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Marcus Auke Topinka

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E. M. Eisenstein
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THE EFFECTS OF DIELDRIN AND CHLORDIMEFORM ON LEARNING AND MEMORY IN THE COCKROACH, PERIPLANETA AMERICANA

by

Marcus Auke Topinka

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ABSTRACT

THE EFFECTS OF DIELDRIN AND CHLORDIMEFORM ON LEARNING AND MEMORY IN THE COCKROACH, PERIPLANETA AMERICANA

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A one-session T-maze training procedure for cockroaches was utilized to investigate the effects of dieldrin and chlordimeform on learning and memory. Animals were trained and then tested five hours later. In control animals the number of correct choices, the runway time, and the choice point time increased with succeeding trials during training. Control animals showed retention of correct choice behavior from training to testing.

A non-toxic dose (no overt symptoms) of dieldrin was injected two hours before training or 15 minutes after training. Pre-training injections of dieldrin eliminated correct choice behavior but did not eliminate increased runway or choice point times. Post-training dieldrin administration did not interfere with retention of correct choice behavior upon testing. Non-toxic doses of chlordimeform injected one hour before training eliminated correct choice learning and facilitated increased runway times during training.

Dedicated with love to my Parents,
Karel and Erika Topinka, who have given
moral support throughout my life.

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INTRODUCTION

Pesticides are used extensively worldwide. In the United States alone production tonnage in 1975 for all pesticides was 1.61 billion pounds, a gain of 13.5% over 1974. Furthermore, it is estimated that tonnage sold will increase 5%/year (Ouellette and King, 1977). Assessment of the toxicology of this chemical load on living systems usually involves measures of body residues, organ weight changes, biochemistry studies, histopathologies, and gross overt behaviors (Smith et al., 1976). Rarely is research directed at the effects of toxicants on behaviors such as learning and memory. This is especially of concern since many pesticides, in this case dieldrin and chlordimeform, are neurotoxicants. The study of toxicant-behavior interactions has been termed behavioral toxicology and includes such disciplines as toxicology, neurophysiology, and experimental psychology (Van Gelder et al., 1973). A recent example of behavioral toxicology is a study which tested the learning and memory performance of chemical workers exposed to Polybrominated Biphenyls (PBB) (Brown et al., 1981).

One major concern in behavioral toxicology is the selection of a model system which will reveal subtle

behavior alterations to toxicants. Certain behaviors in mammals, for example, may be neuronally distributed in different regions of the brain such that a toxicant-produced biochemical or histological lesion could be masked. Such a possibility is suggested by the fact that to see pesticide-induced behavioral effects in mammals and birds often requires toxic (overt symptomology) chronic doses. In addition, mammalian behavioral studies are often mechanically difficult, time consuming, and more difficult to interpret. "Simpler" neural systems, such as invertebrates, offer the possibility of fewer variables involved in analysis due to at least a 10^5 decrease in the number of neurones involved (Eisenstein, 1972). In addition, behavioral plasticity to sensory input is less in invertebrates than mammals. These factors plus the less distributed nature of neuronally stored information should result in easier detection of lesion-induced behavioral alterations. It is assumed that the results of such studies with "simpler" systems will apply to more "complex" organisms (see Literature Review).

This study was undertaken to test effects of low (no overt symptomology) acute doses of pesticides (dieldrin, chlordimeform) on T-maze learning and memory in the cockroach, Periplaneta americana. The development of a one-session T-maze training procedure for the cockroach could prove to be a reliable, inexpensive, quick,

and highly sensitive method for evaluating pesticide-behavior interactions.

LITERATURE REVIEW

Dieldrin

Dieldrin is a member of the cyclodiene group of chlorinated hydrocarbon pesticides and consists of not less than 85% of the compound HEOD. Dieldrin has a relative low oral LD_{50} (60 mg/kg) and is very persistent in the environment. Caro et al. (1976) estimated that it takes approximately 13 years for 95% disappearance of dieldrin from soil. This estimation was made by measuring its persistence in soil and losses due to runoff, plant uptake, and volatilization. Dieldrin is insoluble in water and only treatments with strong acid and long exposure to ultraviolet light are known to decompose it (Matsumura, 1975). Due to this persistence and the notoriety of organochlorine compounds (lipid solubility, bioaccumulation, known DDT toxicity), dieldrin use in the United States has been banned since 1974 except for subsurface insertion for termite control, dipping of non-food roots and tops, and for moth-proofing by closed system manufacturing processes.

However, because of dieldrin's long life span in the environment and the unrestricted use elsewhere in the world, it still remains a potential threat to the

biosphere. For example, residue levels of DDT, dieldrin, and PCB in brain tissue of birds correlates well with death (Sielo et al., 1977). The route of toxic dieldrin accumulation through the food chain is complex. The death by dieldrin poisoning in 1976 of a zoological collection of owls was traced to insecticide-treated timber (Jones et al., 1978). The sawdust used by the mouse supplier as bedding was obtained from a builder who was using timber preservative containing dieldrin to protect against woodworm. As late as 1977 the death of gray bats was linked to dieldrin poisoning from prey insects that developed in dieldrin treated Missouri cornfields (Clark et al., 1978).

This toxicity is largely due to dieldrin's neurotoxicity. Convulsions are a general overt sign of dieldrin intoxication throughout much of the animal kingdom. It is thought that these convulsions are a response to decreasing inhibitory or increasing excitatory inputs to cortical cells rather than altered stimulus input. Joy (1976) used recovery cycle analysis as an indirect assessment of cortical excitability changes subsequent to stimulus presentation in cats. Pairs of stimuli pulses separated in time allowed changes in the neuronal population to be seen upon reception of the second stimulus. Using this analysis, Joy found that dieldrin reduced or abolished the early facilitative (5-10 msec) and later

depressive (40-320 msec) consequences of paired stimulation on visual cortical cells. Abolishment of early facilitative responses is explained on the basis that more cortical cells are responding to the first stimulus than normally would and therefore leaving fewer neurons to respond to the second stimulus. Abolishment of the later depressive responses is explained by dieldrin-increased excitatory activity feeding into the cell population. Joy (1974) cites support of this by the fact that the amplitude of single responses is increased by dieldrin. It is possible that these changes to sensory stimuli increase excitatory cortical processes rather than decrease inhibitory processes. Dieldrin has been shown to have no effect on recurrent collateral inhibition in motor cortex pyramidal cells (Joy, 1975).

Axonic membrane seems to be the primary target for dieldrin in the nervous system. In the cockroach, B. germanica, 50 to 60% of all dieldrin entering the nervous system is bound to axonic membrane (Matsurmura, 1975). Likewise, Coons et al. (1973) found that tritated dieldrin was localized as silver grain deposits in neural lamella and membranes surrounding axons in the neurophile region of housefly thoracic ganglia. Also, it has been shown that isolated myelin from susceptible and resistent fish treated with dieldrin showed greater retention by susceptible fish (Wells and Yardbrough, 1972).

Furthermore, it is known that the mean concentration of dieldrin in human brain white matter is significantly higher than that found in grey matter (de Vlieger et al., 1968).

Electrophysiologically, there is conflicting evidence as to whether dieldrin or aldrin-transdiol (a metabolite of dieldrin) is responsible for recorded neurotoxic actions. Van der Berken and Narahashi (1974) showed that aldrin-transdiol caused a blockade of the action potential and a depolarization of squid giant axon membrane. Dieldrin itself had no effect. Application of aldrin-transdiol to isolated spinal chords of toads produced a potentiation of spinal reflex activity and an increase in spontaneous activity of ventral and dorsal roots (Akkermans 35 al., 1975). Aldrin-transdiol also produced a marked reduction of spinal inhibitory mechanisms in this preparation. The excitatory effects of aldrin-transdiol were followed by a strong depressant action on spinal excitability. Again, dieldrin had no effect on the isolated spinal chord. On the other hand, Joy (1977) implicates dieldrin as the major toxicant. He intravenously injected dieldrin and aldrin-transdiol to cats to test whether dieldrin is directly active or whether it requires conversion to aldrin-transdiol for CNS effects. Two to four mg/kg of dieldrin produced convulsive activity in 2-15 minutes and was preceeded by

changes in the EEG and in responses evoked by sensory stimuli. Aldrin-transdiol when administered in doses up to 200 mg/kg had no effect. Joy accounts for reported dieldrin/aldrin-transdiol differences by noting that the previous experiments were in vitro. In vitro alcoholic solutions of dieldrin form opalescent suspensions which make the effective concentrations only an estimate. Also, brain sensitivity to dieldrin is much greater than it is for the spinal chord or peripheral nerves.

The events in the mechanism for dieldrin's neural toxicity seem to be time dependent. Walsh and Fink (1972) observed that the concentration of dieldrin reached equilibrium before the appearance of the seizure components. One possibility that may influence time of toxicity is inhibition of ATPases. ATPases associated with oxidative phosphorylation and cation transport (Mg^{++} ATPase and Na^{+} - K^{+} ATPase, respectively) are known to be inhibited by dieldrin and other chlorinated hydrocarbons (Koch, 1969). In experimental fish, the amount of inhibition of the ATPase system was noted maximum in brain followed by gill, kidney, and liver tissue (Verma et al., 1978). This is further evidence that the primary toxicity from dieldrin is directed at nerve tissue. Verma further notes that increased inhibition is seen with increased concentration of the pesticide. However, an unusual stimulation of Na^{+} - K^{+} ATPase activity has

been reported (Desaiah and Koch, 1975). Desaiah and Koch (1975) also report that aldrin-transdiol had no effect on ATPase activity from fish brain homogenates. Since dieldrin binds to membrane, and if inhibition of ATPases is a major mode of the observed toxicity, then a mechanism can be postulated. Dieldrin binding to membrane could alter the configuration of the ATPases. An inhibition of the ATPases would reduce the available ATP needed for active transport of metabolites and cations needed for nervous transmission.

Another biochemical effect of dieldrin is reduction of certain biogenic amines. Wagner and Greene (1977) demonstrated that a single oral dose of dieldrin at 50 mg/kg to male rats produced overt neurotoxicity and was accompanied by a significant decrease in norepinephrine. No change in dopamine concentration was observed. Serotonin concentrations decreased in both sexes two hours after acute dieldrin exposure. Kohli (1977) demonstrated an unusual increase in rat brain serotonin levels 24 hours after exposure to 30 mg/kg. Heinz et al. (1979) found depletion of both dopamine and norepinephrine in brains of ring doves fed doses of 4 and 16 ppm dieldrin. At 16 ppm dieldrin, dopamine and norepinephrine were depressed to 41.4 and 62.0% of controls, respectively. Wagner and Greene (1977) also showed that chronic administration of dieldrin produced norepinephrine depletion

in selected brain areas (hippocampus) rather than whole brain tissue. It is a possibility that alteration of biogenic amines could produce sensory, motor, or endocrine dysfunction leading to overt toxicological symptoms or more discrete behavioral changes.

It is not totally agreed upon whether dieldrin toxicity also produces structural degeneration at a cellular or organ level of organization. Harr et al. (1970) found focal degeneration and necrobiosis of the endothelium and motor neurons in rats fed varying doses (0.08-40.0 ppm) of dieldrin. However, he states that although as much as 10% of the neurons, glia, and vasculature of the affected brain was degenerated, lesion scores did not correlate well with the incidence of convulsions, cranial edema, or the concentration of dieldrin residues in the brain. Uzoukwu et al. (1972) did not find any of these histopathological lesions in the central nervous system of dieldrin treated guinea pigs but did notice swelling of cerebral cortex mitochondria. Bergen (1972) reported dieldrin diets of one and five ppm to young rats had no effect on brain growth. Brain weight was unaffected by dieldrin treatment. Furthermore, RNA and DNA concentrations in the brain of young growing rats fed five ppm dieldrin were either similar to or higher than the concentrations found in the controls after two and four weeks. Higher nucleic acid levels usually express

improvements in protein nutrition. Bergen et al. (1974) found similar results in more sensitive suckling rats.

