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The Nervous System Influenced by Toxaphene

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MODE OF ACTION OF CYCLODIENE INSECTICIDES: THE NERVOUS SYSTEM INFLUENCED BY TOXAPHENE

by

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FOREWARD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

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The report discusses the mode of action, excretion, metabolism, and behavioral effects of toxaphene and combinations of toxaphene, methyl parathion, and/or chlordimeform in various insects, the mouse and the rat.

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ABSTRACT

This report contains information concerning the mode of action, excretion, metabolism, and behavioral effects of toxaphene and combinations of toxaphene, methyl parathion, and/or chlordimeform in various insects, the mouse and rat.

By employing radioisotopes of ions involved in neural functioning, toxaphene was shown to alter ionic movements to produce an increase of internal K^+ and Ca^{++} within the central nervous systems of the American cockroach, Periplaneta americana. Toxaphene increased the levels of cAMP and cGMP in tissues of the cockroach, Leucophaea maderae. These increases were also observed in tissues of mice poisoned with toxaphene, with the highest levels occurring in mice displaying advanced symptoms of poisoning. ATPase enzymes were inhibited by toxaphene in tissues of P. americana and the mouse; inhibition of Na^+-K^+ ATPase and Mg^{++} ATPase was observed in mouse tissues both in vivo and in vitro.

Rat pups peri- or postnatally exposed to sublethal doses of toxaphene and methyl parathion in combination or alone showed few significant changes in motor skills, behavior, or learning ability as measured by a simple T-maze.

Combining chlordimeform and/or methyl parathion with ^{36}Cl-toxaphene produced some significant changes in the amount of ^{36}Cl recovered in feces and the amount deposited in tissues of orally-dosed mice. Toxaphene and toxaphene-methyl parathion in combination with ^{14}C-chlordimeform resulted in lower recoveries of ^{14}C in tissues of mice than from those dosed with ^{14}C-chlordimeform alone. Toxaphene also decreased the quantity of para-nitrophenol excreted in the urine of mice dosed with a toxaphene-methyl parathion combination.

Dosage-mortality studies indicated that toxaphene does not potentiate methyl parathion in several hemipteran predators or lepidopterous pests under laboratory conditions. Methyl parathion was more toxic to the predators than target insects.

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SECTION I

CONCLUSIONS

Toxaphene increased the internal levels of K^+ and Ca^{++}, increased cAMP and cGMP quantities, but inhibited ATPases in the central nervous system of the cockroach. These changes may or may not be interrelated. Increases in cAMP and cGMP, as well as the inhibition of ATPases, were also observed in other tissues of the cockroach and in tissues of the mouse.

Oral exposure to sublethal doses of toxaphene appeared not to cause impairment of learning abilities of adult rats, as well as when the exposure was received perinatally. Perinatal exposure to toxaphene, methyl parathion, or a combination of both produced only minor changes in motor skills and behavior of the neonates.

Combining toxaphene with methyl parathion did not potentiate the toxicity of methyl parathion under laboratory conditions. This was true for various lepidopterous pests and hemipteran predators. The toxicity of methyl parathion with or without toxaphene was much greater to the predators than the pests.

Combinations of toxaphene, methyl parathion and/or chlordimeform caused only slight differences in excretion and tissue retention of the individual insecticides in mice. Important differences were (1) methyl parathion and/or chlordimeform combined with toxaphene increased quantities of toxaphene retained by some tissues, and (2) toxaphene reduced the amount of para-nitrophenol (a major metabolite of methyl parathion) excreted in the urine.

SECTION II

RECOMMENDATIONS

Although many data have been collected on several facets of the use and properties of toxaphene, many important questions still remain. The most striking questions are toxaphene's exact mode and site of action, as well as it's metabolism. Although the continued use of toxaphene is currently being questioned, answers to the above questions might still increase our knowledge of the compounds that have been released into the environment and eventually become incorporated into the food chain.

Indications were obtained that toxaphene does not interfere with the learning ability of adult rats but produces slight changes in motor functions and behavior of rats exposed perinatally. These changes were not drastic, but indicated the need to pursue these questions to determine the potential effect of toxaphene on the behavior and neuro-muscular coordination of mammals chronically exposed.

The use of toxaphene in combination with methyl parathion, and/or chlordimeform on crops should be further examined. The toxicity of methyl parathion appeared not to be potentiated by addition of toxaphene, but such combinations might restrict the metabolism, excretion, and tissue retention of other insecticides in mammals.

SECTION III

INTRODUCTION

Among the chlorinated hydrocarbon insecticides, toxaphene ($C_{10}H_{10}Cl_8$, chlorinated camphene with a chlorine content of 67-69%), is of importance and interest because of its tremendous use on cotton. Information regarding this compounds mode of action, excretion, and metabolism in animal systems is imperative to better evaluate the effects on man and other non-target species. Unfortunately, there is a paucity of such information with which to make a proper judgement concerning toxaphene's continued usage.

Although it is generally accepted that toxaphene acts on the nervous system, a precise locus of action has not been defined. LaLonde and Brown[1] observed the action of toxaphene on sensory nerves of Periplaneta americana (L.) isolated from the central nervous system. Following a latent period, toxaphene generated 100 mV spikes for 30 min. Intense forms of activity were also observed in isolated nerve preparations from this same species treated with toxaphene in a study by Dary and Crowder[2]. Latent periods between introduction of toxaphene and onset of intense activity decreased as the concentrations were increased. Wang and Matsumura[3] observed toxaphene neurotoxicity; it appeared to be more highly neurotoxic (threshold concentration 6×10^{-7}M) than was indicated by in vivo toxicity.

There is considerable evidence suggesting cyclic AMP (adenosine 3', 5'-cyclic monophosphate) and ATPases (adenosine triphosphatase enzymes) are involved in neural transmission. The transport of Na^+ and K^+ ions across the membrane through the active transport mechanism is thought to be controlled by ATPase (Skou[4]). This led to investigations of ATPase as the mediator of insecticide action on ion fluxes, dominated by the research of Koch[5], Koch et al.[6], Desaiah and Koch[7,8,9], and Yap et al.[10] They demonstrated that ATPase was inhibited by DDT, cyclodienes, and toxaphene in numerous tissues of a variety of animals.

Cyclic nucleotides have been shown to be involved in nerve function (Rall and Gilman[11], and Bloom[12]) and as mediators of the active transport mecha-

nism (Horwitz and Eaton[13]). The potential relationships between insecticides and the action of cyclic AMP has been explored by several investigators (Casida and Maddrell[14], Kacew and Singhal[15,16], and Rojakovick and March[17]).

But the effects of insecticides, and specifically toxaphene, on cyclic nucleotides and ATPase activity are still unresolved. It was hypothesized that toxaphene may interfere with transport mechanisms in nerve membranes thus causing the effects observed electrophysiologically. Since these transport mechanisms are related to cyclic nucleotides and ATPase, the objectives of the project were to:

(1) Measure ionic fluxes across nerve membranes in cyclodiene- and toxaphene-treated animals.

(2) Examine the effects of cyclodienes and toxaphene on cAMP, cGMP, adenyl cyclase, guanyl cyclase, and ATPase in nerves.

(3) Determine the effects of cyclodienes and toxaphene on synaptic and axonal transmission.

To generate further information about toxaphene, an additional objective was to:

(4) Determine the uptake, metabolism, and excretion of toxaphene in combination with methyl parathion and chlordimeform.

SECTION IV

METHODS AND MATERIALS

A. EFFECTS OF TOXAPHENE ON THE NERVOUS SYSTEM

1. Ion Movements in the Nervous System of the American Cockroach

a. Experimental Animals and Dissection Procedures

American cockroaches were maintained in the rearing facilities of the Department of Entomology, University of Arizona. The cockroaches were fed Purina® Dog Chow coated with honey and glycerin (12:1:1, v/v), with water supplied *ad libitum*. Conditions within the rearing facility were: 24 ± 3° C, 30 to 60% relative humidity, and a 9:15 hr light-dark cycle.

Adult male cockroaches were removed from the colony and anesthetized with carbon dioxide. The head, legs, and wings were clipped from the body, and a dorsal, longitudinal incision made along the length of the body. The gut, fat body, and reproductive organs were removed, and three drops of saline were placed in the body cavity to prevent desiccation of the ventral nerve cord (VNC). The VNC was severed at the cercal nerves, posterior to the sixth abdominal ganglion, and between the metathoracic and first abdominal ganglia. Nerve sections produced by these cuts were designated: thoracic (TH), consisting of the 3 thoracic ganglia and connectives, and abdominal (AB), consisting of the 6 abdominal ganglia and connectives. The third section, brain (BR), consisted of the ganglia from the head capsule.

The head was cut into two halves, pulled apart with forceps, and BR dissected from the head capsule, often without the suboesophageal ganglion. Therefore, BR consisted of the 3 major lobes (proto-, deuto-, and tritocerebrum), and in some cases, the suboesophageal ganglion.

b. Ions and Solutions

Four radioactive ions were employed: calcium-45 ($^{45}Ca^{++}$, ICN Chemical and Radioisotope Division, Irvine, Calif.), chlorine-36 ($^{36}Cl^{-}$, New England Nuclear, Boston, Mass.), potassium-42 ($^{42}K^{+}$), and sodium-24 ($^{24}Na^{+}$). The $^{42}K^{+}$ and $^{24}Na^{+}$ were produced in a Triga Mark I reactor by the Nuclear Engineering Department, University of Arizona.

The saline of Yamasaki and Narahashi[18] was used throughout this study,

and prepared with demineralized distilled water, with a final pH of 7.4.

c. Experimental -

The methods employed were adapted from those of Matsumura and O'Brien[19], Hayashi and Matsumura[20], Treherne[21], and Eldefrawi and O'Brien[22]. Exposure of the nerve sections to toxaphene was entirely *in vitro*. The experiment was divided into 2 main areas of evaluation: uptake and efflux of ions.

(1). Uptake of Ions

For uptake measurements, 10 of each CNS section (BR, TH, and AB) were used at each of the 7 time periods for exposed and a like number for control evaluations of each ion. Once dissected, TH and AB sections were pulled across a piece of filter paper to remove extraneous fat body, tracheae, and saline. The third section, BR, was placed directly into the saline.

Once dissected, BR, TH, and AB were placed into scintillation vials containing 1.0 ml of saline composed of 1 of the 4 radioactive ions. The saline had 1.0 µl of 10^{-2}M technical grade toxaphene (batch number X16189-49) in acetone added, for a final concentration of 10^{-5}M toxaphene for exposed samples. This concentration was selected because it had been used for DDT and dieldrin in similar experiments[19,20].

Nerve sections were incubated in the radiolabelled saline for the following time periods: 1, 2.5, 5, 10, 15, 30 and 60 min. Following the prescribed incubation period, nerve sections were removed from the saline and pulled along the vial wall to remove as much extraneous saline as possible. Each section was then transferred into a preweighed dry scintillation vial, reweighed, and 1.0 ml of TS-1® tissue solubilizer (Research Products International Corp., Elk Grove Village, Ill.) added to each for digestion.

(2). Efflux of Ions

Ten of each CNS section (BR, TH, and AB) were used for the entire evaluation of control as well as exposed efflux of each ion. Dissected nerve sections were placed into scintillation vials containing 1.0 ml of radiolabelled saline for 30 min. This saline contained neither acetone nor toxaphene. Following the incubation period, BR, TH, and AB were pulled along the vial wall to remove excess saline and passed through a series of 1.0 ml rinses of non-labelled saline containing either 10^{-5}M toxaphene for exposed samples, or 1.0 µl of acetone for controls. The sections were allowed to remain in each rinse vial for a specified time interval, then transferred to the next without being pulled along the vial wall.

The different rinses represented the amount effluxed after: 1, 2.5, 5, 10, 15, 30 and 60 min. The nerve sections were pulled along the wall of the last rinse vial and placed into individual, dry scintillation vials. Efflux data were expressed as the percentage of radioactivity remaining within the sections over time. Percentages were based upon the sum of rinse values for each section plus that remaining within the section. This represented the total sum of the activity within the section at the beginning of efflux measurements. Each rinse value, added to those before it and divided by the total sum, resulted in the percent efflux up to that time period. Each section had 1.0 ml of TS-1® tissue solubilizer added for digestion.

d. Radioassay

Radioassay was performed on a Nuclear Chicago Liquid Scintillation Spectrophotometer (Model 6822). To each of the digested nerve samples from uptake and efflux measurements, 10 ml of scintillation cocktail were added. The cocktail was composed of: 4.0 grams PPO and 0.05 grams POPOP (Research Products International Corp.) per liter toluene. Rinses from efflux measurements had 10 ml of a cocktail for aqueous samples, consisting of 4.0 grams PPO and 0.05 grams POPOP per liter of a 2:1 mixture of toluene and the surfactant Triton X-100® added to each. All samples were assayed for 10 min for a counting efficiency of >90 percent.

2. Cyclic Nucleotides in Several Tissues of the Cockroach and Mouse

a. Animals

Male and female cockroaches, *Leucophaea maderae* (Fab.), were maintained as a mixed laboratory population with free access to water and food consisting of honey and glycerin-coated dog chow. Male Swiss mice (Charles River Laboratories, Inc.) were maintained on a diet of rat chow and water *ad libitum*. The rearing room was maintained as previously described.

b. Experimental Design

Adult cockroaches were injected with 207 μg/animal of toxaphene in 0.05 cc mineral oil under the third tergite, and held for 24 to 168 hr without food and water for sacrifice of 2 males and 2 females at each of 8 time intervals. Controls, injected with 0.05 cc mineral oil, and untreated individuals were also held without food and water for 24 to 168 hr. One male and 1 female from each of these latter 2 groups of insects were sacrificed at each of the 8 time intervals. Additionally, cockroaches (15 males and 15 females) from the laboratory stock were sacrificed immediately to determine normal cAMP

and cGMP levels. Brain, VNC, Malpighian tubules, gastric caeca, fat body, rectum, and testes or ovaries were dissected from the sacrificed individuals and frozen immediately to be assayed for cAMP and cGMP. Tissues were analyzed separately on an individual animal basis.

Fifteen-week old male mice weighing approximately 37 g were orally dosed with 112 mg/kg of toxaphene in 0.16 cc Mazola® corn oil and held with free access to food and water. Controls were dosed with 0.16 cc of corn oil. Five treated and 5 control mice were sacrificed at each time period: 15, 45, 90, and 240 min. The 240 min treated sample included 2 mice that died between 110-145 min. Brain, spinal cord, liver, kidney, and testes were dissected from the animals and immediately frozen to be assayed for cAMP and cGMP. Tissues were analyzed separately on an individual animal basis.

c. Assay Procedure

A single sample containing approximately 10 mg of a frozen tissue was homogenized with 1 ml 5% (w/v) TCA. The homogenate was centrifuged at $0^{o}C$, 4000 G for 15 min. The supernate was extracted with 5 ml of acidified ether (3 ml 0.1 N HCl/100 ml ethyl ether). The aqueous phase was air-dried in a stream of compressed air and the residue reconstituted with 1 ml Tris-EDTA buffer, pH 7.5

Competitive protein-binding assay kits for cAMP and cGMP (Diagnostic Products Corp., Culver City, CA. 90230) were used following extraction of the tissues. The assay procedure consisted of the competition of (^{3}H)-cyclic nucleotide and unlabelled cyclic nucleotide for binding sites on a binding protein. Unbound nucleotides were separated from bound by adsorption onto dextran-coated charcoal. Bound radiolabelled nucleotide was then radioassayed. Cyclic nucleotide concentrations were determined from standard curves prepared from serial dilutions of stock standards contained in the assay kits. The curves, 3 replicates for cAMP and 4 replicates for cGMP (1 replicate for each kit), were analyzed by weighted linear regression and plotted using the logit log method (Rodbard and Lewald[23], Felman and Rodbard[24], and Rodbard[25,26]). Protein concentrations were determined by the method of Lowry et al.[27] employing a standard curve prepared in triplicate with bovine albumin serum. Unknowns were calculated from the respective standard curve equations.

3. ATPases in Several Tissues of the Cockroach and Mouse

a. Experimental Animals

Mice used for *in vivo* experiment I were male Swiss mice. Male Swiss Webster

mice used in both *in vivo* experiment II and the *in vitro* investigations were obtained from the University of Arizona Department of Veterinary Science. Adult male cockroaches, *Periplaneta americana* (L.), were removed from the Department's cultures and held separately in the rearing room.

b. Experimental Design

(1) *In vivo* Experiment I

Male mice were used at 20-30 weeks of age and weighing approximately 38 g. Eighteen mice, each constituting a replicate, were dosed by oral gavage with 112 mg/kg of toxaphene in 0.16 cc of corn oil. Mice surviving the dose were sacrificed 60-90 min later during the second symptom of the poisoning as described in the cyclic nucleotide study, i.e. hyperactivity indicated by kicking, chewing, and coordinated jerky movements with rapid breathing tremors. If death occurred within the first hour, mice were immediately processed. Controls (N = 18, 1 mouse/replicate) were dosed with 0.16 cc corn oil and sacrificed 60-90 min later.

(2) *In vivo* Experiment II

Male mice were employed when 9-11 weeks old and weighing approximately 28 g. Treated (N = 35, 1 mouse/replicate) and the control mice (N = 28, 1 mouse/replicate) were exposed to toxaphene and corn oil, respectively, in the manner described above.

(3) *In vitro* Experiments

Twenty mice (each constituting a replicate) were used to study the effects of *in vitro* toxaphene exposure on mouse tissues. Each tissue was subjected to 4 treatments and 2 controls: 10^{-4}M, 10^{-5}M, 10^{-6}M and 10^{-7}M toxaphene; 10 µl ethanol and untreated controls. Three subsamples were run for each treatment in each of the 3 tissues. Tissues from 3 cockroaches were combined to constitute a replicate. Fourteen replicates were performed in the manner described above for mice.

c. Tissue Collection and Homogenate Preparation

Brain, whole liver and the right kidney were dissected immediately after sacrificing the mouse, washed with ice cold homogenization solution (pH 7.6) containing 0.25 M sucrose, 10 mM Tris (hydroxymethyl) aminomethane and 0.5 mM EDTA (ethylene diamine tetraacetic acid) (Clark and Nicklas[28]), and cut into small pieces with scissors. Each tissue was then transferred to a preweighed beaker containing 2 ml ice cold homogenization solution and weighed. The Malpighian tubules and central nervous system were dissected from adult male cockroaches and transferred immediately to ice cold homogenization solutions.

The method used in tissue fractionation was after Koch[5]. All tissues were homogenized by a motor-driven homogenizer at 500 rpm for 2 min. The liver was homogenized in 15 ml homogenization solution, brain and kidney in 10 ml each, and cockroach tissues in 5 ml each. The whole homogenate was centrifuged for 10 min, with 2 washings, at $0^{o}C$ and 900 G in a Sorvall RC2-B refrigerated centrifuge. The supernate from cockroach tissues was retained as the homogenate fraction. Mouse tissues were further centrifuged for 20 min at $0^{o}C$ and 13,000 G; the resulting pellet was resuspended in homogenization solution to a volume of 5 ml and used as the homogenate fraction. Glassware used in the fractionation procedure was maintained ice cold using crushed ice.

d. Electron Microscopy

The pellets of homogenate fractions for liver, brain and kidney were prepared as previously described and immediately fixed in 0.1 M sodium cacodylate buffer (pH 7.4) containing 2% glutaraldehyde for 2 hr. The tissues were post fixed in the same buffer containing 2% OsO_4 (osmium tetroxide) and dehydrated in ethanol. They were than embedded in Epon 812 and sectioned with a Sorvall MT-2 microtome. The sections were examined on a Philips EM 200 electron microscope at a magnification of 11,600.

e. Enzyme Assay

(1) *In vivo* Experiment I

The reaction mixture was prepared as a stock solution and kept refrigerated. It contained (as final concentrations in the stock solution) 5mM ATP (adenosine 5'-triphosphate, disodium salt; Eastman Kodak Co.), 5 mM Mg^{++} (as Cl salt; from Merck Chemical Co.), 90 mM Na^{+}, 22 mM K^{+} (both Na and K as Cl salts; Matheson Coleman and Bell Manufacturing Chemists), 118 mM imidazole buffer (pH 7.5; J. T. Baker Co.), 0.19 mM NADH (diphosphopyridine nucleotide "reduced NADH disodium"; ICN pharmaceuticals, Inc.), 0.5 mM PEP (phosphoenol pyruvate; Sigma Chemical Co.) and 3 units of pyruvate kinase (ICN pharmaceuticals, Inc.) Another stock reaction mixture was prepared as above except for the addition of 1 mM ouabain (ICN Pharmaceuticals, Inc.). Ouabain is a cardiac glycoside which specifically inhibits the Na^{+}-K^{+} ATPase (McIlwain[29]). One hundred microliters of each homogenate fraction were added to 3 ml of reaction mixture. Enzyme activities were measured at $37^{o}C$ for 40 min using a water bath. Stoppered 12 ml centrifuge tubes were used as reaction vessels. The reaction was stopped by addition of 100 µl ice cold 30% TCA (trichloroacetic acid).

