Terriere, Kjgema, and England, 1958) and because dieldrin
is used more often and in greater quantities than endrin (Wolfe,
Durham, and Armstrong, 1963). Both compounds undergo
photochemical alterations which render dieldrin more toxic and
endrin less toxic to insects (Soto and Deichmann, 1967; Rosen,
Sutherland, and Lipton, 1966).

Research in the second category has investigated the nature
of the acute and chronic toxicity of chlorinated hydrocarbons in
several species, including mammals, birds, fish, and insects.
The majority of studies have been on chronic toxicity. It has
been shown in mammals that long term feeding of endrin and
dieldrin can produce degenerative changes in the liver and
kidneys, capillary-venous congestion, and loss of weight (Boyd
and Stefec, 1969). Lesions in hepatic cells may occur while
other organs remain unaffected (Sowell et al., 1968), and such
lesions appear to be nonspecific (Conley, 1960; Princi; 1957;
Hodge, Boyce, Deichmann, Kraybill, 1967).

Fat contains the largest concentration of stored insecticide,
followed by the liver, brain, and then blood (Robinson et al.,
1969). Zavon et al. (1965) examined perirenal fat in the human
population of the United States and were able to detect dieldrin in concentrations ranging from 0.07 to 2.82 ppm. Interestingly enough, they were not able to detect any endrin. Other investigators have shown that dieldrin accumulates in the fat more readily than endrin (Kunze and Laug, 1953). It has been estimated that dietary exposure to dieldrin is minor, but individuals employed in plants manufacturing this compound will absorb 578 micrograms per person per day (Robinson and Roberts, 1969).

Dieldrin is stored in the fat unchanged (Bann et al., 1956), and it is thought that clinical symptoms of intoxication appear when mechanisms for detoxification and excretion of dieldrin are saturated and exceeded (Patel and Rao, 1958). It has been shown that dieldrin is absorbed through the skin to a greater extent than DDT, and that protection against dermal absorption is necessary to prevent intoxication (Hayes, 1959). Individuals with occupational exposure to dieldrin show a correlation between blood and fat levels of this compound (Robinson and Hunter, 1966). A correlation also exists between blood levels and clinical symptoms and signs (Keane and Zavon, 1969). Thus it has been possible to obtain an indication of the body burden of dieldrin in persons with occupational exposure and to estimate what level of intoxication they are approaching.

An unanswered question is: What are the long term effects
of these compounds in the body? It still remains unknown what potential hazards lie in the constant exposure and ingestion of chlorinated hydrocarbon insecticides. Princi (1954) felt that much of the fear of insecticide exposure was based on opinion and hearsay. He described extravagant use of DDT and other organochlorines in Mexico with poor preventive measures against human contamination. Even under these conditions there were no significant numbers of intoxication. In Princi's opinion, these compounds were safe to use. Other authors (Wolfe et al., 1963) have shared this view, stating that the hazard of exposure to endrin and dieldrin is relatively low compared to the hazard from the more toxic organophosphates.

In order to determine the potential dangers of lifetime body burdens of organochlorines, recent studies have attempted to establish a correlation among these compounds with carcinogenesis and errors in reproduction. Radomski, Deichmann, and Clizer (1968) found highly significant elevations of pesticide concentrations in cases of carcinoma of various tissues in terminal hospital patients. Animal studies in which dieldrin, 10 ppm, was fed to mice for two years resulted in a shortening of the life span by two months and a significant increase in the incidence of histologically benign liver tumors (Davis and Fitzhugh, 1962). Treon (as cited by Sowell et al., 1968) did not
find any such increase of tumors in animals subjected to endrin feeding, when they were compared to a control group which had not received endrin. At the present time carcinogenicity of these compounds does not appear to be resolved.

Chlorinated hydrocarbon insecticides have been detected in maternal and placental blood specimens of humans (Selby et al., 1969). They have also been detected in the tissues of stillborns, of infants dying in the very early neonatal period, and in the cord blood of normal neonates. The detected residue levels were within the same range observed in adults (Curley, Copeland, and Kimbrough, 1969). Experimentally, it has been shown that exposure of female rats to dieldrin results in a lower percentage of conceiving females (Harr et al., 1970). Good and Ware (1969) found that endrin and dieldrin fed to mice 5 ppm for 120 days significantly reduced litter size. Endrin also caused a significant increase in parent mortality. It remains inconclusive, however, what hazard the organochlorines present to humans in terms of growth and reproduction.

In contrast to chronic intoxication, acute intoxication by chlorinated hydrocarbon insecticides produces a relatively well-defined spectrum of effects. The mechanism through which these effects are mediated remains unknown. According to Princi (1957), despite the difference in structure and physiological effectiveness of the chlorinated hydrocarbon insecticides, their
effects on mammals are so similar that their toxicology and clinical symptomology can be considered collectively. There appear to be three degrees of clinical illness: 1) The initial stage of intoxication includes headaches, blurred vision, dizziness, slight involuntary muscular movements, sweating, insomnia, and nausea: 2) This progresses to stronger clonic jerking and momentary loss of consciousness: 3) Severe intoxication may develop, which includes epileptiform convulsions (Conley, 1960). Pulmonary edema and endocarditis may be produced, but these sequelae may follow convulsions of any origin. These signs of acute poisoning are related primarily to central nervous system stimulation (Hodge et al., 1967). Death can occur in poisoning that results from large dosage, with little evidence of histopathological changes in the liver or kidney. If death is delayed several hours, degenerative changes can occur in both organs. Convulsions which result from intoxication can be controlled, but not prevented, by the use of barbiturates (Conley, 1960).

Despite the chemical similarity of endrin and dieldrin, endrin is significantly more toxic, in terms of potency, than dieldrin. Why this is so is unknown, but the answer could very well lie within the basic mechanism of action of these compounds. In this respect it would be very helpful to know, in detail, the
qualitative and quantitative differences and similarities of endrin and dieldrin in living systems. Only a few articles have been published that directly compare endrin and dieldrin. Approximately half of them are concerned only with acute lethal doses (Treon, Cleveland, and Cappel, 1955; Treon and Cleveland, 1955; Luckens and Davis, 1965; Gaines, 1960; Wolfe et al., 1963; Henderson, Pickering, and Tarzwell, 1959; Res sang et al., 1958; Sherman and Rosenberg, 1953), while the rest describe fat distribution, excretion, metabolism or effects on reproduction. (Kunze and Lauge, 1953; Zavon et al., 1965; Rosen et al., 1966; Cole, Klevay, Zavon, 1970; Soto and Deichmann, 1967; Good and Ware, 1969).

According to Treon et al. (1955) and Treon and Cleveland (1955) the median lethal doses (LD50s in mg/kg, plus 95% confidence limits) of orally administered endrin and dieldrin in rats are as follows:

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<th>dieldrin</th>
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<tr>
<td>male</td>
<td>28</td>
<td>47</td>
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<tr>
<td></td>
<td>(16.2-51.2)</td>
<td>----</td>
</tr>
<tr>
<td>female</td>
<td>16.8</td>
<td>38.3</td>
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<tr>
<td></td>
<td>(13.0-21.7)</td>
<td>(32.7-44.8)</td>
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Similar data have been reported by Gaines (1960) suggesting that endrin and dieldrin were more toxic to females than males. Other species besides the rat show a greater sensitivity to endrin than to dieldrin. In chicks (Sherman and Rosenberg, 1953) and
bats (Luckens and Davis, 1965) endrin is 8 to 10 times more potent than dieldrin. A similar relationship holds true for fish (Henderson et al., 1959) and cats (Ressang et al., 1958).

In mammals other than humans, descriptions of acute intoxications by dieldrin and endrin are similar to those described in humans, but are more precise and informative. In 1954, Gowdey et al. (1954) described the pharmacological properties of dieldrin in cats and rabbits. Dieldrin, when injected intravenously, caused central nervous system stimulation resulting in increased reflex excitability, convulsions, bradycardia, and some vasodepression. Dieldrin potentiated the effects of acetylcholine on the central nervous system, on the circulatory system, and on intestinal motility. These manifestations were abolished by surgical section of the vagus nerves, which suggested that the action of dieldrin was on higher central nervous system structures. Dieldrin had no effect on salivary secretion produced either by stimulation of the chorda tympani or by injections of acetylcholine in the decentralized submaxillary gland. Gowdey and coworkers concluded that although dieldrin had a marked parasympathetic action, this effect was mediated through stimulation of central mechanisms and not peripherally. Gowdey obtained equivalent results with aldrin, the epoxide of which is dieldrin (Gowdey et al., 1952).
Similar experiments with endrin in dogs were later carried out by Emerson and coworkers. Intravenous injections of endrin produced bradycardia, hypertension, salivation, hyperexcitability, tonic-clonic convulsions, an increase in body temperature, leukocytosis, hemoconcentration, a decrease in blood pH, and an increase in cerebral venous and spinal fluid pressures (Emerson, Brake, and Hinshaw, 1964). Experiments on renal hemodynamics revealed that intravenous administration of endrin resulted in increased renal vascular resistance and afferent arteriolar vasoconstriction. No evidence was provided that renal failure which occurred was attributable to the direct effects of endrin on the kidney (Reins, Holmes, and Hinshaw, 1964). In-situ studies revealed large increases in total limb vascular resistance resulting from an increased level of circulating catecholamines (Emerson and Hinshaw, 1965). In venous return experiments, pulmonary vascular resistance and left atrial and pulmonary artery pressures increased after endrin administration (Emerson, 1965). When succinylcholine was used to prevent convulsions the rise in systemic arterial pressure was shown to depend on an increased cardiac output measured as an increased venous return. There was no change in total peripheral resistance. The abdominal viscera were the primary source of the increased venous return after the administration of endrin (Reins et al., 1966).
In their final experiments with endrin in the dog, they lowered the dosage and did not use any agents to prevent convulsions (Hinshaw et al., 1966). Once again, an increased venous return was measured following endrin administration, but total peripheral persistence fell and remained low. There were no changes in pulmonary peripheral resistance. An elevation of left atrial pressure occurred regularly, which was interpreted as a sign of left heart failure. The results of the experiments can be summarized by stating that endrin acts directly on the central nervous system and on the left heart. By acting on the former, there is an intense parasympathetic discharge and release of catecholamines, accompanied by convulsions, which account for the effects observed.

Other, more diverse, areas of research, involving studies on the general chemistry, biochemistry, metabolism, binding, and structural relationships of the organochlorines, have attempted to define insecticide activity more precisely. Hosein and Proulx (1960) showed the presence of damaged brain mitochondria and increased levels of the CoA derivatives of betaines, following dieldrin-induced convulsions. They postulated that the CoA derivatives attach to the acetylcholine receptors and activate them. The work of Hathaway (1965) upon the effect of acute dieldrin intoxication on the biochemistry of the brain suggested disturbances associated with brain cell mitochondria. It appeared also that
dieldrin inhibited glutamine synthesis leading to an accumulation of ammonia in the brain.

Other biochemical studies with chlorinated hydrocarbon insecticides have dealt with the metabolism and excretion of these compounds. Bann et al. (1956), in their studies on the fate of dieldrin in the animal body, found that dieldrin was stored in the body chemically unchanged. Hunter et al. (as cited by Cole et al., 1970) later showed that the fecal excretion of endrin and dieldrin was greater than the urinary excretion. Morsdorf et al. (1963) then demonstrated the presence of high concentrations of dieldrin, plus a hydrophilic metabolite of dieldrin, in the bile of dieldrin-treated rats. Heath and Vandekar (1964), by cannulating the bile ducts, were able to demonstrate the enterohepatic circulation of dieldrin. By elimination of enterohepatic circulation using biliary fistulae, the proportion of dieldrin excreted unaltered in the bile could be increased from 3% of the total excretion to 10%. Soto and Deichmann (1967), in their review of the major metabolism of aldrin, endrin, and dieldrin, point out that metabolites of endrin and dieldrin appear in both urine and feces, but mostly in feces. The metabolites remain unidentified except for the principal urinary metabolite of dieldrin, which is an aldrin trans diol. While in-vitro preparations of rat liver microsomes are capable of converting isodrin
and aldrin to endrin and dieldrin, respectively, no one has yet obtained any metabolites of endrin and dieldrin with this microsomal preparation. Cole et al. (1970) measured the rates of hepatic excretion of radioactive endrin and dieldrin in rats. Over 90% of the excreted activity was found in the feces of the intact animals and in the bile of animals with biliary fistulae. Endrin was excreted more rapidly than dieldrin in the bile, and the authors postulated that this may explain why dieldrin is stored in fat to a greater extent than dieldrin. Street (1969), in his review of insecticides and microsomal systems, described the ability of DDT to enhance the metabolism of endrin and dieldrin, thereby decreasing their storage. It would thus appear that the cyclodiienes are metabolized by liver microsomes and respond in a predictable manner, but why it has not been possible to metabolize these compounds in in-vitro systems remains unrec-
ocented with this postulation.

The most recent research on the organochlorine insecticides has attempted to expand and amplify earlier experiments which were designed to test specific hypotheses on the exact mechanisms of action of these compounds. It was initially observed that a certain concentration of chlorinated hydrocarbon insecticide had to be attained in either the blood or tissues before convulsions occurred (Speck and Maaske, 1958). It was later established
that the degree of central nervous system toxicity was related
to the concentration of the insecticide in the brain. (Dale,
Gaines, and Hayes, 1963; Hayes and Dale, 1964). Since it had
become obvious that the site of action of the organochlorines was
on nerves or nerve components, neurophysiological research
became emphasized. More attention was payed to the early
theories on the molecular interactions of organochlorine insec-
ticides with biological components. It was first postulated by
Gunther et al. (1954) that the DDT type molecule slips into a
cavity in an apoenzyme or other protein and is held there, inhib-
iting the normal subsequent chemistry. A similar theory was
put forth by Mullins (1955) to explain the toxicity of lindane and
its isomers. It differed slightly in that Mullins described the
molecule fitting into a membrane "pore" rather than combining
with an enzyme. More recently, Soloway (1965) has described
a partial mechanism of action for the cyclodienes on the basis
of their molecular topography and toxicity to insects. These
compounds have two electron rich sites, both of which become
attached to a biological entity. Physical interaction as a basis
for the toxic action of the cyclodienes is supported by the corre-
lation of the size and shape of their electronegative centers with
toxic activity.

These theories stimulated research on the interactions of
insecticides with nerves and membranes. Hoogendam, Versteeg and Blieger (1962) measured EEG activity in rats treated with endrin. Severe bursts of multiple spikes were associated with clonic convulsions whereas moribund animals showed only minimal changes of irregular, slow wave activity. Because bilateral synchronous spikes persisted after recovery, it was possible that this activity was associated with brain stem damage. Parallel results were obtained by Revzin (1968) in monkeys treated chronically with endrin. The EEG remained abnormal more than one month after the last dose. According to Revzin, the generalized changes in the EEG seen in all leads and the relative lack of effect on evoked potentials is consistent with the hypothesis that endrin selectively poisons centrencephalic structures in mammals.

The effects of cyclodienes on cellular membrane systems have been studied by Weikel et al. (1958) using the red cell membrane. They found that dieldrin inhibited the phosphate exchange rate, but that endrin did not have this effect. DDT appeared to cause a selective increase in the permeability of the erythrocytes to sodium. A most recent and interesting study by Hinton and O'Brien (1970) revealed that the potassium conductance is induced in a lecithin-decane membrane by valinomycin was reversed by $3 \times 10^{-6}$ M DDT, but that dieldrin and lindane did not affect valinomycin-induced conductance of lecithin
membranes.

In 1964 O'Brien and Matsumura (1964) put forth the hypothesis that chlorinated hydrocarbon insecticides owe their activity to the formation of charge-transfer complexes with components of the nerve axon, which lead to a consequent disturbance of function. Such binding was demonstrated in insect nerve (Matsumura and O'Brien, 1966a, 1966b; O'Brien and Matsumura, 1964; Hayashi and Matsumura, 1967) and in rat brain (Matsumura and Hayashi, 1969). Matsumura and Hayashi, in pursuing this hypothesis, reasoned that if binding was related to poisoning, then an increase or decrease in binding to nerve components would be reflected as an increase or decrease in toxicity in the organisms from which the nerve components were derived. They found in the German cockroach that both DDT (Hayashi and Matsumura, 1967) and dieldrin (Matsumura and Hayashi, 1966) bound less to the nerves of resistant cockroaches. They felt that whether this binding played an indispensable part in insecticide poisoning was a problem that required further study.