Although brain structure does not seem to be altered, there are definite changes in EEG recordings of brain after injection of dieldrin. Burchfiel et al. (1976) demonstrated in Rhesus monkeys that dieldrin treatment caused significant increases in the relative amount of beta voltage after a single symptomatic exposure or a series of subclinical exposures. Moreover, these changes persisted for one year. Burchfiel also mentions that potent psychoactive agents increase beta activity comparable to that seen in his study.

The striking possibility exists that dieldrin's effect on ATPases, biogenic amines, and electrophysiologic nerve function could lead to behavioral disorders. Bildstein and Forsyth (1979) studied the effect of dieldrin on the anti-predator response of the white footed mouse. Using hawk flyover experiments, they observed a decrease in the usual freeze response. Dahlgren and Linder (1974) found that offspring of parent pheasants given dieldrin chose the deep side of a visual cliff more often and were more susceptible to hand capture than controls. These effects persisted throughout the second and third generation suggesting a genetic mechanism. Virgo and Bellward (1977) found that dieldrin produced a congenital inviability in mice caused by a reduced tendency

of the dam to nurse. Sharma et al. (1976) demonstrated a decline in the tendency for dieldrin exposed mallard ducks (drakes) to take the initiative and establish rights of access in approach confrontations. Gesel et al. (1979) trained bobwhite quail to peck a green light to receive food. Responses were monitored for 14 days at which time the birds were given dieldrin for 42 days and their response performance was followed. All birds that had at least 5.73 ppm of dieldrin in their brain tissue underwent change in peck behavior. These changes were increased response time and misdirected pecks.

All experiments mentioned thus far used high chronic dieldrin dosages. Mortality, and overt toxic symptoms were observed in many of the test animals. Under these conditions it is difficult to determine if dieldrin's effect on behavior is due to discrete neural processing involved with that behavior. It is possible that at toxic doses changes in behavior are seen because of general sickness. Only one study to my knowledge has tested effects of low dieldrin dosages on behavior. Smith et al. (1976) examined successive discrimination reversal in squirrel monkeys during dieldrin exposure. Two zero-dose controls were included with four monkeys and three monkeys fed 0.10 and 0.01 mg/kg per day, respectively. Dieldrin at these doses was administered for 55 days, during which time each monkey was tested.

Control and low-dose monkeys learned the task while high-dose monkeys did not. To test for effects on memory the low-dose group was shifted to the high-dose for another 54 days. No effect on memory is suggested by the fact that no decrement in acquisition was observed after the switch. However, the high to low group never recovered to the level of acquisition seen in the controls.

Chlordimeform

Compared to dieldrin, much less is known about chlordimeform. This is because it is a relatively new compound; introduction of this compound occurred in 1966 under the trade names "Fundal" and "Galecron." Chlordimeform, unlike the cyclodiene structure of dieldrin, is a chloro-methyl-substituted formamdine. Also, it is generally classified as a nitrogen containing acaricide rather than a chlorinated hydrocarbon insecticide. Interest in chlordimeform as a pesticide was generated because it is selective, exhibits low mammalian toxicity (rat oral LD_{50} = 340 mg/kg), and is easily broken down. It is generally active against ticks, mites, and insect orders Lepidoptera and Hemiptera, but does not usually harm beneficial insects such as parasites, predators, and pollinators (Hollingworth, 1976). In contrast to dieldrin, chlordimeform is quickly metabolized by mammalian systems

(Knowles, 1970; Tsu Hui Lin et al., 1976; Knowles and Benezet, 1977) with approximately 90% elimination within 1-3 days. Chlordimeform also does not easily penetrate into plant tissue (Lizuka and Masuda, 1979). However, due to its lack of persistence, chlordimeform often is used in multiple application programs.

Lethality due to chlordimeform is partially attributed to neural toxicity as observed by behavioral alterations in treated animals. Field action in arthropods include an anti-feedant action, hyperexcitation and detachment from food material, and disturbed flight, mating, and oviposition (Hollingworth, 1976). Matsumura and Beeman (1976) have shown treated rats and mice undergo an initial hyperexcitation (1-3 hours) followed by a state of sedation. Also observed were dilation of pupils and locomotor difficulties. Nerve related disorders in poisoned humans include feeling hot, an urgency to void, and sleepiness (Kimbrough, 1980). Electrophysiologic evidence for chlordimeform nerve interaction is seen when exposed cockroach nerve cords are exposed to a chlordimeform hydrochloride solution. Ten to 20 minutes after exposing the nerve to this solution, short volleys of action potentials are observed followed by long trains of repetitive discharge (Matsumura and Beeman, 1976).

The mechanism of action for chlordimeform is unknown at this time although several distinctive effects

can be recorded. It has been shown in rat livers that chlordimeform is an inhibitor of monoamine oxidase (MAO) (Beeman and Matsurmura, 1973). They also observed a resultant large increase of the biogenic amines serotine and norepinephrine. Disruption of biogenic amine levels can be correlated to nerve toxicity (see dieldrin section). Aziz and Knowles (1973) found similar MAO inhibition in rat livers with strongest inhibition exhibited by demethylchlordimeform, a chlordimeform metabolite. The inhibitory effect is reversible (Benezet and Knowles, 1975) and type B MAO is affected more than type A as is evident from the relative extent to which phenethylanaine and serotonin are exempt from deamination (Maitre, 1978).

Although MAO inhibition by chlordimeform is not questioned, whether inhibition correlates to toxicity is. Holden and Hadfield (1975) have shown that ticks survive with low MAO activity when metabolism of chlordimeform is inhibited by the presence of piperonyl butoxide. Neither blockade of serotonergic, alpha-adrenergic receptors nor depletion of tissue stores of these amines reduced the lethality of male rats injected with chlordimeform (Robinson et al., 1975). Also, administration of phenylephrine, a directly acting alpha-adrenergic agonist, with chlordimeform did not increase lethality in male rats (Robinson and Smith, 1977). Furthermore, no correlation

between inhibition potencies and toxicities of various formamidines was found (Neumann and Voss, 1976).

There are ways other than MAO inhibition to disrupt biogenic amine levels. Possibilities include blocking receptor sites and influencing secretory mechanisms. Pento et al. (1979) has shown chlordimeform decreases plasma calcium levels. Demethylchlordimeform has been shown to inhibit calcium, acetycholine, and high potassium evoked secretion of catecholamines from isolated bovine adrenals (Emran et al., 1980). They suggest that chlordimeform toxicity is due to local anesthetic effects.

Little research with clordimeform has been done at the behavior level. Olson et al. (1978) recorded retarded maturation in a chlordimeform-fed group of rats with respect to swimming behavior. No differences were found between control and chlordimeform rat groups in maze or motivational tests. Furthermore, no histological alterations were observed in any organ systems.

The Cockroach as a Model System

Classical and instrumental learning have been shown throughout much of the invertebrate phyla including coelenterates, platyhelminthes, annelids, arthropods, and mollusks (for a review, see Eisenstein, 1967). Habituation, a simpler form of learning, is also seen throughout the invertebrates including aneural systems such as protozoa (Eisenstein and Peretz, 1973).

Invertebrates are often chosen as systems for behavioral studies since "simpler" neural systems often are easier to work with and reduce the variables involved. Eisenstein (1972) states that "fundamental to the use of a model system (simplified) is the notion that the variables and their interaction in the model also apply to the intact and more complex biological system which is being modelled." Owing to the similarity of neural function throughout the animal kingdom, such a comparison is not unreasonable. For example, shorter and longer term memory as observed in mammals also have been seen in goldfish (Riege and Cherkin, 1971) as well as in cockroaches (Lovell and Eisenstein, 1972). Also, the time parameters for shorter and longer term memory were surprisingly similar for both the rat and cockroach (Eisenstein, 1970).

Taking advantage of the "simpler" system approach may be useful in studying pesticide alteration of behavior. Arthropods provide a selection of behaviors that should be a sensitive assay for toxicant-induced behaviroal changes. A T-maze training procedure was chosen as it has proven to be a quick one-step procedure (approximately 1½ hours) and produces reliable results. The training procedure described in this paper was modelled after the system first devised and described by Barraco et al. (1980).

METHODS AND MATERIALS

Experimental Animals

All experiments used adult male cockroaches of the species Periplaneta americana. Since initial studies suggested that animal vigor is important in learning, an effort was made to select the largest and healthiest animals. A large male cockroach was approximately 40 mm in length, 10-12 mm at the widest portion of the abdomen, and .7-.8 gm in weight. Coloring, wing structure, movement, and appendage quality were parameters in judging health.

Cockroaches used for experimentation were maintained in colonies of under 50. They were given ad lib dogfood and water. The temperature was approximately 21°C and the animal bins were exposed to light-dark periods of 14 and 10 hours, respectively. The breeding colonies housing both male and female animals were kept under similar conditions except the temperature was 24°-25°C. When transfer of adult males from the breeding to the experimental colony was needed, five days were allowed for acclimation before any experimentation was begun.

Training Apparatus and Procedure

A plexiglas T-maze was used for all training procedures (Figure 1). The cockroaches were trained to turn either left or right in the maze. Electric shock was used as a negative reinforcer for an incorrect response and a dark goal box was used as a positive reinforcer. The maze consisted of a start box, a runway, two choice arms with shock grid floors, and opaque goal boxes. To facilitate repetition of training, the goal box containing an animal could be moved to the start box at the beginning of a new trial. Manually operated doors, as shown in Figure 1, prevented backtracking as the animal progressed through the maze. An incorrect response to a selected choice arm would be punished by a 10 V shock which produced immediate escape but without noticeable injury.

A day before training, an animal was removed from the lab colony and put in a darkly covered container with access to food and water. At the time of the experiment, the animal was placed in the goal box for two minutes. Then by operating doors on the goal and start box, and using a plunger in the goal box, the animal could be prodded into the start box. The empty goal box was then moved to the choice end. After 15 seconds in the start box, the door to the runway was opened (A in Figure 1). If the cockroach did not leave the start box within one

Figure 1.--Schematic representation of the T-maze used for shock avoidance training and testing of cockroaches. The dotted lines located at the entrance to the start box, runway, and at points labeled by letters A, B, and C indicate sliding doors which can be raised or lowered. All goal boxes contain a plunger and can be placed either at the start box or at the end of either arm of the maze. The floors of the arms are covered with a shock grid. The maze, constructed of plexiglas, is 22 cm long (excluding the goal box) and 19 cm wide across the arms. The runway is 3.2 cm wide and 3.8 cm high. Abbreviations used: G, goal box; P, plunger; S, start box; R, runway; A, B, C, sliding doors. (From Barraco, 1980.)

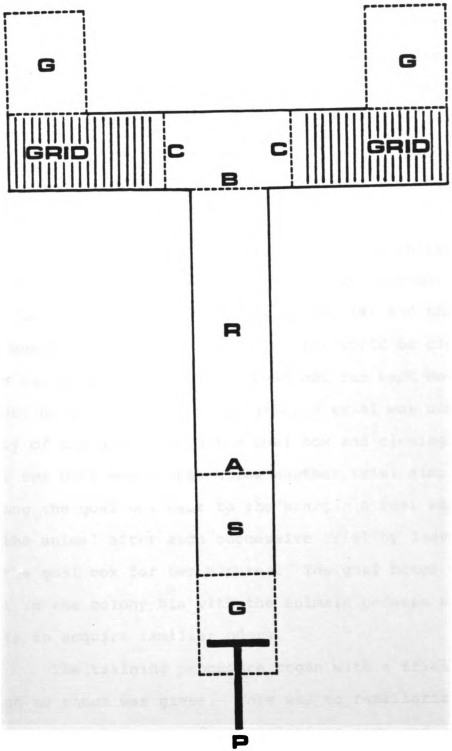

Figure 1

minute, it was prodded by a light stroke of a brush on the dorsal side of the abdomen. Also, the animal was prodded every 30 seconds as needed if it stopped in the runway or choice point area for more than one minute. Care was taken to avoid prodding in a particular direction.