(2) In vivo Experiment II

The enzyme activities were determined by the procedures of Skou[30,31]. The reaction mixture (3 ml) contained, in final concentrations, 5mM ATP, 5 mM Mg^{++}, 90 mM Na^{+}, 19 mM K^{+}, 120 mM imidazole buffer (pH 7.5), and 100 µl homogenate fraction. Ouabain (where added) was 3 mM and 1mM for mouse and cockroach tissues, respectively. The reaction was stopped by the addition of 200 ml ice cold 30% TCA and the enzyme activity measured at $37^{o}C$ for 30 min.

(3) In vitro Experiments

The reaction mixture used was identical to that of in vivo experiment II except for the addition of toxaphene. Toxaphene stock solutions were prepared in ethanol in such a way that when added in 10 µl aliquots would give a final concentration of 10^{-4} M, 10^{-5}M, 10^{-6}M or 10^{-7}M toxaphene in 3 ml reaction mixture.

Mg^{++} - ATPase activity was measured in the presence of ouabain in the reaction mixture. Na^{+}-K^{+} ATPase activity was calculated as the difference between total ATPase and Mg^{++}-ATPase measured activities. ATPase activities were obtained in terms of quantities of inorganic phosphate (Pi) released during the reaction. One milliliter of the sample was treated according to the method of Ohnishi et al.[32] for ATPase and read at 750λ in a Bausch and Lomb Spectronic 20. The Pi concentration of the sample was then determined from a standard curve with concentrations ranging from 0.001-0.1 mg Pi. This method was found unreliable for concentrations above 0.1 mg Pi. Protein concentrations of the samples were determined after Lowry et al.[27]

f. Statistical Analysis

Enzyme activities were calculated for all experiments as mgs Pi released/ mg protein/hr. A one-way ANOVA followed by mean separations (SNK procedure) was performed on data at an alpha level of 0.05.

(4) Neonatal Development and Postnatal Learning in the Rat

a. Perinatal Exposure to Toxaphene-Methyl Parathion and Methyl Parathion

Inseminated, adult female rats (Sprague-Dawley) were obtained from Hilltop Lab Animals (Chadworth, Calif.). Beginning on day 7 of pregnancy and continuing through the fifteenth, the experimental females received daily oral (gavage) doses of methyl parathion (MP) or methyl parathion and toxaphene (MP-T). The doses administered were 1.0 mg/kg MP and 1.0 mg/kg MP + 2.0 mg/kg toxaphene in 0.1 ml corn oil. Control females were dosed under the same regime with 0.1 ml corn oil. The rats were caged in pairs until 2 or 3 days prior to parturition then caged individually. The animals had access

to food (Wayne's Lab BLOX®) and water ad libitum.

Once all females had given birth, the pups were distributed among the females in a group (control or experimental) so that each had 10 pups. Once the pups were 3 days old and until they reached 15 days, daily measurements of weight and mortality were recorded for each litter.

(1) Maternal Behavior - Beginning on day 5 postpartum, maternal behavior was observed by measuring retrieval time (Paulsen et al.[33]). Following weighing, pups were scattered about the bottom of the cage and the amount of time necessary for the mothers to initiate the return of the pups to their nests was recorded.

Behavioral tests to measure neonatal development commenced on the seventh day postpartum and consisted of the grasp-hold reflex (Davenport and Gonzalez[34]). righting reflex (Ibid.), startle reflex (Eayrs and Lishman[35]) and placing reflex (Ibid.). Beginning at 18 days of age, the pups were tested in the open-field test for emotionality (Candland and Nagy[36]). They were tested for maze learning transfer upon reaching an age of 66-68 days. Description of these tests follows:

(2) Grasp-Hold Reflex - Each pup received 3 daily tests of grasp-hold reflex ability on days 7 through 15. In this test a 0.5 mm diameter wire was suspended 15 cm above a bed of wood shavings. Each pup was "hung" on the wire by its forepaws, released, and the length of time the pup held on to the wire before falling was measured.

(3) Startle Response - Each pup was held upright and a sharp clicking sound was made behind its head. The age at which the first response occurred was recorded, as well as the age at which 90% of the litter scored a positive response.

(4) Placing Reflex - The pups chin was touched to a 0.5 mm wire, suspended directly in front of it. The first appearance of the response was recorded at the age at which the animal first placed either forepaw on the wire on either side of its chin. This test was performed concurrently with grasp-hold.

(5) Righting Reflex - Each pup received 3 daily righting reflex trials beginning on day 7. On each trial, the pup was held by the head and tail end upside down and dropped, approximately 35 cm onto a bed of wood shavings. The response was scored positive when the pup landed on all fours. The age at which the first positive response occurred, and the age at which 90% of the trials by the litter were positive were recorded.

(6). Open Field Test - The open field consisted of a circular area, 1.8 m in diameter marked off in 11.3 cm. squares. The field was contained by a 69 cm high wall constructed of unpainted corrugated aluminum roofing. A single 200 W light was suspended 76 cm above the center of the field. Pups were tested in the open field at the following ages: 18, 23, 30, 44, 54 and 65 days of age. Each pup was placed individually into the open field and observed for a period of 10 min. Counts were made of the numbers of squares crossed and the numbers of boluses deposited in defecation during each 10 min period. Each pup was tested only once at each of the appropriate ages.

(7). Maze Learning Transfer - When the pups were 55 days old, 5 males and 5 females from each group (control, experimental) were randomly selected for maze learning ability. These rats had the food removed from their cages, and were fed for 1 hr each day in a T-shaped maze (Fig. 1) to condition them to associate feeding with the maze. By the time the actual test began, they were at approximately 85% of their weight when the conditioning began, and remained within this range throughout the evaluation. When they were between 66 and 68 days old the actual testing began.

Each rat was placed in the start box and allowed to travel to the T junction where it had to make a decision of turning to the right or the left. A one-way swinging gate insured that a decision was made. If the rat turned to the right (arbitrarily established as the first correct direction) it received 2 food pellets (Noyes 45 mg) but nothing for a left turn. Each rat received 10 trials per day, then allowed to feed ad lib following the trials, as well as all during the week-end.

The criterion for "learning" was established as 9 out of 10 consecutive correct trials. Once a rat had met the criterion, the "correct" side of the maze was reversed to measure "learning transfer". Again the number of trials necessary to meet the criterion in this new direction were recorded. This alternation of "correct" side occurred 3 more times, so that each rat had met the "learning" criterion 3 times to the right, and twice to the left.

b. Peri- and Postnatal Exposure to Toxaphene

Rats of the postnatal group (5-6 wk adult males and females) were dosed daily by oral gavage with 6 mg/kg toxaphene (T) on 0.1 ml corn oil for 21 days. The perinatal group (14 wk females) received daily oral doses of 6 mg/kg in 0.1 ml corn oil from day 7 following introduction of males until parturition. Controls of both groups were dosed on the same schedules with 0.1 ml corn oil.

(Scale: 1/10 actual size)

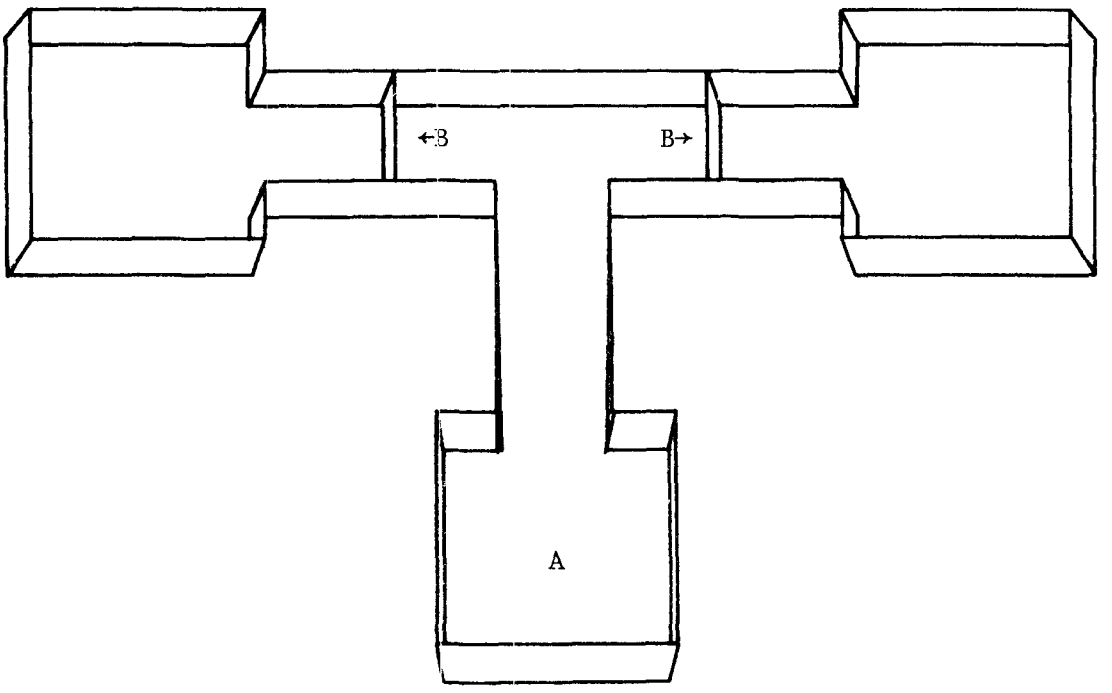


Figure 1. T-Maze employed in learning transfer analysis of rats postnatally exposed to toxaphene, and perinatally exposed to toxaphene, methyl parathion, and toxaphene:methyl parathion. A. Start box. B. One way swinging gates through which the rat must pass to ensure that a choice was made.

Once the pups of the perinatal group were 7 days old, behavioral tests consisting of the grasp-hold reflex, righting reflex, and startle reflex were initiated as previously described. When rats of both the perinatal and postnatal groups were between 14-16 weeks old, they were tested for "learning" and "learning transfer" abilities as described above.

B. TOXAPHENE AND METHYL PARATHION AGAINST NON-TARGET AND TARGET INSECTS

1. Non-Target Insects

Several adult hemipteran predators were tested from three different areas in Southern Arizona. During the summer of 1977, *Nabis* spp., *Geocoris* spp., and *Orius tristicolor* White were collected from alfalfa fields at University of Arizona Agricultural Experiment Stations in Tucson, Safford, and Yuma with a sweep net. Individual *Nabis* spp. and *Geocoris* spp. or several *O. tristicolor* were placed in 10 dram glass vials with snap-on plastic caps and transported in an ice chest to prevent dehydration and overheating. After return to the laboratory, the vials were removed from the ice chest and warmed to room temperature. A small square of paper toweling (approx. 2 x 2 cm) with a drop of approx. 0.5 M sucrose solution (just enough to moisten the paper) was placed into each vial for the insects to feed upon.

After being held in the laboratory overnight at room temperature, the insects were dosed by topical application. The insects were anesthetized with carbon dioxide. Solutions of methyl parathion or toxaphene were then applied to the dorsal surface of the thorax after the technique of Hopkins et al.[37], using a motor-driven microapplictor. All *Nabis* spp. and *Geocoris* spp. received 0.25 µl of a specific concentration of insecticide dissolved in acetone while *O. tristicolor* received 0.10 µl. Controls were either untreated or dosed with acetone in an amount comparable to the treatments. The insects were returned to their vials and held in the laboratory at room temperature after dosing. Mortality was determined by probing the insects to observe a lack of reaction or movement. Mortality values were obtained at 24 hr and based on 10-30 insects in groups of 10 per concentration. LD-50 values (µg insecticide/g insect) were determined for each insecticide by logarithm-probit analysis (Finney[38,39]) and compared statistically.

A similar procedure was used during the summer of 1978. *Nabis alternatus* Parshley, *N. americoferus* Carayon, *Geocoris punctipes* (Say), *G. pallens* Stal, and *O. tristicolor* were collected from the same alfalfa fields as used in 1977.

However, a D-Vac® was employed because it was less injurious than a sweep net. Nabis spp. were placed singly in each vial to avoid cannibalism. Since no aggression was observed among Geocoris spp. or O. tristicolor, up to 5 Geocoris spp. or 10 O. tristicolor were placed in each vial. Paper toweling and sucrose solution were placed in the vials as previously described.

The day following collection each insect was dosed topically with the appropriate amount of a specific concentration of MP, T, or MP-T mixture (1:2). After determining the MP LC-50 levels (ng insecticide/insect), each species was dosed with MP at or near the LC-50 level, T twice that of the MP, and a 1:2 MP-T mixture. Nabis spp. and Geocoris spp. received 0.25 µl of solution while O. tristicolor received 0.10 µl. Controls were untreated or dosed with acetone in the appropriate amount. After dosing, one Nabis spp., 5 Geocoris spp., or 10 O. tristicolor were placed in each vial. Mortality values were obtained at 24 and 48 hr. Depending on the abundance of each species, the values were based on 10-80 insects in groups of 10 per concentration for MP, and 10-90 insects in groups of 10 for T and MP-T.

2. Target Insects

a. Lepidopterous Larvae

Newly hatched larvae of the tobacco budworm, Heliothis virescens (Fab.); corn earworm, H. zea (Boddie); beet armyworm, Spodoptera exigua (Hübner); and salt marsh caterpillar, Estigmene acrea Drury, were obtained from the USDA Cotton Insects Laboratory in Tucson. A modified Shorey and Hale[40] diet was used to rear the larvae. Cups containing the larvae were held in a humidified environator at 27°C with a 15:9 hr light:dark cycle until the larvae attained the proper size.

Third instar larvae weighing 15-30 mg (10-20 mg for S. exigua) were selected. Either 20% of the larvae to be dosed or at least 10 individuals, were weighed and the average weight calculated. H. virescens and H. zea larvae were placed individually into 30 ml clear plastic cups filled halfway with medium and enclosed with paper lids. Plastic petri dishes containing medium, prepared by first slanting the dishes and filling halfway with medium, were used to treat 15 S. exigua or 10 E. acrea per dish. E. acrea were anesthetized with CO_2 prior to dosing to slow their rapid movements. Larvae were dosed by topical application to the thorax with 0.5 µl of MP, T, or MP-T (1:2) as described for non-target insects and returned to the environator. Controls were either untreated or dosed with acetone. Mortality values were obtained at 24 and 48 hr and based on 40 insects in groups of 10 per concentration.

b. Houseflies

For reference material, houseflies, *Musca domestica* L., were obtained from the laboratory culture of the Department of Entomology at the University of Arizona, Tucson.

At 4-5 days of age, flies were sexed under CO_2 anesthesia. Female flies were dosed by topical application on the thorax with 1.0 µl of MP, T or MP-T as previously described. After dosing, the flies were placed in tall cylindrical paper containers (17 x 9 cm) fitted with screen lids and kept at room temperature. A sugar cube and small glass vial filled with water and fitted with a cotton plug were provided to each cage. Mortality values, were obtained at 24 and 48 hr and based on 75 insects in groups of 25 for each concentration.

C. FATE OF TOXAPHENE, CHLORDIMEFORM AND METHYL PARATHION IN THE MOUSE

To determine if combinations of MP-T-chlordimeform (C) affect the excretion, retention, or mortality of any of the individual compounds when orally fed to mice, the following experiment was conducted in 3 parts: (1) Toxaphene - T alone and in combination with the other compounds. The combinations were T-MP, T-C, and T-MP-C. (2) Chlordimeform - C-T, C-MP, and C-T-MP. (3) Methyl-Parathion - MP and MP-T.

Mortality data were collected from the excretion-retention dosing of each part. Additional mortality dosing was performed for each combination to obtain 3 replicates of 12 each.

1. Toxaphene

Male Swiss mice, 16-17 weeks, and an average of 39.98 g were orally dosed via a syringe and feeding tube attached to a motor driven microapplicator with 1.0 mg (25 mg/kg) ^{36}Cl-Toxaphene (42 µCi/g) in 0.16 ml corn oil. For the T-MP combination, the dose included 0.5 mg MP (12.5 mg/kg), the T-C included 0.125 mg C (3.2 mg/kg), and T-MP-C had 0.5 mg MP and 0.125 mg C added to the 1.0 mg T.

For excretion studies, 3 replicates of 4 mice each were placed into glass metabolism chambers following dosing. The metabolism cages provided for separate collection of urine and feces, and were maintained in an air conditioned environment (22-25°C, 50% RH; L:D - 11:13). The animals were provided laboratory chow and water *ad libitum*.

Urine and feces were collected and mortality counts made at 3, 6, 12, 24, 48, 72, 96, 168, and 192 hr. Feces and urine were stored at 20°C to await further

analysis. One mouse from each replicate was sacrificed at 192 hr and frozen to by analyzed for tissue retention. The remaining mice in each replicate were given an additional dose; these animals were referred to as "redosed". Urine and feces were collected in the previously described manner. Likewise, 1 mouse from each replicate of redosed animals was sacrificed at the last sample time and retained for tissue storage determination. Another 3 mice from each treatment were held until the 33rd day following redose and sacrificed to obtain tissue retention data at that point. At each sampling period cages were rinsed to remove any urine residues; the rinses were frozen until analyzed.

Feces samples were thawed, air dried, weighed, and ground to a powder, and 2, 50 mg subsamples digested in 3 ml of TS-1® tissue solubilizer using heat to aid digestion. Bleaching was accomplished with 0.3 ml benzoyl peroxide (1g/5 ml toluene). Tissues (brain, liver, lipid, kidney, skeletal muscle, and testes) were weighed and 50 mg subsamples digested in 3 ml of TS-1®; radioassay was performed. Quench was corrected using the external standard method. Female mice, 16-17 weeks, average weight 29.41 g, were orally dosed in the same manner and at the same levels as the males except with non-labelled T and their lower weights resulted in the following dosages: T (34 mg/kg), MP (17 mg/kg) and C (4.3 mg/kg). Also ovaries were substituted for testes.

Twelve mice were used for each treatment (T, T-MP, T-C, T-MP-C and control) and placed in holding cages. Urine and feces were not collected, but mortality counts were taken at 3, 6, 12, 24, 48, 72, 96, 168, and 192 hr. After 192 hr all were redosed.

Following this mortality study, the 12 controls and the 12 T-dosed females were dosed with 1.0 mg of the radio-labelled T and placed in metabolism cages. This gave a group of 12 mice that had not received T and a group that was receiving its third dose. Urine and feces were collected at 24, 48, 72, and 96 hr for both groups, and also at 168 and 192 hr for the single dosed group. The tri-dosed group was sacrificed at 96 hr and 3 carcasses retained for tissue analysis. After 192 hr the remaining group was redosed, and urine and feces collections made at 24, 48, 72, and 96 hr, and 3 carcasses retained for tissue analysis. Therefore urine, feces, and tissues were available from mice which received single, double, and triple doses of T. Cages were rinsed at every collection with acetone and 1:1 mixture of water: methanol.

2. Chlordimeform

All methods employed and dosages applied in this part of the experiment were almost identical with those used for male mice in part (1) Toxaphene. The only difference was in the dosing combinations and the use of ^{14}C-chlordimeform (15.3 μCi/mg; Ciba-Geigy Corp.). The dosing combinations were C, C-T, C-MP, and C-T-MP.