An assumption that seems to run through most of the literature on chlorinated hydrocarbon insecticides is that these compounds all act by the same mechanism or interact with the same biological site. They are also generally considered to be convulsants. However, as Soloway points out, DDT and dieldrin
have different sites of action and even produce different toxic symptoms (Soloway, 1965). Hilton and O'Brien (1970) have demonstrated that DDT and dieldrin affect artificial membranes differently. Understanding these differences may be fundamental to solving the problem of the mechanisms of action of chlorinated hydrocarbon insecticides.

In conclusion, endrin and dieldrin represent two chemically related compounds that possess a powerful influence on biological activity. In this respect, endrin is three to five times more potent than dieldrin. An explanation for this difference in potency is presently not available, but may be fundamental to the basic mechanism of action of the cyclodienes and other chlorinated hydrocarbon insecticides. The purpose of this thesis is to define precisely the differences in potency and duration of action between endrin and dieldrin under a variety of experimental conditions. Differential distribution of these compounds into various tissues is considered as a possible explanation for these differences. The proposition put forth in this thesis is that endrin and dieldrin have a similar mechanism of action, but different intrinsic activities and/or affinities for active sites in the central nervous system.
II GENERAL METHODS

**Animals**

Animals used in all experiments were either CF #1 mice, Sprague-Dawley rats, or mongrel dogs, of either sex. Mice, raised in our own animal quarters from a stock strain of CF #1 mice obtained from Carworth Farms, were housed in groups of eight in plastic cages (27 cm X 16 cm X 10 cm). This type of cage was used in all experiments involving mice. Bedding for the mice was oven-dried wood chips. Rats raised in our animal quarters from stock animals obtained from Simonsen Laboratories, Inc., were housed five per cage (16" X 10" X 7"). Dogs were obtained from a local pound and housed separately from mice and rats in a large room. Free access to food (Purina Lab Chow) and water was available at all times. Ambient temperature in the animal quarters ranged from 21-23° C.

**Insecticides and Solvents**

The cyclodiene insecticides were obtained in the form of Technical Endrin (95%) and Technical Dieldrin (HEOD 95%) from Velsicol Chemical Corporation, Chicago, and used in this form. Several solvents were tried, but the most effective and least toxic was dimethylsulfoxide (DMSO). Fortunately, the effects of DMSO on mammalian systems have been well investigated. Dixon et al.
(1965) have shown that the lethality of drugs dissolved in DMSO was not significantly different than when dissolved in saline. DMSO also failed to alter penetration of drugs into the CSF. These investigators concluded that DMSO appeared to be a very good solvent with little or no effect on pharmacological action. Worthly and Schott (1966) demonstrated that DMSO was less toxic to mice than ethanol, 100% polyethylene glycol-200, and 100% propylene glycol. Weiss and Orzel (1967) reported that the oral toxicities of dieldrin and other pesticides were similar whether administered in DMSO, corn oil, or as an aqueous suspension. They noted that undiluted DMSO produced a depression of spontaneous motor activity without an effect on hexobarbital sleep time or the conditioned-avoidance response. The depression was abolished by dilution. Because of the low acute toxicity and apparent lack of interaction, it was concluded that DMSO was a useful solvent for studying the effects of pesticides in animals.

In all in-vivo experiments, endrin and dieldrin were administered intravenously. Because of DMSO's relatively low toxicity this procedure was satisfactory, especially if the amount injected was minimal. The total dose of DMSO for mice was always one microliter per gram of body weight; for dogs, 0.5 milliliters per kilogram of body weight. In no instances did this quantity of DMSO cause death. Once the cyclodiene was mixed with the blood, there was little problem of the compound
precipitating out, since endrin and dieldrin have been reported
to be up to 4,000 times more soluble in serum than in water
(Hathaway, 1965).

**Estimation of Toxicity and Intensity of Action**

Potency, as an index of the toxicity of endrin and dieldrin,
was expressed as the median lethal dose (LD50), estimated by
the method of Litchfield and Wilcoxon (1949). This method is
a rapid, graphic estimation approximating LD50, the potency
ratio of any two LD50s, the slope of the dose-percent deaths
curves, and the 95% confidence limits of these three parameters.
The LD50 is defined as the dose required to kill 50% of a
population. The slope of the dose-percent deaths curve is a
measurement of variance in the population. In order to deter-
mine the LD50 of endrin or dieldrin, four to five dose levels were
administered to groups of 10 mice, each group receiving one of
the dose levels. At the end of 24 hours, the percent of deaths
in each group was recorded and the LD50s plus 95% confidence
limits calculated.

When the LD50s had been calculated for endrin and dieldrin
under various conditions (described in Chapter I), it was then
possible to obtain information on the intensity of action of these
compounds. The intensity of action was measured by the time
required for the compound to produce death. This parameter is
inversely related to the time to death: the longer the time to death, the less intense is the action of the compound. The median lethal time (LT50) was used as an indication of intensity of action, estimated by the method of Litchfield (1949). This rapid graphic method estimates the time required for half the population to die from a given dose, the reaction time ratio of any two LT50s, the slope of the time-percent effect curve, and the 95% confidence limits of these three parameters. Estimations of LT50s require doses that are lethal to at least 50% of the population. Thus, it is necessary to know the LD50 of a given compound before measuring the LT50 of any given dose of that compound. In these experiments an LD90 was selected from the dose-percent deaths curve for endrin or dieldrin, and, under any one set of conditions, was administered to a group of 10 mice. The time to death for each mouse was recorded, and the LT50 plus 95% confidence limits calculated by log-probit analysis.

**Convulsant and Anticonvulsant Agents**

Pentylenetetrazol and picrotoxin were selected as prototype classical convulsants with which endrin and dieldrin could be compared. These two drugs were also dissolved in DMSO and administered intravenously. The LD50s and LT50s for pentylenetetrazol and picrotoxin were then determined in the same
manner as for endrin and dieldrin.

Several depressants and anticonvulsants were selected for the pretreatment of mice prior to dieldrin or endrin exposure. The drugs included hypnotics, sedatives, anticonvulsants, muscle relaxants, tranquilizers, and miscellaneous agents. The duration of pretreatment times that would approximate the peak time activity of the various drugs were obtained from several sources in the literature (Tedeschi and Swinyard, 1958; Fink and Swinyard, 1959; Fink and Swinyard, 1962) or from gross observations of motor activity and the development of ataxia. The effects of pretreatment with these drugs on the lethality and LT50s of endrin and dieldrin were determined.
III. THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE POTENCY AND INTENSITY OF ACTION OF ENDRIN AND DIELDRIN

Introduction

In evaluating the toxicity of any compound, it is important to consider the possible influence of environmental factors. Fuhrman and Fuhrman (1961), in their review of the effects of temperature on drug action, pointed out that the study of toxicity at different temperatures is not informative when the object of the research is an understanding of the fundamental action of a drug. A point may be made, however, that when two drugs having a similar dose-effect relationship produce parallel responses in animals under a variety of experimental conditions, there is a good possibility that they act by a similar mechanism. On the other hand, when two drugs have a similar dose-effect relationship but do not produce parallel responses under the same variety of experimental conditions, one is less justified in postulating that both compounds act by the same mechanism. In the case of the cyclodiene insecticides, if it can be shown that they have a similar dose-effect relationship and produce parallel responses in animals under a variety of environmental conditions, then one can safely postulate that they act by similar mechanisms. The experiments described in this chapter have, therefore, compared the potencies
and durations of action of endrin and dieldrin under several controlled environmental conditions. The general purpose was to determine how closely the actions of these stereoisomers resembled each other under conditions of altered environmental parameters.

Endrin and dieldrin, as described in the General Introduction, produce central nervous system stimulation which can lead to death. This type of stimulation is similar to that produced by the classical convulsants picrotoxin and pentylenetetrazol (PTZ), i.e., they all produce epileptiform convulsions. The fact that picrotoxin and PTZ act by different mechanisms (Goodman and Gilman, 1970) may explain why these compounds do not produce parallel responses in animals exposed to a variety of environmental conditions. It has been shown that aggregation (Greenblatt and Osterberg, 1961) and induced hypothermia (Bogdanovic, 1956, as cited by Fuhrman and Fuhrman, 1961) increased the lethal potency of picrotoxin but not that of PTZ. Keplinger, Lanier, and Deichmann (1959) described the lethality of PTZ as being unaffected by lower ambient temperatures, but that it increased at a temperature of 36°C. Hall, Nelson, and Edlin (1967) showed that the median convulsive dose (CD50) for PTZ increased with increasing ambient temperature. It appeared that with PTZ, at higher ambient temperatures, it was more difficult to produce convulsions, but easier to produce death. An
almost opposite relationship was found when mice were aggregated: the lethality of PTZ was not affected by aggregation (Greenblatt and Osterberg, 1961) but aggregation lowered PTZ seizure threshold (Swinyard et al., 1961). It seems that seizure threshold and lethality are not parallel phenomenon in PTZ-treated mice. It may very well be that changes in the seizure threshold of PTZ reflect changes in the intensities of action of the drug. Goldberg and Salama (1969) have implied the same idea when they concluded that stress does not increase the lethality of amphetamine, but that amphetamine enhances the effects of stress. Thus, in other forms of stress which lower PTZ seizure threshold, such as shock, immobilization, and postural disequilibrium, there may actually be an increase in the intensity of action of PTZ, rather than an increase in its lethality (Swinyard et al., 1963).

In summary, the lethality of picrotoxin is increased in aggregated animals and in hypothermic animals. Under the same conditions the lethality of PTZ is not changed. The lethality of PTZ is increased in animals maintained at high ambient temperatures, and the seizure threshold of PTZ is decreased by low ambient temperatures, aggregation, and other forms of stress.

The objectives of the experimental work described in this chapter were to 1) determine possible differences in toxicity of
endrin and dieldrin in isolated and aggregated male and female mice, and in male and female mice exposed to high and low environmental temperatures; 2) select an appropriate dose of endrin and dieldrin to determine the LT50s of these compounds; 3) determine if aggregation or ambient temperature influenced the LT50s of endrin and dieldrin; and 4) determine the toxicity and duration of action of endrin and dieldrin in hypothermic mice.

Methods

Lethality Tests:

LD50 estimations (see General Methods) for endrin and dieldrin were obtained in male and female mice under several conditions to determine the effects of ambient temperature and aggregation on the lethality of these compounds. Male and female mice, selected randomly and housed separately, were placed one and ten mice per cage at 23° C, and ten per cage at 10 and 34° C, following appropriate dosage with endrin or dieldrin.

Animals were dosed with the compounds intravenously. Endrin and dieldrin were dissolved in DMSO and injected via the tail vein with a Hamilton 50 microliter syringe. The site of injection was taped to prevent loss of solution from the wound.

Animals housed at 10° C were placed in a refrigerator (13 cubic feet) for 24 hours, with temperature ranging between 8 and
12° C. Control studies showed that periodic opening of the refrigerator would provide adequate ventilation for the mice. Animals housed at 34° C were placed in a drying oven (15 cubic feet) for 24 hours with temperature varying less than a degree. Ventilation was adequate without periodic opening of the doors. The oven was provided with a set of glass doors which permitted continual observation. Under these conditions, untreated mice, or mice treated with DMSO alone, could survive for 24 hours in the oven or refrigerator.

Dosage Selection for LT50 Studies:

In order to obtain an optimum lethal dose for LT50 determinations (5 mg/kg and 15 mg/kg for endrin and dieldrin respectively), five or six different lethal doses of endrin and dieldrin were selected and the LT50 for each was determined in male mice housed 10 per cage. This permitted a "dose-response" comparison between endrin and dieldrin, as well as providing a wide range of doses to select from.

Influences of Age and Choice of Solvent:

To be sure that the age of the mice was not critical, and that DMSO was the best choice of solvents, appropriate experiments were carried out. Several age groups of male mice were
selected for LT50 determinations of endrin. Three solvents, propanol, acetone, and ethyl alcohol, were compared with DMSO by using them to administer endrin and calculating a respective LT50 for each. Mice in this experiment were housed ten per cage.

**Median Lethal Time Studies:**

LT50 estimations for endrin and dieldrin were determined in female mice under various conditions, to determine the effects of ambient temperature and aggregation on the intensity of action of these compounds. Female mice were selected randomly, and housed one or ten per cage at 10, 23, 34° C, after receiving endrin and dieldrin 5 mg/kg and 15 mg/kg, respectively, as described. LT50 determinations were made as outlined in General Methods. Body temperatures were recorded rectally with a yellow springs telethermometer and thermistor immediately prior to dosing and again prior to death.

The details for the refrigerator and oven were as described, except that variations in temperature were slightly larger, since the doors were frequently opened and closed. Temperatures in the refrigeration unit varied from 7-13° C and 33-35° C in the oven.

Tests on the effects of hypothermia on the toxicity and
intensity of action of endrin and dieldrin were made in hypothermic female mice. Hypothermia was induced by the submersion of mice into water which had equilibrated with room temperature. The water level was adjusted so that mice were able to stand on the bottom of the vessel and keep just their heads above water. Exposure of this kind caused the body temperatures to fall to room temperature within fifteen minutes. At this time the mice were removed from the vessel and dried. The body temperature of these animals would remain at room temperature for approximately two hours, and then rise slowly to normal. Once hypothermia was induced, the animals were housed ten per cage and treated with endrin or dieldrin for LD50 determinations. LT50s were determined under the same conditions, using endrin 5 mg/kg and dieldrin 15 mg/kg. Body temperatures immediately prior to death were recorded.

Results

Toxicity Tests:

Table 3.1 shows the results of the LD50 determinations. Generally, if animals did not die within an hour after exposure, they survived the remainder of the 24 hour exposure. LD50s were analyzed statistically by comparing the ratio of any two LD50s with a statistical value obtained by the method of Litchfield
Table 3.1. The effects of alterations in ambient temperature and aggregation\(^a\) on the medial lethal doses (LD\(_{50}\)) of endrin and dieldrin in mice.

<table>
<thead>
<tr>
<th>compound</th>
<th>sex</th>
<th>23° C</th>
<th>34° C</th>
<th>10° C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/cage</td>
<td>10/cage</td>
<td>10/cage</td>
</tr>
<tr>
<td>endrin</td>
<td>female</td>
<td>2.3</td>
<td>(2.2-2.4)(^b)</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>2.1</td>
<td>(2.0-2.3)</td>
<td>2.3</td>
</tr>
<tr>
<td>dieldrin</td>
<td>female</td>
<td>11.6</td>
<td>(11.1-12.1)</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>10.8</td>
<td>(10.5-11.1)</td>
<td>10.5</td>
</tr>
</tbody>
</table>

\(^{a}\) number of animals per unit area

\(^{b}\) the numbers in parentheses are 95% confidence limits

\(^{c}\) significantly larger than corresponding LD\(_{50}\) in male, \(p < 0.05\)

\(^{d}\) significantly smaller than corresponding LD\(_{50}\)s at 10 and 23° C, \(p < 0.05\)
and Wilcoxon (1949). When the ratio, referred to as a potency ratio, exceeded this value, then the two LD50s were significantly different at \( p < 0.05 \). In order to perform this comparison, it must be shown that the variances in each LD50 determination are not significantly different. This is done by the test for parallelism" which indicates whether the two regression curves for the LD50s are parallel. Parallelism between two curves indicates that both have a similar variance. Employing this analysis on the LD50 determinations indicated that aggregation and low ambient temperatures had no effect on the lethality of endrin or dieldrin. Increased ambient temperatures of \( 34^\circ C \) significantly increased the lethality of dieldrin, compared to animals housed at either 10 or \( 23^\circ C \). Dieldrin was also found to be significantly more lethal to males, when housed 1 or 10 per cage at \( 23^\circ C \). The difference in lethality was small (0.7 to 0.8 mg/kg), but consistent in three separate LD50 determinations. Under all conditions, endrin had approximately five times the lethal potency of dieldrin.