After the cockroach proceeded down the runway it would then make a choice to either arm. A choice was defined as having two legs on either grid surface. If an incorrect choice was made, the animal would receive a shock and run to the opposite arm. After a correct choice, or after an incorrect choice with subsequent movement to the other arm, the runway door (B) and the door at the beginning of the choice point (C) would be closed. This was done so the animal would not run back down the runway or into the other arm after a trial was completed. Entry of the animal into the goal box and closing of the goal box door would then allow another trial simply by moving the goal box back to the start. A rest was given to the animal after each successive trial by leaving it in the goal box for two minutes. The goal boxes were kept in the colony bin with the animals between experiments to acquire familiar odors.

The training procedure began with a trial in which no shock was given. This was to familiarize the animal with the maze and manipulations involved in a trial. This would then be followed by 20 trials in which

a shock was given in one arm. In the first of these 20 trials, the animal would receive a shock to the first side it turned. Therefore, the first training trial always involved an incorrect response. In the following trials it would be trained to go in the opposite direction it initially chose. This was done so that any initial direction preference would not influence the learning curve. It will be shown later that the cockroaches do indeed show a "handedness."

Retention of the training procedure was tested five hours after training. The testing procedure consisted of 20 trials administered as described above without an initial no-shock trial. Shock was again given on the same side as that of the training procedure. All no-injection and dieldrin experiments employed both training and testing procedures. Only the training procedure was used in the chlordimeform experiments.

All procedures for training and testing emphasized methods that would reduce handling and prodding of the animals. An animal that has been excessively handled will become hyperreactive. This reactivity will cause the cockroach to exhibit a quick fleeing response in the maze without regard to other sensory stimuli. Therefore, handling (transfers, injections) and prodding were done as gently as possible. Also, after any handling or prodding, a period of time was allowed before any further

manipulation of the animal was done. This would allow time for the animals to return to their normal excitation level. Careful attention was given to avoiding startle stimuli such as noise, vibrations, and shadows during the training procedure.

Measurement of T-maze Behavior

Three measurements were recorded for all trials during both training and testing procedures. These included the choice behavior or direction turned, the runway time, and the choice point time. The runway time is the time taken by the animal to proceed down the runway (A to B in Figure 1) to the 90 degree connection with the choice arms. The choice point time is the time taken by the animal to proceed from the choice area to either grid surface. Any erratic behavior was recorded after each trial.

For analysis of coice behavior, the 20 trials were divided into blocks of ten. Correct choices within each block of ten were summed and a comparison for change of behavior over time was done by a two-tail Wilcoxon matched-pairs signed-ranks test or a two-tail Mann-Whitney U test. If previous work suggested a direction of change in behavior, a 1-tail test could be used. For analysis of runway and choice point time, the 20 trials were divided into five blocks of four trials. The times within each

block of four trials were then summed. Statistical comparisons between the first and last block of four trials also were done by the Wilcoxon or Mann-Whitney U tests. When a 12-trial training procedure was used, choice behavior was analyzed in blocks of six trials. Runway and choice point times were compared between the first and third block of four trials. P was set at .05 for all comparisons unless otherwise stated.

Determination of Drug Dosage, Volume and Route of Administration

Dieldrin and chlordimeform were obtained by courtesy of Dr. Fumio Matsumura, Director of the Pesticide Center at Michigan State University. Toxicity studies were performed by injecting different animals (groups of 5-10) with varying doses of dieldrin or chlordimeform in acetone and ethanol, respectively. Each group of animals was observed for behavioral effects for five days. Four categories of behavior were measured:

1. General coordination--included presence or absence of convulsions, sluggishness, hypersensitivity, alertness, and the ability to cling to the sides of a box.

2. Cercal response--how effective was the ability to flee when the cerci were touched?

3. Righting behavior--the presence or absence of a righting reflex when turned over.

4. Antennae behavior--the presence or absence of the ability to clean the antennae when smeared with vaseline and dogfood crumbs.

The dosage arrived at for all experiments was the largest amount of dieldrin or chlordimeform that could be injected without mortality or behavioral abnormalities over the observation period. A similar study was done on animals injected with one µl of either acetone or ethanol.

All injections were given into the hemolymph under the ventral abdomenal cuticle with a five µl Hamilton microsyringe. Animals were anesthetized for pre-training injections. Anesthesia was achieved by treatment with CO_2 for 45 seconds. No anesthesia was given for post-training injections since application of CO_2 after training in cockroaches has been shown to disrupt memory (Lovell and Eisenstein, 1973).

When the effect of dieldrin on learning was investigated, the drug was administered two hours before training. Two hours was chosen because previous work suggested that was the time lag between application and appearance of electrophysiological symptoms in cockroaches (Matsumura, 1975). One hour was arbitrarily chosen as the pre-injection time for chlordimeform.

For the studies of the effect of dieldrin on memory, only 12 trials for both training and testing were given in order to facilitate any drug interference with the memory process. Furthermore, dieldrin was administered 15 minutes after the end of training. Waiting a longer time before administration of the drug, or

increasing the number of training trials to 20, might allow for the establishment of a longer-term memory which might be susceptible to disruption.

Each time a dieldrin or chlordimeform animal was tested for learning or memory, a control animal also was run. Acetone was injected in controls for dieldrin and ethanol was injected in controls for chlordimeform. This was because acetone and ethanol served as the vehicles for dieldrin and chlordimeform, respectively. The experimentor did not know which treatment the animal received.

RESULTS

Drug Dosages, Behavior, and Mortality

The amount of dieldrin given to the animals for both pre- and post-training experiments was .9 µg. This amount produced no unusual behavior or mortality during the observation period as measured by the four categories of behavior outlined in the Methods section. Increasingly higher dosages resulted in decreased coordination (measured as above), depressed response to stimuli, and tremors (in that order). Some "poisoned" animals would "sit" higher on their legs, seemingly by flexion of the coxal-trochanter joints. At a dose of four µg, 100% mortality was observed after five days.

Chlordimeform was much less toxic. A dose of 150 µg was needed to produce 100% mortality over a five-day period. Dosages from 60 to 130 µg produced an initial lethargy and an approximately 10% mortality rate. The 90% survivors would recover after 6 to 25 hours. Less than 60 µg produced no abnormal behavior in the toxicity study. Fifty-five µg was chosen as the dosage for chlordimeform.

Since both drugs are relatively nonpolar, it was not possible to use an insect Ringer solution. Therefore,

the vehicles acetone and ethanol were chosen. Neither one µl of acetone nor one µl of ethanol were toxic to the animals over the observation period. Since previous studies indicated that acetone might depress learning, dieldrin was delivered in the smallest volume (0.25 µl) that could feasibly be injected. Chlordimeform was delivered in .50 µl of ethanol.

After all training and testing procedures, control and drug animals were put in separate bins and observed for two weeks. During the entire course of the experiments two dieldrin, one dieldrin control, and zero chlordimeform animals died. One dieldrin animal's death exhibited classical poisoning symptoms with death occurring approximately 24 hours after testing. In the control and the other dieldrin animal, death occurred approximately 72 hours after testing. Since a large N (50) was observed, and only three deaths were recorded, I attribute the two non-poisoning cases to normal attrition. Also, all animals used were non-molting adult males but the exact age was unknown. Experimental error such as dosage administration deveiation, could have produced the poisoning case.

Pre-Training Injection of Dieldrin

Choice Behavior

The effects of dieldrin and acetone administration two hours before training on T-maze behavior is shown in

Figure 2. Also included in this figure for comparison is a group of animals which received neither CO_2 nor an injection. Comparison of trials 1-10 with trials 11-20 in either the training or testing procedures gives an indication of change of behavior over time. During training, both the non-injection and acetone groups learn the maze equally well, showing a statistically significant rise of 75%. The 12.5% rise shown by the dieldrin group during training is significant.

The non-injection group shows another significant 21% rise during testing. The 14.3% rise for the acetone group during testing is not significant. No increase for the dieldrin group is seen for the testing procedure. The percentage improvement is calculated by subtracting the median correct choices for trials 1-10 from the median correct choices for trials 11-20 and dividing by the median number of correct choices for trials 1-10.

Another useful measure is the memory retention and memory loss of learning acquired during training. This is seen by comparing the level of correct choices in trials 1-10 of testing (five hours later) with the level of correct choices for trials 11-20 of training. The percentage retention is calculated by subtracting the median number of correct choices for trials 1-10 of training from the median number of correct choices for trials 1-10 of testing and dividing by the absolute difference

Figure 2.--Choice behavior learning curve as measured by the median number of correct choices during training and testing for animals receiving no injection (n=13), or injected two hours before shock avoidance training with acetone (n=10) or dieldrin (n=10). During training trials 11-20, the non-injection and acetone animals made significantly more correct choices than the dieldrin animals. There was no significant learning during training in the dieldrin group. During testing five hours later, the non-injection and acetone animals exhibit a significant retention of the training.

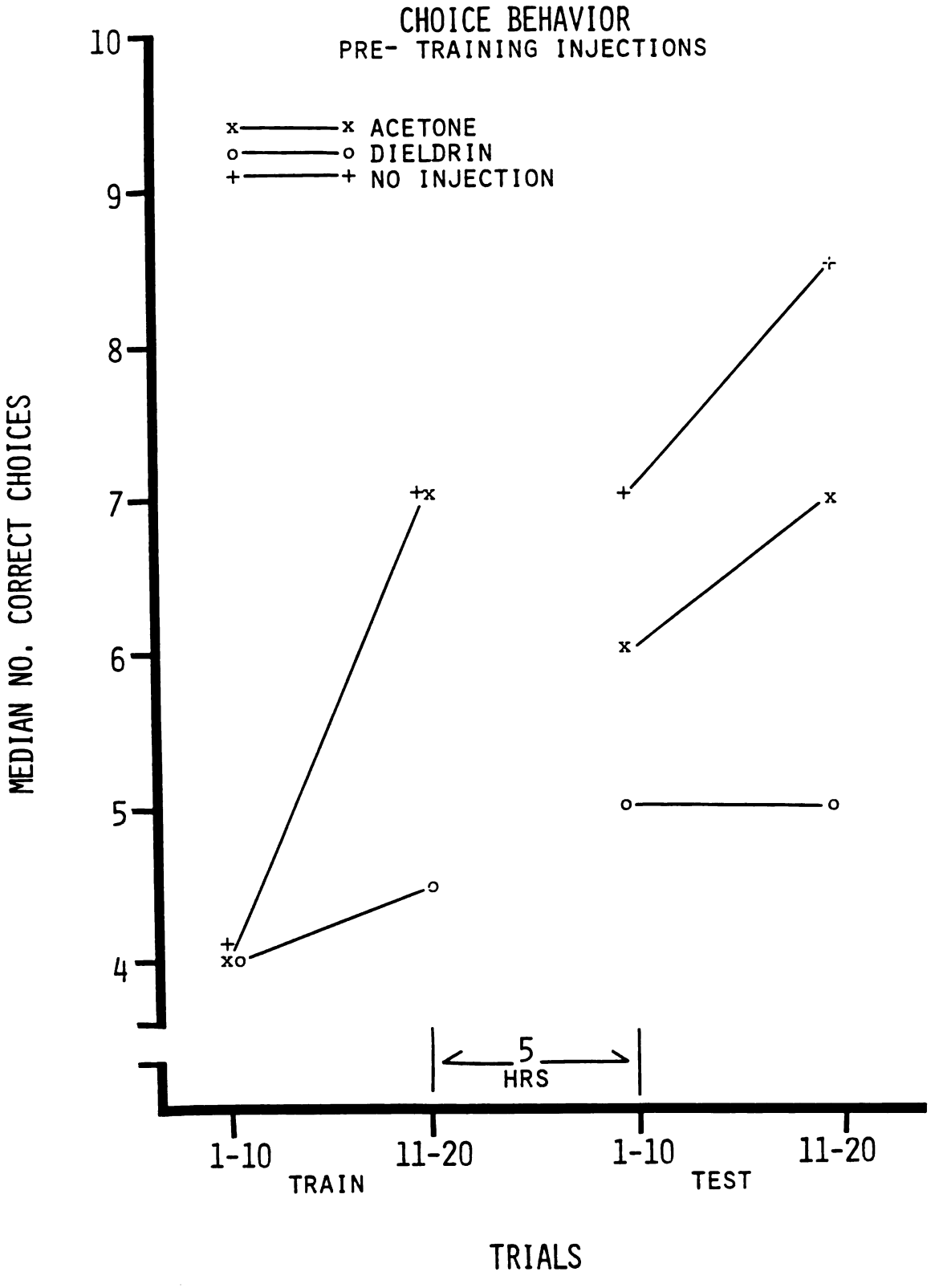
CHOICE BEHAVIOR
PRE- TRAINING INJECTIONS
x——x ACETONE
o——o DIELDRIN
+——+ NO INJECTION
MEDIAN NO. CORRECT CHOICES
10
9
8
7
6
5
4
5
HRS
1-10
11-20
1-10
11-20
TRAIN
TEST
TRIALS