3. Methyl Parathion

Methods employed and dosages applied were the same as previously described with the following exceptions. Only MP and T-MP combinations were evaluated, with neither compound being radio-labelled. Urine and feces were collected at 3, 6, 12, 48, 72, and 96 hr only, and no redose was made. Four mice from each treatment were retained for tissue analysis, and only liver, kidney, and lipid were sampled.

Urine was analyzed for para-nitrophenol (PNP) by electron capture gas chromatography following the complete method of Cranmer[41]. Feces and tissues were extracted in hexane, dried through sodium sulfate and concentrated to 1.0 ml. Lipid samples were partitioned between acetonitrile and hexane (4:1) after the initial hexane extraction, dried, reconstituted in hexane, dried through sodium sulfate, and then concentrated to 1.0 ml. Analysis for MP in feces and tissues is being evaluated by flame photometric gas chromotography.

In addition to the mortality data collected from the excretion-retention studies, the possible potentiation of MP by T was studied in this evaluation. Male mice received doses of one half the LD_{50} (56T and 16MP mg/kg) and a 2:1 combination based upon the MP one half LD_{50} (32T and 16 MP mg/kg). The groups were: T (56 mg/kg), T (32 mg/kg), T (56) + MP (16 mg/kg), T (32) + MP (16 mg/kg), MP (16 mg/kg), and corn oil-dosed controls. Each group consisted of 4 replicates of 12 (48 total/dose). Mortality counts were made up to 48 hr.

SECTION V

DISCUSSION

A. CHANGES IN THE NERVOUS SYSTEM INFLUENCED BY TOXAPHENE

1. Ion Movements in the Nervous System of the American Cockroach

a. Methodology -

A primary criticism of the procedures employed is that by evaluating ion fluxes *in vitro*, innumerable openings are created for the exchange of ions when connectives and peripheral nerves are severed (O'Brien[42]). Therefore, this procedure does not distinguish between fluxes through these openings and those through the natural channels of the extraneuronal and axonal membranes. This would be a valid point if the observed changes were presented in absolute terms. The results herein, although based upon numerical data, describe significant changes relative to controls under the same circumstances. Therefore, regardless of the numerical values, toxaphene did cause significant changes in some ion movements. However, it must be reemphasized that the values presented for uptake and efflux should not be considered as representative of the actual values for ionic movements that would occur in the intact nervous system.

(1) $^{24}Na^{+}$ - Overall, there were no changes in $^{24}Na^{+}$ movements (Table 1). Relative uptake of $^{24}Na^{+}$ between control and toxaphene-exposed sections was very inconsistent. However, evaluation of each time interval uncovered a significant reduction at 5 min for AB, and 10 min for all 3 sections (Table 2). Efflux of $^{24}Na^{+}$ also displayed few changes. $^{24}Na^{+}$ efflux was slightly accelerated, becoming significant for AB and TH by 60 min. BR displayed no significant changes (Table 2).

(2) $^{36}Cl^{-}$ - As with $^{24}Na^{+}$, overall there were no changes in $^{36}Cl^{-}$ movements (Table 1). However, evaluation of each time period showed the relative uptake of $^{36}Cl^{-}$ by toxaphene-exposed BR as elevated over controls between 2.5 and 10 min, but lower after 60 min, with no changes in TH and AB (Table 3), Efflux of $^{36}Cl^{-}$ from toxaphene-exposed BR was significant only at the 15 min sampling period, with no changes in TH or AB (Table 3).

(3) $^{42}K^+$ - Movements of $^{42}K^+$ were significantly altered by toxaphene in all but TH (Table 1). Differences in uptake of $^{42}K^+$ by toxaphene-exposed BR and AB occurred after 30 min. All 3 sections displayed inconsistent rates of uptake for the first 10 to 15 min; then exposed sections became accelerated over controls through the remainder of the evaluation (Fig. 2a, 3a, 4a).

TABLE 1. TWO-WAY ANALYSIS OF VARIANCE FOR CONTROL VS. EXPOSED SECTIONS. F VALUES FOR UPTAKE AND EFFLUX OF IONS BY EACH NERVE SECTION. (*) INDICATES SIGNIFICANT DIFFERENCES AT THE 0.05 LEVEL. VALUES WERE SIGNIFICANT IF GREATER THAN 3.92

	$^{24}Na^+$	$^{42}K^+$	$^{36}Cl^-$	$^{45}Ca^{++}$
Uptake				
Brain	0.36	12.90*	0.01	15.72*
Thoracic	0.87	1.83	2.22	19.54*
Abdominal	3.37	7.02*	0.27	16.61*
Efflux				
Brain	0.69	6.19*	0.01	0.14
Thoracic	0.71	2.51	0.08	8.50*
Abdominal	0.41	9.29*	3.02	18.01*

The influence of toxaphene upon $^{42}K^+$ efflux was similar to that for uptake, in that BR and AB displayed significant changes, while TH did not (Fig. 2b, 3b, 4b). This decrease in efflux became significant for BR after 30 min, whereas AB displayed a decrease throughout the entire evaluation (Table 4).

TABLE 2. RESULTS OF T-TEST EVALUATION OF SODIUM-24 MOVEMENTS BETWEEN CONTROL AND EXPOSED NERVE SECTIONS AT EACH TIME INTERVAL

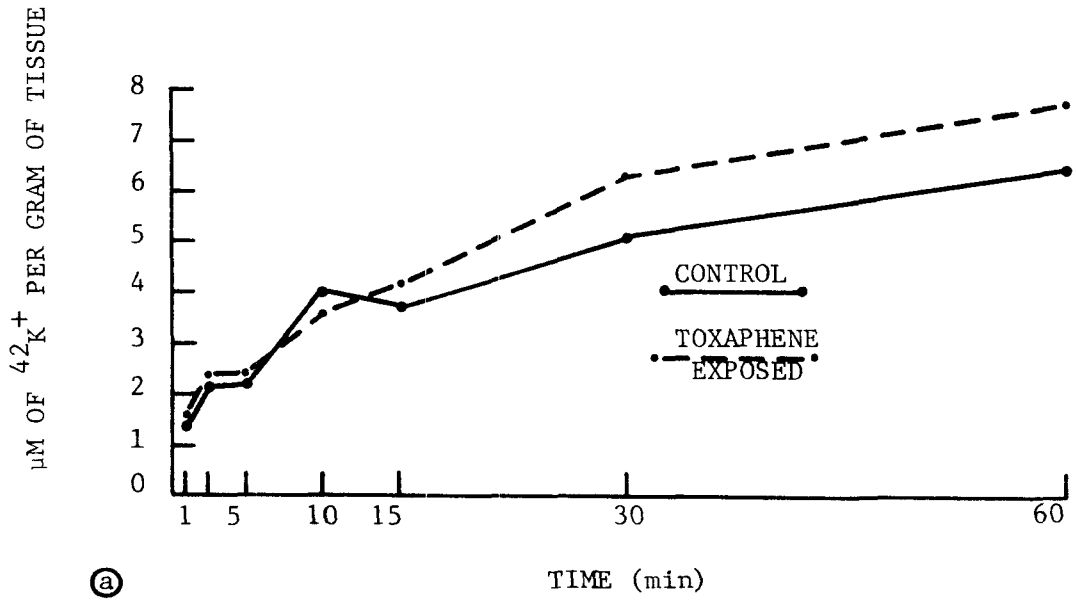
	Brain	Thoracic	Abdominal
Uptake (min)			
1	NS	NS	NS
2.5	NS	NS	NS
5	NS	NS	S
10	S	S	S
15	NS	NS	NS
30	NS	NS	NS
60	NS	NS	NS
Efflux (min)			
1	NS	NS	NS
2.5	NS	NS	NS
5	NS	NS	NS
10	NS	NS	NS
15	NS	NS	NS
30	NS	NS	NS
60	NS	S	S

Significance determined at the 0.05 level. (S) significant. (NS) not significant

TABLE 3. RESULTS OF T-TEST EVALUATION OF CHLORINE-36 MOVEMENTS BETWEEN CONTROL AND EXPOSED NERVE SECTIONS AT EACH TIME INTERVAL

	Brain	Thoracic	Abdominal
Uptake (min)			
1	NS	NS	NS
2.5	S	NS	NS
5	S	NS	NS
10	S	NS	NS
15	NS	NS	NS
30	NS	NS	NS
60	S	NS	NS
Efflux (min)			
1	NS	NS	NS
2.5	NS	NS	NS
5	NS	NS	NS
10	NS	NS	NS
15	S	NS	NS
30	NS	NS	NS
60	NS	NS	NS

Significance determined at the 0.05 level. (S) significant. (NS) not significant



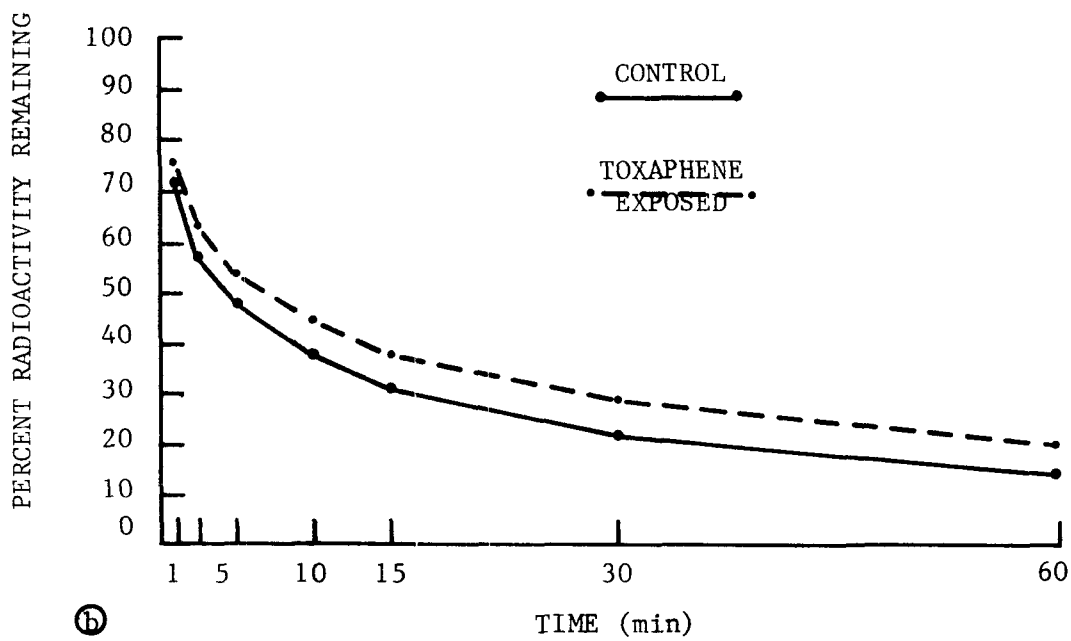
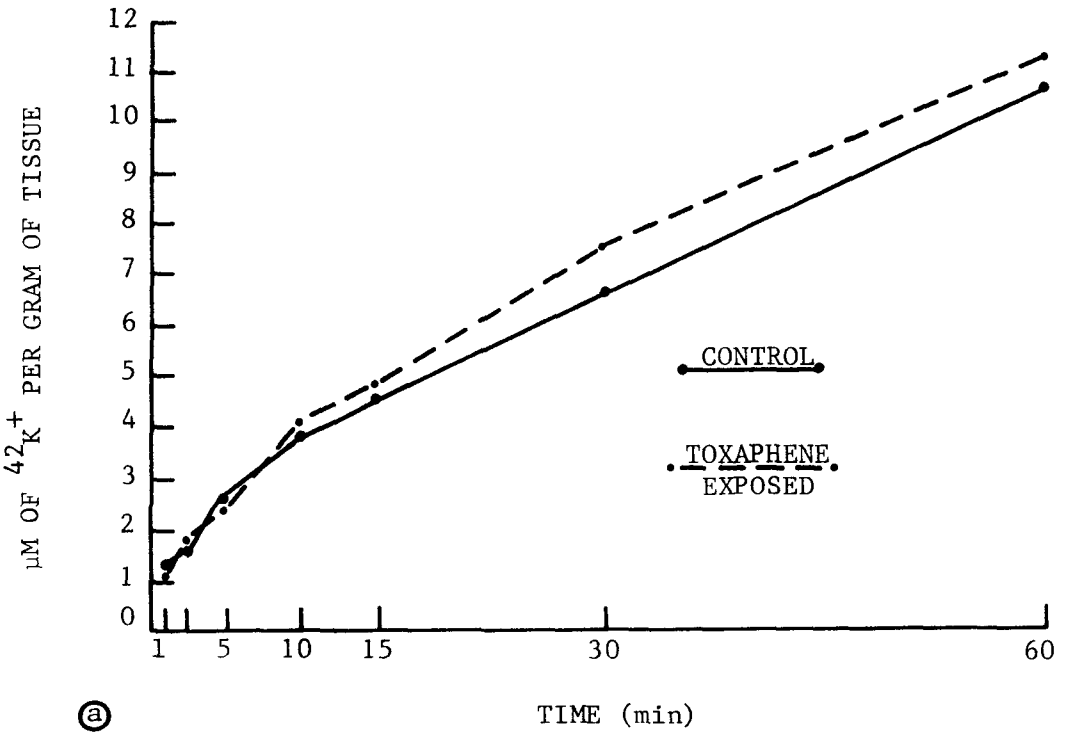


Fig. 2 Rates of $^{42}K^+$ flux measured from the brain section of the central nervous system of P. americana exposed to 10^{-5}M toxaphene in vitro.

a. Uptake.
b. Efflux.



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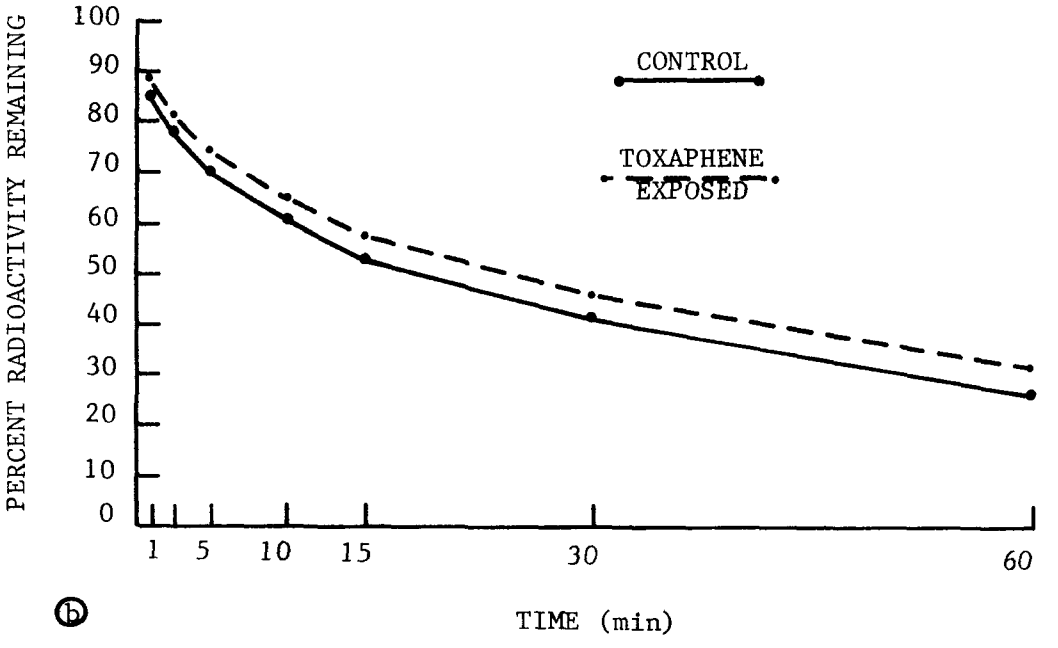

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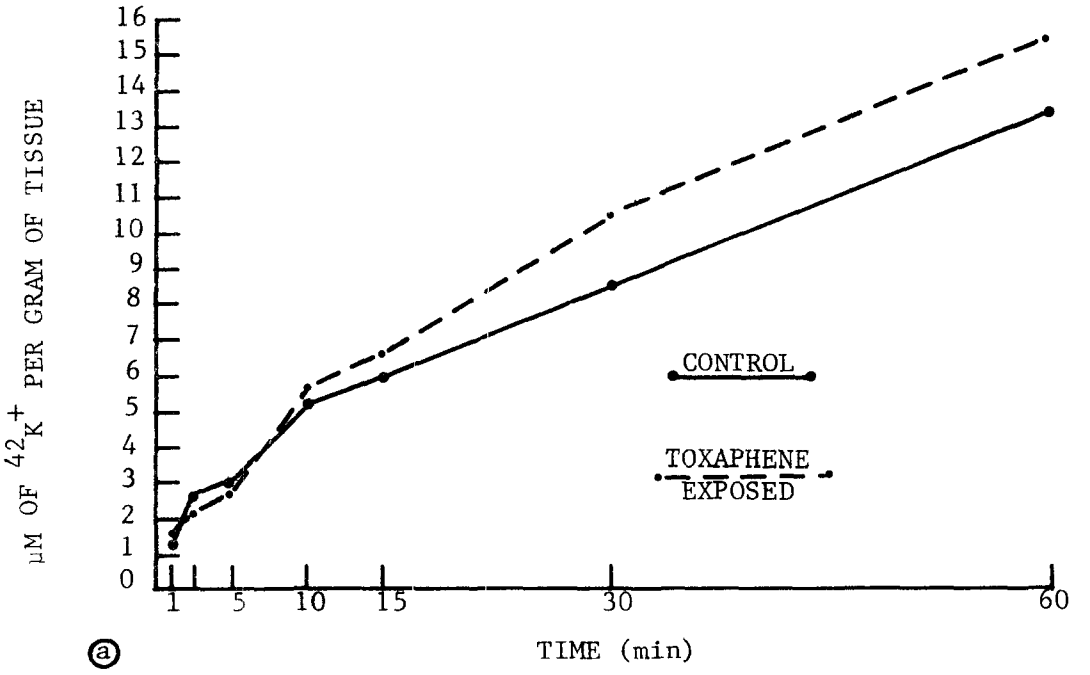
Fig. 3 Rates of $^{42}K^+$ flux measured from the thoracic section of the central nervous system of P. americana exposed to 10^{-5}M toxaphene in vitro.

a. Uptake.
B. Efflux.



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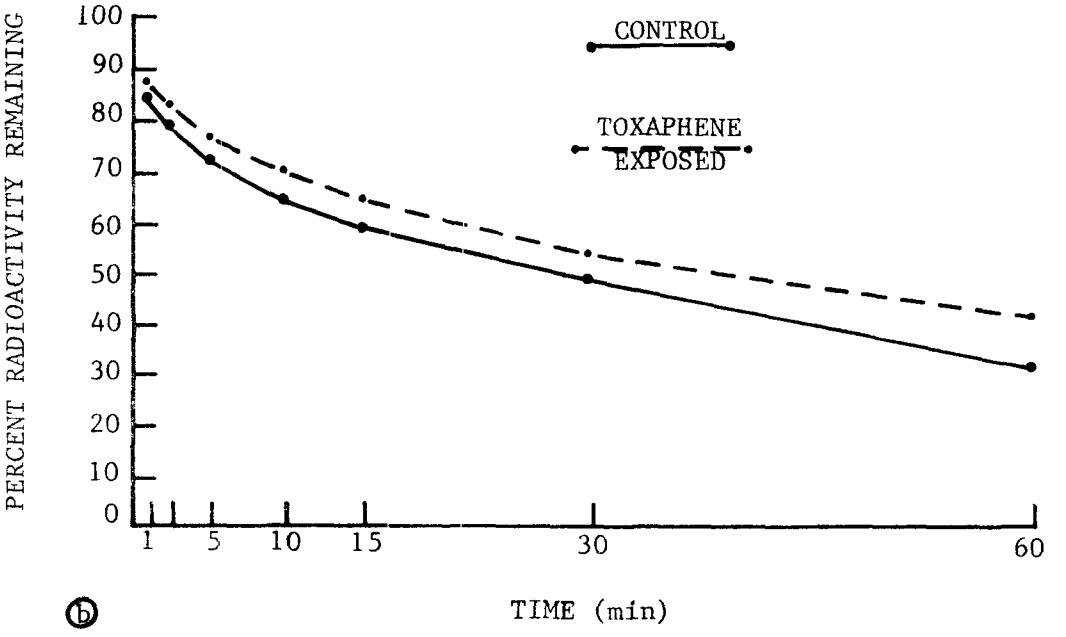

ⓑ

Fig. 4 Rates of $^{42}K^+$ flux measured from the abdominal section of the central nervous system of P. americana exposed to 10^{-5}M toxaphene in vitro.

a. Uptake.
b. Efflux.