**Dosage Selection for LT50 Studies:**

Table 3.2 and Figure 3.1 show the relationship between the dose of endrin or dieldrin and the LT50. 3 mg/kg of endrin represented an LD80, while 5 mg/kg represented an LD90. The remaining doses for endrin were LD100s. The 3 mg/kg dose
Table 3.2. A comparison of doses with corresponding median lethal times for endrin and dieldrin in mice.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose mg/kg</th>
<th>LT50 in minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>endrin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17.5 a</td>
<td>(15.2-20.1)</td>
<td>11.0 (8.3-14.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dieldrin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Numbers in parentheses are 95% confidence limits.

b Significantly greater than corresponding endrin value, p < 0.05.
Figure 3-1. The relationship of dose to the intensity of action of endrin and dieldrin in mice. The intensity of action is expressed as the reciprocal of the LT50 multiplied by 100. Increasing values on the ordinate represent increasing intensities of action or shorter times to death.
did not consistently produce 80 to 90 percent lethality, which resulted in a lack of sensitivity in the statistical analysis. The 5 mg/kg dose, on the other hand, consistently produced at least 90% lethality and provided a more precise measurement of time to death. This dose was useful in that it represented a known level of lethality. It was possible to calculate a similar level of lethality for dieldrin and use the corresponding dose (LD90), which was approximately 15 mg/kg.

Each insecticide had an inverse relationship between the dose and resulting LT50. The LT50 is a measurement of the duration of action and reflects the intensity of action at a particular dose. Shorter durations represent greater intensities of action. As seen in Figure 3.1, by plotting the reciprocal of the LT50 (multiplied by 100) it is possible to show a direct, rather than inverse, relationship between the dose and intensity of action. The data then become analogous to a classical dose-response relationship. The resulting curves for endrin and dieldrin are roughly parallel over the 10 to 40 mg/kg dose range. The data reveal that at equimolar doses, endrin had a significantly greater intensity of action than dieldrin, \( p < 0.05 \). Since the LD90s of endrin and dieldrin produced a minimum intensity of action with a maximum amount of precision, they were used for further LT50 determinations. Significant differences between any two LT50s were determined in a similar fashion as the LD50s,
by the method of Litchfield (1949).

Influence of Age and Choice of Solvent:

The LT50s of endrin, 5 mg/kg, in male mice of various ages and weights, are presented in Table 3.3. The LT50s were not significantly different in mice ranging from 57 to 122 days. The LT50 in 25 day old mice was significantly larger than all other LT50s. Table 3.4 shows the effects of different solvents on the LT50 of endrin, 5 mg/kg. The LT50 of endrin dissolved in DMSO was significantly smaller than LT50s of endrin dissolved in propanol, acetone, or ethyl alcohol, $p < 0.05$. The LT50 of endrin in ethyl alcohol was significantly greater than that of endrin dissolved in propanol, $p < 0.05$. Since the confidence interval was smallest for LT50s obtained with endrin dissolved in DMSO, maximum sensitivity was obtained using this solvent. Further experiments, therefore, utilized DMSO, and mice were at least 75 days of age prior to use.

LT50 Studies:

The effects of aggregation and ambient temperature on the LT50s of endrin and dieldrin are presented in Table 3.5a. Shown also are rectal temperatures measured prior to injection (control) and again shortly before death (test). Tables 3.5b and 3.5c list statistical differences among LT50s and test body
Table 3.3. The effects of age on the median lethal time (LT50) of endrin in male mice.

<table>
<thead>
<tr>
<th>age (days)</th>
<th>weight (grams)</th>
<th>LT50 (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>10 ± 0.35</td>
<td>22.0 (18.0-27.0)</td>
</tr>
<tr>
<td>57</td>
<td>28 ± 0.65</td>
<td>9.6 (7.2-12.7)</td>
</tr>
<tr>
<td>75</td>
<td>28 ± 0.86</td>
<td>11.0 (8.9-13.5)</td>
</tr>
<tr>
<td>81</td>
<td>31 ± 1.0</td>
<td>8.4 (6.3-11.1)</td>
</tr>
<tr>
<td>108</td>
<td>31 ± 1.0</td>
<td>13.5 (10.8-16.8)</td>
</tr>
<tr>
<td>122</td>
<td>29 ± 1.0</td>
<td>9.5 (7.3-12.4)</td>
</tr>
</tbody>
</table>

a mean ± S. E. M.; n = 10
b numbers in parentheses are 95% confidence limits
c significantly larger than other LT50s, p < 0.05.

Table 3.4. The variability of the median lethal times of endrin in female mice, associated with the administration of endrin in various solvents.

<table>
<thead>
<tr>
<th>solvent</th>
<th>LT50 (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>9.3 (7.0-12.2)</td>
</tr>
<tr>
<td>propanol</td>
<td>18.5 (12.7-26.8)</td>
</tr>
<tr>
<td>acetone</td>
<td>26.5 (19.9-35.2)</td>
</tr>
<tr>
<td>ethyl alcohol</td>
<td>37.0 (28.9-47.3)</td>
</tr>
</tbody>
</table>

a numbers in parentheses are 95% confidence limits.
b significantly smaller than other LT50s, p < 0.05.
c significantly greater than the LT50 of endrin in propanol, p < 0.05.
Table 3.5a. The effects of ambient temperature and aggregation on the median lethal times (LT50) of endrin and dieldrin in female mice.

<table>
<thead>
<tr>
<th>compound</th>
<th>ambient temp. °C</th>
<th>number of mice per cage</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LT50</td>
<td>Body temp °C</td>
<td>LT50</td>
<td>Body temp °C</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>test</td>
<td>control</td>
<td>test</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>endrin</td>
<td>34 (9.8-13.1)b</td>
<td>38.4 (0.19)</td>
<td>36.6d (0.18)</td>
<td>8.2 (7.2-9.3)</td>
</tr>
<tr>
<td></td>
<td>23 (18.2-24.3)</td>
<td>38.0 (0.41)</td>
<td>35.6d (0.76)</td>
<td>13.0 (10.8-15.6)</td>
</tr>
<tr>
<td></td>
<td>5 (45.0-99.8)</td>
<td>37.0 (0.44)</td>
<td>29.0d (0.27)</td>
<td>22.5 (19.1-26.6)</td>
</tr>
<tr>
<td>dieldrin</td>
<td>34 (5.3-7.7)</td>
<td>38.1 (0.43)</td>
<td>38.0</td>
<td>6.6 (5.108.5)</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>39.2 (0.43)</td>
<td>35.4d (0.67)</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>23 (5.4-9.0)</td>
<td>31.0 (0.20)</td>
<td>29.6d (0.67)</td>
<td>27.0 (5.0-7.7)</td>
</tr>
<tr>
<td></td>
<td>5 (23.8-40.3)</td>
<td>38.5 (0.16)</td>
<td>29.6d (1.62)</td>
<td>27.0 (20.5-35.6)</td>
</tr>
</tbody>
</table>

a body temperature recorded just prior to injection and again prior to death, expressed as the means of 5 observations; numbers in parentheses are standard errors of the means.

b numbers in parentheses represent 95% confidence intervals.

c significantly different from corresponding LT50 in aggregated mice, p < 0.05.

d significantly different from control body temperatures, p < 0.01.
Table 3.5b. A statistical comparison among LT50s in isolated or aggregated groups of mice. The abbreviation "sig." is presented when the potency ratio for two LT50s from aggregated or isolated mice housed at two separate ambient temperatures indicates that the LT50s are significantly different, \( p < .05 \). NSD = no significant difference. Refer to Table 5a.

<table>
<thead>
<tr>
<th></th>
<th>Endrin</th>
<th>Dieldrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>isolated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34° vs 5°</td>
<td>sig.</td>
<td>sig.</td>
</tr>
<tr>
<td>23° vs 5°</td>
<td>sig.</td>
<td>sig.</td>
</tr>
<tr>
<td>23° vs 34°</td>
<td>sig.</td>
<td>NSD</td>
</tr>
<tr>
<td>aggregated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34° vs 5°</td>
<td>sig.</td>
<td>sig.</td>
</tr>
<tr>
<td>23° vs 5°</td>
<td>sig.</td>
<td>sig.</td>
</tr>
<tr>
<td>23° vs 34°</td>
<td>sig.</td>
<td>NSD</td>
</tr>
</tbody>
</table>
Table 3.5c. A statistical comparison among mean test body temperatures in isolated or aggregated groups of mice. The level of significance is presented when two mean test body temperatures from isolated or aggregated groups of mice housed at two separate ambient temperatures are significantly different. NSD = no significant difference. Refer to Table 5a.

<table>
<thead>
<tr>
<th></th>
<th>Endrin</th>
<th>Dieldrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>isolated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$34^\circ$ vs $5^\circ$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>$23^\circ$ vs $5^\circ$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>$23^\circ$ vs $34^\circ$</td>
<td>NSD</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>aggregated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$34^\circ$ vs $5^\circ$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>$23^\circ$ vs $5^\circ$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>$23^\circ$ vs $34^\circ$</td>
<td>$p &lt; 0.01$</td>
<td>$p &lt; 0.01$</td>
</tr>
</tbody>
</table>
temperatures, respectively, in isolated or aggregated groups of mice. The reciprocals of the LT50s vs. ambient temperature are plotted in Figure 3.2; increasing numbers on the ordinate represent relative increases in the intensity of action of endrin or dieldrin. Control studies indicated that rectal temperatures in mice did not fluctuate significantly over a one hour period for mice housed at 23° or 34° C, after an injection of the DMSO only. Mice housed at 5° C after injection with DMSO had a significant decrease in rectal temperature, p < 0.001, of 2.5° C (38.2 at zero time exposure vs. 35.7 after 60 minutes exposure). Untreated mice were able to maintain control body temperatures at a 5° C ambient temperature exposure.

The data indicate that increases in ambient temperature (from 5° C to 34° C) decreased the LT50 of endrin. Increases from 5° to 23° decreased the LT50 of dieldrin, but a further increase in ambient temperature to 34° C had no apparent effect on the LT50. Aggregation of mice decreased the LT50 on endrin at all ambient temperatures, but had no significant effect on the LT50 of dieldrin. Body temperatures were significantly lowered by endrin treatment in all groups, except in mice aggregated at 34° C; body temperatures were significantly lowered by dieldrin treatment only in aggregated and isolated mice housed at 23° and 5° C, when compared to control body temperatures. Test
Figure 3-2. The effects of ambient temperature and aggregation on median lethal times (LT50) of endrin and dieldrin in mice. The reciprocals of the LT50s multiplied by 100 are plotted on the ordinate, bracketed by the 95% confidence limits.
body temperatures were highest in mice housed at 34° C; test
temperatures in mice housed at 23° C were greater than those
of mice housed at 5° C. Only in the case of endrin-treated
mice isolated at 23° and 34° C was there no significant differ-
ence in test body temperatures (see Table 3.5c).

The effects of hypothermia on the lethality and intensity of
action of endrin and dieldrin are shown in Table 3.6, including
alterations in body temperature measured just prior to death.
Hypothermia caused a significant decrease in lethality and intensity
of action for endrin and dieldrin. Since less than half of the
mice died from the 15 mg/kg LD90 of dieldrin, an LT50 could
not be calculated. The lethality of dieldrin appeared to be
decreased to a greater extent than endrin. Test body temperatures
were significantly higher than the initial control temperatures,
p < 0.001, but were still hypothermic. Endrin-treated hypothermic
animals had significantly higher test body temperatures than
corresponding dieldrin-treated animals.

Discussion

The lethal potency of endrin was found to be approximately
4 to 5 times greater than that of dieldrin in all experimental
situations. This indicates that endrin and dieldrin produce
parallel lethality in animals under a variety of environmental
Table 3.6. Alterations in median lethal doses (LD50) and median lethal times (LT50) of endrin and dieldrin in hypothermic female mice.

<table>
<thead>
<tr>
<th>compound</th>
<th>normothermic animals (from Table 5a)</th>
<th>hypothermic animals b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD50 (mg/kg)</td>
<td>LT50 (min.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>endrin</td>
<td>2.4</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>(2.2-2.6)c</td>
<td>(10.8-15.6)c</td>
</tr>
<tr>
<td>dieldrin</td>
<td>11.2</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>(10.9-11.5)</td>
<td>(5.0-7.7)</td>
</tr>
</tbody>
</table>

a body temperatures, recorded just prior to injection and again prior to death are expressed as the mean of 5 measurements.

b hypothermia was induced by immersing mice in water up to their necks at room temperature for 15 minutes.

c numbers in parentheses are 95% confidence limits.

d the number in parentheses is the standard error of the mean.

e significantly different from corresponding normothermic value, p < 0.05.

f significantly different from control value, p < 0.001.

g significantly different from dieldrin value, p < 0.001.
conditions. The fact that dieldrin was significantly more lethal to males at 23° C or to either sex at 34° C may not reflect true biological differences between endrin and dieldrin. The difference in lethality for dieldrin was small and barely significant at p 0.05.

Our studies have indicated that the dose-response relationship for time of death is roughly parallel for the two compounds, suggesting that endrin has greater intrinsic activity and affinity for active sites that are involved in the mechanism of initiating and maintaining seizure activity. Since the curves for endrin and dieldrin were approximately parallel, it is likely that the compounds interact with the same active sites.

The duration of action of dieldrin does not appear to be modified by environmental variables to the same extent as endrin. The data suggest that the central nervous system is more sensitive to the actions of endrin and hence these actions are more easily modified. This is consistent with the concept that endrin and dieldrin interact with the same active site. In the case of endrin, however, it would appear that aggregation and increases in ambient temperature above 23° increase the efficiency of seizure spread through the central nervous system.

Endrin and dieldrin cause parallel changes in the regulation of body temperature. Mice treated with either compound became
hypothermic, except at high ambient temperatures, in the case of dieldrin, and at high ambient temperatures in an aggregated situation, in the case of endrin. Mice rendered hypothermic resist the lethal action of dieldrin more so than endrin, evidenced by the fact that a greater percent of animals die from endrin than from an equilethal dose of dieldrin.

Since the toxicities of picrotoxin and PTZ were not measured in our studies, it is difficult to compare the actions of the cyclo-dienes with those of the classical convulsants. It would be of interest to see how the lethality or duration of action of PTZ and picrotoxin are modified by the methods described in this chapter.

It is concluded from the data that the intravenous administration of endrin in mice has approximately 5 times more lethal potency than the intravenous administration of dieldrin. This relationship holds true under a variety of environmental conditions. Endrin and dieldrin have a parallel dose-duration relationship, and cause parallel changes in body temperature. These data suggest that endrin and dieldrin interact with similar active sites in the central nervous system, but with different affinities and/or intrinsic activities. Differences in the durations of action of these insecticides resulting from variations in environmental
parameters may reflect differences in the efficiency with which endrin and dieldrin induce seizures.
IV. THE NATURE OF ENDRIN AND DIELDRIN INDUCED
CONVULSIONS, AND THEIR MODIFICATION
BY VARIOUS AGENTS

Introduction

Studies on the toxicity and intensity of action of endrin
and dieldrin have revealed that these compounds induce a charac-
teristic convulsive activity in mice. Since endrin and dieldrin
differ from each other in terms of the LD50 and LT50, it is of
interest whether they also differ from each other by the type of
convulsion they induce. Many chemicals besides the chlorinated
hydrocarbon insecticides are capable of inducing seizures, and
the convulsive activity of some of these have been studied in
detail. Thus, it is also of interest to compare the insecticide-
induced convulsions to other chemical-induced convulsions.

Pentylenetetrazol (PTZ) and picrotoxin represent two clas-
sical convulsant drugs whose mechanisms of action have been
investigated. Galindo (1969) has demonstrated that picrotoxin
may interfere with pre- or postsynaptic inhibition in the mamma-
lian central nervous system through a specific blockade of the
inhibitory effects of gamma-amino butyric acid. Lewin and Esplin
(1961) have shown that PTZ decreased the time for recovery in
the monosynaptic pathway of the spinal cord. They suggested
that PTZ stimulates excitatory and inhibitory neurons and that the convulsions are due to excitation of cerebral structures relatively unopposed by inhibition. Whether endrin or dieldrin act by the same mechanism as PTZ or picrotoxin has not been investigated.

Convulsive seizures variously induced can theoretically be altered by anticonvulsant drugs. Such alteration is dependent on the mechanisms by which the convulsant and anticonvulsant agents act. It may be postulated that two convulsants which are affected differently by a single anticonvulsant may each act by a different mechanism. A study of the interaction of dieldrin and endrin with anticonvulsants would show if these insecticides act by a common mechanism, and thus respond similarly to anticonvulsant treatment.