Figure 2

in the median number of correct choices between training trials 1-10 and 11-20. Percent loss can then simply be calculated by subtracting the percentage retention from 100. The 100% retention shown in Figure 2 for the non-injection group is statistically significant. In other words, there has been no significant memory loss for this group. Likewise, the acetone group shows a significant 66.6% retention. The 33.3% loss indicated is not significant; therefore, no memory loss has occurred. Measurement of retention in the dieldrin group is not meaningful since no learning occurred during training.

Figure 2 shows the trials combined in blocks of ten. A more detailed learning curve of Figure 2 is shown in Figure 3. This compares the percentage of acetone and dieldrin animals making correct choices trial by trial during training. The curves separate after the tenth trial with the acetone animals reaching a percentage of correct choices as high as 90% on the thirteenth trial. Trial by trial variation occurs with some leveling off after the thirteenth trial in the acetone group.

Runway Time

Figure 4 shows the amount of time taken for acetone, dieldrin, and no-injection groups to proceed down the runway (runway time) from A to B (see Figure 1). Each point is the mean summation time for each group of

Figure 3.--Trial by trial learning curve during training for animals injected two hours before training with acetone (n=10) or dieldrin (n=10). A difference between the percentage of correct choices occurs after the tenth trial. Cyclic fluctuations are seen for both. (Same data as Figure 2).

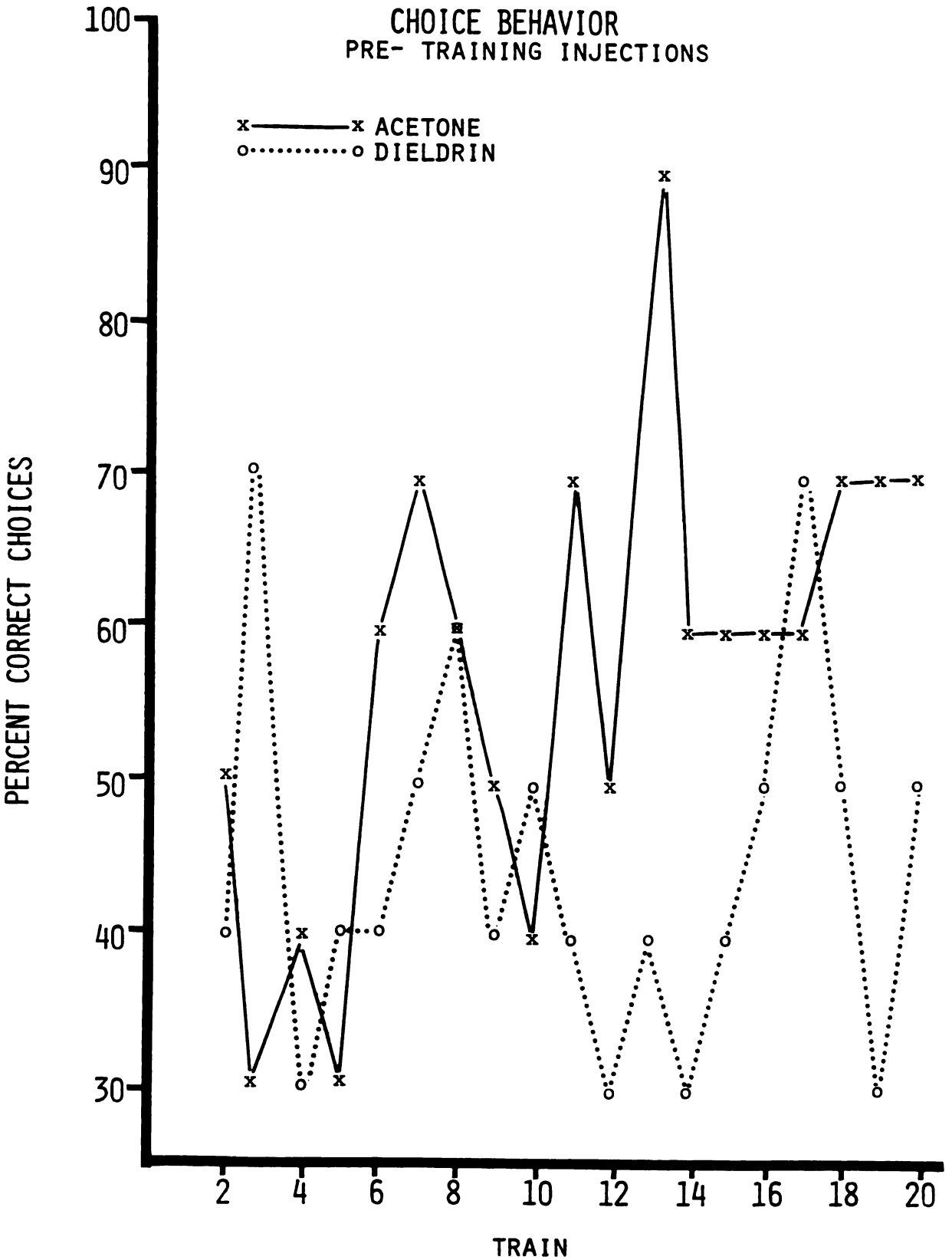
CHOICE BEHAVIOR
PRE- TRAINING INJECTIONS
x——x ACETONE
o·····o DIELDRIN
PERCENT CORRECT CHOICES
100
90
80
70
60
50
40
30
2
4
6
8
10
12
14
16
18
20
TRAIN
TRIALS

Figure 3

Figure 4.--Mean runway times for animals receiving no injection (n=20) or injected two hours before shock avoidance training with acetone (n=10) or dieldrin (n=10). Both the acetone and dieldrin groups show a statistically significant increase in runway times with succeeding trials during training. Both groups also show an increase in runway time during testing but neither group exhibits significant retention of this behavior from training to testing.

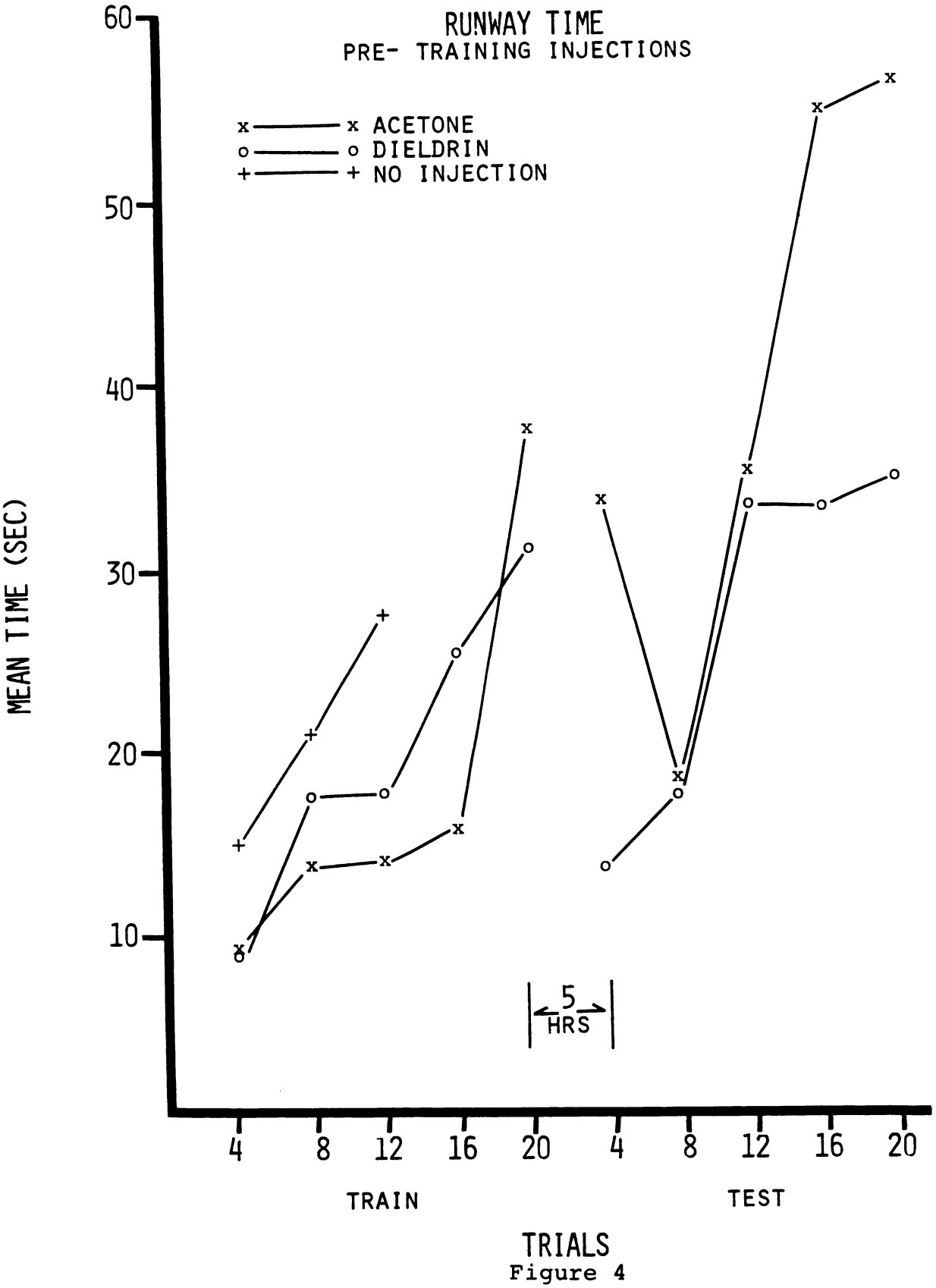
RUNWAY TIME
PRE- TRAINING INJECTIONS
x——x ACETONE
o——o DIELDRIN
+——+ NO INJECTION
MEAN TIME (SEC)
60
50
40
30
20
10
5 HRS
4
8
12
16
20
4
8
12
16
20
TRAIN
TEST
TRIALS

Figure 4

animals for succeeding blocks of four trials. The general trend for all groups is a progressive increase in runway time from trial block 4 through trial block 20. Both the acetone and dieldrin groups show a statistically significant increase in runway time during training. Moreover, they are very similar. The non-injection group is not significant but it was only given 12 trials. The non-injection times were taken from the average non-injection times in Figure 7 where only 12 trials were used. A non-injection group run at an earlier time of the year was highly significant when trained for 20 trials.