TABLE 4. RESULTS OF T-TEST EVALUATION OF POTASSIUM-42 MOVEMENTS BETWEEN CONTROL AND EXPOSED NERVE SECTIONS AT EACH TIME INTERVAL

	Brain	Thoracic	Abdominal
Uptake (min)			
1	NS	NS	NS
2.5	NS	NS	NS
5	NS	NS	NS
10	NS	NS	NS
15	NS	NS	NS
30	S	NS	S
60	S	NS	S
Efflux (min)			
1	NS	NS	S
2.5	NS	NS	S
5	NS	NS	S
10	NS	NS	S
15	NS	NS	S
30	S	NS	S
60	S	NS	S

Significance determined at the 0.05 level. (S) significant. (NS) not significant

The effect of toxaphene upon $^{42}K^{+}$ movements was opposite that induced by DDT or dieldrin. Toxaphene caused an increase of internal $^{42}K^{+}$ levels while DDT and dieldrin were reported as causing reductions of same (Matsumura and O'Brien[19], and Hayashi and Matsumura[20]). Both DDT and dieldrin induced a decrease of internal $^{42}K^{+}$ levels with in vivo preparations, while few changes were evident from tissues exposed in vitro.

A possible explanation for the increase of internal K^{+} levels by toxaphene might be the inhibition of a Na^{+}-K^{+} ATPase. It was reported by Desaiah and Koch[7] that toxaphene inhibited this enzyme in catfish brain. This could result in a reduction of $^{42}K^{+}$ efflux as was observed herein. If uptake proceeded as normal in all samples, but efflux was reduced by toxaphene, it is possible that an accumulation of $^{42}K^{+}$ would occur in these samples, giving the illusion of increased uptake (Fig. 2a, 3a, 4a).

(4) $^{45}Ca^{++}$- Of the ions investigated, $^{45}Ca^{++}$ displayed the most acute changes as a result of toxaphene exposure (Table 1). During the initial 10 min of evaluation, uptake by exposed sections was greatly accelerated over controls (Fig. 5a, 6a, 7a). This difference became significant between 5 and 10 min for AB and BR, and within 10 min for TH (Table 5).

Between 10 and 15 min, $^{45}Ca^{+}$ uptake displayed an abrupt and dramatic reduction. Following this reduction the difference in uptake between control and exposed sections increased, becoming significant by 60 min for all 3 sections.

Toxaphene was responsible for a significant drop in $^{45}Ca^{++}$ efflux from all but BR (Table 1). A significant decrease in efflux from AB occurred throughout the entire 60 min (Fig. 7b), while TH displayed significant reduction from 2.5 min through the remainder of the evaluation (Fig. 6b). The increase in uptake and decreased efflux would lead to significant increases in the internal levels of $^{45}Ca^{++}$.

Changes in $^{45}Ca^{++}$ movements due to toxaphene, bore little resemblance to those changes induced by DDT or dieldrin. Dieldrin was reported as causing a net reduction of internal $^{45}Ca^{++}$ levels, while DDT was reported as reducing efflux in vivo, yet increasing it in vitro (Matsumura and O'Brien[19], and Hayashi and Matsumura[20]).

Involvement of cyclic AMP in the uptake of $^{45}Ca^{++}$ is possible. Functioning of cyclic AMP, and the enzyme responsible for its breakdown (phosphodiesterase),

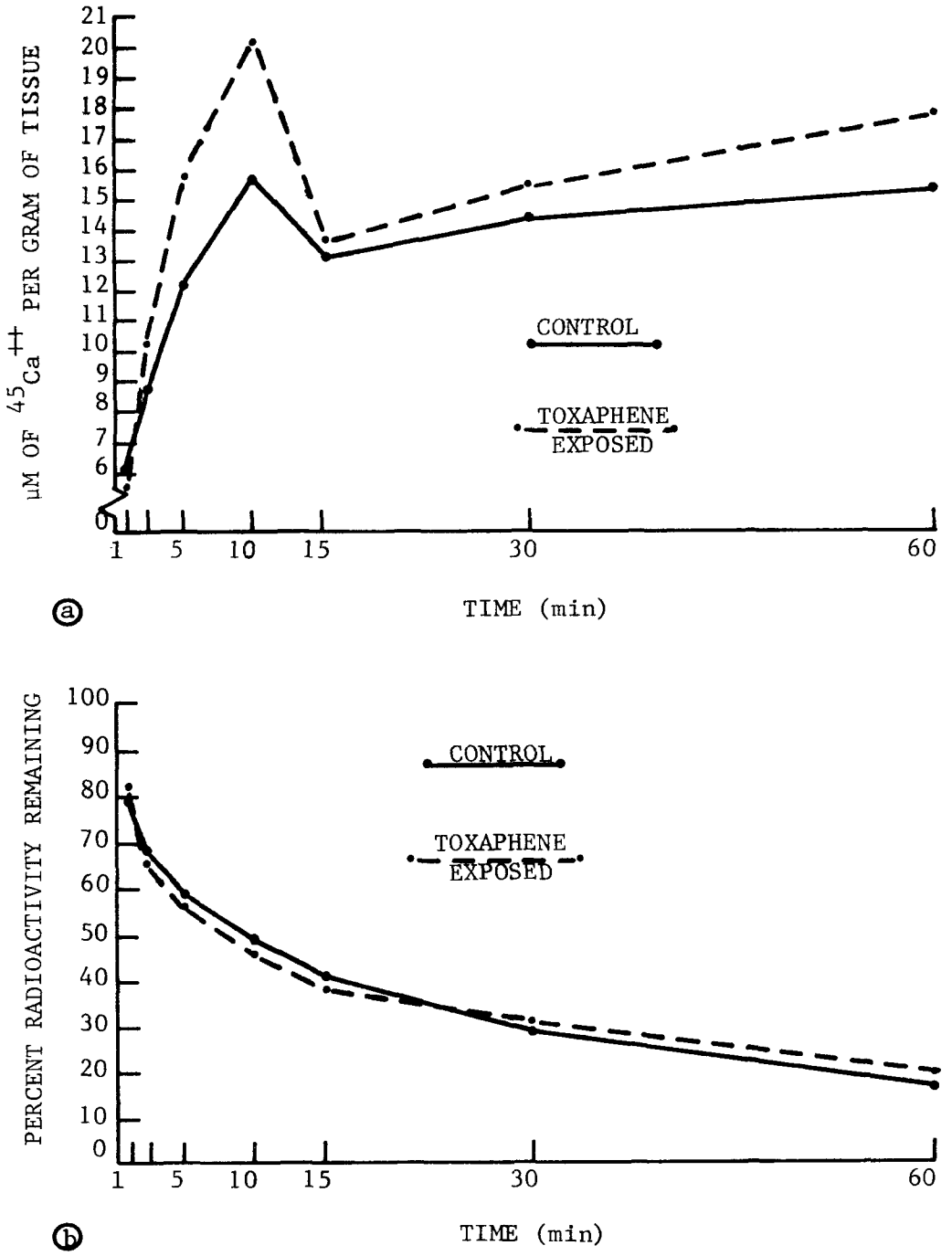

Fig. 5 Rates of $^{45}Ca^{++}$ flux measured from the brain section of the central nervous system of P. americana exposed to 10^{-5}M toxaphene in vitro.

a. Uptake.
b. Efflux.

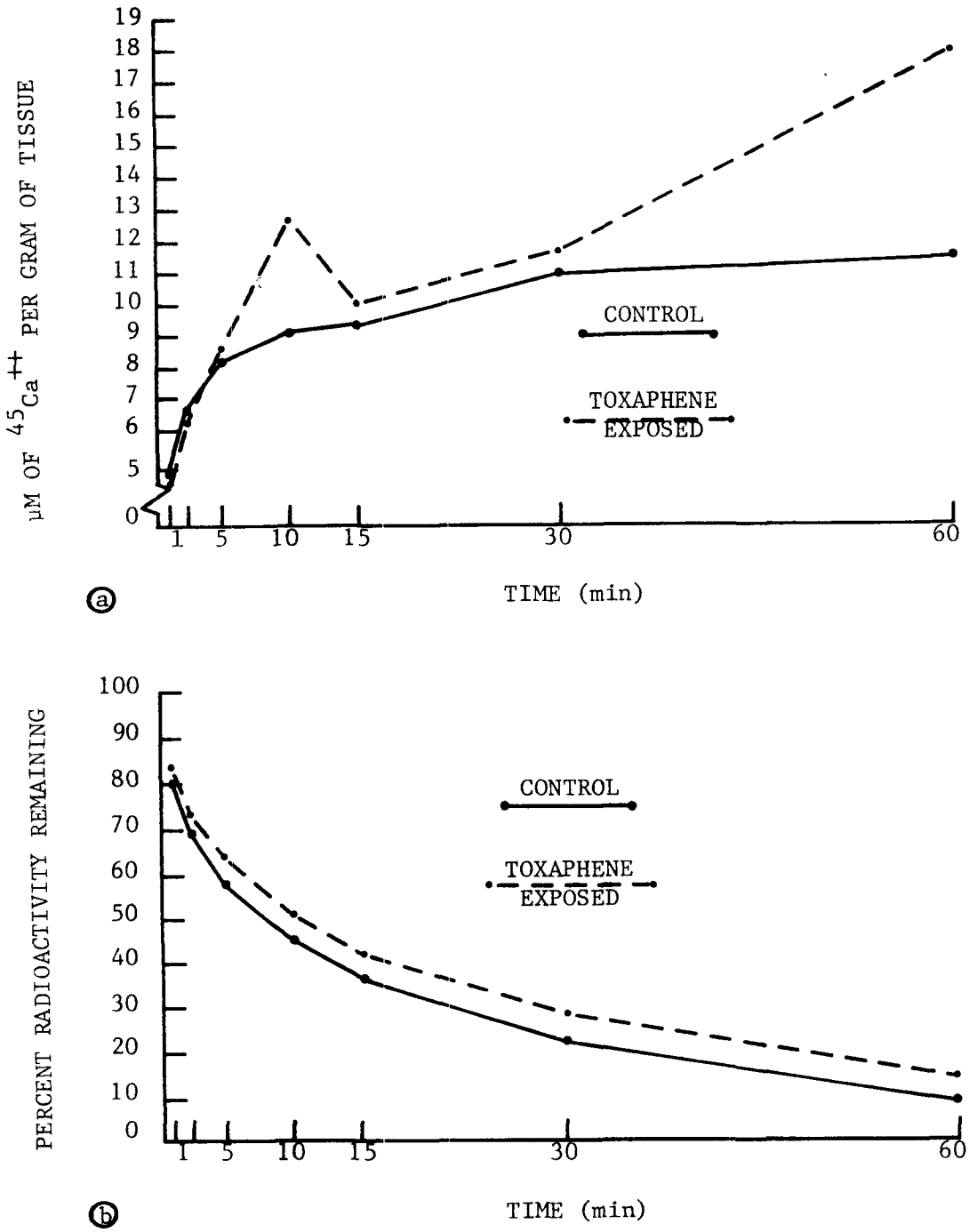

Fig. 6 Rates of $^{45}Ca^{++}$ flux measured from the thoracic section of the central nervous system of P. americana exposed to 10^{-5}M toxaphene in vitro.

a. Uptake.
b. Efflux.

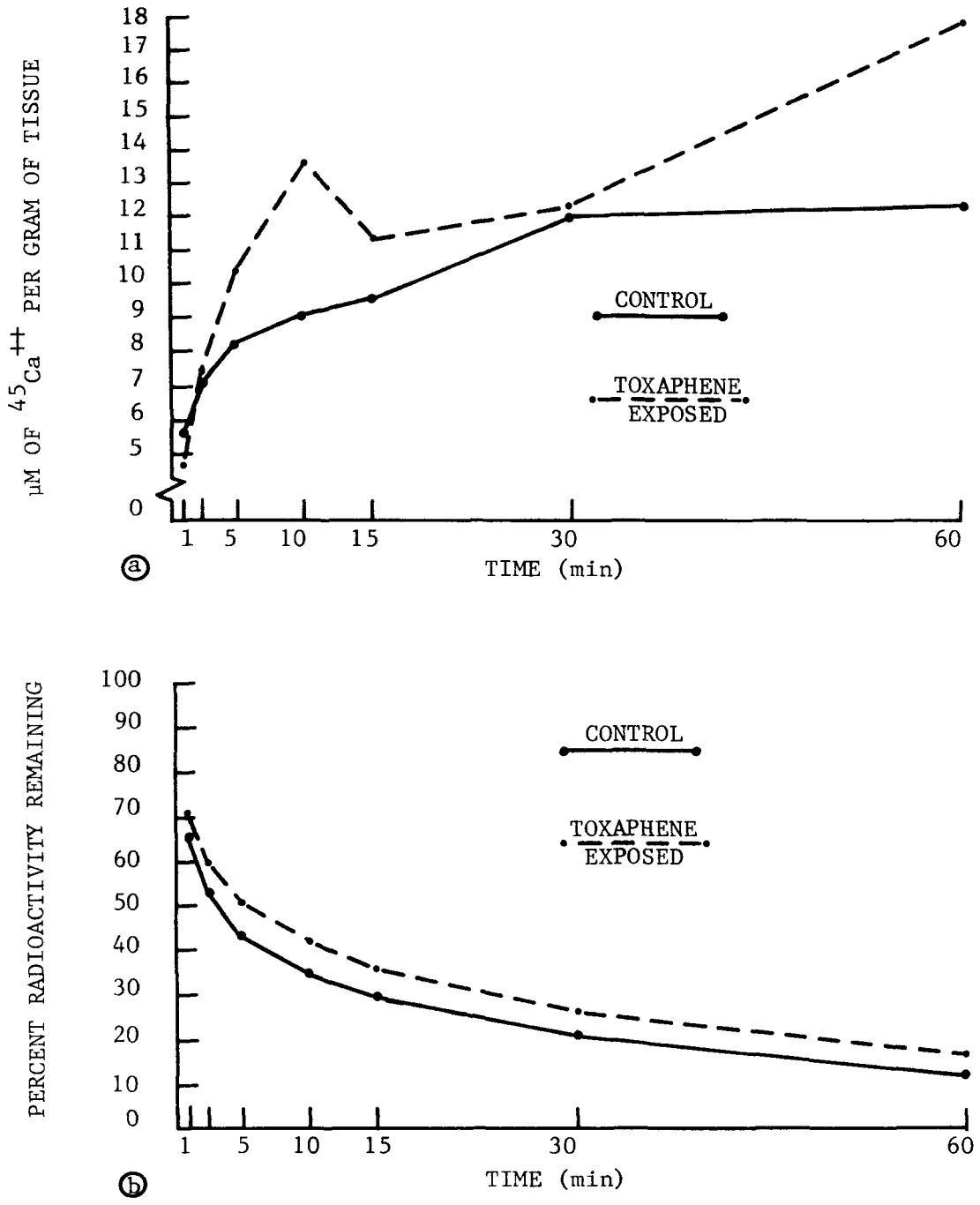

Figure 7. Rates of $^{45}Ca^{++}$ flux measured from the abdominal section of the central nervous system of P. americana exposed to 10^{-5} M toxaphene in vitro.

a. Uptake.

b. Efflux.

TABLE 5. RESULTS OF T-TEST EVALUATION OF CALCIUM-45 MOVEMENTS BETWEEN CONTROL AND EXPOSED NERVE SECTIONS AT EACH TIME INTERVAL

	Brain	Thoracic	Abdominal
Uptake (min)			
1	NS	NS	NS
2.5	NS	NS	NS
5	S	NS	S
10	S	S	S
15	NS	NS	NS
30	NS	NS	NS
60	S	S	S
Efflux (min)			
1	NS	NS	S
2.5	NS	S	S
5	NS	S	S
10	NS	S	S
15	NS	S	S
30	NS	S	S
60	NS	S	S

Significance determined at the 0.05 level. (S) significant. (NS) not significant

have been observed to be Ca^{++} dependent (Rasmussen[43], and Kakiuchi et al.[44]). Increases in cyclic AMP have been observed to be associated with Ca^{++} uptake (Rasmussen[43]) while brain and nerve cord of L. maderae displayed significant increases in cyclic AMP after exposure to toxaphene in vivo (Butler and Crowder[45]). Therefore, increased uptake of $^{45}Ca^{++}$ may be in response to this elevation of cyclic AMP by toxaphene.

b. Relationship of the Observed Changes to Neural Activity

The initial effect of toxaphene may have been upon the membranes of the glial and perineural cells and the ion regulation controlled by them. Dary and Crowder[2] observed that isolated abdominal nerve sections of P. americana exposed to 10^{-5}M toxaphene, displayed an average latent period of 26 min before intense activity was observed electrophysiologically. However, changes in ion movements by toxaphene became significant before this time, especially efflux of $^{42}K^{+}$ and $^{45}Ca^{++}$.

Therefore, the initial response to toxaphene by the neurons may be due to the altered ionic concentrations within the extracellular spaces. The concentrations of K^{+} and Ca^{++} would have been greater, while those of Na^{+} and Cl^{-} could have been unchanged or possibly reduced. These concentration changes could account for the nerve activity observed with toxaphene exposure. For example, high internal concentrations of K^{+} would lead to a reduction in the resting potential (Yamasaki and Narahashi[18]). Such a lowering of the resting potential could make the neuron more receptive to a stimulus, and able to produce high frequency activity, as has been observed during the early stages of toxaphene poisoning (Lalonde and Brown[1], Dary and Crowder[2], and Wang and Matsumura[3]). Elevated Ca^{++} concentrations could stabilize the membrane against this depolarization (Ulbricht[46]), enabling the nerve to continue functioning rather than leading to conduction block due to the high K^{+} levels. Since there was no overall significant change in $^{24}Na^{+}$ and $^{36}Cl^{-}$ movements, it is possible that these ions play only a small part in the initial disruption of the CNS following toxaphene exposure. However, they may have a role in this process during the later stages of poisoning.

The processes involved in ion movement changes brought about by toxaphene are uncertain. The stimulation of cyclic AMP production (Butler and Crowder[45]), or the inhibition of a Na^{+}-K^{+} ATPase (Desaiah and Koch[7]) by toxaphene might explain the increases and decreases of the ions as observed. However, these two processes may not be mutually exclusive, as ouabain (inhibitor of Na^{+}-K^{+} ATPase) and high K^{+} levels have been observed to increase cyclic AMP levels

in brain slices of guinea pigs (Shimizu et al[47]). Also, high levels of K^+ and Ca^{++} have been observed to inhibit Na^+-K^+ ATPase of crab peripheral nerve (Skou[30]). Therefore, stimulation of cyclic AMP formation by toxaphene could accelerate Ca^{++} uptake, resulting in the indirect inhibition of Na^+-K^+ ATPase, or an increase of internal K^+ by blockage of Na^+-K^+ ATPase could stimulate cyclic AMP production. Either would result in an increase of internal concentrations of Ca^{++} and K^+ and a reduction of Na^+, as was observed in this study.

c. Differences Between Nerve Sections

The difference in response to toxaphene by the 3 nerve sections was primarily in the time it took for one section to display symptoms, relative to the others. The earliest and most numerous significant changes occurred with AB. Its large surface to volume ratio may be responsible for this difference.