The purpose of the experiments presented in this chapter is twofold: 1) to compare endrin and dieldrin induced seizures with each other, and with seizures induced by PTZ and picrotoxin; and 2) to compare alterations in endrin and dieldrin induced seizures by various agents. The results of such experiments should support or contradict an hypothesis that endrin and dieldrin act by a common mechanism.
Methods

Studies on Seizure Patterns:

Intravenous LD50s were determined for pentylenetetrazol and picrotoxin in male mice, housed ten per cage at room temperature (23°C) and the LD90s for each were selected (see General Methods).

To compare seizures induced by the insecticides and convulsants, an LD90 of each was administered i.v. to male mice in groups of 10 (see General Methods). According to the literature, chemically induced seizures can be divided into several components: latency, pretonic clonic activity, hindleg tonic flexion and extension, post-tonic clonic activity, and death (E. A. Swinyard et al., 1963: Iturrian and Fink, 1969). The duration of each component was therefore measured in each mouse, immediately following the i.v. LD90 of either endrin, dieldrin, PTZ, or picrotoxin. By using several electric timers, it was possible to measure latency, pretonic activity, and post-tonic activity in the same mouse. It was necessary to repeat the experiment in order to obtain values for the hindleg tonic component. The duration of all components was measured in seconds.

Drug Antagonism of Endrin and Dieldrin Toxicity:
Representative drugs from various classifications were used to pretreat animals, prior to measurements of LT50s of endrin or dieldrin. Effective doses of these drugs were selected as described in General Methods. Groups of 10 male mice were pretreated intraperitoneally with each drug dissolved or suspended in 0.9% saline. Mice were then housed 10 per cage at room temperature (23°C) and administered endrin or dieldrin for LT50 determinations (see General Methods). The experiment was duplicated for endrin, housing animals one per cage at room temperature. Control LT50s for either insecticide were run simultaneously in mice pretreated with 0.9% saline, and repeated when the experiment carried over to the following day.

Results

Studies on Seizure Patterns:

The durations of the various seizure components for each compound are listed in Table 4.1. Latency (a period immediately following injection of the compound) involved no convulsive actions and ended with the first clonic convulsive activity. At this point a period of pretonic activity began which included intermittent clonic seizures. The pretonic component ended with the beginning of hindleg tonic extension, or maximal seizure. The hindleg tonic
component was divided into two phases: 1) a period of tonic flexion of the limbs, followed by 2) tonic extension of the limbs. Animals which did not die at this point entered a period of post-tonic activity, which involved continual clonic seizures terminating in death. The mean values for these components were compared by the Student's "t" test, or the corrected form thereof when variances between values were not equal (Li, 1965).

Data in Table 4.1 reveal that the duration of the latent components fell into the following order of relative magnitudes at p < 0.05: endrin > dieldrin > picrotoxin > PTZ. In the case of pretonic activity duration, the order was endrin > picrotoxin > dieldrin > PTZ, p < 0.05. The durations of hindleg tonic flexion were similar for endrin, dieldrin, and picrotoxin, but were significantly longer (p < 0.05) than that of PTZ. The durations of hindleg tonic extension fell into the order of dieldrin > picrotoxin > endrin = PTZ, p < 0.05. Dieldrin- and picrotoxin-treated mice generally did not survive tonic extension. PTZ-treated mice demonstrated a relatively larger degree of variability of time to death. During the post-tonic period some animals would die very soon after tonic extension, while others lingered and occasionally underwent a second tonic seizure. The total time to death in endrin-treated mice was significantly longer (p < 0.01) than in dieldrin- or picrotoxin-treated mice. An observation was made
Table 4.1. A comparison of the durations of seizure components induced by equitoxic doses (LD90s) of endrin, dieldrin, pentylenetetrazol, and picrotoxin.

<table>
<thead>
<tr>
<th>Compound</th>
<th>latency</th>
<th>pre-tonic activity</th>
<th>hindleg tonic flexion</th>
<th>tonic extension</th>
<th>post-tonic activity</th>
<th>total time to death</th>
</tr>
</thead>
<tbody>
<tr>
<td>endrin 5 mg/kg</td>
<td>300\textsuperscript{a}</td>
<td>260\textsuperscript{a}</td>
<td>1.68</td>
<td>12.98\textsuperscript{b}</td>
<td>342</td>
<td>733\textsuperscript{a, e}</td>
</tr>
<tr>
<td></td>
<td>± 28</td>
<td>± 35</td>
<td>± 0.85</td>
<td>± 0.85</td>
<td>± 64</td>
<td>± 82</td>
</tr>
<tr>
<td>dieldrin 15 mg/kg</td>
<td>194\textsuperscript{c}</td>
<td>85\textsuperscript{c}</td>
<td>1.81</td>
<td>19.8\textsuperscript{c}</td>
<td>0\textsuperscript{d}</td>
<td>318</td>
</tr>
<tr>
<td></td>
<td>± 26</td>
<td>± 40</td>
<td>± 0.16</td>
<td>± 0.42</td>
<td></td>
<td>± 42</td>
</tr>
<tr>
<td>pentylenetetrazol</td>
<td>4.9\textsuperscript{a}</td>
<td>1.4\textsuperscript{a}</td>
<td>1.19\textsuperscript{b}</td>
<td>12.4\textsuperscript{b}</td>
<td>623</td>
<td>644</td>
</tr>
<tr>
<td>70 mg/kg</td>
<td>± 0.39</td>
<td>± 0.11</td>
<td>± 0.06</td>
<td>± 1.22</td>
<td>± 248</td>
<td>± 248</td>
</tr>
<tr>
<td>picrotoxin</td>
<td>94</td>
<td>151</td>
<td>1.5</td>
<td>15.8</td>
<td>0</td>
<td>262</td>
</tr>
<tr>
<td>6 mg/kg</td>
<td>± 4.5</td>
<td>± 24</td>
<td>± 0.07</td>
<td>± 1.07</td>
<td></td>
<td>± 25</td>
</tr>
</tbody>
</table>

\textsuperscript{a} significantly different from picrotoxin (t test, p < 0.01)

\textsuperscript{b} significantly different from picrotoxin (t test, p < 0.05)

\textsuperscript{c} significantly different from endrin, pentylenetetrazol, and picrotoxin (t test, p < 0.05)

\textsuperscript{d} dieldrin and picrotoxin-treated animals did not survive tonic extension

\textsuperscript{e} significantly different from dieldrin (t test, p < 0.01)
that mice receiving 10 mg/kg of endrin produced a seizure pattern identical to that of dieldrin.

**Drug Antagonism of Endrin and Dieldrin Toxicity:**

Table 4.2 lists the drugs effective in decreasing the toxicity or intensity of action of endrin and dieldrin in mice. Drugs tested but not found effective in this respect were atropine sulfate, scopolamine hydrobromide, propantheline bromide, hexamethonium ethdichloride, propanolol hydrochloride, phenoxybenzamine, phen tolamine hydrochloride, trimethadione, guanethidine sulfate, ergotamine tartrate, reserpine acetate, chlorpheniramine maleate, doxylamine succinate, diphenhydramine hydrochloride, CaCl₂, phensuximide, and acetazolamide. Data in Table 4.2 illustrate that drugs most effective in antagonizing endrin and dieldrin were anticonvulsants, sedatives, muscle relaxants, and tranquilizers. Calcium lactate and lactic acid produced sedation in the doses used. One significant finding resulting from pretreatment with these drugs was that dieldrin was considerably more amenable to changes in lethality than endrin. Only two compounds, (Librium and Trancopal) altered the lethality of endrin, while five of the compounds (propylene glycol, librium, trancopal, pentobarbital, and phenobarbital) altered the lethality of dieldrin. Another significant finding was that pretreatment with all the drugs listed, with the exception of lactic acid, was more effective against endrin when animals were
Table 4.2. The effects of pretreatment with various agents on the LT50s of endrin and dieldrin in male mice.

<table>
<thead>
<tr>
<th>drug</th>
<th>dose mg/kg</th>
<th>1/cage</th>
<th>endrin 5 mg/kg</th>
<th>10/cage</th>
<th>dieldrin 15 mg/kg</th>
<th>10/cage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LT50a</td>
<td>RRb</td>
<td>LT50</td>
<td>RR</td>
<td>LT50</td>
</tr>
<tr>
<td>ethyl alcohol</td>
<td>789</td>
<td>-</td>
<td>-</td>
<td>13.8</td>
<td>1.33</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(minutes)</td>
<td></td>
<td>(12.2-15.5)</td>
<td>(0.96-1.8)</td>
<td>(8.1-13.6)</td>
</tr>
<tr>
<td>propylene glycol</td>
<td>5180</td>
<td>122</td>
<td>6</td>
<td>28.5</td>
<td>3.39</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(66-223)c</td>
<td>(3-12)c</td>
<td>(24.9-32.6)</td>
<td>(2.85-4.06)</td>
<td>mortality</td>
</tr>
<tr>
<td>lactic acid</td>
<td>1039</td>
<td>32</td>
<td>1.6</td>
<td>41.5</td>
<td>4.06</td>
<td>29.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(25-40)</td>
<td>(1.16-2.2)</td>
<td>(35.5-48.6)</td>
<td>(2.74-6.0)</td>
<td>(21.3-40.7)</td>
</tr>
<tr>
<td>calcium lactate</td>
<td>700</td>
<td>27</td>
<td>1.5</td>
<td>16.2</td>
<td>4.4</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(19.7-37)</td>
<td>(0.8-2.6)</td>
<td>(12.3-21.3)</td>
<td>(2.91-6.64)</td>
<td>(9.9-16.2)</td>
</tr>
<tr>
<td>chlorpromazine</td>
<td>5</td>
<td>76</td>
<td>3.5</td>
<td>25</td>
<td>2.97</td>
<td>18.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(51-96)</td>
<td>(2.37-5.2)</td>
<td>(19.8-31.6)</td>
<td>(2.28-3.86)</td>
<td>(15.3-22.4)</td>
</tr>
<tr>
<td>Librium</td>
<td>5</td>
<td>50%</td>
<td>-</td>
<td>20</td>
<td>1.96</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mortality</td>
<td></td>
<td>(15.9-25.2)</td>
<td>(2.33-6.26)</td>
<td>mortality</td>
</tr>
</tbody>
</table>

a median lethal times, determined by the method of Litchfield (1949)--see general methods.

b reaction ratio--the ratio of experimental to control LT50s. When the RR plus 95% confidence limits are greater than 1, the experimental LT50 is significantly greater than control.

c numbers in parentheses are 95% confidence limits.
<table>
<thead>
<tr>
<th>drug</th>
<th>dose mg/kg</th>
<th>1/cage</th>
<th>endrin 5 mg/kg</th>
<th>10/cage</th>
<th>dieldrin 15 mg/kg</th>
<th>10/cage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LT50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>RR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>LT50</td>
<td>RR</td>
<td>LT50</td>
</tr>
<tr>
<td>Trancopal</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0%</td>
</tr>
<tr>
<td>mortality</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pentobarbital</td>
<td>30</td>
<td>90</td>
<td>5</td>
<td>32.8</td>
<td>3.21</td>
<td>30%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(33-243)</td>
<td>(1.6-15)</td>
<td>(21.9-49.2)</td>
<td>(1.95-5.26)</td>
<td>-</td>
</tr>
<tr>
<td>phenoobarbital</td>
<td>30</td>
<td>73</td>
<td>4</td>
<td>45.0</td>
<td>3.82</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(43-124)</td>
<td>(1.8-9.1)</td>
<td>(32.4-62.6)</td>
<td>(2.33-6.26)</td>
<td>-</td>
</tr>
<tr>
<td>control 1</td>
<td>-</td>
<td>20</td>
<td>1</td>
<td>8.4</td>
<td>1</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(16-25)</td>
<td>(7.5-9.8)</td>
<td></td>
<td>(5.7-7.1)</td>
<td></td>
</tr>
<tr>
<td>control 2</td>
<td>18</td>
<td>1</td>
<td>10.2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(12.5-27)</td>
<td>(7.7-12.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
isolated. Librium and Trancopal appeared to be the most effective
drugs against either endrin or dieldrin.

Discussion

The seizures studied in these experiments are classified
as maximal, based on the occurrence of a tonic extensor convul-
sion. The neurological components involved in the maximal
seizure have been reviewed by Iturrian and Fink (1968), and
briefly include a neuronal oscillator, a mediating neuronal network,
and final common motor pathways. The maximal tonic-clonic
seizure involves the entire brain: the oscillator, triggered by
some external or epileptogenic stimulus, serves to disperse the
neuronal discharge to the cortex. From there it enters the spinal
cord through pyramidal and extrapyramidal pathways, and hence
to motor nerves. The oscillator is loosely defined as a collec-
tion of neurons discharging over a finite period of time to suffi-
ciently cause a minimal or clonic seizure (Woodbury and Esplin,
1959). Evidence indicates that the anatomical substratum of the
oscillator is the centrencephalic system, which includes the
thalamus, diencephalon, mesencephalon, and rhombencephalon

The components of the seizures induced by endrin, dieldrin,
PTZ, and picrotoxin can be related to the concept of a discharging
oscillator. Latency can be interpreted as the time required to trigger the oscillator; pretonic activity as the time required to build up sufficient discharge and dispersion through the oscillator and mediating system; hindleg tonic extension as a result of intense input to cortical motor areas by the mediating system; and post-tonic activity as a period of neuronal depression in which the oscillator cannot sustain enough discharge to precipitate recurrent tonic seizures.

Interpretation of the data in view of the oscillator concept indicates that at equitoxic doses a rate limiting step is present between the injection of endrin, dieldrin, or picrotoxin and the appearance to the first clonic seizure; such a step does not appear to be present for PTZ. Considerable time is required for the oscillator to discharge sufficiently for tonus in the presence of endrin, dieldrin, or picrotoxin, but such oscillator discharge is very short in the presence of PTZ. The resulting tonic seizure is more intense with dieldrin, compared to endrin, indicating a more intense discharge of the oscillator in the presence of dieldrin. Dieldrin and picrotoxin induce death immediately following tonus, while endrin and PTZ induce a period of post-tonic depression, suggesting that dieldrin and picrotoxin both cause a more intense activation of the oscillator than endrin or PTZ.

The difference in action between endrin and dieldrin appears
to be simply one of intensity of action. Dieldrin triggers the oscillator to discharge sooner and more intensly. As a result, at equitoxic doses dieldrin-treated animals die sooner than endrin-treated animals. The relatively long latent and pretonic periods for endrin, dieldrin, and picrotoxin may be explained by the following hypotheses: 1) Duration of latency and pretonic activity is dependent upon the ability a compound to cross the blood-brain barrier; 2) A metabolite is the active component of the convulsant compounds, and time is required for sufficient metabolism; 3) A biochemical lesion is induced over a definite period of time, which is the cause of convulsive activity; 4) A definite period of time is required for the interaction of these compounds with nerve membrane components, the result of which leads to increased neuronal activity. The validity of these hypotheses will be discussed in the following chapter.

According to Swinyard et al. (1963), drugs can prevent experimental seizures by three mechanisms: 1) stabilization of the neuronal membrane; 2) decrease in the tendency to repetitive discharge; and 3) reduction in the spread of neuronal discharge. Trimethadione acts by the first mechanism, dilantin by the third, and phenobarbital by all three. In the data presented, dilantin modified endrin and dieldrin induced seizures by abolishing the tonic component, but neither dilantin or trimethadione altered the
total time to death or lethality of either compound. All drugs
effective in altering LT50s or LD50s caused some degree of
overt central nervous system depression, with the exception of
phenobarbital. The fact that over half the drugs altered the
lethality of dieldrin but not endrin suggests a direct antagonism
of the insecticides by the drugs, rather than suppression of
oscillatory mechanisms. It would appear that the effective drugs
tend to inhibit the process of oscillatory excitation by endrin and
dieldrin, rather than simply inhibiting the oscillator. Endrin
could then be considered more effective in exciting the oscillator
than dieldrin, and, at the same time, less susceptible to antag-
agonistic drugs.

Since it was observed that propylene glycol was effective in
altering the LT50 of endrin and dieldrin, lactic acid and lactate
were tested. Propylene glycol is metabolized to lactic acid, and
the possibility presented itself that propylene glycol was effective
following its metabolic conversion. The results indicated that
propylene glycol was a more effective antagonist of endrin and
dieldrin, and lactic acid or lactate were effective only in aggre-
gated mice. Hathaway, Mullinson, and Akintonwa (1965) reported
that increases in brain lactate accompany dieldrin-, telodrin-, and
picrotoxin-induced convulsions. The formation of lactate naturally
under these conditions may act as a form of negative feedback
resulting in decreased seizure severity.