The dieldrin group increase is significant during testing at a P value of .08. The acetone testing group increase is also significant at P = .08 for trials 8 through 20. Both memory losses shown are significant but neither memory retentions are significant even though the acetone group indicates an 86% retention. Furthermore, there is no significant difference between the testing acetone and dieldrin curves at the first (trials 1-4) and last (trials 17-20) points where the largest differences occur.

Choice Point Time

Figure 5 shows the mean choice point times over succeeding trials for non-injection, acetone, and dieldrin groups. The non-injection times are taken from the

Figure 5.--Mean choice point times for animals receiving no injection (n=20) or injected two hours before shock avoidance training with acetone (n=10) or dieldrin (n=10). The no injection, acetone, and dieldrin groups show a statistically significant increase in choice point times during training. Neither group exhibits an increase or decrease in times during testing. There is no significant memory loss of this behavior from training to testing for either group.

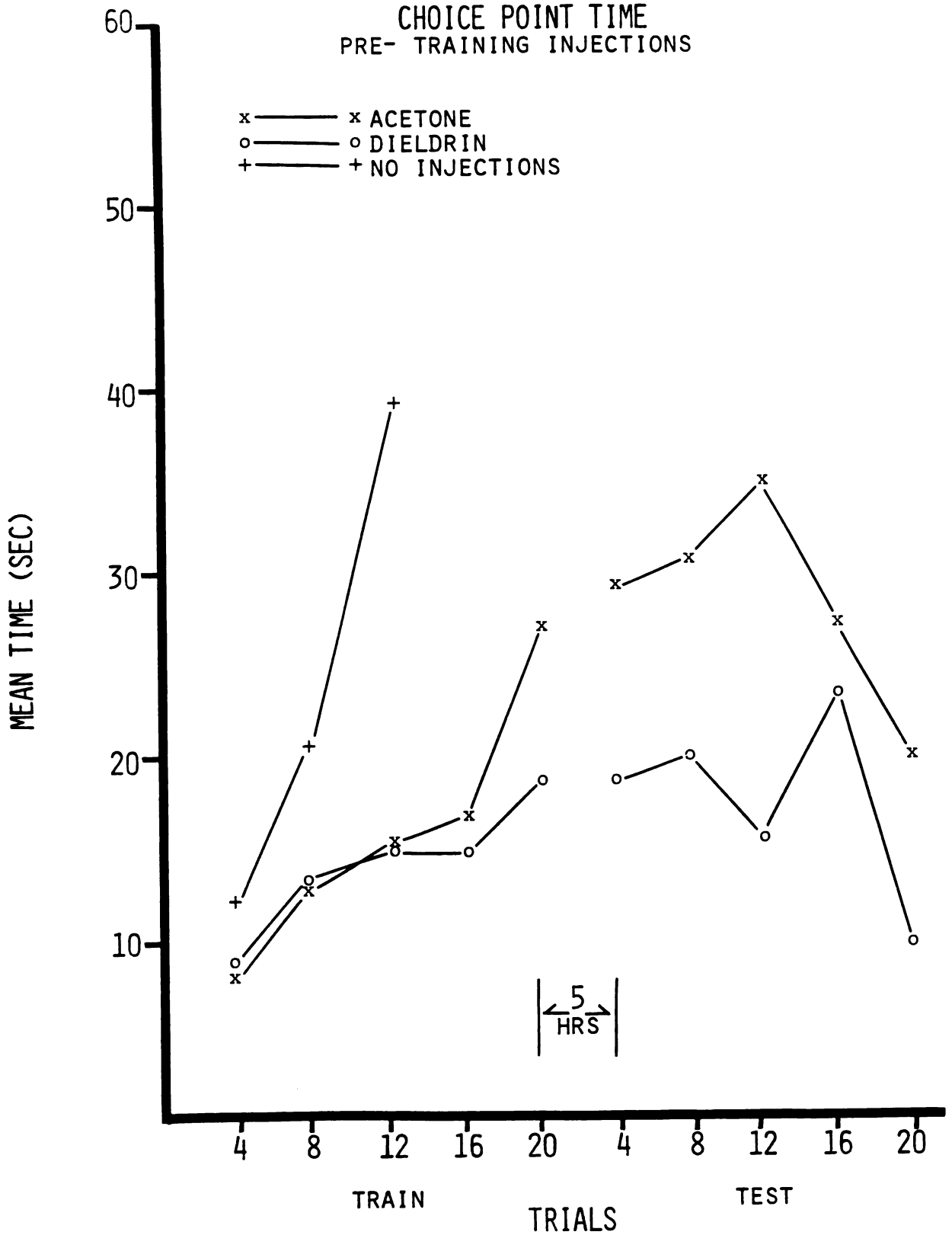
CHOICE POINT TIME
PRE- TRAINING INJECTIONS
x ACETONE
o DIELDRIN
+ NO INJECTIONS
MEAN TIME (SEC)
60
50
40
30
20
10
5 HRS
4
8
12
16
20
4
8
12
16
20
TRAIN
TEST
TRIALS

Figure 5

average no-injection times in Figure 8 for comparison. The choice point time is the time taken to proceed from B in Figure 1 to either point C. As in the runway times, a general increase in choice point times during training is exhibited over succeeding trials. During training all three groups show a statistically significant rise. This increase over time is similar for both the acetone and dieldrin groups.

During testing, there is no significant increase or decrease in choice point times. However, both show no significant memory loss of the increase in choice point time behavior from training to testing. Also, there is no significant difference between the acetone and dieldrin testing curves for the first point (trials 1-4).

Post-Training Injection of Dieldrin

Choice Behavior

Pre-training injection of a drug investigates effects it may have on learning in a training situation. Post-training injection tests the effects of a drug on the memory of such training. Only 12 trials were used in post-training experiments (reasons outlined in the Methods section (page 27) so the blocks of trials are now 1-6 and 7-12. Figure 6 shows the effects of post-training injection of acetone and dieldrin on choice behavior. During training a statistically significant

Figure 6.--Choice behavior learning curve as measured by median number of correct choices during training and testing for animals injected 15 minutes after shock avoidance training with acetone (n=10) or dieldrin (n=10). No statistically significant memory loss in choice behavior is seen for either the acetone or dieldrin group from training to testing. During testing trials 7-12 the acetone group makes significantly (1 tail P = .08) more correct choices than the dieldrin group.

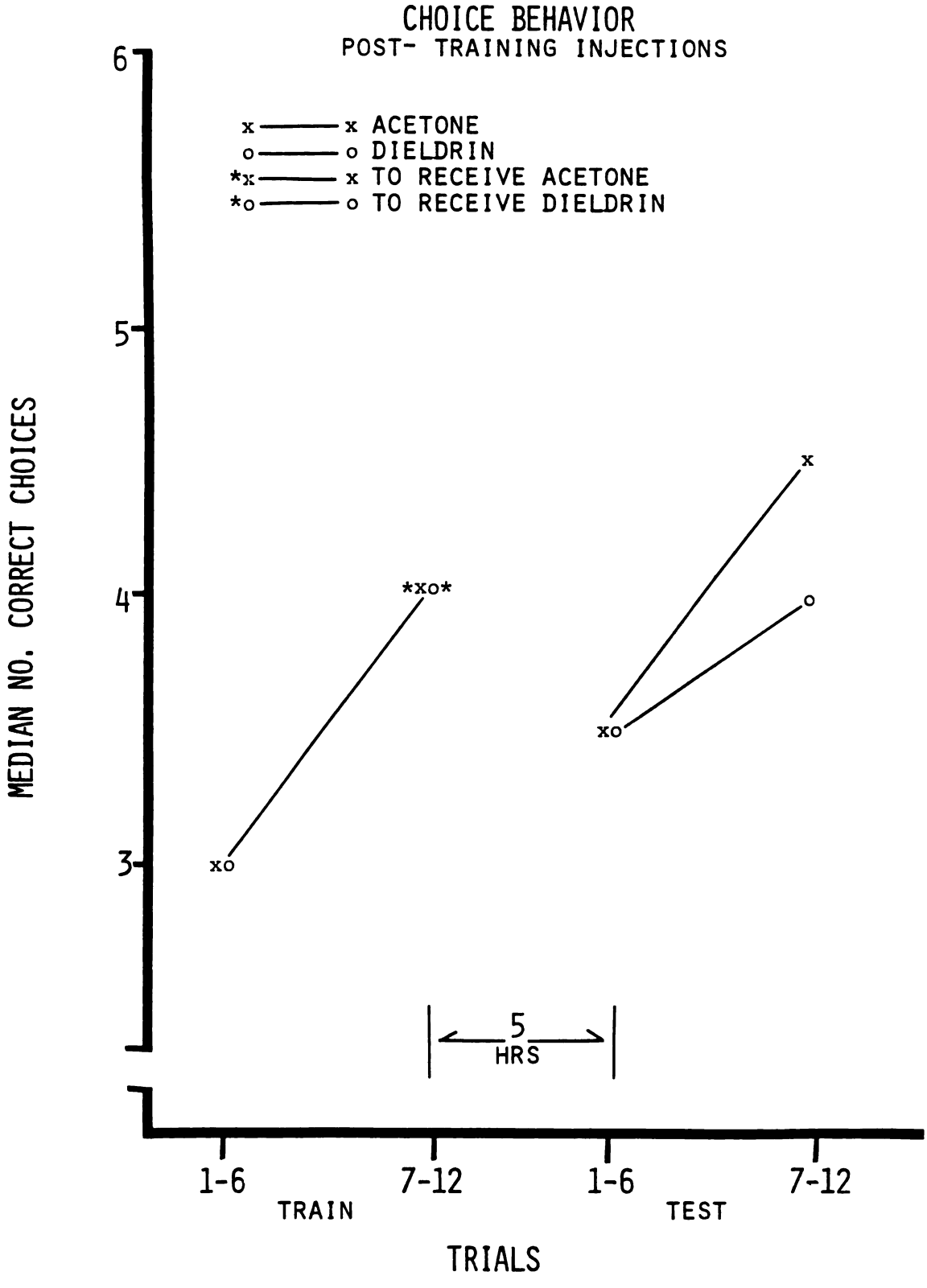
CHOICE BEHAVIOR
POST- TRAINING INJECTIONS
x —— x ACETONE
o —— o DIELDRIN
*x —— x TO RECEIVE ACETONE
*o —— o TO RECEIVE DIELDRIN
MEDIAN NO. CORRECT CHOICES
6
5
4
3
xo
xo
x
o
xo
5
HRS
1-6
7-12
1-6
7-12
TRAIN
TEST
TRIALS

Figure 6

(P = .067) 33.3% increase in number of correct choices is exhibited from trials 1-6 to trials 7-12. Since no animals received injections, the percentage increase is calculated for all animals in a single group. However, to test for differences between the groups that would receive either acetone or dieldrin after training, a distinctive training plot was made for each. As can be seen from Figure 6, both groups show the same learning curve.*

Acetone or dieldrin was administered 15 minutes after training. Four hours and 45 minutes later the animals were tested. Although a 50% memory loss between training and testing is shown in Figure 6 for both groups, this loss is not statistically significant. Therefore, neither acetone nor dieldrin affects long term memory. Both groups show a significant increase in correct choices during testing with succeeding trials. The acetone group made significantly (P = .08) more correct choices during testing trials 7-12 than did the dieldrin group, again indicating the interference with learning by dieldrin.