2. Cyclic Nucleotides in Several Tissues of the Cockroach and Mouse

Male and female cockroaches selected at random from a laboratory population were compared for differences in cAMP and cGMP levels of various tissues (Table 6). Cyclic AMP was significantly greater in males for the gastric caeca and rectum, and cGMP was significantly greater in testes than ovaries. The greatest concentration of cAMP was found in Malpighian tubules, which was the only tissue significantly different from other tissues for either sex. There was no significant difference between tissues for cGMP for either sex.

Cyclic AMP (3.0-36.0 picomoles/mg protein) and cGMP (1.7-3.9 picomoles/mg protein) found in all tissues of untreated cockroaches were comparable to concentrations of these cyclic nucleotides in vertebrates (Robinson[48], Ferrendelli et al.[49,50,51], Hepp et al.[52], and Steiner et al.[53]). Cyclic GMP was less than cAMP, as was also the case with mice (Tables 8 and 9). Fallon and Wyatt[54] working with male reproductive organs of crickets, showed that cyclic nucleotide concentrations, except for *Acheta domesticus* L. used by Ishikawa et al.[55], were comparable to those in vertebrates. Levels reported herein for *L. maderae* agreed with the results of Fallon and Wyatt[54].

Analysis of variance showed no significant differences between untreated and oil control levels of cAMP and cGMP for any tissue, or between cAMP and cGMP controls (Table 7). Cyclic nucleotide levels in tissues were compared between oil control and T-treated cockroaches. T-treated cockroaches produced a large amount of variability in cyclic nucleotide levels; however, significant differences were still observed between T-treated and control samples. Symptoms of poisoning from T were not noted during the course of the experiments.

TABLE 6. CYCLIC NUCLEOTIDE CONCENTRATIONS IN SEVERAL TISSUES OF A LABORATORY CULTURE OF THE COCKROACH, *L. MADERAE*, AS DETERMINED BY COMPETITIVE PROTEIN-BINDING ASSAY *

Cyclic Nucleotide	Sex	Brain	Nerve Cord	Gastric Caeca	Malpighian Tubules	Fat Body	Rectum	Gonads
cAMP	Male	16.8 ± 4.6	23.7 ± 7.9	16.9 ± 4.6a	32.4 ± 10.9b	20.3 ± 9.8	15.7 ± 2.6a	9.8 ± 2.6
	Female	12.6 ± 2.6	9.8 ± 3.7	3.0 ± 1.3a	36.0 ± 15.5b	10.2 ± 1.8	6.6 ± 1.4a	8.4 ± 6.2
cGMP	Male	1.9 ± 0.6	2.4 ± 0.8	2.4 ± 0.5	3.1 ± 1.1b	1.7 ± 0.7	3.1 ± 0.8	3.9 ± 0.8a
	Female	1.9 ± 0.5	2.5 ± 0.8	2.0 ± 0.4	3.8 ± 1.8b	2.0 ± 0.5	2.8 ± 0.5	1.7 ± 0.3a

* Values expressed as mean ± S.E. (picomoles/mg protein) for 15 males and 15 females for each cyclic nucleotide.

[a] $p < 0.05$, values are significantly different between sexes according to ANOVA.

[b] $p < 0.05$, values are significantly different from other tissues according to ANOVA.

TABLE 7. CYCLIC NUCLEOTIDE CONCENTRATIONS, DETERMINED BY COMPETITIVE PROTEIN-BINDING ASSAY, OF THE COCKROACH, *L. MADERAE*, TREATED WITH 207 μg/ANIMAL OF TOXAPHENE *

Cyclic Nucleotide	Sample	Brain	Nerve Cord	Gastric Caeca	Malpighian Tubules	Fat Body	Rectum	Gonads
cAMP	Control	74.5±25.4	33.6±10.3	28.5±13.4	42.2±8.1	5.9±0.9	24.8±4.3	27.2±7.1
	Toxaphene	742.8±532.5	1834.0±927.0	1091.0±518.0a	1302.0±523.0a	1155.0±841.0	895.1±475.0	860.2±773.1
cGMP	Control	8.8±3.2	13.0±4.3	4.7±1.5	14.2±4.2	2.6±1.4	7.4±2.3	6.5±1.6
	Toxaphene	13.6±2.8a	31.7±6.7a	27.1±6.0a	52.1±17.8a	39.6±18.9	39.5±7.9a	36.5±12.6a

* Values expressed as means ± S.E. (picomoles/mg protein) for 8 combined time intervals, each interval comprising 2 males and 2 females.

[a] $p < 0.05$, values are significantly different from controls values, ANOVA

TABLE 8. CYCLIC AMP CONCENTRATIONS, DETERMINED BY COMPETITIVE PROTEIN-BINDING ASSAY, IN SEVERAL TISSUES OF THE MALE MOUSE ORALLY DOSED WITH 112 mg/kg OF TOXAPHENE *

Sample	Time (Min)	Brain	Nerve Cord	Kidney	Liver	Testes
Control		67.1±20.0	59.6±24.2	64.6±20.4	39.8±19.8	26.8±7.3
Toxaphene						
Asymptomatic	15	28.6±13.3	14.2±5.7	6.7±1.8	13.8±5.5	75.4±62.4
Symptomatic	45	199.2±141.8	502.7±444.3	27.7±3.6	54.2±33.8	118.9±111.5a
Symptomatic	90	1136.0±846.0a	744.2±362.8a	647.0±48.6a	450.2±41.6a	653.3±197.8a
Symptomatic	240	237.5±104.2	29.1±7.8	528.7±474.5	907.4±851.5	232.7±200.8

* Values expressed as mean ± S.E. (picomoles/mg protein). Each time interval consists of 5 animals. The 4 time intervals are combined for the control and toxaphene:asymptomatic mice.

[a] $p < 0.05$, Values are significantly different from control and toxaphene:asymptomatic, ANOVA

TABLE 9. CYCLIC GMP CONCENTRATIONS, DETERMINED BY COMPETITIVE PROTEIN-BINDING ASSAY, IN SEVERAL TISSUES OF THE MALE MOUSE ORALLY DOSED WITH 112 mg/kg OF TOXAPHENE *

	Time (min)	Brain	Nerve Cord	Kidney	Liver	Testes
Control		12.3±4.8	25.3±18.3	7.8±3.1	2.7±0.5	3.4±0.7
Toxaphene:						
Asymptomatic	15	3.2±1.3	3.2±1.1	3.9±2.3	2.0±0.8	2.2±0.7
Symptomatic	45	4.3±0.0	5.3±0.0	5.3±0.0	3.4±0.0	17.1±0.0a
Symptomatic	90	6.5±1.2	8.0±5.2	5.5±2.0	6.2±0.1	9.9±2.1
Symptomatic	240	4.1±0.6	1.6±0.7	13.0±11.2	2.0±0.6	2.8±0.9

* Values expressed as mean ± S.E. (picomoles/mg protein). Each time interval consists of 5 animals. The 4 time intervals are combined for the controls and toxaphene:asymptomatic mice.

[a] $p < 0.05$, values are significantly different from control and toxaphene:asymptomatic, ANOVA

The greatest and most significant increase in cAMP in all tissues samples occurred at 24 hr after T treatment. Following this time cAMP fluctuated until 144 hr after treatment, when cAMP again increased significantly. Cyclic GMP was slightly increased over controls at 24 hr after T treatment, with only brain, nerve cord, and fat body being significant. Cyclic GMP continued to fluctuate until 168 hr after treatment, when cGMP increased in all tissues sampled.

Analyzed over time, cyclic nucleotide levels for a particular tissue were found not to be significantly different between time intervals; therefore, values for that tissue for all time intervals were pooled. On this basis (Table 7) it was found that in the presence of T, cAMP increased in all tissues, significantly in gastric caeca and malpighian tubules, and cGMP increased significantly in all treated tissues except fat body. Since the presence of T was associated with increased levels of both cAMP and cGMP in tissues of the cockroach, both nucleotides might be involved in the mode of action or metabolism of T by the insect.

T directly or indirectly increased cAMP and cGMP in malpighian tubules. This result supports the idea of Casida and Maddrell[14] that insecticide poisoning may increase malpighian tubule secretion through the mediating action of cAMP. They reported that insect diuretic hormone induced adenyl cyclase at the receptor sites in malpighian tubules resulting in increased levels of cAMP. Rojakovick and March[17] tested several insecticides *in vitro* on the cockroach, *Gromphadorhina portentosa*, brain adenyl cyclase and phosphodiesterase enzymes. They could not demonstrate to any degree of certainty, whether insecticides have any direct effect on the enzymes. However, it appears that the presence of T *in vivo* has a direct or indirect relationship with cyclic nucleotide levels in several tissues of *L. maderae*.

Cyclic AMP and cGMP in several tissues of the mouse were compared between oil controls and T-treatments, the latter being subdivided into observable symptomatic and asymptomatic groups (Tables 8 and 9). These comparisons were made at 4 time periods and correlated with symptoms. At 15 min, no symptoms were observed. At 45 min, the first symptom appeared to be mild, exhibited by mice lying quietly in a prone position with labored breathing. At 90 min after treatment the symptoms consisted of hyperactivity illustrated by kicking, chewing, and uncoordinated body movements. The third symptom, lasting only 2-5 min and appearing 100-180 min after being dosed, was expressed by mice lying on their

backs in convulsions, leading immediately to death or reversal to the first symptom. Mice that died from the dose were combined with those sacrificed at 240 min.

Cyclic nucleotide levels for a particular tissue of controls were not significantly different between time intervals. Asymptomatic mice were not significantly different from controls at any time. Cyclic AMP was increased significantly over controls in all symptomatic tissues (Table 8), but cGMP was increased significantly only in symptomatic testes (Table 9). It appeared that T poisoning was associated with increased levels of endogenous cAMP, but not with cGMP. Fifteen min after application of T, no symptoms were observed and no difference between T-treated and controls was found. At 45 min there was a slight, but non-significant, increase in cAMP in nervous tissue (brain and spinal cord) and liver. However, there was a significant increase in testicle cAMP and cGMP. The greatest symptomatic reaction to T occurred at 90 min. This time also reppresented the greatest, and significant, increase in cAMP in all tissues examined. At 240 min cyclic nucleotide levels were not significantly different from controls.

Increased hepatic and renal cAMP in the presence of T was similar to the effect found by Kacew and Singhal[15,16] for cyclodienes and DDT. They reported that *in vitro* and *in vivo* administration of insecticides to rats increased cAMP levels and adenyl cyclase activity. Increased cAMP associated with the presence of T appeared to be related to the degree of symptomatic poisoning. This might implicate cAMP in the mode of action or metabolism of T by the mammal as well as the insect.

3. ATPases in Several Tissues of the Cockroach and Mouse

a. Electron Microscopy

Electron micrographs of kidney, brain and liver homogenates were taken to insure that the homogenate fractions were rich in plasma membrane vesicles. The micrographs demonstrated this point. The kidney homogenate was the richest in membrane vesicles and had the lowest protein content per ml. The brain homogenate fraction appeared to contain larger vesicles than the kidney homogenate and some structures similar to what Koch[5] termed nerve endings. The liver fraction had the highest protein content, but the lowest specific activity for ATPases. It was densely contaminated with blood cells and other cellular debris. This fraction was very rich in mitochondria.

TABLE 10. MEANS OF TOTAL ATPase SPECIFIC ACTIVITY ± S.E. IN KIDNEY AND LIVER OF CONTROL AND TOXAPHENE-DOSED MICE USED IN *IN VIVO* EXPERIMENT I. SPECIFIC ACTIVITY EXPRESSED IN μM Pi/mg PROTEIN/HR.

	Kidney	Brain	Liver
Control (corn oil)	66.61 ± 1.07b*	36.12 ± 0.64b	10.78 ± 0.23b
Toxaphene (LD_{50})**	45.01 ± 1.71a	31.57 ± 0.30a	9.26 ± 0.15b

* Means followed by the same letter in each tissue are not significantly different (LSD, $\alpha = 0.05$)

** 112 mg/kg of toxaphene

TABLE 11. PERCENTAGE OF INHIBITION OF ATPase ACTIVITIES DUE TO TOXAPHENE (112 mg/kg) IN MICE *IN VIVO*.

	Total ATPase	Na^+-K^+ ATPase	Mg^{++} ATPase
Experiment I			
Kidney	32.4	-	-
Brain	12.6	-	-
Liver	14.1	-	-
Experiment II			
Kidney	14.0	12.6	15.0
Brain	5.2	0.6	8.8
Liver	7.5	0.2	10.6

b. *In vivo Experiments*

In *in vivo* experiment I, homogenates from T-treated mice exhibited significant decreases in total ATPase activity of the kidney, brain and liver (Table 10). The LD_{50} dose (112 mg/kg) did not display 50% inhibition of the activity; the highest inhibition reached was 32.5% in kidney homogenates (Table 11).

In *in vivo* experiment II, Na^+-K^+ ATPase was inhibited significantly only in the kidney, and the Mg^{++} ATPase was inhibited significantly in all three tissues (Table 12). The highest inhibition percentage was observed in the kidney for both Na^+-K^+ and Mg^{++} ATPases, 12.6% and 15.0% respectively (Table 11). In general, the Mg^{++} ATPase activity was more sensitive to T than the Na^+-K^+ ATPase in all tissues.

The mice used in experiment I were observed to have lower specific ATPase activities in all tissues, and their ATPases were more vulnerable to inhibition by T than of those used in experiment II. However, the LD_{50} dose did not exhibit more than 32.4% inhibition.

c. In Vitro Experiment

(1) Mouse - The solvent control treatment (10 µl ethanol) reduced the activity significantly in all ATPases tested except the Na^+-K^+ ATPase of the brain, and displayed an insignificant stimulation of the liver Mg^{++} ATPase (Table 13). The highest inhibition due to ethanol was in the Na^+-K^+ ATPase of liver (10.9%) and the only stimulation was a 2.2% increase in Mg^{++} ATPase of the same tissue (Table 14).

Na^+-K^+ ATPase activity was significantly inhibited only in the kidney homogenates by all levels of T (Table 13). The highest concentration (10^{-4}M) exhibited the highest degree of inhibition (with a maximum of 44%) and the lowest concentration produced the lowest inhibition (Table 14). Na^+-K^+ ATPase activity was not inhibited in the brain homogenates. In liver homogenates the activity was variable in response to T with a significant reduction caused by the 10^{-6}M concentration, while higher concentrations (10^{-4}M and 10^{-5}M) inflicted no significant reduction (Tables 13 and 14).

Mg^{++} ATPase activity was significantly reduced by all T concentrations in both the kidney and the brain (Tables 13 and 14). In the liver homogenates only the highest concentration exhibited a significant reduction in Mg^{++} ATPase activity (Table 13). The total ATPase activity was inhibited 28.9%, 6.0% and 14.7% in kidney, brain and liver, respectively, by the highest concentration which produced the highest degree of inhibition.

(2) *P. americana* - The solvent control treatment (ethanol) inhibited the activity in Na^+-K^+ ATPase of the CNS (Table 15). Otherwise, it did not have a significant effect (Table 16) with the exception of the total ATPase activity of the Malpighian tubules which showed a slight stimulation of the Mg^{++} ATPase activity by 9.6% (Tables 15 and 16).

TABLE 12. MEANS OF Na^{+}-K^{+} AND Mg^{++} ATPase SPECIFIC ACTIVITIES IN KIDNEY, BRAIN AND LIVER FROM CONTROL AND TOXAPHENE-DOSED MICE USED IN IN VIVO EXPERIMENT II. -- SPECIFIC ACTIVITY EXPRESSED IN μM Pi/mg PROTEIN/HR.

	Kidney		Brain		Liver	
	Na^{+}-K^{+} ATPase	Mg^{++} ATPase	Na^{+}-K^{+} ATPase	Mg^{++} ATPase	Na^{+}-K^{+} ATPase	Mg^{++} ATPase
Control (corn oil)	36.30±0.96 b*	47.30±0.74 b	16.75±0.38 a	21.12±0.34 b	4.15±0.12 a	10.03±0.31 b
Toxaphene (LD_{50})**	31.71±0.45 a	40.21±0.58 a	16.65±0.31 a	19.29±0.29 a	4.14±0.10 b	8.97±0.23 a

* Means followed by the same letter in each tissue are not significantly different (LSD, = 0.05)

** 112 mg/kg of toxaphene

TABLE 13. MEANS OF Na^+-K^+ AND Mg^{++} ATPase SPECIFIC ACTIVITIES IN HOMOGENATES OF KIDNEY, BRAIN AND LIVER, FROM MICE, SUBJECTED IN VITRO TO VARIOUS CONCENTRATIONS OF TOXAPHENE. -- SPECIFIC ACTIVITY EXPRESSED IN μM Pi/mg PROTEIN/HR.

	Kidney		Brain		Liver	
	Na^+-K^+ ATPase activity ± S.E.	Mg^{++} ATPase activity ± S.E.	Na^+-K^+ ATPase activity ± S.E.	Mg^{++} ATPase activity ± S.E.	Na^+-K^+ ATPase activity ± S.E.	Mg^{++} ATPase activity ± S.E.
Controls						
Untreated	36.69±0.72e*	48.19±0.51d	16.91±0.26a	21.05±0.24d	4.33±0.11be	10.20±0.19b
Solvent*	34.81±0.55d	45.05±0.47c	16.89±0.21a	19.97±0.26b	3.86±0.09a	10.42±0.13b
Toxaphene (M)						
10^{-4}	20.55±0.59a	40.25±0.58a	16.30±0.36a	19.52±0.23a	4.02±0.11ac	8.37±0.23a
10^{-5}	27.68±0.40b	40.16±0.51a	16.28±0.17a	20.61±0.26c	4.03±0.11ac	10.05±0.14b
10^{-6}	27.71±0.32b	42.60±0.30b	16.43±0.14a	20.43±0.29c	3.80±0.08a	10.10±0.15b
10^{-7}	32.46±0.63c	42.78±0.28b	16.84±0.23a	20.54±0.25c	4.54±0.11b	10.29±0.13b

* Means followed by the same letter in each tissue are not significantly different (LSD, = 0.05)

** Solvent control is 10 μl ethanol

TABLE 14. PERCENTAGE OF REDUCTION OR INCREASE IN ACTIVITY OF THE ATPase OF MOUSE TISSUES TESTED IN RESPONSE TO ETHANOL AND VARIOUS CONCENTRATIONS OF TOXAPHENE *IN VITRO*. -- MINUS AND PLUS SIGNS INDICATE REDUCTION AND INCREASE, RESPECTIVELY.

Tissue	ATPase activity	Solvent* control	Toxaphene concentration (M)			
			10^{-4}	10^{-5}	10^{-6}	10^{-7}
Kidney	Total	-5.9	-28.9	-20.8	-17.7	-12.0
	Na^{+}-K^{+}	-5.1	-44.0	-24.6	-24.5	-11.6
	Mg^{++}	-6.5	-16.5	-16.7	-11.6	-11.2
Brain	Total	-2.9	-6.0	-2.9	-2.9	-1.6
	Na^{+}-K^{+}	-0.1	-3.8	-3.8	-2.8	-0.4
	Mg^{++}	-5.1	-0.8	-2.1	-2.9	-2.4
Liver	Total	-0.2	-14.7	-3.0	-4.3	+2.0
	Na^{+}-K^{+}	-10.9	-7.0	-6.9	-12.2	+4.8
	Mg^{++}	+2.2	-18.0	-1.4	-1.0	+0.9

* 10 µl ethanol

TABLE 15. MEANS OF Na^{+}-K^{+} AND Mg^{++} ATPase SPECIFIC ACTIVITIES IN HOMOGENATES OF *P. AMERICANA* CNS AND MALPIGHIAN TUBULES SUBJECTED *IN VITRO* TO VARIOUS CONCENTRATIONS OF TOXAPHENE. SPECIFIC ACTIVITY EXPRESSED IN μM Pi/mg PROTEIN/HR.