It can be concluded from the data that dieldrin and endrin resemble picrotoxin rather than pentylenetetrazol in the pattern of induced seizures. At equitoxic doses, dieldrin-induced seizures are more severe than endrin-induced seizures, but the former are more easily antagonized by several kinds of drugs. The data are consistent with an hypothesis that endrin, dieldrin, and picrotoxin combine with a common receptor in the central nervous system, and that endrin and dieldrin have different intrinsic activities and/or affinities for the receptor.
V. A COMPARISON OF THE DISTRIBUTION OF ENDRIN AND DIELDRIN IN MICE

Introduction

The previous chapters have presented data which delineate certain distinct differences between the toxicity of endrin and dieldrin. These include differences in potency, duration of action, alterations by environmental variables, and antagonism by depressant drugs. Four hypotheses were presented to explain some of these differences. One of these postulated that the metabolites of endrin and dieldrin were the toxic components; i.e., conversion to active metabolites were essential for convulsive activity. In the light of previous research this hypothesis may not be tenable. According to Soto and Deichmann (1967) the recovered metabolites of dieldrin were considerably less toxic than dieldrin. Bioassay methods for endrin also indicated that endrin was degraded to compounds less toxic. Hathaway (1965) emphasized the fact that dieldrin and telodrin were the toxic agents and not their metabolic products.

Another hypothesis suggested that the differences in toxicity between endrin and dieldrin were caused by differences in the permeability of these compounds to the blood-brain barrier. A corollary of this hypothesis is that seizures are related to brain concentrations of insecticides. It has been shown for chlorinated
hydrocarbon insecticides that signs of poisoning are related to the concentrations of toxicant in the brain or other tissues. Speck and Maaske (1958), using rats, demonstrated that the concentration of endrin had to reach specific levels in blood or other tissues before convulsions occurred. Dale, Gaines, and Hayes (1963), after giving single doses of DDT to rats, found that the severity of signs of poisoning was directly proportional to the concentration of the compound in the brains. Continuing this work, Hayes and Dale (1964) found that the severity of intoxication corresponded with the concentration of DDT in the plasma, liver, and kidneys, as well as in the brain. Mount, Vigor, and Shafer (1966) discovered a critical concentration for endrin in fish blood: above this concentration (0.3 micrograms/gram) fish consistently died. From this previous work, it appears that tissue levels reflect the degree of intoxication by chlorinated hydrocarbon insecticides. It may be that some compounds reach a site of action sooner or in higher concentrations and for this reason are more toxic.

The purpose of the experiments in this chapter is to test the hypothesis that differences in toxicity between endrin and dieldrin might be caused by differences in permeability of these compounds in the blood-brain barrier. According to this hypothesis, it could be predicted that at equitoxic doses endrin would exist in the brain in equal or smaller concentrations than dieldrin.
It might be further predicted that dieldrin would appear sooner and in greater concentrations in fat and bile. Since Cole et al. (1970) have shown that endrin is excreted faster than dieldrin in the bile, while dieldrin tends to be stored in fat, the possibilities of differential distribution into liver, fat and bile were considered.

**Methods**

**Tissue Studies:**

In order to measure the distribution and concentration of endrin or dieldrin in blood, brain, liver, and fat tissues of mice at selected intervals after intravenous administration, the following experiments were performed. Groups of five male mice were injected i.v. with an LD90 of endrin or dieldrin and killed by cervical dislocation at the end of a given time interval (50, 100, 150, 200, 300, 450, or 600 seconds). At any particular time blood was removed from each mouse immediately following cervical dislocation. Samples of omental fat and liver were taken and the whole brains were removed. The entire process of tissue retrieval took less than two minutes for each mouse. Each sample was then weighed, extracted, and analyzed for endrin or dieldrin content. The brains from groups of 5 mice receiving equimolar doses of endrin and dieldrin (15 mg/kg) were also extracted and
analyzed five minutes after injection. Mean values were analyzed statistically by the student's t test, or when applicable, the corrected student's t (Li, 1965).

Since most determinations were made following lethal doses, it was considered necessary to use some toxic endpoint other than death and relate this to brain levels of insecticide. Thus a comparison could be made between lethality and a less severe toxic symptom. The toxic symptom selected was ataxia, measured as the inability of a mouse to stay on a rotating rod (one inch in diameter 6 rev./min.) for 60 seconds, following an i.v. dose of endrin or dieldrin dissolved in DMSO (see General Methods). Four or five dose levels of endrin or dieldrin were administered to groups of ten male mice, each group receiving one dose level. If a mouse failed to stay on the bar within the time limit after three consecutive trys, then the mouse was considered ataxic. Mice were tested every five minutes until they recovered. A median toxic dose (ED50) and 95% confidence limits were calculated for endrin and dieldrin by the method of Litchfield and Wilcoxon (1949). From the ED50 curves a ED90 was selected for endrin and dieldrin, and administered to groups of five male mice. After fifteen minutes, mice were killed by cervical dislocation, whole brains were removed, and treated as described. Control mice for all experiments were treated similarly but
received no insecticides.

Bile Studies:

In order to measure possible biliary excretion of endrin and dieldrin, male mice were prepared with biliary fistulae by the method of Becker and Plaa (1965). Male mice were anesthetized with pentobarbital sodium, 45 mg/kg, i.p.. Midventral incisions were made and the common bile duct exposed. After ligating the gall bladder, the common bile duct was incised about 6 mm below the hilum of the liver. A PE-10 polyethylene tube was passed through the incision and guided towards the hilum for a distance of about 3 mm. A ligature was placed above the incision so that the cannula was held in place. The body wall was then closed with wound clips and the mouse restrained in the vertical position by binding with adhesive tape to a wooden tongue depressor. From groups of five mice so prepared, bile was collected in calibrated Wintrobe tubes 1/2, 1, and 2 hours after i.v. injection of a ED90 of endrin, or dieldrin. Control animals received DMSO alone. Bile samples were then extracted and analysed for insecticide content. The preparation was checked by injecting fluorescein (20 mg in 10 ml 0.17 N NaHCO₃; 0.1 ml/10 g body weight) i.v., and examining the gall bladder and bile duct in a darkened room for fluorescence under ultraviolet light.
This indicated if the ligature was effective in preventing bile from collecting in the gall bladder.

**Extraction Procedures:**

Extraction of endrin and dieldrin from tissues was carried out by a modification of the method described by Jain *et al.* (1965). Each tissue was homogenized in 2 ml of acetone, and the homogenate transferred to a test tube. The homogenizing vessel was rinsed with an additional 2 ml of acetone and added to the homogenate. The sample was then centrifuged and the supernatant was transferred to a clean test tube. The residue was redissolved in another 2 mls of acetone and centrifuged a second time. The second supernate was added to the first, making a total of 6 mls of acetone extract. This was then placed in an Evapo-mix shaker-bath, and the acetone was evaporated off. The final residue was redissolved in one ml of hexane and 2 microliters of this were used for analysis.

**Insecticide Analysis:**

Analyses were made on Varian-aerograph gas chromatograph model 200, with an electron capture detector and Westronix recorder. The chromatographic column was a Pyrex glass coil, 0.125 inch outside diameter, and 5 feet in length. The solid
support material was Chromosorb W 80 mesh, acid washed and treated with hexamethyldisilizane. This was coated with 5% QF-1 (fluorosilicone fluid-Dow Corning). Conditions of the gas chromatograph were as follows: inlet temperature 205°, column temperature 185°; flow rate of nitrogen-60-70 mls/min. Inlet pressure was approximately 100 pounds per square inch.

Results

Tissue Studies:

A typical standard curve for dieldrin is shown in Figure 5.1. The standard curve for endrin was similar, and each compound had a retention time of approximately six minutes. The efficiencies of extraction for fat, brain, liver and blood were 95.4, 99.8, 94.8, and 91.4%, respectively, for dieldrin, and 100, 90, 82, and 90% respectively, for endrin. Peak areas for both compounds were analyzed by triangulation. In the case of endrin, the relationship between peak area and quantity injected on the column formed a linear standard curve which intersected the X and Y axis at the origin. A single standard was therefore utilized for every five unknown samples in order to obtain, by proportion, the endrin content of each sample. In the case of dieldrin, the relationship between peak area and quantity injected was linear.
Figure 5-1. A typical standard curve for dieldrin analysis by gas chromatography. The largest to smallest peaks were generated by 90, 60, 30 and 15 nanograms, respectively.
only over the 15ng to 300ng range. A single standard was therefore utilized for every five samples as a correction factor for changes in sensitivity of the detector. Sample dieldrin values were then read directly from the standard curve.

The pattern of tissue distribution of endrin in the mouse is shown in Table 5.1 and Figure 5.2. Tissue concentrations at 50 seconds for brain, liver, and fat were significantly smaller than corresponding values at 150, 300, 450 seconds, tonus and death; blood concentrations at 50 seconds were significantly larger than the values at the other intervals, p < 0.005. At 50 seconds, the respective tissue concentrations had the following relative order of magnitudes: blood > liver > brain > fat, p < 0.025. At the remaining intervals, the relative orders of magnitudes were liver > brain = fat > blood, p < 0.025.

The pattern of tissue distribution for dieldrin is shown in Table 5.2 and Figure 5.3. In the case of fat analyses, the quantities of dieldrin extracted from the 50, 100 and 200 second interval samples fell on the non-linear portion of the standard curve and could not be accurately analysed. Since 15ng was the lower limit of detection for dieldrin and fat samples weighed approximately 150 mg, a concentration of 50 micrograms/gram of fat was required before fat tissue could be analyzed accurately. Thus, at intervals of 50, 100, and 200 seconds the concentration
Table 5.1. The concentrations of endrin\(^a\) in brain, liver, fat, and blood after i.v. administration of an LD90 of endrin in male mice.

<table>
<thead>
<tr>
<th>post-injection time intervals (seconds)</th>
<th>50(^d)</th>
<th>150(^e)</th>
<th>300(^e)</th>
<th>450(^e)</th>
<th>tonus(^e)</th>
<th>death(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>brain</td>
<td>12.4(^c)</td>
<td>18.0</td>
<td>22.6</td>
<td>22.7</td>
<td>23.9</td>
<td>22.6</td>
</tr>
<tr>
<td></td>
<td>± 1.42</td>
<td>± 1.56</td>
<td>± 4.3</td>
<td>± 2.2</td>
<td>± 4.5</td>
<td>± 2.3</td>
</tr>
<tr>
<td>liver</td>
<td>17.5(^c)</td>
<td>43.3</td>
<td>52.2</td>
<td>61</td>
<td>53.6</td>
<td>43.3</td>
</tr>
<tr>
<td></td>
<td>± 5.4</td>
<td>± 13.6</td>
<td>± 17.7</td>
<td>± 6.3</td>
<td>± 4.2</td>
<td>± 7.5</td>
</tr>
<tr>
<td>fat</td>
<td>4.6(^c)</td>
<td>17.3</td>
<td>25</td>
<td>23</td>
<td>22.9</td>
<td>20.6</td>
</tr>
<tr>
<td></td>
<td>± 1.76</td>
<td>± 4.8</td>
<td>± 7.7</td>
<td>± 4.4</td>
<td>± 3.3</td>
<td>± 5.9</td>
</tr>
<tr>
<td>blood</td>
<td>22.2</td>
<td>6.1</td>
<td>6</td>
<td>6.5</td>
<td>6.3</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>± 5.6</td>
<td>++ 1.43</td>
<td>± 2.8</td>
<td>± 1.8</td>
<td>± 3.8</td>
<td></td>
</tr>
</tbody>
</table>

---

\(^a\) extracted in acetone and analyzed by gas chromatography.

\(^b\) mean value of 5 determinations from 5 individual mice.

\(^c\) significantly different from corresponding value at 150 seconds, \(p < 0.005\).

\(^d\) order of magnitudes of tissue concentrations: blood = liver > brain > fat, \(p < 0.025\).

\(^e\) order of magnitudes of tissue concentrations: liver > brain = fat > blood, \(p < 0.025\).
Figure 5-2. The tissue distribution of endrin in mice at various intervals after i.v. administration of an LD90 (5 mg/kg). Each point represents the mean of 4 or 5 determinations, plus or minus one standard deviation.
Table 5.2. The concentrations of dieldrin in brain, liver, fat, and blood after i.v. administration of an LD90 of dieldrin in male mice.

<table>
<thead>
<tr>
<th>tissue</th>
<th>post-injection time intervals (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>micrograms dieldrin/gram tissue (± S.D.)</td>
</tr>
<tr>
<td>brain</td>
<td>53.8 ± 11.1</td>
</tr>
<tr>
<td>liver</td>
<td>35.0 ± 19.2</td>
</tr>
<tr>
<td>fat</td>
<td>50</td>
</tr>
<tr>
<td>blood</td>
<td>84.8 ± 10.8</td>
</tr>
</tbody>
</table>

a extracted in acetone and analyzed by gas chromatography.
b mean values of 5 determinations from 5 separate mice.
c significantly different from corresponding value at 50 seconds, p < 0.025.
d significantly different from corresponding value at 100 seconds, p < 0.025.
e significantly different from corresponding value at 200 seconds, p < 0.025.
f order of magnitudes of tissue concentrations: blood > brain > liver, p < 0.025.
g order of magnitudes of tissue concentrations: liver > brain > blood, p < 0.025.
Figure 5-3. The distribution of dieldrin in mice at various intervals after i.v. administration of an LD90 (15 mg/kg). Each point represents the mean of 4 or 5 determinations, plus or minus one standard deviation.
of dieldrin in fat was at least smaller than 50 micrograms per gram of tissue. Brain concentrations of dieldrin were equal at all intervals. In the case of liver, concentrations increased significantly at 100 and 300 seconds, \( p < 0.025 \), and in the case of blood, decreased significantly at 100 and 200 seconds, \( p < 0.025 \). At 50 seconds, the tissue concentrations of dieldrin had the following relative order of magnitudes: blood \( > \) brain \( > \) liver, \( p < 0.025 \). At the remaining intervals, the relative orders of magnitudes were liver \( > \) brain \( > \) blood, \( p < 0.025 \). At 300 seconds brain and fat concentrations were equal. Table 5.2 does not contain separate values for tonus at approximately 300 seconds. Zero time values for blood concentrations of endrin and dieldrin were extrapolated to zero from 50 seconds assuming blood volume equaled 8% of body weight. (Altman and Dittmer, 1964a, b).

The data indicate that dieldrin equilibrated with brain more quickly than endrin, and that endrin disappeared from the blood sooner and entered fat and liver at a faster rate than dieldrin. Endrin equilibrated with all tissues by 150 seconds, but dieldrin appeared to equilibrate only with brain and blood over the time intervals analyzed. Neither in the case of endrin nor dieldrin did the rate of distribution into brain correlate with the onset and duration of the seizure pattern.
A comparison of tissue concentrations of endrin and dieldrin at the 300 second sampling interval reveals that there is approximately three times as much dieldrin in brain, liver, and blood than endrin. A comparison of brain concentrations resulting from almost equimolar doses of endrin and dieldrin, 5 and 4.5 mg/kg, respectively, is presented in Table 5.3. Although dieldrin was administered in a smaller amount and analyzed 5 minutes later than endrin, the brain concentration of dieldrin is still significantly higher, $p < 0.01$. This suggested that at equimolar doses, dieldrin concentration in the brain would be equal to or greater than endrin concentration. Analyses of brains from mice treated with 15 mg/kg of endrin or dieldrin revealed that the concentrations of the compounds in the brains at 300 seconds were equal. These particular analyses were performed in a different laboratory than our own, utilizing the same species of mouse. A $^{53}$N detector was employed instead of a tritium foil. The mean concentrations of endrin and dieldrin, one standard deviation, were found to be $19.8 \pm 5.1$ and $22.3 \pm 2.21$ micrograms per gram of tissue, respectively. The fact that the dieldrin value was one third the value obtained in our laboratory is presently unexplained.

Table 5.4 lists the ED50s for endrin and dieldrin, the respective ED90s, and the brain concentrations of either
Table 5.3. A comparison of brain concentration of endrin and dieldrin after approximately equimolar i.v. dosage in mice.