---

*Similarity of behavior during training for groups of no-injection animals earmarked for different treatment after training also can be seen in Figures 7 and 8. Again both groups in runway and choice point training times are very similar. This should be expected since neither group has yet received a distinctive treatment. It is important to note though because it demonstrates that two groups constructed from randomly selected animals will behave similarly. Because of such similarity, any change in behavior after drug administration can be explained as drug effects and not group variation.

Runway Time

Post-training injection animal mean runway times are illustrated in Figure 7. Although both groupw show an increase in runway times during training, neither is significant. It is possible that 12 trials does not establish this change of runway time behavior. Figure 3 indicates it takes more than 10 trials to establish a change in choice behavior. It is also possible that significant change in runway behavior occurs during later trials. No-injection animals trained for 20 trials undergo a highly significant increase in runway times with succeeding trials. Therefore, I suspect the insignificant rise shown in Figure 7 during training is due to the 12 trial procedure.

During testing, however, a significant increase in runway time with succeeding trials is shown by both groups. Also, there is no significant difference between the two curves. Percentage retention or loss from training to testing of this behavior is not meaningful since no significant increase occurred during training.

Choice Point Time

Mean choice point time behavior for animals receiving post-training injections is shown in Figure 8. Again the animals are only receiving 12 training trials but choice point time increases during training for both groups are significant (the control group at a $P = .08$).

Figure 7.--Mean runway times for animals injected 15 minutes after shock avoidance training with acetone (n=10) or dieldrin (n=10). Both the acetone and dieldrin groups show a statistically significant increase in runway times with succeeding trials during testing. Furthermore, the slope of the curves are similar.

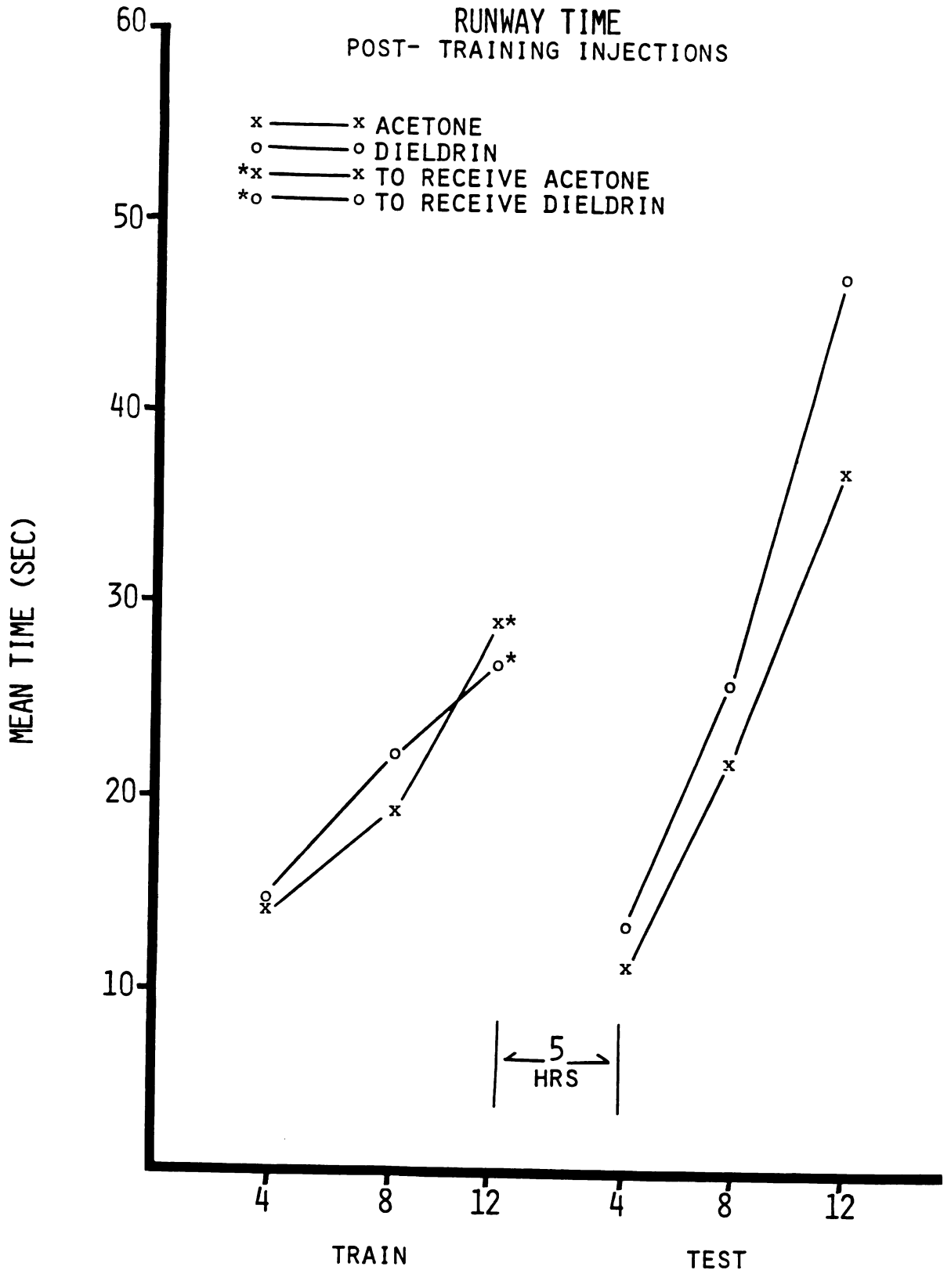
RUNWAY TIME
POST- TRAINING INJECTIONS
x ——— x ACETONE
o ——— o DIELDRIN
*x ——— x TO RECEIVE ACETONE
*o ——— o TO RECEIVE DIELDRIN
MEAN TIME (SEC)
60
50
40
30
20
10
5 HRS
4
8
12
4
8
12
TRAIN
TEST
TRIALS

Figure 7

Figure 8.--Mean choice point times for animals injected 15 minutes after shock avoidance training with acetone (n=10) or dieldrin (n=10). Both the acetone and dieldrin groups show a statistically significant increase in choice point times with succeeding trials during training and testing. Furthermore, the rate of change of those curves is similar.

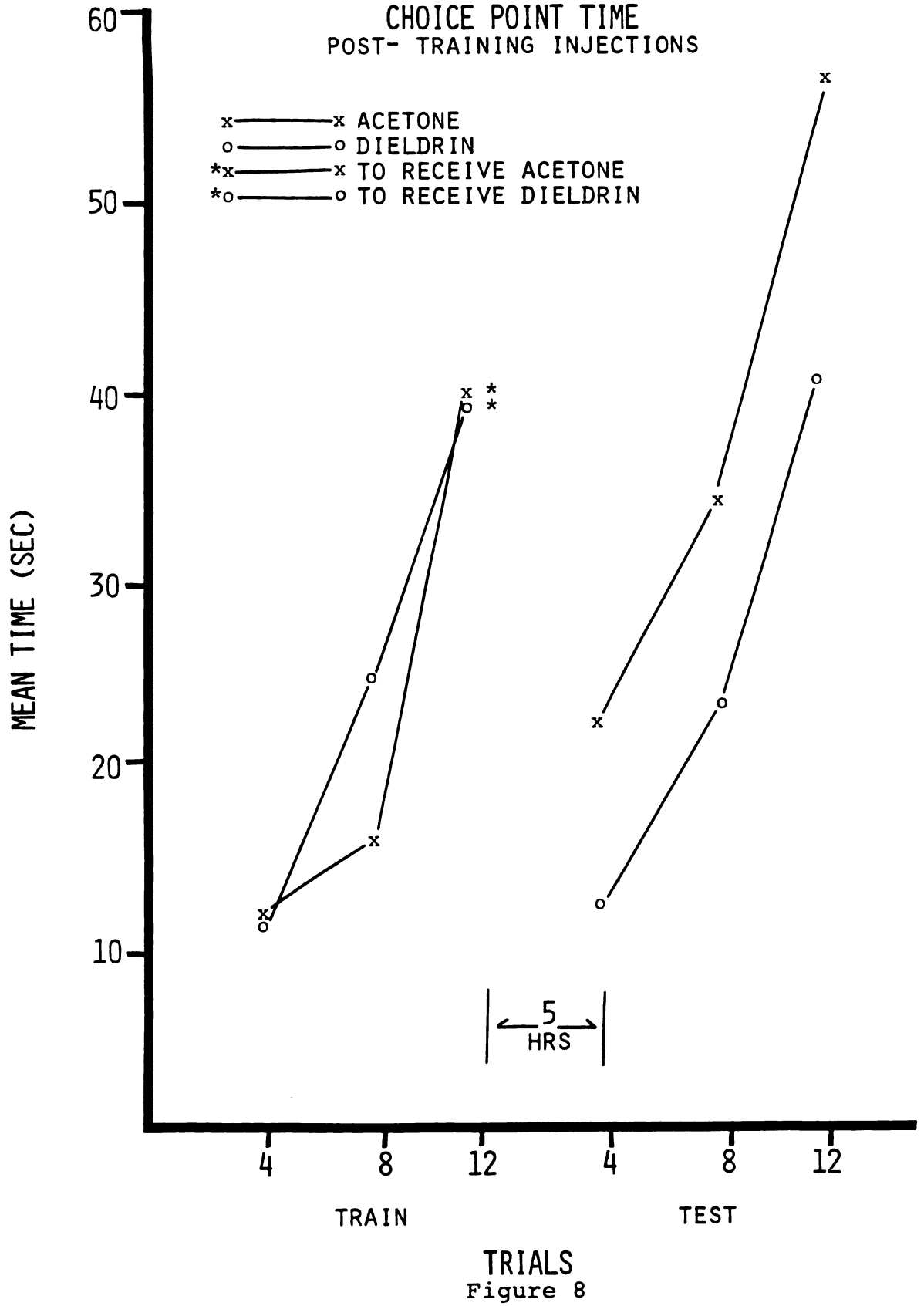
CHOICE POINT TIME
POST- TRAINING INJECTIONS
x——x ACETONE
o——o DIELDRIN
*x——x TO RECEIVE ACETONE
*o——o TO RECEIVE DIELDRIN
MEAN TIME (SEC)
60
50
40
30
20
10
5 HRS
4
8
12
4
8
12
TRAIN
TEST
TRIALS

Figure 8

No statistically significant retention of increased choice point time from training to testing is exhibited by either group. Furthermore, the two curves do not differ significantly during testing. Both groups show similar statistically significant increases in choice point time over succeeding trials during testing.

Pre-Training Injection of Chlordimeform

Choice Behavior

Figure 9 indicates that there is a significant increase in the number of correct choices from trials 1-10 to trials 11-20 for animals injected with ethanol one hour before training. No increase is seen for animals injected with chlordimeform. Furthermore, the ethanol group makes significantly more correct choices than does the chlordimeform group during trials 11-20.

Runway Time

As can be seen from Figure 10, the chlordimeform animals undergo a larger change of runway time with succeeding trials than do the ethanol animals. During both the first and last block of four trials there is a significant difference in runway times between the chlordimeform and ethanol groups. The increase in runway times for the chlordimeform animals is significant while the increase shown for the ethanol animals is not.