	CNS		Malpighian tubules	
	Na^{+}-K^{+} ATPase activity ± S.E.	Mg^{++} ATPase activity ± S.E.	Na^{+}-K^{+} ATPase activity ± S.E.	Mg^{++} ATPase activity ± S.E.
Controls				
Untreated	73.40 ± 2.13 e*	40.07 ± 1.88 c	48.34 ± 1.89 c	48.82 ± 1.45 a
Solvent**	63.05 ± 1.70 d	39.30 ± 1.31 c	45.96 ± 1.42 c	53.51 ± 1.55 ab
Toxaphene (M)				
10^{-4}	30.18 ± 0.61 a	28.35 ± 1.07 a	22.93 ± 0.92 a	48.88 ± 1.45 a
10^{-5}	37.66 ± 0.98 b	36.79 ± 1.31 b	27.83 ± 1.44 b	50.73 ± 1.19 ab
10^{-6}	37.88 ± 1.61 b	37.36 ± 1.16 bc	46.74 ± 1.22 c	53.30 ± 1.43 ab
10^{-7}	60.24 ± 1.46 c	38.79 ± 1.33 bc	52.24 ± 1.44 d	55.23 ± 1.56 b

* Means followed by the same letter in each tissue are not significantly different (LSD, = 0.05)

** Solvent control is 10 μl ethanol

TABLE 16. PERCENTAGE OF REDUCTION OR INCREASE IN ATPase ACTIVITY OF *P. AMERICANA* TISSUES TESTED IN RESPONSE TO ETHANOL AND VARIOUS CONCENTRATIONS OF TOXAPHENE *IN VITRO*. -- MINUS AND PLUS SIGNS INDICATE REDUCTION AND INCREASE, RESPECTIVELY.

Tissue	ATPase activity	Solvent* control	Toxaphene concentrations (M)			
			10^{-4}	10^{-5}	10^{-6}	10^{-7}
CNS	Total	-9.8	-47.8	-34.5	-34.0	-12.5
	Na^{+}-K^{+}	-14.1	-58.9	-48.7	-48.4	-17.9
	Mg^{++}	-1.9	-29.2	-8.2	-6.8	-3.2
Malpighian tubules	Total	+2.4	-26.8	-19.9	+1.1	+9.6
	Na^{+}-K^{+}	-4.9	-52.6	-42.4	-3.3	+8.1
	Mg^{++}	+9.6	+0.1	+3.9	+9.2	+13.1

* 10 µl ethanol

Na^+-K^+ ATPase activity of the CNS was significantly reduced by all T concentrations used (Table 15). The 10^{-4} concentration produced the highest inhibition (58.9%); 10^{-5} and 10^{-6} were equipotent at 48.7% and 48.4%, respectively; and the lowest concentration (10^{-7}M) resulted in 17.9% inhibition (Table 16). In Malpighian tubules, the Na^+-K^+ ATPase activity was significantly reduced by 10^{-4} and 10^{-5}M; was not affected by 10^{-6}; and was significantly stimulated by 10^{-7}M concentration (Table 15).

Mg^{++} ATPase activity of the CNS was reduced by 10^{-4} and 10^{-5}M. In Malpighian tubules, Mg^{++} ATPase activity was not inhibited but displayed a trend of stimulation which became significant at 10^{-7}M. However, the initial increase in this activity in this tissue was as high as 9.6% due to ethanol alone (Table 16). The higher dose (10^{-4}M) inflicted a significant reduction on the stimulation by ethanol (Tables 15 and 16). The lower doses were unable to reverse the ethanol effect.

(3) Regressions - Two regression analyses were conducted to determine the significance of the dose response of each ATPase activity in each tissue of both animals. The linear regression included the solvent treatment (ethanol) as the zero T treatment, but the linear $\log_{10}$ regression was made for the four T concentrations only. The significance of dose response based upon both regressions is presented in Table 17.

Table 18 lists the prediction equations for the activity of the ATPase against $\log_{10}$ of T concentrations. The linear regression indicated that there was a dose response in all ATPases of the kidney, malpighian tubules, total and Na^+-K^+ ATPases of the CNS and the Na^+-K^+ ATPase of the liver. With linear $\log_{10}$ regressions (where data for control treatment was omitted) only the Na^+-K^+ ATPase of the brain did not show a significant dose response to T.

4. Neonatal Development and Postnatal Learning in the Rat

a. Perinatal Exposure to Toxaphene - Methyl Parathion and Methyl Parathion

(1) Pup Mortality - Mortality of rat pups was significantly greater among litters that had been perinatally exposed to the insecticides. Total percent mortalities for 15 days postpartum were: 30% for pups exposed to MP vs. 10% for controls, and 76.7% for MP-T pups vs. 23.3% for controls. Perinatal exposure to MP or MP-T caused no significant difference in the mean total weight gained by the rat pups from day 5 through day 15 postpartum. Mortality of rats pups perinatally exposed to MP and MP-T may or may not be directly attributed to the insecticides. Although no significant differences were observed in the analysis of retrieval times by the mothers, the mortality may have been as a result of poor mothering

TABLE 17. SIGNIFICANCE OF LINEAR AND LINEAR-LOG REGRESSIONS OF THE DIFFERENT ATPases WITH THE TREATMENTS. -- S = SIGNIFICANT, NS = NOT SIGNIFICANT.

Tissue	ATPase activity	Linear regression	Linear-log regression
Kidney			
	Total	S	S
	Na^{+}-K^{+}	S	S
	Mg^{++}	S	S
Brain			
	Total	NS	S
	Na^{+}-K^{+}	NS	NS
	Mg^{++}	NS	S
Liver			
	Total	NS	S
	Na^{+}-K^{+}	S	S
	Mg^{++}	NS	S
CNS			
	Total	S	S
	Na^{+}-K^{+}	S	S
	Mg^{++}	NS	S
Malpighian tubules			
	Total	S	S
	Na^{+}-K^{+}	S	S
	Mg^{++}	S	S

TABLE 18. PREDICTION EQUATIONS OF LINEAR LOG_{10} REGRESSION BETWEEN TISSUES OF THE MOUSE AND *P. AMERICANA* AND THE FOUR TOXAPHENE CONCENTRATIONS.

Tissue	Total ATPase	Mg^{++} ATPase	Na^{+}-K^{+} ATPase
Mouse			
Kidney	$\hat{y}$= 43.40 - 4.59X*	$\hat{y}$= 35.79 - 1.03X	$\hat{y}$= 7.44 - 3.58X
Brain	$\hat{y}$= 33.86 - 0.52X	$\hat{y}$= 18.46 - 0.32X	$\hat{y}$= 15.40 - 0.20X
Liver	$\hat{y}$= 9.75 - 0.73X	$\hat{y}$= 6.42 - 0.60X	$\hat{y}$= 3.32 - 0.14X
P. americana			
CNS	$\hat{y}$= 11.23 - 11.88X	$\hat{y}$= 17.64 - 3.27X	$\hat{y}$= 7.47 - 8.84X
Malphighian Tubules	$\hat{y}$= 25.12 - 11.89X	$\hat{y}$= 42.88 - 1.86X	$\hat{y}$=18.07 - 10.13X

*X= LOG_{10} dose.

by the exposed females. During the first 3 days of retrieval testing (pups 5-7 days old), exposed mothers took longer (although not statistically significant) to retrieve the pups than the control mothers. This is important, as at this age the pups are rather dependent on the mother for retrieval to be fed. After 1-2 days of insufficient feeding, the pups became very weak and died. Therefore a change in maternal behavior brought about by exposure to MP or MP-T may have been responsible for the mortality.

(2) Coordination Tests - Results of the righting, startle, and placement reflex tests are presented in Table 19. The only significant change from perinatal exposure was an extension of the time required by litters exposed to MP-T to successfully master the righting reflex. The results of the grasp-hold reflex also uncovered no significant differences. MP litters were observed to grasp-hold for a slightly shorter period of time as they aged compared to controls (Fig. 8) but the MP-T litters displayed no such trend (Fig. 9).

(3) Open Field Testing - This test for emotionality indicated a possible change due to MP exposure but not with MP-T. MP rats moved about the open field more than the controls, and the number of defecations (boluses) were more constant (Figures 10 and 11). Activity by MP rats was significantly higher only at 23 and 54 days. It is not clear in the literature whether high activity means high or low emotionality, but it does appear that MP caused some change in the emotionality of the neonates.
The effect of perinatal exposure to MP-T on emotionality of the rat pups was very small. Activity in the open field was almost indistinguishable between MP-T and control rat pups. There appeared to be a slight difference in the number of boluses deposited by each group, but the patterns were so variable that no inferences could be drawn.

(4) Maze Learning Transfer - There were no overall nor consistent differences in maze learning or transfer as a result of perinatal exposure to MP or MP-T. However, as with the emotionality tests, MP rats displayed greater differences over controls than did MP-T animals.

The MP group required about 2 X as much time to meet the first criterion as the controls (Fig. 12). However, the exposed group took longer to master the transfer to the left. The groups then handled the next right about the same, but the MP rats again had difficulty transferring to the second left as well as the final transfer back to the right.

TABLE 19. RESULTS OF COORDINATION TESTS PERFORMED ON RAT PUPS PERINATALLY EXPOSED TO METHYL PARATHION, AND METHYL PARATHION: TOXAPHENE (1:2)

Experiment	Methyl Parathion[a] start[1]	finish[2]	Methyl Parathion:Toxaphene start	finish
Righting Reflex				
Control	13.3a	18.7a	14.0a	17.7a
Experimental	14.5a	18.0a	16.0a	22.0b
Startle Reflex				
Control	11.0a	11.7a	11.3a	12.7a
Experimental	11.5a	12.5a	13.0a	14.0a
Placement Reflex				
Control	9.7a	14.0a	7.0a	14.3a
Experimental	10.0a	13.5a	7.3a	13.7a

[a]Values followed by the same letter are not significantly different at the 0.05 level.

[1]Mean number of days for observation of first correct response.

[2]Mean number of days for the daily number of correct responses of each litter to equal or exceed 90%.

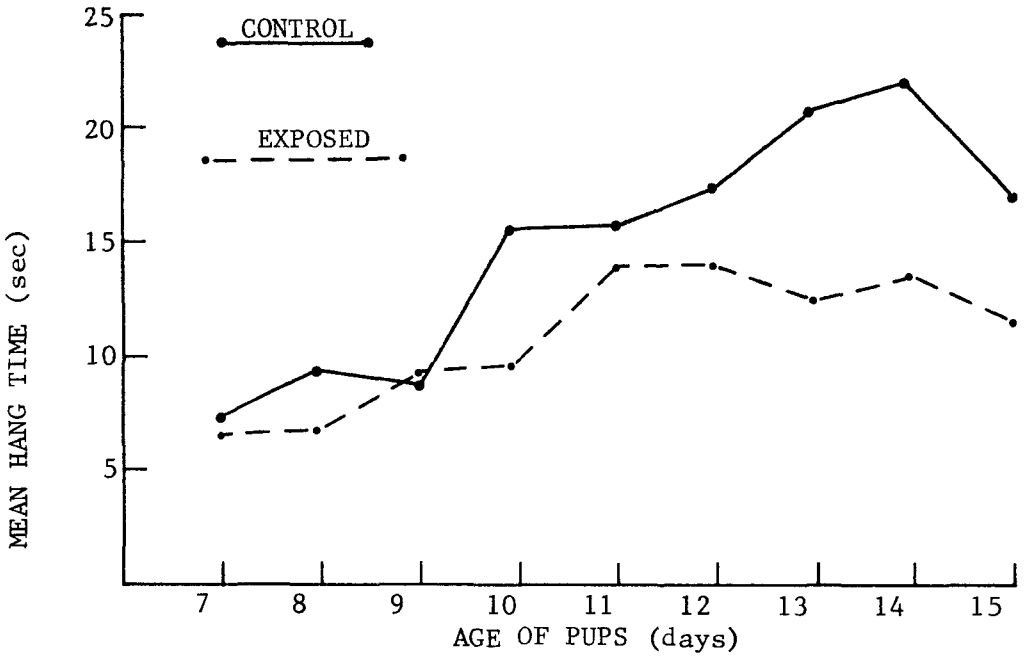

Fig. 8. Mean hang time (grasp-hold reflex) of rat pups perinatally exposed to methyl parathion.

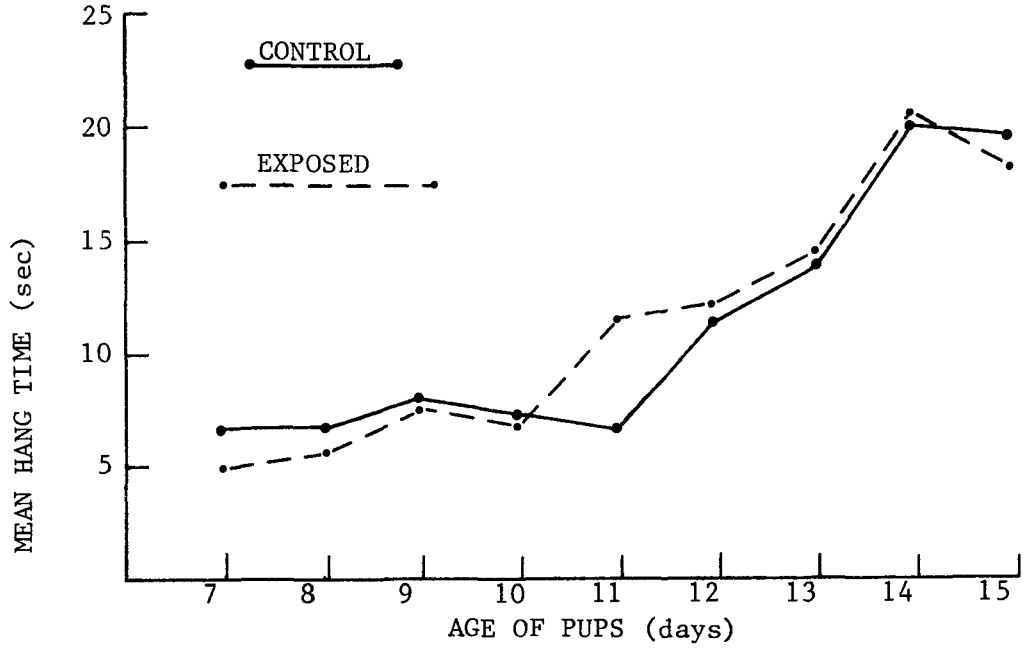

Fig. 9 Mean hang time (grasp-hold reflex) of rat pups perinatally exposed to methyl parathion: toxaphene (1:2).

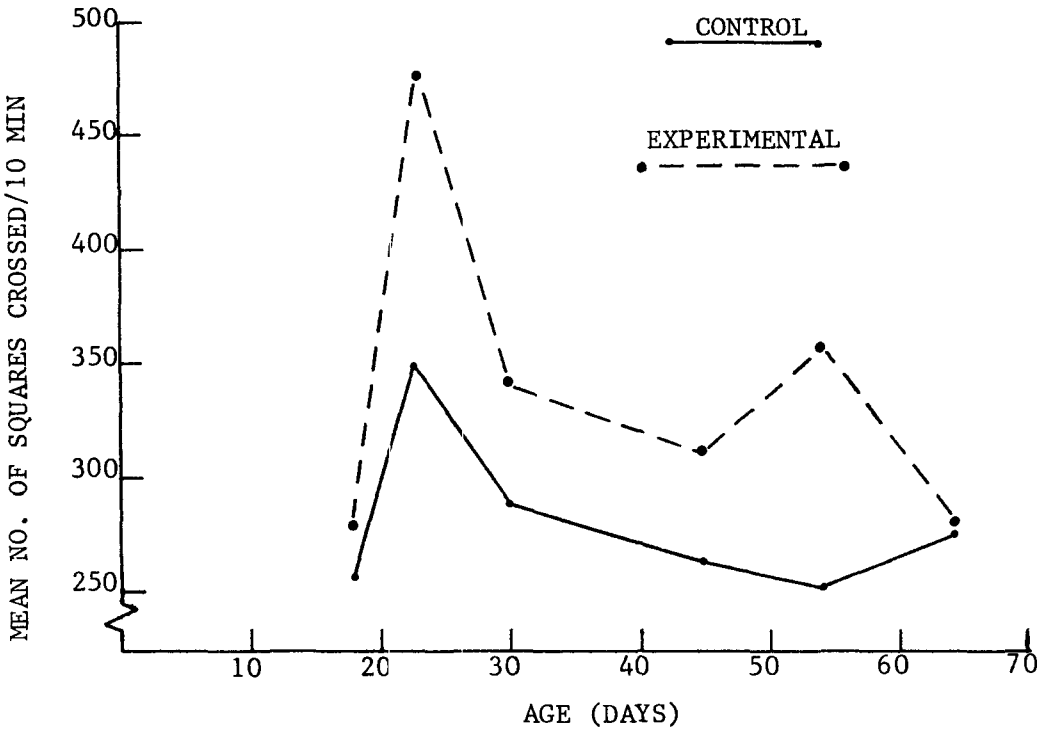

Fig. 10 Activity in the open field test by rat pups perinatally exposed to methyl parathion.

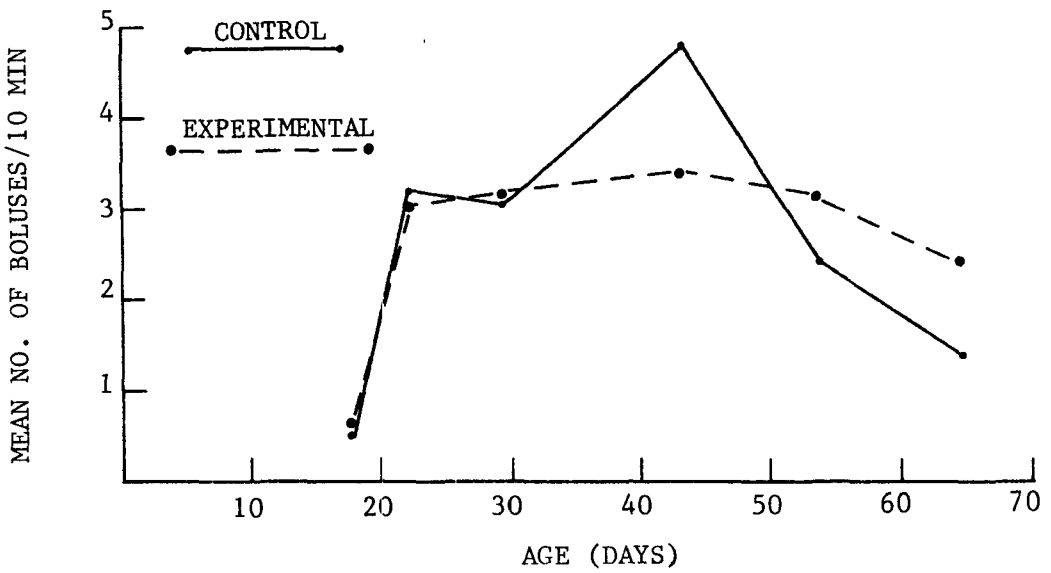

Fig. 11 Number of boluses deposited in the open field by rat pups perinatally exposed to methyl parathion.