<table>
<thead>
<tr>
<th>insecticide</th>
<th>brain concentration micrograms/gram of tissue $\pm$ S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>endrin</td>
<td>22.6$^a$ $\pm$ 2.3 (at 10 minutes post injection)</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td></td>
</tr>
<tr>
<td>dieldrin</td>
<td>31.1 $\pm$ 1.6 (at 15 minutes post injection)</td>
</tr>
<tr>
<td>4.5 mg/kg</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ significantly smaller than dieldrin value at $p < 0.01$. 
Table 5.4. A comparison of brain levels of endrin and dieldrin, after i.v. administration of an ED90 of either compound in mice.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ED50 mg/kg</th>
<th>ED90 mg/kg</th>
<th>brain concentration micrograms/gram tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>endrin</td>
<td>0.75(^b)(^c) (0.59-0.98)</td>
<td>1.5</td>
<td>9.4(^a),(^b) ± 2.0</td>
</tr>
<tr>
<td>dieldrin</td>
<td>2.6 (2.08-3.25)</td>
<td>4.5</td>
<td>31.1 ± 1.6</td>
</tr>
</tbody>
</table>

\(^a\) mean value ± standard deviation of 5 determinations from 5 separate mice.

\(^b\) significantly different from dieldrin value (p < 0.05).

\(^c\) numbers in parentheses are 95% confidence limits.
insecticide 15 minutes after i.v. administration of the ED90s. Endrin has approximately three times the potency of dieldrin for producing ataxia in mice. Approximately 3 times as much brain concentration of dieldrin is required to produce the same degree of ataxia as endrin. This is in agreement with the finding that three times as much brain concentration of dieldrin is required to produce the same percentage lethality as endrin.

**Bile Studies:**

Fluorescein studies indicated that the ligature around the gall bladder was effective in preventing bile flow into the bladder. Bile flow ranged from 0.04 to 0.09 mls/hr, which was consistent with flow rates reported by Plaa and Becker (1965). Neither endrin nor dieldrin appeared in the 1/2, 1, or 2 hour collection samples of bile.

**Discussion**

A different rate or degree of accumulation into brain tissue is not the basis for the difference in potency between endrin and dieldrin, nor for the difference in the intensity of action after the administration of equimolar doses. The larger concentration of dieldrin in the brain does explain the greater intensity of action of dieldrin after the administration of equitoxic doses of
either compound. The different rates of distribution for each compound into liver fat and brain may result from a difference in solubility in tissue lipids. If endrin is more soluble than dieldrin in tissue lipids, its distribution would be less dependent on blood supply and it would, therefore, tend to enter all organs at equal rates. Dieldrin, on the other hand, would tend to enter organs which had the highest blood supply and lipid content. Negherbon (1959) has tabulated the solubilities of endrin and dieldrin in various lipids and solvents. No consistent pattern was obvious, however, since dieldrin was more soluble in some, while endrin in others. It is possible that endrin is more soluble in tissue lipids than dieldrin, and that the mouse brain represents an organ high in blood supply and lipid concentration. If so, this would account for the more rapid equilibration of dieldrin with brain, and the more rapid equilibration of endrin with liver, fat, and blood. Woolley and Runnells (1967) have shown that lipids in gray matter of the rat take up DDT faster than lipids in white matter. The authors point out that blood flow is 5-10 times higher in gray matter and, hence, the faster equilibration with blood.

The fact that neither endrin nor dieldrin appeared in the bile over a two hour interval indicated that biliary excretion does not influence the potency or action of the insecticides during the
seizure period. The possibility remains that a polar metabolite of endrin or dieldrin was present but was not detected.

The data in these experiments lead one to hypothesize that differences in potency or intensity of action between endrin and dieldrin result from the interactions of these insecticides with active sites in the central nervous system. It would follow from this hypothesis that endrin might combine with the active site to a greater extent than dieldrin, or induce a certain discrete change in physiological function with greater potency than dieldrin. If one postulates, for instance, that endrin and dieldrin inhibit Na-K-Mg-ATPase leading to altered membrane conduction and consequent seizure excitability, then one must show that endrin has a greater affinity for the enzyme, or that it has greater potency than dieldrin as an inhibitor of the enzyme. When it is possible to show such a relationship or a molecular basis, then the mechanism of action of the cyclodienes and other chlorinated hydrocarbons will begin to reveal itself.
VI. CARDIOVASCULAR RESPONSES TO ENDRIN- AND DIELDRIN-INDUCED SEIZURES

Endrin- and dieldrin-induced convulsions considered in the previous chapters have been skeletal motor manifestations of CNS stimulation. It is possible that the autonomic nervous system also responds to the central nervous stimulation resulting from the action of endrin or dieldrin. Gowdey et al. (1952) demonstrated that central nervous stimulation by aldrin (the epoxide of which is dieldrin) produced bradycardia, vasodepression, miosis, and potentiation of vagal stimulation, reflecting alterations in the autonomic nervous system. Gowdey et al. (1954) repeated these experiments with dieldrin and obtained similar results. They were able to show that the parasympathetic action of dieldrin was exerted through stimulation of central mechanisms rather than peripheral mechanisms.

Between 1964 and 1966, several investigators performed extensive experiments on the cardiovascular effects of endrin (Emerson et al., 1964; Reins et al., 1964; Emerson and Hinshaw, 1965; Emerson, 1965; Reins et al., 1966; Hinshaw et al., 1966). From their work they concluded that endrin had direct effects in two places; the central nervous system and the left heart. Stimulation of the CNS produced activation of sympathetic and parasympathetic nerves, and convulsions of the skeletal muscles.
The latter, in turn, produced a release of metabolites which resulted in decreased vascular resistance, and a decrease in effective gaseous transport in the lungs. This lead to acidosis and hypoxia. Generally, nothing was reported about phasic irregularities in the autonomic nervous system, except that this system was stimulated or activated. These workers made no comment as to whether the effects they measured were specific for endrin or were common to any agent that produced prolonged intense central nervous stimulation. An interesting finding that remained unexplained was that in succinylcholine-treated dogs, blood pressure increased and remained high after i.v. administration of endrin. However, in dogs that were allowed to convulse, blood pressure rose only briefly and then fell to hypotensive levels.

Bircher et al. (1963, 1968) have observed the effects of i.v. PTZ and picrotoxin on blood pressure in dogs. Immobilizing the animals with succinylcholine, they demonstrated that the convulsants produced hypertension, and a hypotensive response was produced in animals without functional sympathetic nervous systems. It was further observed that blood pressure remained constant in sympathectomized and vagotomized animals after treatment with the convulsants.

Bonnevaux et al. (1968) cite several reports on the stimulation of autonomic nerve centers by PTZ. In their studies
with PTZ they demonstrated that sympathetic activation pre-
dominated over parasympathetic, and that anticonvulsants which
blocked convulsions produced by PTZ did not block the effects
of PTZ on the autonomic nervous system.

None of the authors cited have discussed phasic irregularities
induced in autonomic centers. A recent study by Polosa et al.
(1969) examined the effects of picrotoxin on blood pressure in cats.
The purpose of the investigation was to examine oscillations in
blood pressure induced by picrotoxin. They pointed out that
only one observation of this phenomenon was reported in experi-
mental work on the effects of PTZ on the autonomic nervous
system (Gellhorn and Darrow, 1939). Other authors referred to,
who had worked with picrotoxin, PTZ, endrin, or dieldrin, had
never reported the existence of oscillations in the blood pressure.
Polosa et al. were able to show sustained picrotoxin-induced
oscillations of arterial blood pressure in cats anesthetized with
pentobarbital sodium and paralyzed with Gallamine. These
oscillations were considered analogous to vasomotor waves or
Mayer waves. The latter occur in hemorrhage, metabolic acidosis,
and cerebral ischemia. It appeared to the investigators that
the oscillation was a result of epileptiform activity of sympathetic
neurons.

The experiments presented in this chapter were designed to
compare the effects of endrin and dieldrin on the blood pressure of dogs to determine 1) if oscillatory behavior was present during the period of intoxication; and 2) if the responses of the cardiovascular system to exogenous catecholamines were altered.

**Methods**

**Cardiovascular Experiments:**

Mongrel dogs of either sex were anesthetized with sodium pentobarbital, 30 mg/kg body weight, and prepared surgically for arterial blood pressure measurements in intact animals, adrenalectomized animals, and in spinal animals. In all preparations drugs were injected via a cannula in the femoral vein. Endrin and dieldrin were administered in DMSO, using the LD90s of each, determined from the LD50 measurements in mice. The effects of endrin and dieldrin on blood pressure in intact animals, and of endrin in adrenalectomized animals, were recorded from the femoral artery, utilizing a statham pressure transducer. The interactions of curare and endrin were determined in intact animals; the interactions of catecholamines with endrin and dieldrin were determined in spinal animals. Tracheotomies were performed in all experiments.

**Studies in Intact Dogs:**
Groups of five dogs were anesthetized and given endrin 5 mg/kg or dieldrin 15 mg/kg, i.v. in DMSO. Animals were allowed to convulse over a 2-hour period, or until death occurred spontaneously. Blood pressure was continuously monitored during this time.

In order to determine the role of convulsant activity on blood pressure responses, the experiments were repeated using the neuromuscular blocking agent curare. Endrin, 5 mg/kg, was administered i.v. to 5 dogs, and convulsions were prevented by injecting curare whenever necessary. Animals were artificially respired; blood pressure was monitored continuously.

Studies in Adrenalectomized Dogs:

In three dogs, a flank incision was made over the superior aspect of the kidney region. The adrenals were exposed, tied off, and surgically removed. The wound was closed with two or three stitches of surgical silk thread. After adrenalectomy, the dogs received endrin, 5 mg/kg i.v. in DMSO. The animals were allowed to convulse, and blood pressure was monitored continuously.

Studies in Spinal Dogs:

In two groups of 5 dogs the cervical vertebrae were exposed by blunt dissection. Bone was carefully chipped away, and the
exposed spinal cord was severed between C-3 and C-5. The animals were artificially respired and allowed to recover for 45 minutes. At this time they received injections of epinephrine (1 μg/kg), norepinephrine (1 μg/kg), isoproterenol (1 μg/kg), and tyramine (100 μg/kg), and the resulting blood pressure responses were recorded. Control studies in 3 spinal dogs indicated that DMSO did not alter these responses. Animals were then treated with endrin 5 mg/kg or dieldrin 15 mg/kg, the injections of catecholamines were repeated.

Toxicity Studies:

Estimations of the toxicity of endrin were made in a separate group of intact dogs, in order to obtain information on the lethal potency of endrin in untreated animals. Dogs received endrin in DMSO i.v., and were allowed to convulse without restraint.

Results

Studies in Intact Dogs:

Endrin and dieldrin treated animals exhibited several similar tonic and clonic convulsions during the testing period. Endrin induced a slightly higher frequency of convulsions than dieldrin. Spontaneous urination, defecation, and salivation accompanied all
convulsions.

The solvent, dimethylsulfoxide, had little effect on blood pressure by itself, when injected slowly over a ten minute interval. Figures 6.1 and 6.2 show the effects of endrin and dieldrin on the blood pressure of anesthetized dogs. The endrin-treated animals developed a large pulse pressure associated with bradycardia and a rise in mean blood pressure, immediately after the infusion period. During this hypertensive phase, oscillations occurred in the arterial blood pressure, which were apparently not associated with respiration. The oscillations disappeared after 5-15 minutes, at which time the animals became hypotensive and died. Death usually occurred between 20 and 45 minutes after treatment. The oscillations had a frequency of 1-3 per minute. The frequency and duration of the oscillations varied from dog to dog.

Dieldrin-treated dogs did not show the oscillatory response in blood pressure at the dose tested. A brief, biphasic response in blood pressure did occur with the onset of each tonic convolution, but blood pressure remained relatively stable throughout the duration of the experiment. Dieldrin-treated animals did not die during this 2-hour interval.

When dogs were immobilized with curare after endrin administration, blood pressure increased and remained elevated.
Figure 6-1. The effects of endrin (5 mg/kg, i.v.) on systemic arterial blood pressure in the anesthetized dog. Infusion of endrin in DMSO (50 mg/ml) over a 10 minute period began at point 1 and ended at point 2. A = 5 minute recording following infusion; B - F = continuous 30 minute recording, beginning 10 minutes after infusion.
Figure 6-2. The effects of dieldrin (15 mg/kg, i.v.) on systemic arterial blood pressure in the anesthetized dog. Preparation as in Figure 6-1. A thru Z = continuous recordings.
Figure 6.3 shows that immobilization prevented animals from becoming hypotensive, and sustained the oscillatory response of the blood pressure. The frequency of oscillations were relatively constant for any one animal, ranging from 1-3 oscillations per minute among the animals tested.

**Studies in Adrenalectomized Dogs:**

The effects of bilateral adrenalectomy on the cardiovascular response to endrin is shown in Figure 6.4. After endrin administration, animals became hypotensive without passing through a hypertensive phase. Adrenalectomy appeared to facilitate endrin-induced oscillations in blood pressure. Adrenalectomized animals survived endrin treatment longer than intact animals (2 hours vs. 1 hour).

**Studies in Spinal Dogs:**

The effects of endrin and dieldrin in the spinal dog are shown in Figures 6.5 and 6.6. The animals did not convulse in any portion of the body caudal to the level of spinal transection. Blood pressure oscillations did not occur after treatment with the insecticides. Endrin produced a noticeable alteration in the response of the cardiovascular system to injected catecholamines. The responses to norepinephrine and tyramine were approximately
Figure 6-3. The effects of endrin (5 mg/kg, i.v.) on systemic arterial blood pressure in anesthetized dogs immobilized with curare. The infusion of endrin began at point 1 and ended at point 2. A thru C are continuous recordings.
Figure 6-4. The effects of endrin (5 mg/kg, i.v.) on systemic arterial blood pressure in anesthetized, adrenalectomized dogs. The infusion of endrin began at point 1 and ended at point 2. A and B are continuous recordings.
Figure 6-5. The effects of endrin (5 mg/kg, i.v.) on the systemic arterial blood pressure responses to injected catecholamines in the spinal dog. 1 = norepinephrine, 1 ug/kg; 2 = norepinephrine, 2 ug/kg; 3 = isoproterenol, 1 ug/kg; 4 = isoproterenol, 2 ug/kg; 5 = tyramine, 100 ug/kg; 6 = tyramine 200 ug/kg. A = recording prior to endrin administration; B = 20 minutes after endrin administration.
Figure 6-6. The effects of dieldrin (15 mg/kg, i.v.) on the systemic arterial blood pressure responses to injected catecholamines in the spinal dog. 1 thru 5 same as in Figure 6-5. A = recording prior to dieldrin administration; B = 20 minutes after dieldrin; C = 40 minutes after dieldrin.
50% reduced, and the response to isoproterenal was reversed. Diastolic blood pressure fell from 50 mm Hg to 25 mm Hg within 10 minutes after endrin administration, and remained relatively uninfluenced by injections of catecholamines.

Dieldrin did not appear to produce any noticeable alterations in the response of the cardiovascular system to injected catecholamines. A decrease in heart rate was observed during the pressor response to norepinephrine and tyramine, as seen in panels B and C of Figure 6.6. Blood pressure in dieldrin-treated dogs remained stable through the duration of the experiment.

Toxicity Tests:

The results of tests on the lethal potency of endrin in unanesthetized dogs are presented in Table 6.1. The data indicate that the LD50 for endrin in dogs lies between 0.35 and 0.75 mg/kg, i.v. If the animals did not die within one hour, they survived. Each dog exhibited at least two to three tonic seizures, with intermittent clonus.

Discussion

In the intact anesthetized dog, blood pressure responds dramatically to a dose of endrin that is approximately 8-10 times the i.v. LD50 of endrin in dogs. Systemic arterial blood pressure
Table 6.1. The lethality of several dose levels of endrin in unanesthetized dogs. Endrin was dissolved in DMSO (2.5-40 mg/ml) and administered i.v..