Figure 9.--Choice behavior learning curve as measured by the median number of correct choices during training for animals receiving injection one hour before shock avoidance training with ethanol (n=5) or chlordimeform (n=5). The ethanol animals make significantly more correct choices during trials 11-20 than do the chlordimeform animals.

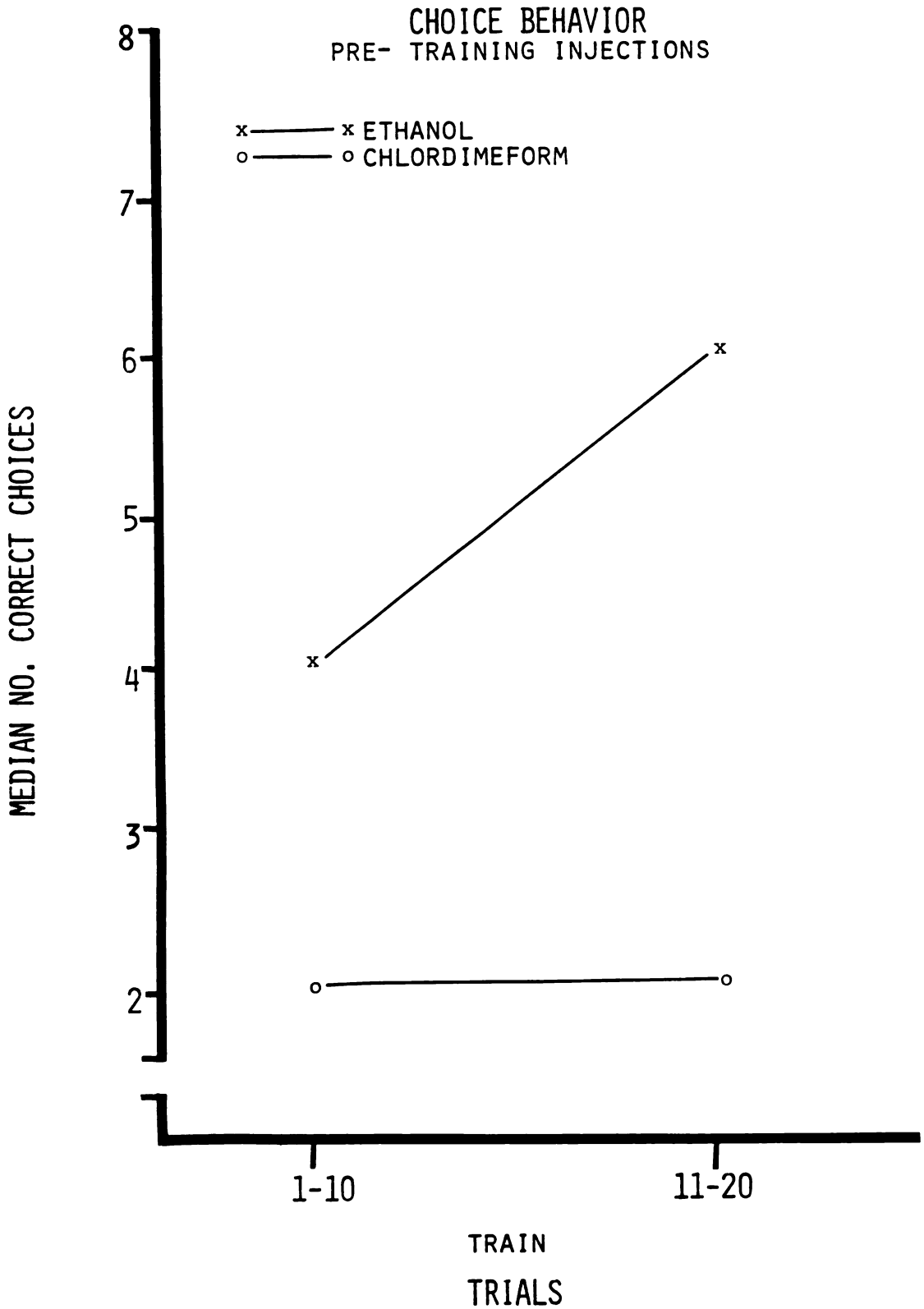
CHOICE BEHAVIOR
PRE- TRAINING INJECTIONS
x——x ETHANOL
o——o CHLORDIMEFORM
MEDIAN NO. CORRECT CHOICES
8
7
6
5
4
3
2
1-10
11-20
TRAIN
TRIALS

Figure 9

Figure 10.--Mean runway times for animals injected one hour before shock avoidance training with ethanol (n=5) or chlordimeform (n=5). The chlordimeform animals exhibit a significant increase in runway times with succeeding trials whereas the ethanol animals do not. During trial blocks 4 and 20 the chlordimeform animal times are significantly longer than the ethanol animal times.

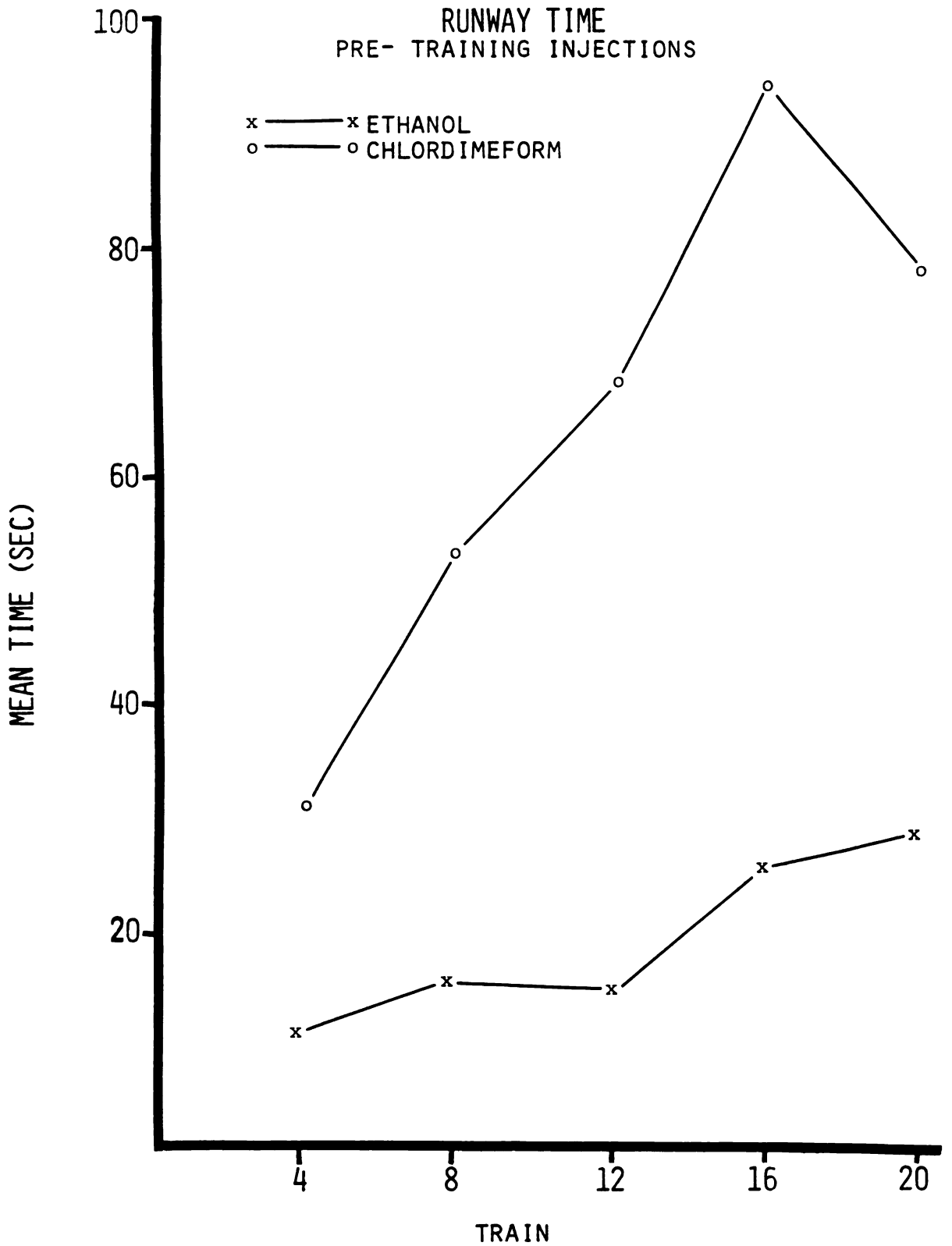
RUNWAY TIME
PRE- TRAINING INJECTIONS
x ETHANOL
o CHLORDIMEFORM
MEAN TIME (SEC)
100
80
60
40
20
4
8
12
16
20
TRAIN
TRIALS

Figure 10

Choice Point Time

Figure 11 shows the choice point time behavior for ethanol and chlordimeform animals during training. There is no significant increase or decrease in choice point times between trial blocks 4 and 20 for either group. Furthermore, there is no significant difference between the ethanol and chlordimeform curves at the first point (trials 1-4) where the largest difference is observed.

Figure 11.--Mean choice point times for animals injected one hour before shock avoidance training with ethanol (n=5) or chlordimeform (n=5). No significant change of behavior is seen between trial blocks 4 and 20 for either curve. Furthermore, there is no significant difference between the curves at trial block 4.

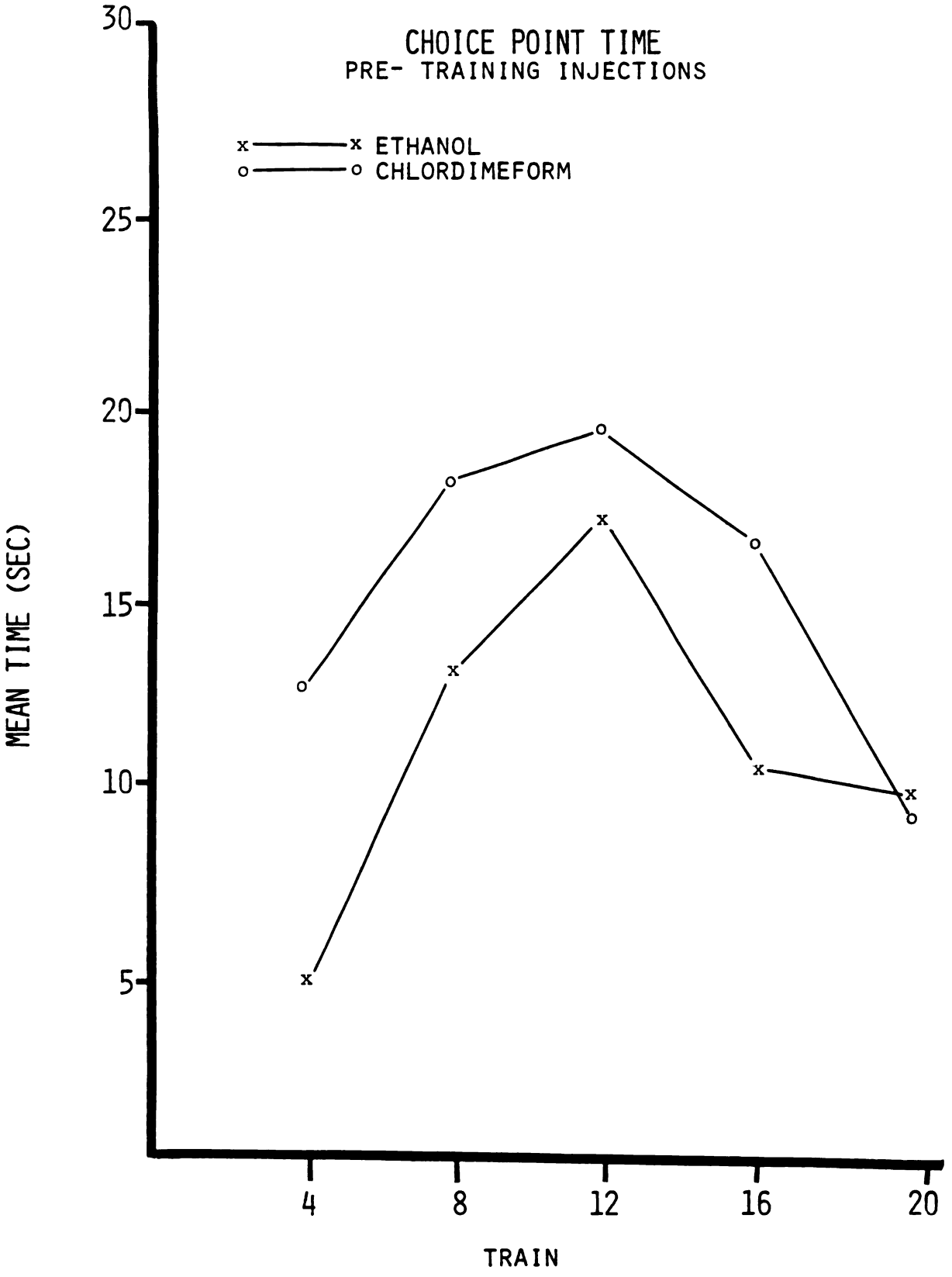
CHOICE POINT TIME
PRE- TRAINING INJECTIONS
x——x ETHANOL
o——o CHLORDIMEFORM
MEAN TIME (SEC)
30
25
20
15
10
5
4
8
12
16
20
TRAIN
TRIALS