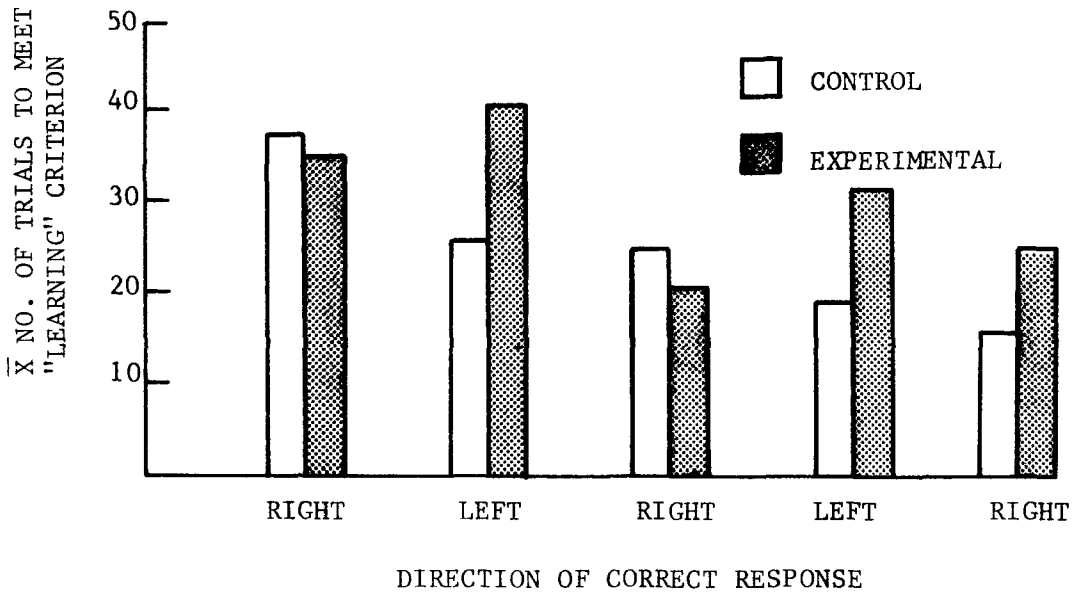

Fig. 12 Results of maze learning transfer by rats perinatally exposed to methyl parathion.

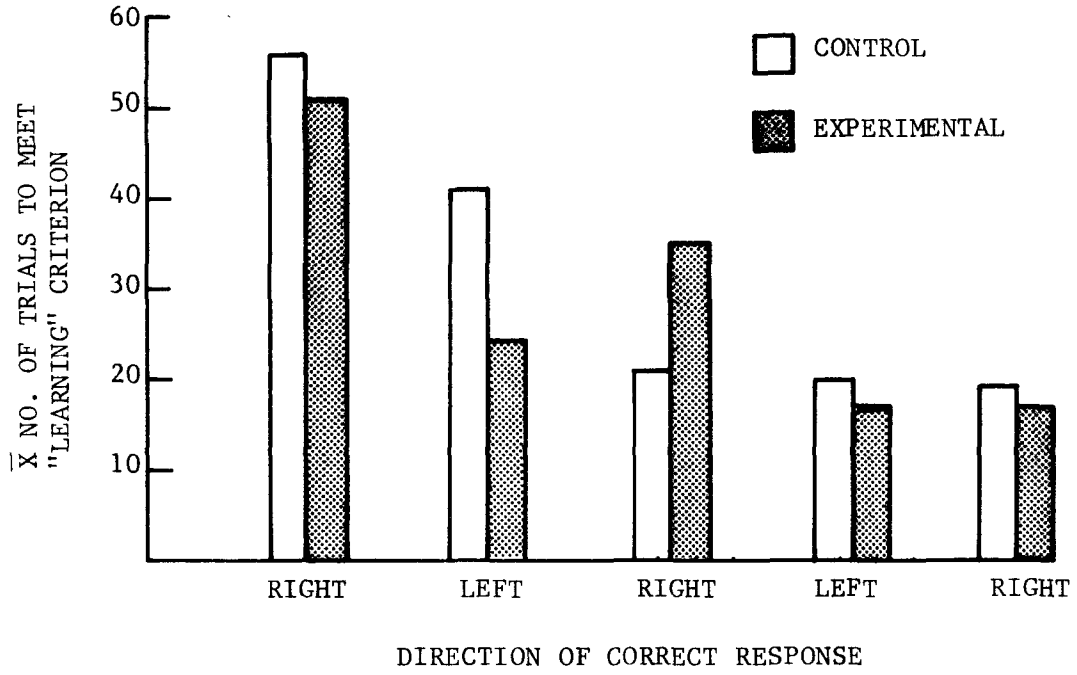

Fig. 13 Results of maze learning transfer by rats perinatally exposed to methyl parathion and toxaphene (1:2)

Since the MP group performed about as well on the first and second evaluation to the right, it may be inferred that their learning ability was not severely impaired, but perhaps the ability to transfer this knowledge (i.e. turn to the left) was.

Rats perinatally exposed to MP-T did not appear to be impaired in maze learning or transfer. As with the MP group, MP-T rats performed as well as controls on the first criterion to the right (Fig. 13).

b. Peri- and Posnatal Exposure to Toxaphene

(1) Coordination Tests - Perinatal exposure to T caused no significant differences in grasp-hold (Fig. 14), startle, or the initiation of the righting reflex. However, there was a significant difference observed in the length of time required for 90% of the trials to be positive by T litters; it required more time for these pups to master the skills than the controls (Table 20).

(2) Maze Learning Transfer - There were no significant differences in maze learning and transfer of this learning between postnatally exposed and control rats (Fig. 15). Perinatally exposed rats did not display any difference in learning or transfer as compared to controls (Fig. 16). The early patterns were similar in both exposed groups. Both groups mastered the first criterion to the right earlier than controls, and later on the first left. For the last 3 changes the postnatal group improved on each change, while the perinatal and controls of both groups had more difficulty with the second left than with the remaining right turns. But this was offset by controls mastering the second right before the exposed rats. The final 2 turns were handled easily and about equally by both groups.

In the maze evaluation of both MP and MP-T, controls of both displayed an overall reduction in the number of trials for meeting each consecutive criterion. This would be expected, for in learning one criterion, transfer of this learning would make later learning easier. However, the exposed rats did not display this trend.

B. TOXAPHENE AND METHYL PARATHION AGAINST NON-TARGET AND TARGET INSECTS

1. Non-Target Insects

In 1977 the number of predators collected from each location (Tucson, Safford, and Yuma) varied considerably and in some cases few or none of particular species were obtained. Almost all MP LD_{50} values (μg insecticide/g insect) at 24 hr

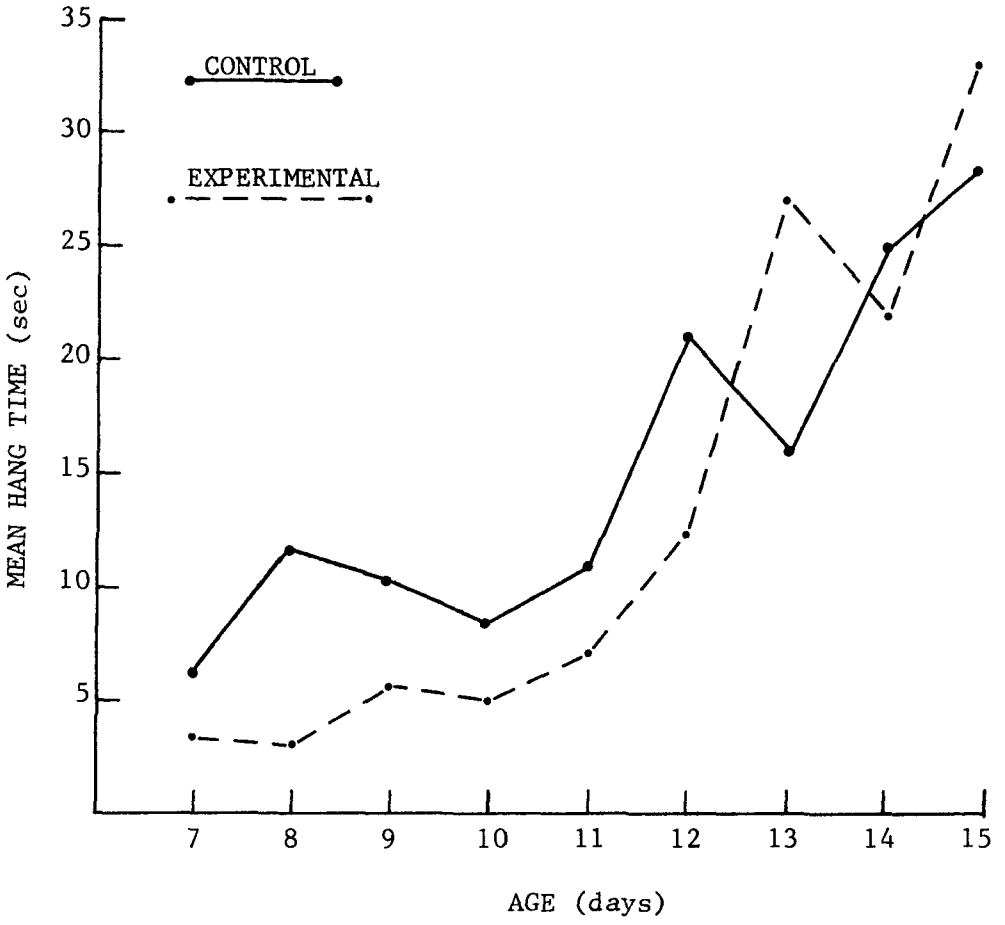

Fig. 14 Mean hang time of rat pups perinatally exposed to toxaphene.

Table 20. RESULTS OF COORDINATION TESTS PERFORMED ON RAT PUPS PERINATALLY EXPOSED TO TOXAPHENE.

Experiment	Start[1]	Finish[2]
Righting Reflex		
Control	13.5a	15.5a
Experimental	14.0a	18.5b
Startle Reflex		
Control	10.5a	11.0a
Experimental	11.0a	11.5a

a. Values followed by the same letter are not significantly different at the 0.05 level.
1. Mean number of days for observation of first correct response.
2. Mean number of days for the daily number of correct responses of each litter to equal or exceed 90%.

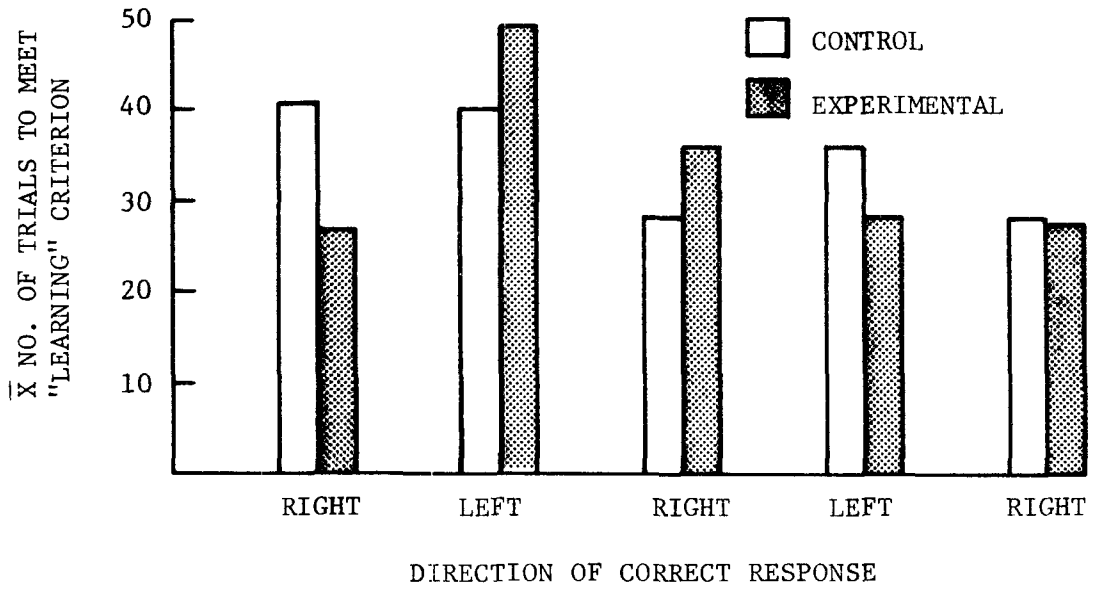

Fig. 15 Results of maze learning transfer by rats postnatally exposed to toxaphene.

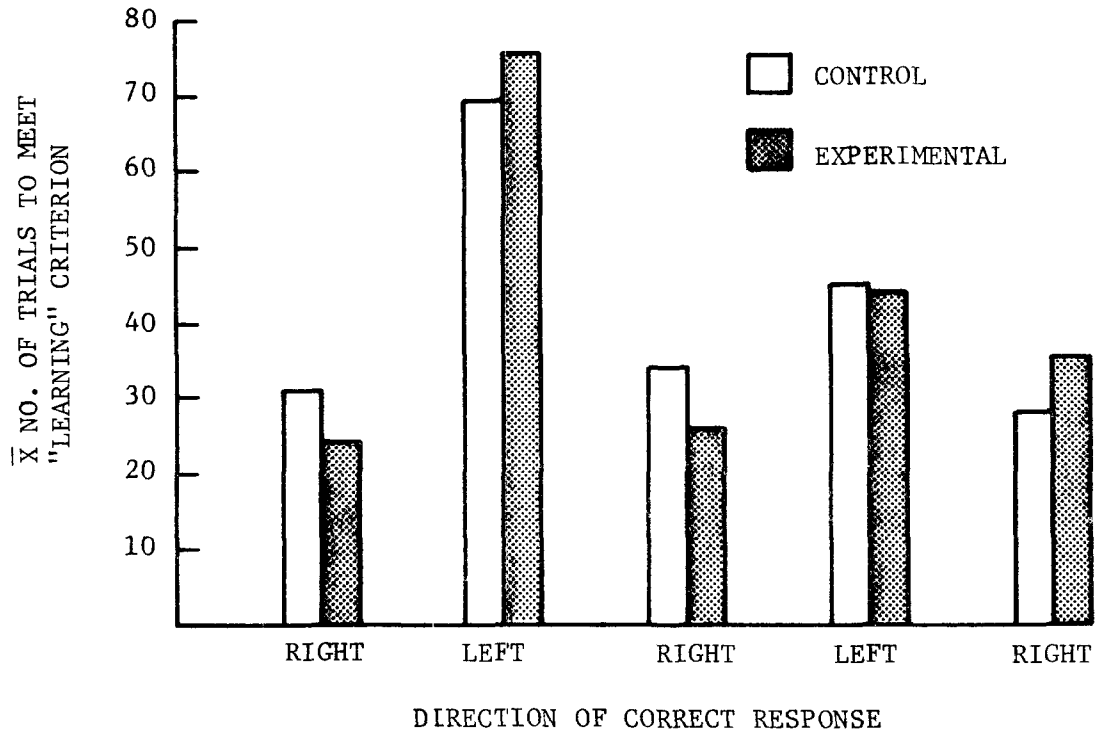

Fig. 16 Results of maze learning transfer by rats perinatally exposed to toxaphene.

were obtained and are presented in Table 21. Tucson and Safford Nabis spp. displayed nearly the same LD-50 values of 1.65 and 1.56, respectively. Yuma Nabis spp. produced a non-significant regression due to the few insects tested and the erratic results, but the estimated LD-50 was 1.01. Tucson and Safford Georcoris spp. also displayed similar LD-50 values with 1.95 and 2.34 respectively, while the Yuma population produced a non-significant regression with an estimated LD-50 of 2.85. Too few O. tristicolor were collected from Safford and Yuma for tests and the Tucson population results were too erratic for a significant regression, but the estimated LD-50 for the Tucson population was 0.68.

The LD-50 values at 24 hr for T are presented in Table 22. Attempts to establish dosage-mortality values for T all failed to give significant regressions. Only Tucson Nabis spp; Tucson and Yuma Geocoris spp; and Safford O. tristicolor were tested to an extent to give LD-50 estimates of over 10,000 for Nabis spp. and 282, 89, and 90 for the Geocoris sp. populations and O. tristicolor, respectively.

The 1978 MP LD-50 values at 24 and 48 hr are presented in Tables 23 and 24, respectively. N. alternatus, N. americoferus, and O. tristicolor displayed the highest intraspecific values for the Yuma populations. The values for G. pallens were higher for Tucson than Yuma populations. These intraspecific differences were not significant between locations. When comparing the species, generally G. pallens displayed the highest LD-50 values (except for Yuma N. alternatus) followed by G. punctipes and N. alternatus, N. americoferus, and O. tristicolor.

At 24 hr, only Safford N. alternatus displayed a significant regression with an LD-50 of 1.39. Tucson and Yuma populations displayed non-significant regressions, with estimated LD-50 values of 1.81 and 2.62, respectively. It was observed that in each set of data for the Tucson and Yuma populations there was a value that appeared to be an anomaly. If the respective values were removed, significant regressions were obtained (Table 25) and the LD-50 values became 1.38 and 2.19, respectively. At 48 hr, Safford had the only significant regression with an LD-50 of 1.01, while the LD-50 estimate was 1.63 for the Tucson population and 2.12 for the Yuma population. If the anomalous values removed from the 24 hr values were also removed at 48 hr (Table 26) the Yuma population displayed a significant regression and identical LD-50 value of 2.12, while the Tucson population failed to produce a significant regression and the estimated LD-50 dropped to 1.25

TABLE 21. METHYL PARATHION LD-50 VALUES (μg/g) AT 24 HR FOR PREDATORS FROM SEVERAL AREAS IN ARIZONA IN 1977

Insect[1]	Area Collected	LD-50[2]	95% Fiducial Limits	Slope
Nabis spp.	Tucson	1.65	1.33 - 2.74	3.90
	Safford	1.56	1.19 - 2.23	7.10
	Yuma	(1.01)	---	1.64
Geocoris spp.	Tucson	1.95	0.28 - 2.81	2.45
	Safford	2.34	1.51 - 3.13	4.47
	Yuma	(2.85)	---	2.41
Orius tristicolor	Tucson	(0.68)	---	16.25
	Safford	---	---	---
	Yuma	---	---	---

[1]Adult average weights (mg): *Nabis* spp. 7.48, *Geocoris* spp. 3.28, *O. tristicolor* 0.15.

[2]Values in parentheses are estimated values due to non-significant regressions.

TABLE 22. TOXAPHENE LD-50 VALUES (μg/g) AT 24 HR FOR PREDATORS FROM SEVERAL AREAS IN ARIZONA IN 1977

Insect[1]	Area Collected	LD-50[2]	95% Fiducial Limits	Slope
Nabis spp.	Tucson	(>10,000)	- - -	0.12
	Safford	- - -	- - -	- - -
	Yuma	- - -	- - -	- - -
Geocoris spp.	Tucson	(282)	- - -	1.32
	Safford	- - -	- - -	- - -
	Yuma	(89)	- - -	0.76
Orius tristicolor	Tucson	- - -	- - -	- - -
	Safford	(90)	- - -	1.65
	Yuma	- - -	- - -	- - -

[1]Adult average weights (mg): *Nabis* spp. 7.48, *Geocoris* spp. 3.28, *O. tristicolor* 0.15.

[2]All values are estimated values due to non-significant regressions.

TABLE 23. METHYL PARATHION LD-50 VALUES (μg/g) AT 24 HR FOR PREDATORS FROM SEVERAL AREAS IN ARIZONA IN 1978

Insect	Area Collected	LD-50[2]	95% Fiducial Limits	Slope
Nabis alternatus	Tucson	(1.81)	-	2.85
	Safford	1.39	0.90 - 1.70	4.10
	Yuma	(2.62)	-	2.81
Nabis americoferus	Tucson	(0.97)	-	3.71
	Safford	(1.37)	-	13.83
	Yuma	(1.61)	-	2.59
Geocoris pallens	Tucson	(2.43)	-	21.03
	Safford	-	-	-
	Yuma	2.13	1.82 - 2.40	4.67
Geocoris punctipes	Tucson	-	-	-
	Safford	-	-	-
	Yuma	1.93	1.56 - 2.19	3.91
Orius tristicolor	Tucson	(0.60)	-	4.15
	Safford	(0.73)	-	0.51
	Yuma	(0.91)	-	2.64

[1]Adult average weights (mg): N. alternatus 6.31, N. americoferus 10.48, G. pallens 3.28, G. punctipes 3.72, O. tristicolor 0.2.

[2]Values in parentheses are estimated values due to non-significant regressions.