<table>
<thead>
<tr>
<th>Dose mg/kg</th>
<th>number of animals</th>
<th>% mortality</th>
</tr>
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<tbody>
<tr>
<td>0.25</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>0.35</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>0.50</td>
<td>5</td>
<td>60</td>
</tr>
<tr>
<td>1.00</td>
<td>2</td>
<td>100</td>
</tr>
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<td>2.00</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>4.00</td>
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generally rises to a maximum and then declines to hypotensive levels. During the rising and/or declining phase oscillations are observed in blood pressure which are not associated with respiration. These oscillations can be made to occur more frequently and uniformly, if the animals are paralyzed with curare and artificially ventilated. Such a procedure reduces, or prevents entirely, anoxia and acidosis in animals treated with convulsant compounds (Straw, 1968; Munson and Wagman; Beresferd, 1969; Collins, Posher and Plum, 1970a). This suggests that anoxia and/or acidosis inhibit rather than cause oscillations in blood pressure following administration of endrin. As long as animals are paralyzed and ventilated they do not become hypotensive, indicating that convulsive skeletal muscular activity leads to terminal hypotension or shock. In adrenalectomized animals, only progressive hypotension occurs after endrin, without an initial hypertensive phase, and oscillations in blood pressure are facilitated. In spinal transected dogs, endrin does not induce any oscillations in blood pressure. These data suggest that endrin acts directly in the central nervous system to stimulate autonomic centers. This, in turn, leads to a slowing of the heart and a release of catecholamines from the adrenal medulla, which both lead to an increased pulse pressure. In the presence of skeletal muscle convulsions, anoxia and acidosis occur
which depress the heart and lead to decreased peripheral resistance and hypotension.

Skeletal muscle convulsions that occur following endrin administration are oscillatory in nature, occurring every 1 to 3 minutes. These may result from an oscillatory waxing and waning of neuronal activity in the central nervous system. Similar phenomenon may occur in central autonomic nerve centers, reflected as oscillations in systemic arterial blood pressure.

Oscillations occur in dieldrin-treated dogs, but are only single, biphasic responses that occur with each tonic convulsion. Blood pressure otherwise remains stable following a 15 mg/kg i.v. dose of dieldrin. If dieldrin had been administered in larger doses, perhaps sustained oscillations would have been present.

In the spinalectomized preparation, the effects of endrin and dieldrin on blood pressure can be observed without the complications of skeletal muscle convulsions or neuromuscular blocking agents. It can be shown under these conditions that endrin causes a decrease in systemic arterial pressure, and significantly alters the response of the cardiovascular system to injected catecholamines. The results suggest that endrin may have some alpha adrenergic blocking activity, and may also have a direct action on the heart. These actions are reflected by a decrease in both
the inotropic response of the heart and peripheral vasoconstriction resulting from injected or released norepinephrine. The reversal of the isoproterenal response may result from the low diastolic pressure following endrin treatment. Peripheral vessels may be fully dilated at this point, and stimulation of beta receptors cannot produce further dilatation. The heart, however, is still sensitive to the beta stimulation of isoproterenal. As a result, only an increase in blood pressure is seen, uninfluenced by active peripheral vasodilation. The finding that endrin alters the response of the heart to catecholamines is consistent with the finding that endrin causes left heart failure (Sowell et al, 1968; Hinshaw et al; 1966).

Dieldrin does not produce this alpha blocking action with the dose used. Possibly at higher dose levels, dieldrin may produce this effect. It would be worthwhile to duplicate these experiments utilizing various doses of endrin and dieldrin, in order to determine if the induced alterations in the cardiovascular system are qualitatively the same for each compound. Previous studies by other investigators using dieldrin intravenously in cats, 7.5 mg/kg, did not report any oscillations in blood pressure or the development of hypotension (Gowdey and Stravraky, 1955). The possibility remains that endrin may affect the cardiovascular system by different mechanisms than dieldrin.
The actions of the anesthetic used may alter the responses of the cardiovascular system to endrin and dieldrin differently. The toxicity tests indicate the i.v. LD50 of endrin in unanesthetized dogs is about 0.5 mg/kg. According to Hinshaw et al, (1966), the i.v. LD50 of endrin in anesthetized dogs is between 2 and 3 mg/kg. It is possible that the LD50 of dieldrin is shifted to an even greater degree by pentobarbital. Data in Chapter IV indicate that the LD50 of dieldrin in mice is changed by 30 mg/kg of pentobarbital, while that of endrin is not. This suggests that pentobarbital prevents dieldrin from influencing the cardiovascular system to the same degree as endrin, but that larger doses of dieldrin would produce the same effects as endrin.

It may be concluded that endrin induces hypertension in dogs, followed by terminal hypotension. During this response, oscillations in blood pressure occur. The hypertensive response can be eliminated by adrenalectomy, and the hypotensive response by curare. Adrenalectomy and curare both sustain the endrin-induced oscillations in blood pressure. Endrin appears to induce a toxic effect on the heart and cause alpha blockade. Dieldrin, at three times the dose of endrin, produces none of these effects, with the exception of a single, biphasic oscillation in blood pressure associated with a tonic convolution. It is suggested that dieldrin is considerably less potent than endrin with respect to insecticide-
induced alterations in blood pressure, or that endrin produces qualitatively different responses on the cardiovascular system than dieldrin.
VII. THE EFFECTS OF ENDRIN ON ISOLATED RAT VENTRICULAR MUSCLE

Introduction

The altered response of the dog heart to injected catecholamines, after endrin administration, led to the conclusion in Chapter VI that endrin may affect the heart directly. It has generally been known that chlorinated hydrocarbons, such as chloroform, sensitize the myocardium to catecholamines. Philips et al. (1946) have shown this to be true for DDT. These investigators found a causal relationship between brain electrical activity and cardiac arrhythmias, induced by sympathetic stimulation during DDT intoxication. Imbesi (1958), in his studies on isolated rabbit heart, was able to inhibit cardiac contractility with aqueous dispersions of DDT in concentrations as small as 0.5 ppm. The only report of a cyclodiene insecticide affecting the heart directly was by Hinshaw et al. (1966), who described left heart failure in dogs intoxicated with endrin. By using an isolated heart-lung preparation they were able to show that the effect of endrin on the heart was direct. In these experiments, left atrial pressures increased, while right atrial pressures remained constant, indicating a failing left ventricle. These investigators did not measure this effect in the intact animal, nor did they attempt to explain what part left heart
failure played in the overall pattern of toxicity.

The purpose of experiments described in this chapter was to determine if the finding in-vivo, that endrin altered the performance of the heart, could be duplicated in-vitro. By utilizing trabecular muscle of the rat, contractile force was measured in the presence of endrin. The influence of endrin on the response of the myocardium to norepinephrine was also determined.

Methods

Hearts were removed from 5 male rats and placed in warm (38° C), modified Krebs-Hensliet solution (Na⁺ 148 mEq, K⁺ 4 mEq, Ca ++ 5 mEq, Mg + 2.5 mEq, Cl⁻ 128 mEq, HCO₃⁻ 25 mEq, HPO₄⁻² 1.2 mM, and glucose 5.6 mM/liter). The left ventricle was opened along the intraventricular septum, held taut with steel pins, and the largest of the trabeculae carneae was dissected out. The muscle was secured at both ends with non-capillary silk thread. The top end of the muscle was connected via the thread to an adjustable micrometer; the bottom was connected to a Statham force-displacement transducer, via a thread that passed through a close-tolerance aperture at the bottom of the bath. After mounting the muscle, it was rinsed several times with the modified Krebs-Hensliet solution. The bath was
aerated with 95% O₂ and 5% CO₂; the pH was adjusted to 7.5.

Muscles were stimulated electrically at a rate of 30/minute by means of platinum field electrodes. Electrical stimuli were generated from a Grass square-wave stimulator and were always supramaximal.

Endrin was dissolved in DMSO, 100 mg/ml, and 0.01 ml of this were added to 1 liter of a separate bath solution, to give a final concentration of 100 ppm of endrin. Norepinephrine hydrochloride was added directly to the muscle bath (10 ml volume) in 0.1 ml injections, yielding a final concentration of 50 ng/ml.

Muscles were allowed to equilibrate for two hours at a resting tension of 1.0 gram. Control responses to DMSO and norepinephrine were then obtained in the 5 trabecular muscles. The muscles were then washed and the bath solution changed to the solution containing endrin, 100 ppm. Isometric contractions in the presence and absence of norepinephrine were recorded over a 40 minute period. At the end of each experiment muscle weight and length were determined. Average muscle length was 7mm with approximately 5.0 mm² cross sectional area. Isometric contractions were recorded on a Sanborn polygraph.

Results
Figure 7.1 shows a representative tracing of muscle contractions resulting from 30/min. electrical stimulation. Each muscle tested developed a different degree of contractile force at the given resting tension, but all muscles responded qualitatively the same way to DMSO, norepinephrine, and endrin. The muscle preparation was stable for 4 or more hours after the muscle was allowed to equilibrate for two hours at 1 gram resting tension.

DMSO caused a slight, reversible, decrease in developed tension when added to the muscle bath. Norepinephrine consistently increased contractile force of the trabecular muscle. Figure 7.2 shows the results of exposing the muscle to 100 ppm of endrin. DMSO, again, decreased developed tension, but by 30 minutes the contractile response was similar to pre-endrin exposure. The inotropic response to norepinephrine did not appear altered by exposure of the preparation to endrin for 40 minutes. Exposure times up to 4 hours did not affect the contractile response of the trabecular muscle.

**Discussion**

Endrin did not alter the development of contractile force in the isolated trabecular muscle of the rat: the rate of tension development and duration of the twitch were not influenced by the
Figure 7-1. The effect of DMSO and norepinephrine on the development of isometric force in rat trabeculae carneae. 1 = control response; 2 = 10 minutes after addition of DMSO, 0.01 ml/10 mls; 3 = 20 minutes after DMSO; 4 = wash; 5 = norepinephrine, 50 ng/ml.
Figure 7-2. The effects of endrin on the development of isometric force in rat trabeculae carneae. 1 = control response; 2 = 10 minutes after addition of endrin in DMSO, 100 ppm; 3 = 20 minutes after endrin; 4 = 30 minutes after endrin; 5 = 40 minutes after endrin plus addition of norepinephrine, 50 ng/ml.
insecticide. Since these parameters reflect the intensity and duration of the active state, respectively (Buccino et al., 1967), endrin did not appear to alter the active state of myocardial contractility. Further proof of this would require more precise measurements of the active state, utilizing "quick-release" experiments described by Sonnenblick (1965).

In view of the reports on the cardiac toxicity of chlorinated hydrocarbons (Imbesi, 1958; Philips, 1946; Hinshaw et al., 1966) it appears that simple aqueous suspensions of endrin are not sufficient for the compound to reach sites of action. Imbesi (1958) used sonically dispersed aqueous suspensions of DDT, while the other investigators cited administered the compound intravenously. In-vivo or in-situ experiments in which endrin was administered i.v., the compound was able to reach cardiac muscle internally through the microcirculation. In the in-vitro experiment described in this chapter, endrin probably collected only on the surface of the muscle.

The best approach to this problem would be to examine cardiac muscle tissue from animals chronically exposed to endrin. Tension development can be normalized by expressing tension as grams/mm² (grams per cross sectional area of muscle). This permits the comparison of two separate populations of cardiac tissues (Buccino et al, 1967). Thus, a group of animals treated
with endrin for a specific time could be compared to a control group, and changes in the active state of myocardial contractility could be detected in this manner.

Recent work in mechanisms of cardiac contractility have attempted to correlate mechanical activity with electrical activity. Braveny and Sunbera (1970) have shown the relationships of the action potential to characteristics of the isometric twitch. The relationships observed implicated the role of calcium and potassium in the contractile process. A study of the influence of endrin on contractility and associated membrane phenomenon might indicate more precisely what effect the compound has. Such an experiment was performed in isolated rat atria to assess the influence of nicotine on transmembrane potentials and contractility (Shibata, 1968). Nicotine increased the duration of the action potential and simultaneously increased developed tension. The authors suggested alterations in potassium fluxes as a cause. Experiments such as these might establish a definite correlation between insecticide-induced alterations in membrane activity and alterations in physiological performance. Since pentylenetetrazol has been shown to affect the heart directly (Gillen and Covino, 1962) this approach may offer a tool for comparing the insecticides and metrazol on membrane electrical activity.

In order to confirm conclusively the finding that endrin
causes left heart failure (Hinshaw, et al., 1966), it would be best to compare cardiac function curves in treated and control dogs. Bishop, Stone, and Guyton (1964) have described a method for determining ventricular performance in conscious dogs. Such a study would provide some quantitative information about the degree of failure induced and how this was related to dose. Such a method would allow chronic or acute intoxications to be evaluated.

Results from the experiments presented in this chapter suggested that endrin does not affect the heart directly. The possibility remains that in heart-lung preparations in which heart failure was observed (Hinshaw et al., 1966), endrin may have precipitated in coronary vessels resulting in anoxia and, consequently, failure.
VIII. GENERAL DISCUSSION

The mechanism of action of chlorinated hydrocarbon insecticides on the mammalian central nervous system remains unexplained. As a result, it has not been possible to ascertain the potential toxic hazard that these compounds possess, other than their acute lethal potency. One approach to this problem is to compare the in-vivo effects of the compounds under various conditions in order to determine the possibility that they act by a similar mechanism. If evidence indicates that such a possibility exists the insecticides could be compared at a molecular in-vitro level, such as the natural or artificial membrane. The in-vitro actions of the insecticides should parallel their actions in in-vivo experiments, if the mechanism of action of the compounds truly functions at the proposed in-vitro level.

The purpose of experiments described in this thesis has been to provide in-vivo evidence that two chlorinated hydrocarbon insecticides, endrin and dieldrin, act by the same mechanism. It has been shown that endrin has approximately 5 times the lethal potency of dieldrin in mice after intravenous administration. At several equimolar doses, endrin acts with greater intensity than dieldrin. The greater lethal potency and greater intensity of action of endrin cannot be explained by differential distribution of
these compounds into the brain or other tissues. The difference between these two compounds must be based on the nature of interaction with active sites in the central nervous system. Both compounds produce qualitatively the same type of central nervous system stimulation. The potency of each compound in this respect is not altered by aggregation or changes in ambient temperature, but the potency of each is decreased by induced hypothermia. The intensity with which these compounds produce CNS stimulation is qualitatively and quantitatively different. Aggregation or increased ambient temperatures increase the intensity of action of endrin, but not dieldrin. Decreased ambient temperatures decrease the intensity of action of both. The potency and intensity of action of dieldrin are more susceptible to changes by drug pretreatment than those of endrin. These data indicate that endrin and dieldrin act with a similar active site, but with different intrinsic activity and/or affinity. It has further been shown that endrin may act directly on the cardiovascular system with greater potency or by a separate mechanism than dieldrin. Any interpretation of the data may be based on two possible hypotheses: 1) endrin and dieldrin interfere with some biochemical process which leads to the accumulation, depletion, or inactivation of a substances(s), resulting in altered
neuronal activity; and 2) endrin and dieldrin interact directly with nerve membrane components resulting in altered neuronal activity. The first hypothesis is useful because it accounts for the delay in action, i.e., the latent period. The second hypothesis is useful because it accounts for the electrophysiological alterations these compounds produce in nerve membranes. The second hypothesis does not necessarily exclude an explanation for the latency of endrin and dieldrin. In any event, endrin and dieldrin can be considered to react with a receptor, whether it is an enzyme, a reactant, a product, or a structural component of a membrane. Such an interaction with the receptor leads to a well-defined series of events.

Picrotoxin is thought to exert its effect by interfering with inhibitory processes. The possibility remains that this involves a biochemical lesion, and would thus require a latent period for the effect to occur. In Chapter IV it was observed that endrin and dieldrin induced a seizure pattern that resembled picrotoxin's more so than pentylenetetrazol's. From this it was inferred that the cyclodienes may combine with a similar receptor as picrotoxin. Because there is insufficient data on the mechanism of all these compounds, only speculation is possible as to how they induce CNS stimulation. It would appear that the compounds cross the blood-brain barrier and interact with some receptor leading
to an unknown number of intermediate steps resulting in altered neuronal activity. While this appears obvious and oversimplified, the mechanism of action of the cyclodienes has not yet been considered from a "receptor" point of view. Some authors have considered binding phenomenon, others alterations in neuronal activity, but no one has yet attempted to organize this information into a uniform story that traces the action of these compounds from the moment they enter the blood stream to the time physiological symptoms appear.