Figure 11

DISCUSSION

Dieldrin

As can be seen from Figure 2, a low pre-training dose of dieldrin eliminates acquisition of correct choice behavior in T-maze training of cockroaches. Furthermore, no acquisition of correct choice behavior is seen five hours later during testing. Several observations suggest that disruption of neural processes involved in learning rather than general sickness accounts for this alteration of behavior. One observation is that no-injection, acetone, and dieldrin animals start with the same number (4) of correct choices during the first 10 trials of the training procedure. This indicates that the dieldrin animals are not fixated to one direction since they start at the same level of correct choices as do the control animals.*

---

*It is interesting to note here that no group gets more than 40% correct choices during the first 10 trials. One would expect 50% correct choices if turn direction was completely random through the first 10 trials. One reason for this discrepancy is that the first trial is always an incorrect choice (see Methods and Materials). Therefore, an average of 4.5 correct choices would be expected if turn direction were completely random through the first 10 trials. This is still higher than what is recorded. I believe this is because the cockroaches have a genetic "handedness." Approximately 45% of control and dieldrin animals chose the same turn

Another reason that lack of choice behavior learning acquisition by dieldrin animals is not due to general sickness is that the dose used produced no observable sickness as recorded by the four categories in the Methods and Materials section. Finally, runway time (Figure 4) and choice point time (Figure 5) behaviors show that both dieldrin and control animals are running the maze at equal speeds. This indicates a similar arousal state for dieldrin and control animals. This would be highly unusual if the dieldrin group was generally sick; especially considering the known nervous system effects of dieldrin (see Literature Review).

Although dieldrin eliminates acquisition of learning, it does not effect the memory of a task learned before dieldrin administration; no interference with memory is seen after dieldrin administration (Figure 6). These results agree with dieldrin-induced behavior changes in monkeys (Smith et al., 1976). Control and low dose monkeys learned the task while high dose monkeys did not (see Literature Review). Low dose monkeys were then switched to high dose. Complete retention of the

---

direction during training trials 0, 1, and 2. If no "handedness" existed one would expect only 12.5% ($\frac{1}{2}x\frac{1}{2}x\frac{1}{2}$) of the animals to turn the same direction three times in a row. Therefore, only four correct choices are recorded (instead of 4.5) during the first 10 trials since the animals must "unlearn" their "handedness" before correct choice learning can be accomplished.

increased level of learning acquisition was seen after the switch indicating once a task has been learned dieldrin administration will not disrupt it. Smith mentions that learning acquisition is a more sensitive indicator of toxic effects than is the maintenance of a learned task. Therefore, dieldrin exposure early in a learning process would have the most detrimental effects.

Another cockroach T-maze behavioral change that is not disrupted by dieldrin is the general increase in runway and choice point times over succeeding trials. Dieldrin and acetone groups do not differ significantly in runway (Figures 4 and 7) or choice point (Figures 5 and 8) times during training or testing. Barraco (1980) proved that the general increase in runway and choice point times over succeeding trials is due to habituation rather than associative learning (i.e., association of the end of the runway with a shock). If the recorded increase in runway and choice point times over succeeding trials was due to associative learning, then a group receiving no shock would not be expected to show this behavior. However, a no-shock group run in the maze exhibited a similar increase in runway and choice point times indicating habituation is responsible for the increase in time of running the maze. Habituation represents a decreased response to maze stimuli probably due to acclimation to the new environment.

Barraco (1980) has suggested that cockroach choice behavior (right or left in the T-maze) is mediated at a more complex (i.e., higher) neural level than is habituation. It is interesting that dieldrin's toxicity differenciates between complexity of learning; correct choice behavior acquisition is eliminated but acquisition of habituation is not. It is possible that this simpler form of learning (habituation) is more widespread in the neural network and therefore less prone to disruption.

Unfortunately, conclusions about dieldrin effects on retention of runway habituation cannot be made since neither control nor dieldrin animals retained increased runway times (Figures 4 and 7). Choice point time habituation was retained in the 20 trial procedure (Figure 5) but not in the 12 trial procedure (Figure 8). Since correct choice behavior is retained from training to testing and since choice behavior is more complex than habituation, one might expect habituation also to be retained. Retention of choice point time habituation is seen from training to testing for the 20 trial procedure (Figure 4). This shows that habituation can be retained. Also, Barraco (1980) recorded retention of runway time habituation for cockroaches injected with saline when 20 trials were used. Why retention of runway habituation is not seen for animals injected with acetone and dieldrin is not known. A possibility is that acetone injections

are exciting the animals causing less habituation. This is supported by previous data showing a much steeper habituation curve for animals receiving no injection of any substance. Possibly, the level of habituation attained by acetone and dieldrin animals is not high enough to fixate this behavior in memory. In an attempt to eliminate such possible acetone effects, DMSO (dimethyl sulfoxide) was tried as a substitute dieldrin vehicle. DMSO administered by itself, however, eliminated correct choice behavior learning.

In summary, a pre-training non-toxic dose (no overt symptoms) of dieldrin to cockroaches eliminates correct choice behavior learning but does not interfere with habituation of runway and choice point times. Also not affected by dieldrin is memory of a learned task; dieldrin does not interfere with retention of correct choice behavior from training to testing.

Chlordimeform

Chlordimeform eliminates acquisition of correct choice behavior (slope of the learning curve) and depresses the overall level of correct choices compared to control and dieldrin groups (Figure 9). As can be seen from Figure 9, chlordimeform animals never make more than two correct choices per 10 trials. It seems that chlordimeform not only eliminates more complex behaviors

such as learning but also disrupts "simpler" behavioral functions. Decreased curiosity could explain lack of exploratory behaviors leading to a fixation of maze running patterns.

Support of this wide range of behavior disruption is observed in Figure 10. Maze running speeds of chlordimeform animals are much slower than control animals. Moreover, chlordimeform habituation curves are much steeper. In other words, the chlordimeform animals are running slower but habituating faster. Chlordimeform may well be facilitating inhibitory behaviors (habituation) while depressing excitatory behaviors (correct choice acquisition).

Several observations suggest chlordimeform effects on cockroach T-maze behavior are not due to animal sickness. The dose of chlordimeform being used (55 μg) is well below reported (Matsumura and Beeman, 1976) LD_{50} values (500 μg) for Periplaneta cockroaches and no overt behavioral symptoms were recorded (see Methods and Materials section). Furthermore, although running speeds were affected in runway measurements, no significant difference is observed in choice point time habituation curves (Figure 11). If general sickness was causing slower running speeds and an increased rate of habituation, one would also expect to see this in choice point times. Finally, showing differential effects on correct

choice acquisition (decreasing it) and habituation (increasing it) tends to support discrete neural alteration.

Implications of Dieldrin Exposure

It has been suggested that behavioral tasks which are not sufficiently difficult for the animal will not allow for differential performance after dieldrin exposure (Sandler et al., 1968). In other words, "easy" behavioral tasks for an animal may not be prone to toxicant induced disruption. This is seen by the fact that many mammalian model systems must use toxic dieldrin doses to observe behavioral alterations. On the other hand, the narrow plasticity range of invertebrates allows the cockroach T-maze model system to be a very sensitive assay system for recording low dose toxicant-produced behavior alterations. The results of this work indicate that there is dieldrin induced behavioral alterations at low doses and detrimental effects increase the more complex the behavior and the earlier exposure is to acquisition of a learned task.

The implication of this work is that human learning also may be disrupted to some extent by dieldrin exposure. Hunter and Robinson (1967) found no ill health in human volunteers fed up to 211 μg of dieldrin daily. However, changes in complex behaviors may be hardest to

detect. Possible examples of dieldrin induced changes might be mood changes due to altered biogenic amine levels. Another possibility might be intellectual impairment of young children. For example, placental transfer of dieldrin results in neonatal dieldrin concentration levels that are within the ranges of concentrations that have been found in various tissues and blood of the general adult population (Curley et al., 1969). Furthermore, milk is the major food source of young children. Pesticide exposure is mainly by food ingestion and the highest food dieldrin concentration (0.05 mg/l) is found in milk (Edwards, 1973).

The dieldrin concentration used in this study is not out of range with the dieldrin concentration found in human tissues. A .9 µg dieldrin injection in a .75 g cockroach yields a concentration of 1.2 µg dieldrin per gram of tissue. Aldrin-dieldrin mean concentration in human adipose tissues have been estimated at approximately .3 µg/g (Edwards, 1973; Radomski et al., 1968). Human brain dieldrin concentrations have been found by autopsy to be approximately 0.04 µg/g (Radomski et al., 1968; Casarett et al., 1972). Although 0.04 µg/g is much less than 1.2 µg/g, several points make the concentration used in this study comparable to the concentrations found in humans. Since the .9 µg was injected into the abdomen of the cockroach, the concentration of dieldrin in the

nervous system would be much less than 1.2 μg/g tissue. Also, organochlorine compounds can synergistically (Diechmann and MacDonald, 1970) increase their pharmacodynamic upper limits (Walker et al., 1968; Hunter and Robinson, 1967). Total organochlorine brain concentrations in humans have been estimated at 2.0 μg/g tissue (Casarett et al., 1972). Finally, chemical workers have much higher pesticide concentrations than do most people. For example, men working in a plant manufacturing DDT have up to 647 μg/g in their fat (Edwards, 1973). Thus, pesticide concentrations in some people may be much higher than autopsy estimates from the general population.

It is suggested from these comparisons that low concentrations of pesticides such as those occurring in man have the potential to disrupt discrete neural processes such as those involved in learning. What effect repeated dieldrin exposure has on the learning and later intellectual performance of the fetus, neonate, and young child can only be surmised. It would be interesting to use the cockroach T-maze model system to evaluate behavioral effects of low dose (no overt symptoms) pesticides other than dieldrin and chlordimeform. This model may prove useful in rapidly distinguishing those pesticides that disrupt learning from those that do not. Selecting pesticides for environmental use that have minimal effects on learning and memory may be one way to reduce such a potential environmental danger.

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