The relationships between endrin and dieldrin under various test conditions can be explained most simply if they are considered to act on the same receptor. This would be reasonable when considering their similarity of structure. The single structural difference that exists between these compounds does not suggest that they would act with different receptors, but that they would act differently with the same receptor. The explanation of the actions of endrin and dieldrin is further simplified if the receptor is considered to exist in the neuronal membrane. This is not an unwarranted assumption: DDT and Dieldrin have been shown to have a selective binding capacity with nerve ending particles (Matsumura and Hayashi, 1969), and DDT has been shown to act directly on the nerve to alter its electrophysiological actions (Narahashi, 1969). The fact that the effects of endrin and
Dieldrin can be most easily explained by the hypothesis that they act on a similar receptor in the nerve membrane does not prove that they actually do. It does, however, indicate that the results obtained are consistent with such an hypothesis and therefore, do not eliminate it as a possible mechanism.

Considering, then, the possibility that endrin and dieldrin interact with the same receptor in the nerve membrane, the following series of events would probably occur. After injections of equipotent doses of endrin or dieldrin, dieldrin-treated mice would receive 3 times as much dieldrin as endrin-treated mice would receive endrin. Thus, the dieldrin-treated group would have received 3 units of dieldrin for every unit of endrin in the endrin-treated group. However, for every 3 units of dieldrin that cross the blood-brain barrier, only 1 unit of endrin crosses the barrier. Conceivably, then, 3 times as many receptors bind with dieldrin than endrin after the equitoxic doses. At the same time, it takes X minutes for dieldrin to act, while it takes 2X minutes for endrin to act. This implies that endrin binds more efficiently to the receptor than dieldrin. Consequently, it would have more intrinsic activity and/or more affinity. Dieldrin, by binding with less efficiency, to a larger number of receptors, brings into play many more neuronal circuits. Once the oscillator is triggered by dieldrin, more pathways are involved than when endrin triggers the oscillator. Hence, neuronal activity spreads more quickly
making the LT50 of dieldrin shorter.

Under the same conditions, when equimolar doses are administered, the data indicate that for every 3 units of dieldrin that cross the blood-brain barrier, 3 units of endrin cross the barrier. In this case, an equal number of receptors bind with dieldrin and endrin, and it takes X minutes for dieldrin to act while it takes only \( \frac{2}{3}X \) minutes for endrin to act. This means that if endrin is administered in a dose of 15 mg/kg instead of 5 mg/kg, more neuronal circuits would be affected simply because more receptors are involved. If at the same time endrin has greater intrinsic activity than dieldrin, this would explain why endrin-treated animals die more fast than dieldrin-treated animals after equimolar dosage.

A reason why endrin reaches the brain more slowly than dieldrin could be related to the lipid solubilities of the compounds. As the compounds pass through the circulation dieldrin tends to move into the most readily available lipid sites, while endrin may distribute more evenly in all available lipid sites. One would conclude that given enough time dieldrin distribution might equal endrin distribution. The data in Chapter V indicate that such is the case.

The question that comes to mind is how to apply this hypothesis to alterations caused by ambient and body temperature
fluctuations, aggregation, and drug pretreatment. High ambient temperatures and aggregation act as forms of environmental stress. As such, they may increase the level of neuronal activity in the CNS. This may facilitate the triggering of the oscillator and/or facilitate transmission through mediating circuits that ultimately synapse on the final common motor pathway. The reason that dieldrin is not altered by aggregation or increased ambient temperatures may be due to the comparatively larger number of receptors involved during dieldrin intoxication. Less neurons would be available for facilitation by the effects of environmental stress that were not already under the influence of dieldrin. Endrin, on the other hand, may involve about 1/3 the number of neurons as dieldrin (at equitoxic doses), and the remaining percent of neurons are left free for facilitation by the influence of environmental stress. In support of this, it was also observed in our laboratory that intense auditory stimulation by a door bell caused isolated mice to die as quickly as aggregated mice after endrin treatment.

Hypothermia and low ambient temperatures (which tend to cause hypothermia in endrin or dieldrin-treated mice) may slow down the rate of binding of the insecticide to the receptor, as well as slowing down or attenuating processes in nerve conduction. Hypothermic conditions may inhibit normal processes of nerve
facilitation such that a significant decrease in the usual number of facilitated pathways may occur. Consequently, more insecticide would be required to produce the lethal response, hence the increase in LD50s and LT50s.

Drugs effective against the actions of endrin and dieldrin all have the ability to cause CNS depression. As such, they may be considered to cause some type or degree of decreased neuronal activity. The drugs may be depressing oscillatory and mediating nerve networks directly, as well as interfering with the binding of the drug to the receptor. Since these drugs are significantly more effective against dieldrin than endrin suggests that dieldrin has less affinity for the receptor than endrin and is more easily displaced by the drugs. Such would not be the mechanism for lactic acid or lactate, however. These compounds were effective in aggregated mice but not in isolated mice, indicating their main function was depression of oscillatory and mediating neuronal activity. Since trimethadione was not effective in the dose used, the insecticides probably do not act by the same mechanism as metrazol, which is antagonized very effectively by trimethadione.

The discussion so far has considered mechanisms leading only to somatic activity, but analogous mechanisms could be involved leading to autonomic activity. For example, increased
release of norepinephrine in the hypothalamus may be the cause of the influences of ambient temperature on the thermoregulatory responses to endrin and dieldrin (Cremer and Bligh, 1969). The differences between endrin and dieldrin in their effects on body temperature may reflect a quantitatively greater effect of endrin on the autonomic system. This is most likely the case in blood pressure experiments, recording the induced oscillations in blood pressure. Endrin may be more potent than dieldrin in this respect. According to Polosa et al. (1969), the oscillations appear to be the result of epileptiform activity of sympathetic neurons.

Activity in either the somatic and autonomic systems could very easily arise from the same mechanism of interaction of endrin and dieldrin in the nerve membrane. Differences between the action of endrin and dieldrin could possible reflect their differences in intrinsic activity and/or affinity for the same receptor site. As was stated previously, this is the simplest explanation that could account for the differences and similarities between endrin and dieldrien in the mouse. The alternative hypothesis, that a biochemical lesion is involved in the action of the compounds, does not appear as satisfactory. In the case of other convulsants such as hydrazides, this kind of hypothesis is tenable. According to Abrahams and Wood (1970), a correlation was evident between the rate of decrease in concentration
of cerebral GABA and the susceptibility of chicks to hydrazide-induced seizures. The lag time for these types of seizures was not caused by a slow penetration of the agents into the brain, but by a rate limiting step decreasing the levels of cerebral GABA. However, Colhoun (1960) has found no evidence of biochemical lesions in dieldrin-treated cockroaches. Biologically active substances appeared in the blood of the cockroach during the period of intoxication, but appeared to be a consequence and not a cause of dieldrin intoxication. The appearances of other substances in the blood such as lactate, pyruvate, and products of mitochondrial damage probably also reflect the results of intoxication and not the cause.

One recent finding offers an exception to the criticisms of this alternative hypothesis. Matsumura and Patil (1969) have shown that Na-K-ATPase is selectively inhibited by DDT. Assuming that Na-K-ATPase actually functions as the sodium pump, then it is feasible that inhibition of this enzyme is the basic mechanism of action of DDT and perhaps other chlorinated hydrocarbon insecticides. A significant amount of time would be required following enzyme inhibition (or poisoning of the pump) for the concentration gradients across the nerve membranes to be significantly reduced to cause a decreased membrane potential and, hence, increased membrane excitability. A previous report,
however, presents conflicting data. Koch (1969) reported that DDT had little effect on Na-K-ATPase from rabbit brain. Other insecticides tested affected Mg-ATPase to a greater extent than Na-K-ATPase. The data of Matsumura and Patil thus became questionable.

An even more recent report by Sanders et al. (1970a) suggests that the reverse of Matsumura and Patil's hypothesis is a more correct interpretation of the interaction of DDT with ATPase. According to Sanders et al., brain ATP levels decrease prior to convulsions. This interferes with maintenance of the membrane potential since the main energy source has been eliminated by the action of the convulsant material. The authors suggest that convulsants such as picrotoxin and hydroxylamine (administered i.p.) may interfere with nicotinamide adenine dinucleotide, inhibit glutamic decarboxylase and gamma-amino butyric acid transaminase, or stimulate Na-K-ATPase. A criticism of this work was put forth by Collins and Posner (1970b). They cited available evidence that seizures can begin, proceed, and stop independently of ATP concentrations in the brain. Sanders et al. (1970b), in rebuttle to this criticism, point out exceptions in the evidence cited by Collins and Posner, and stress their opinion that the decrease in ATP prior to convulsive activity is certainly no artifact.
It should be emphasized at this point that the hypothesis considered in this dissertation as most probable with respect to endrin and dieldrin, i.e., that endrin and dieldrin combine with nerve components to alter nerve membrane function, is by no means contradicted by the work of Sanders et al. (1970). If endrin and dieldrin open up "channels" in the membrane and thereby increase the conductance of sodium while decreasing that of potassium (Narashi, 1969), then a natural result of this would be an increase in the rate of formation of ATP. This increased rate would oppose the action of the insecticides by increasing and maintaining pump activity. As long as this occurred, no convulsive activity would be expected. Eventually, however, ATP production would decrease after the synthetic material was depleted. At this point, membrane potential would decrease and convulsive activity would begin. The initial increased production of ATP would, therefore, account for the latent period.

Weikel et al. (1958) compared the effects of endrin, dieldrin and DDT on the red cell membrane. Dieldrin and DDT, but not endrin, inhibited the phosphate exchange rate. DDT, but not endrin or dieldrin, caused a selective increase in the permeability of the erythrocytes to sodium. The insecticides were required in such large amounts (1 mg/ml blood) to produce the effects that the significance of the findings is questionable. The
red cell membrane, on the other hand, would offer an excellent model to test the hypothesis of Matsumara and Patil (1969) and that of Sanders et al. (1970). The red cell membrane ATPase function could be evaluated in the presence of the insecticides and correlated with any changes in ion distribution or membrane potential (determined by chloride distribution). The hypothesis of Matsumura and Patil would predict a positive correlation: altered membrane potential as a direct function of ATPase inhibition. The hypothesis of Sanders et al. would predict no correlation, or perhaps ATPase activation.

The hypothesis that the chlorinated hydrocarbon insecticides act directly on the membrane is further supported by experiments on the action of DDT on artificial membranes. Hilton and O'Brien (1970) have shown that potassium conductance which was naturally induced in the artificial lecithin-decane membrane by addition of $10^{-6}$ Molar valinomycin was reversed by $10^{-6}$ Molar DDT. This was analogous to the DDT-induced decrease of potassium conductance in the nerve membrane reported by Narahashi (1969). An interesting finding was that dieldrin and lindane did not have such an effect on the artificial membrane. In the opinion of Hilton and O'Brien, it was unlikely that an effect on the "pump" could be the cause of the neurotoxic actions of these insecticides. Such an effect would cause depression and failure of axonic
transmission, rather than the excitatory effect which is observed experimentally. On the other hand, failure and depression of axonic transmission may be precisely what occurs during postictal depression.

The action of chlorinated hydrocarbon insecticides may be exerted on membranes other than nerve. Data in Chapter VI, plus other quoted references, suggested that endrin interferes with heart function directly. It is interesting to note that DDT induces in the cockroach giant axon (Narahashi and Yamasaki, 1960) and in the lobster giant axon (Narahashi, 1969) an action potential that is identical to the cardiac action potential. The effects of endrin and other such insecticides on the heart after long term chronic exposure is unknown. According to the U.S. Bureau of the Census (1970): from the ten leading causes of death, diseases of the heart rank first. The death rate due to heart disease has increased steadily from 1949 to 1966. One speculation that comes to mind is whether environmental contamination by chlorinated hydrocarbon insecticides has contributed to this increased rate. This possibility should be examined, since people all over the world are continually exposed to these compounds. In criticism of Hinshaw et al. (1966) with respect to their finding of direct actions of endrin on the heart, it should be kept in mind that endrin was administered intravenously in their
experiments. Klein et al. (1968) have shown that on the last day after 12 days of chronic endrin feeding in rats there was more endrin (ppm) in the spleen, blood, intestine, ovaries, skin and subcutaneous fat than in the heart. Five days after the experiment there was also more endrin in these other tissues than in the heart. However, 24 hours after an i.v. dose of endrin, heart had the highest concentration of all tissues tested. This suggests that endrin may not mix rapidly with the blood and, unless the compound is injected very slowly, it may precipitate or become lodged in the microcirculation of the coronary vessels. This could easily lead to a failing heart, as mentioned in Chapter VII.

It may be concluded from the data presented in this dissertation that endrin and dieldrin have qualitatively and quantitatively different biological effects, in spite of their close chemical structure. These differences occur at the site of action and are not a result of differences in the ability of the compounds to reach the site of action. These differences can be most easily explained by the hypothesis that endrin and dieldrin act on a similar receptor in the nerve membrane with different affinity and/or intrinsic activity. To this extent the data tend to support such an hypothesis, rather than exclude it. Proof of this hypothesis
would require the demonstration of a positive correlation between the physiological effects of endrin and dieldrin in the whole animal with the electrophysiological effects of these compounds on natural and artificial membranes. Such a correlation would have to persist in the presence of manipulations in ambient temperatures, and in the presence of drugs antagonistic to endrin and dieldrin. Data in this dissertation are insufficient to make any conclusive statements about the relationship of the cyclodienes to pentyletetrazol and picrotoxin, except that endrin and dieldrin may act by the same mechanism as picrotoxin. Endrin does not affect the heart tissue when applied to in-vitro preparations, but evidence indicates that intravenous in-vivo exposure depresses heart action.

It is the opinion of this investigator that further research on the chlorinated hydrocarbon insecticides should deal with the precise mechanism of action of these compounds. It remains to be shown whether or not all chlorinated hydrocarbon insecticides act by the same mechanism. If not, one of these compounds may be potentially less of a hazard to the human population than others. The organophosphates may be used as an example: the mechanism of action of these compounds is known with relative certainty. Consequently, they can be used with appropriate precautions, and the danger of these compounds is minimized.
If the mechanism of action of chlorinated hydrocarbon insecticides can be known with the same degree of certainty, then this may represent a significant contribution to the problem of long-term insect eradication and its associated hazard to the human population.
IX. SUMMARY AND CONCLUSIONS

The intravenous toxicity and tissue distribution of endrin and dieldrin have been compared in mice. Endrin has five times the lethal potency of dieldrin, and the potency of each remains relatively unchanged by alterations in ambient temperature or social aggregation. Induced hypothermia decreases the lethal potency of both compounds. The intensity of action of endrin or dieldrin measured as the reciprocal of the time to death after administration of a lethal dose, is decreased by decreases in ambient temperature. Increases in ambient temperature, or aggregation of animals, increases the intensity of action of endrin, but not that of dieldrin.

Endrin and dieldrin produce a similar pattern of convulsions of specific duration that resemble picrotoxin-induced seizures. At equilethal doses, dieldrin induces a shorter duration of seizures than endrin; at equimolar doses, the reverse is true. The effects of dieldrin appear to be antagonized more easily than those of endrin by several types of drugs.

Endrin and dieldrin have similar patterns of tissue distribution in mice after i.v. injections of equilethal doses. Dieldrin equilibrates sooner with brain, while endrin equilibrates sooner with liver, fat, and blood. The rate of accumulation of dieldrin and endrin into the brain does not correlate with the onset and
development of the seizure pattern.

Endrin appears to have alpha-adrenergic blocking properties that lower peripheral resistance and antagonize the effects of injected catecholamines. There also appears to be a direct action of the compound on the heart, depressing its inotropic response to injected catecholamines. Endrin produces recurring oscillations in systemic arterial blood pressure that are not associated with respiration. Dieldrin either does not cause these same effects on the cardiovascular system, or it is much less potent than endrin in this respect. Endrin does not appear to alter the performance of in-vitro preparations of rat heart muscle.

These findings suggest that differences in potency or intensity of action between endrin and dieldrin are not based on differential tissue distribution, but result from the nature of interaction between the insecticides with active sites in the central nervous system. The data provide evidence that endrin and dieldrin interact with the same active sites, but that endrin has greater intrinsic activity and/or affinity for the active sites. Endrin may exert a direct affect on the cardiovascular system resulting in depression of the heart and alpha blockade. Dieldrin may be considerably less potent than endrin in producing these effects, or may not produce them at all. It is suggested that further research on endrin, dieldrin, and other chlorinated
hydrocarbons should correlate the \textit{in-vivo} interactions of these compounds with interactions in \textit{in-vitro} systems.


Zavon, M. R.. The toxicology and pharmacology of endrin. The Kettering Laboratory, Department of Preventive Medicine and Industrial Health, College of Medicine, University of Cincinnati, Cincinnati, Ohio.