TABLE 24. METHYL PARATHION LD-50 VALUES (μg/g) AT 48 HR FOR PREDATORS FROM SEVERAL AREAS IN ARIZONA IN 1978

Insect[1]	Area Collected	LD-50[2]	95% Fiducial Limits	Slope
Nabis alternatus	Tucson	(1.63)	-	1.60
	Safford	1.01	0.003 - 1.30	3.57
	Yuma	(2.12)	-	9.16
Nabis americoferus	Tucson	(0.69)	-	2.60
	Safford	(1.29)	-	10.57
	Yuma	(1.15)	-	1.41
Geocoris pallens	Tucson	(2.40)	-	21.75
	Safford	-	-	-
	Yuma	2.14	1.60 - 2.54	4.07
Geocoris punctipes	Tucson	-	-	-
	Safford	-	-	-
	Yuma	1.85	1.40 - 2.09	4.45
Orius tristicolor	Tucson	(0.45)	-	0.82
	Safford	(0.35)	-	13.27
	Yuma	(0.66)	-	2.28

[1]Adult average weights (mg): *N. alternatus* 6.31, *N. americoferus* 10.48, *G. pallens* 3.28, *G. punctipes* 3.72, *O. tristicolor* 0.2.

[2]Values in parentheses are estimated values due to non-significant regressions.

TABLE 25. METHYL PARATHION LD-50 VALUES (μg/g) AT 24 HR FOR PREDATORS FROM SEVERAL AREAS IN ARIZONA IN 1978. AFTER AN APPARENT ANOMALOUS VALUE WAS REMOVED FROM THE ORIGINAL DATA SET

Insect[1]	Area Collected	LD-50	95% Fiducial Limits	Slope
Nabis alternatus	Tucson	1.38	1.23 - 1.81	8.34
	Yuma	2.19	2.04 - 2.42	8.17
Orius tristicolor	Tucson	0.50	0.42 - 0.87	5.29
	Safford	0.30	0.26 - 0.36	8.46
	Yuma	0.75	0.60 - 13.04	4.44

[1]Adult average weights (mg): *N. alternatus* 6.31, *O. tristicolor* 0.2

TABLE 26. METHYL PARATHION LD-50 VALUES (μg/g) AT 48 HR FOR PREDATORS FROM SEVERAL AREAS IN ARIZONA IN 1978. AFTER AN APPARENT ANOMALOUS VALUE WAS REMOVED FROM THE ORIGINAL DATA SET

Insect[1]	Area Collected	LD-50[2]	95% Fiducial Limits	Slope
Nabis alternatus	Tucson	(1.25)	-	7.90
	Yuma	2.12	1.13 - 4.02	9.31
Orius tristicolor	Tucson	(0.40)	-	1.05
	Safford	(0.34)	-	9.96
	Yuma	0.60	0.49 - 0.69	4.53

[1]Adult average weights (mg): *N. alternatus* 6.31, *O. tristicolor* 0.2

[2]Values in parentheses are estimated values due to non-significant regressions

There were no significant regressions obtained from any data set for N. americoferus. The estimated LD-50 values at 24 hr were lowest for the Tucson population (0.97), slightly higher for Safford (1.37), and highest for Yuma (1.61). At 48 hr the Tucson population displayed the lowest estimated LD-50 of 0.69, Yuma displayed a value of 1.15, and the Safford value was 1.29. In all cases the 48 hr value was less than the 24 hr value.

With G. pallens, Yuma populations produced the only significant regression at both 24 and 48 hr, those from Tucson displayed non-significant regressions, and no values could be obtained from Safford due to the few insects caught there. At 24 hr, the Yuma population had an LD-50 of 2.13, while the estimated value for Tucson was 2.43. However, the Yuma LD-50 increased to 2.14 at 48 hr and the Tucson estimated value dropped to 2.40.

Although few G. punctipes were found in either Tucson or Safford and no LD-50 value was obtained from these areas, they were plentiful in Yuma and significant regressions were obtained at 24 and 48 hr. The LD-50 was observed to be 1.93, at 24 hr and 1.85 at 48 hr.

There were no significant regressions for O. tristicolor since the dosage-mortality values were so erratic. The estimated LD-50 values at 24 hr were 0.60 for Tucson, 0.73 for Safford, and 0.91 for Yuma. At 48 hr, the estimated values were 0.45, 0.35, and 0.66 for Tucson, Safford, and Yuma, respectively. As with N. alternatus, there was at least one anomalous value in the data of each population, but it could not be validly removed. Removal of the appropriate value would produce significant regressions for each population at 24 hr, but only for the Yuma population at 48 hr. The LD-50 values at 24 hr were 0.50 for Tucson, 0.30 for Safford, and 0.75 for Yuma. At 48 hr, the LD-50 was 0.60 for Yuma and an estimated 0.40 for Tucson and 0.34 for Safford.

The dosage-mortality results of MP and T alone and in combination are presented in Table 27. Only the predators from Yuma were examined since enough insects of each species were collected to allow several replications for each test. In all cases T produced less mortality than MP alone or the MP-T mixture, except for O. tristicolor which had high mortalities with T and low mortalities with MP-T. There does not appear to be a discernible difference between MP alone and MP-T (except for O. tristicolor); therefore, a two sample t-test was performed to test for significance bet-

TABLE 27. DOSAGE-MORTALITY AFTER 48 HR OF YUMA PREDATORS IN 1978 USING METHYL PARATHION (MP) AT THE LC-50 LEVEL (ng/INSECT), TOXAPHENE (T) AT TWICE THE METHYL PARATHION CONCENTRATION, AND A 1:2 METHYL PARATHION:TOXAPHENE MIXTURE (MP-T)

Insect	Treatment (Dose in ng)	Number of Replications	Average[1] Mortality (%)
Nabis alternatus	MP(13)	4	46.4
	T(26)	2	-9.3
	MP(13)-T(26)	3	44.3
Nabis americoferus	MP(15)	4	47.9
	T(30)	4	7.4
	MP(15)-T(30)	5	56.0
Geocoris pallens	MP(8)	3	44.3
	T(16)	4	37.3
	MP(8)-T(16)	5	47.1
Geocoris punctipes	MP(8)	3	44.3
	T(16)	7	-13.7
	MP(8)-T(16)	9	45.4
Orius tristicolor	MP(0.13)	6	56.2
	T(0.26)	5	51.4
	MP(0.13)-T(0.26)	5	10.7

[1]Negative mortality values result from mortality being less than controls.

ween the percent mortalities of MP and MP-T, T and MP-T, and MP and T (Table 28). With both Nabis species and G. punctipes MP and MP-T did not produce significantly different mortalities. Although G. pallens displayed lower mortalities from T than from MP or MP-T, they were not significantly different. O. tristicolor displayed abnormally high mortalities from T and very low mortalities from MP-T, resulting in MP and T not being significantly different while MP-T was significantly less than either insecticide alone.

2. Target Insects

a. Lepidopterous Larvae

The MP LD-50 values for lepidopterous larvae are presented in Table 29. H. virescens, H zea, and S. exigua proved to be more susceptible to MP than E. acrea. The values for H. virescens and H. zea were extremely close although H. zea appeared slightly more resistant, but not significantly by comparison of the fiducial limits. MP was significantly more toxic to S. exigua, but significantly less toxic to E. acrea in comparison to the Heliothis species.

At 24 hr, the MP LD-50 values for H. virescens, H. zea and S. exigua were 9.70, 10.38, and 3.47, respectively. The values at 48 hr were only slightly different at 9.08, 9.57, and 3.75, respectively. Although a significant regression was not obtained at any time for E. acrea, the dosages required were significantly greater than for any other insect tested. The estimated LD-50 values at 24 and 48 hr were 91 and 72, respectively.

A dosage-mortality study with MP, T, and MP-T was performed on the larvae as described previously for the predators. The results, presented in Table 30, indicate T was less toxic than MP or MP-T, except for S. exigua at 48 hr. A two sample t-test (Table 31) demonstrated no significant differences between MP and MP-T for lepidopterous larvae. H. virescens displayed a significant difference between MP and T at 48 hr. T mortality increased in S. exigua at 48 hr, while MP mortality decreased with no significant differences between them or MP-T.

b. Houseflies

Houseflies were observed to be very susceptible to MP (Table 28), and were significantly more susceptible than S. exigua when the fiducial limits were compared. Although the houseflies were used to perfect dosing techniques, they also were used as a reference and were more susceptible than the lepidopterous larvae, but about equally as susceptible as the predators. At 24 hr the LD-50 was 1.04 and dropped to 0.87 at 48 hr.

TABLE 28. TWO SAMPLE T-TEST AT THE 95% CONFIDENCE LEVEL ON 1978 PREDATORS FROM YUMA COMPARING METHYL PARATHION (MP) AT THE LC-50 LEVEL (ng/INSECT), TOXAPHENE (T) AT TWICE THE METHYL PARATHION CONCENTRATIONS, AND A 1:2 METHYL PARATHION:TOXAPHENE MIXTURE (MP-T). S - SIGNIFICANT, NS - NOT SIGNIFICANT

Insect	Concentration of MP (ng)	Time After Dosing (hr)	MP vs. MP-T	T vs. MP-T	MP vs. T
Nabis alternatus	13	24	NS	S	S
		48	NS	S	S
Nabis americoferus	15	24	NS	S	S
		48	NS	S	S
Geocoris pallens	8	24	NS	NS	NS
		48	NS	NS	NS
Geocoris punctipes	8	24	NS	S	S
		48	NS	S	S
Orius tristicolor	0.130	24	S	S	NS
		48	S	S	NS

TABLE 29. METHYL PARATHION LD-50 VALUES (μg/g) FOR LABORATORY STRAINS OF LEPIDOPTEROUS LARVAE AND HOUSEFLIES.

Insect[1]	Time After Dosing (hr)	LD-50[2]	95% Fiducial Limits	Slope
Heliothis virescens	24	9.70	8.78 - 11.60	4.32
	48	9.08	8.35 - 10.19	5.04
Heliothis zea	24	10.38	9.14 - 12.39	3.79
	48	9.57	8.57 - 10.92	4.40
Spodoptera exigua	24	3.47	2.91 - 4.20	3.24
	48	3.75	3.17 - 4.47	4.60
Estigmene acrea	24	(91)	---	2.52
	48	(72)	---	2.77
Musca domestica	24	1.02	0.94 - 1.13	5.82
	48	0.87	0.83 - 0.91	9.02

[1]Average larval weights (mg): *H. virescens* 21.91, *H. zea* 20.84, *S. exigua* 15.28, *E. acrea* 23.02; average adult weight (mg): *M. domestica* 26.45

[2]Values in parentheses are estimated values due to nonsignificant regressions.

TABLE 30. DOSAGE-MORTALITY AFTER 48 HR OF LEPIDOPTEROUS LARVAE AND HOUSEFLIES USING METHYL PARATHION (MP) AT THE LC-50 LEVEL (ng/INSECT), TOXAPHENE (T) AT TWICE THE METHYL PARATHION CONCENTRATION, AND A 1:2 METHYL PARATHION:TOXAPHENE MIXTURE (MP-T)

Insect	Treatment (Dose in ng)	Number of Replications	Average[1] Mortality (%)
Heliothis virescens	MP(180)	4	25.0
	T(360)	4	0
	MP(180)-T(360)	4	15.0
Heliothis zea	MP(200)	4	55.0
	T(400)	4	0
	MP(200)-T(400)	4	47.5
Spodoptera exigua	MP(50)	4	28.0
	T(100)	4	28.0
	MP(50)-T(100)	4	47.0
Estigmene acrea	MP(3000)	4	65.0
	T(6000)	4	0
	MP(3000)-T(6000)	4	45.0
Musca domestica	MP(25)	3	65.3
	T(50)	3	10.7
	MP(25)-T(50)	3	84.0

[1]Negative mortality values result from mortality being less than controls.

TABLE 31. TWO SAMPLE T-TEST AT THE 95% CONFIDENCE LEVEL ON LEPIDOPTEROUS LARVAE AND HOUSEFLIES COMPARING METHYL PARATHION (MP) AT THE LC-50 LEVEL (ng/INSECT), TOXAPHENE (T) AT TWICE THE METHYL PARATHION CONCENTRATIONS, AND A 1:2 METHYL PARATHION:TOXAPHENE MIXTURE (MP-T). S - SIGNIFICANT, NS - NOT SIGNIFICANT

Insect	Concentration of MP (ng)	Time After Dosing (hr)	MP vs. MP-T	T vs. MP-T	MP vs. T
Heliothis virescens	180	24	NS	NS	NS
		48	NS	NS	S
Heliothis zea	200	24	NS	S	S
		48	NS	S	S
Spodoptera exigua	50	24	NS	S	S
		48	NS	NS	NS
Estigmene acrea	3000	24	NS	S	S
		48	NS	S	S
Musca domestica	25	24	NS	S	S
		48	S	S	S

Dosage-mortality studies with MP, T, and MP-T were also performed on the houseflies and are presented in Table 29. Although T toxicity was low, the MP-T mixture appeared to be slightly more toxic than MP alone. Results of a two sample t-test are presented in Table 30. At 24 hr no significant differences between MP and MP-T were observed, while significant differences between T and MP-T and MP and T were. The same was true at 48 hr, except for MP vs MP-T due to the increased toxicity of MP-T. The houseflies were the only insects to display a significant difference between MP and MP-T.

C. FATE OF TOXAPHENE, METHYL PARATHION, AND CHLORDIMEFORM IN THE HOUSE

Mortality data from the excretion-retention studies indicated that mortality occurred only in treatments containing methyl parathion, i.e., MP, T-MP, MP-C, and T-MP-C (Table 32). All mortality occurred within 3 hr of dosing. It appeared that MP toxicity was slightly enhanced by C combination, and slightly lowered by T, but none of these differences were significant. No female mortality was observed with any treatment.

The second mortality study using only MP and T combinations verified the above in that no potentiation of MP was produced by T. As before, T actually lowered the mortality (not significantly) even though applied in combination at 2 and 3.5 X that of MP (Table 33).

1. Excretion:Toxaphene

Overall, the excretion of ^{36}Cl (represents T and/or its metabolites recovered following an oral dose of ^{36}Cl-toxaphene) was not altered by combinations of C and/or MP. There were no significant differences in the total amount of ^{36}Cl excreted in the urine with any combination, or at either dose (Table 34).

Total excretion of ^{36}Cl via the feces was significantly altered by the C and MP combinations (Table 34) but a consistent pattern was not established. Following the initial dose more ^{36}Cl was excreted in the feces when combined with C yet less when combined with MP and C-MP. Following the redose ^{36}Cl excretion was lowered when combined with MP but no change when combined with C or C-MP.

Excretion of ^{36}Cl by female mice (Table 35) was lower (significantly via feces as well as total excretion) following a dose 7 days after the first. However, those females which received a third dose 14 days after a redose excreted ^{36}Cl at essentially the same levels as those which had only received one dose.

TABLE 32. MORTALITY FROM EXCRETION-RETENTION STUDIES. DOSAGES PER MOUSE WERE: 1.0 mg TOXAPHENE (T) 0.5 mg METHYL PARATHION[1] (MP), AND 0.125 mg CHLORDIMEFORM(C). MEAN MORTALITY BASED UPON 3 REPLICATES OF 12[2].
(percent mortality)

Treatment	$\bar{X}$ Mortality (%)[3]
T	0 a
T-MP	20.29 b
T-MP-C	29.17 b
T-C	0 a
MP	25.50 b
MP-C	29.17 b
C	0 a

[1]48 of 60 dosed at 0.59 mg/mouse.

[2]T-MP-C and MP-C, 2 replicates of 12 and MP, 5 replicates of 12.

[3]Means followed by the same letter are not significantly different at the 0.05 level.

TABLE 33. MORTALITY OF MICE FROM COMBINATIONS OF TOXAPHENE AND METHYL PARATHION. MEAN % MORTALITY BASED ON 4 REPLICATES EACH CONTAINING, 12 MICE
(percent mortality)

Treatment - (mg/kg)[a]	$\bar{X}$ Mortality (%)[b]
T (32)	0 a
T (56)	0 a
MP (16)	34.09 b
T (32) - MP (16)	20.83 b
T (56) - MP (16)	29.17 b

[a]T - toxaphene; MP - methyl parathion

[b]Means followed by the same letter are not significantly different at the 0.05 level.

TABLE 34. RECOVERY OF ^{36}Cl FROM URINE AND FECES OF MALE MICE ORALLY DOSED WITH ^{36}Cl-TOXAPHENE. SIGNIFICANCE IS IN RELATION TO T AND THOSE VALUES WITHIN THE SAME DOSE ONLY

(percent recovery)

	TREATMENT[a]			
	T[b]	T-C	T-MP	T-MP-C
Urine				
Initial Dose	18.15a	25.01a	21.22a	17.50a
Redose	28.00a	17.36a	23.39a	25.88a
Feces				
Initial Dose	42.45a	54.70a	31.49b	27.52b
Redose	58.76a	46.97a	46.83a	57.16a

[a]Values followed by the same letter are not significantly different at the 0.05 level.

[b]T-toxaphene, 25 mg/kg; MP-methyl parathion, 12.5 mg/kg, C-chlordimeform, 3.2 mg/kg.

TABLE 35. RECOVERY OF ^{36}Cl FROM URINE AND FECES OF FEMALE MICE ORALLY DOSED WITH ^{36}Cl-TOXAPHENE. TOTALS REPRESENT THE EXCRETION THROUGH 96 HR ONLY.

(percent recovery)

	Single Dose[a]	Redose	Triple Dose
Urine	14.87a	11.85a	19.71a
Feces	34.57a	17.20b	31.00a
Excretion (urine + feces)	49.44a	29.05b	50.71a

[a]Values followed by the same letter are not significantly different at the 0.05 level.

2. Excretion:Chlordimeform

Excretion of ^{14}C (represents C and/or its metabolites recovered following an oral dose of ^{14}C-chlordimeform) was not affected by combinations of T and/or MP. The only significant change was a lowering of ^{14}C excretion when combined with MP in the urine following a redose (Table 36). Although the difference between some values were large, the large variance resulted in non-significant differences.

3. Excretion:Methyl Parathion

The excretion of para-nitrophenol (PNP), a metabolite of MP, was lowered by the addition of T. The mean total amount of PNP sampled from urine 96 hr after dosing was 172.5 μg from MP-mice, and 67.5 μg from those treated with MP-T. Analysis of feces is ongoing for the presence of MP and methyl paraoxon. Little of either has been found and analysis of other metabolites may be necessary.

4. Tissue Retention:Toxaphene

Retention of ^{36}Cl in several tissues of male mice is reported in Table 37. Lipid was the only tissue which displayed no significant changes as a result of combining T with C and/or MP. Adding C to T led to more ^{36}Cl being deposited in the brain, muscle and testes. The T-MP combination resulted in significantly more ^{36}Cl in brain, and less in kidney, and liver. When both C and MP were added to T the results were similar to T-MP; brain had significantly more, and kidney and liver had less ^{36}Cl than T alone.

In general the amount of ^{36}Cl retained by tissues increased after a redose. Tissues from mice sacrificed 33 days after the redose had ^{36}Cl levels nearer those of single dosed mice. Following a redose, ^{36}Cl concentrations were significantly elevated in kidney by T-C and T-MP-C combinations and higher in muscle by T-MP and T-C, yet was significantly lower in lipid from T-C and T-MP-C (Table 38). Thirty three days after the redose, T-C still led to reduced ^{36}Cl levels in lipid, while T-MP and T-MP-C induced higher levels in the liver.

After a single dose of ^{36}Cl-toxaphene, female mice accumulated more ^{36}Cl in brain, muscle, and gonads than did males (Table 39). Both sexes were dosed with 1.0 mg/mouse, but the effective dose was 25 mg/kg for males and 34 mg/kg for females, which may explain the higher level of tissue retention. Following a redose, the measured levels were still higher than those from redosed males, yet none of the differences were significant.

TABLE 36. RECOVERY OF ^{14}C FROM URINE AND FECES OF MALE MICE ORALLY DOSED WITH ^{14}C-CHLORDIMEFORM. SIGNIFICANCE IS IN RELATION TO C AND THOSE VALUES WITHIN THE SAME DOSE ONLY.
(percent recovery)

	TREATMENT[a]			
	C[b]	C-T	C-MP	C-T-MP
Urine				
Initial Dose	31.87a	28.07a	38.23a	30.65a
Redose	37.17a	26.40a	19.82b	23.14a
Feces				
Initial Dose	74.96a	77.45a	75.60a	53.23a
Redose	49.66a	50.00a	42.97a	66.19a

[a]Values followed by the same letter are not significantly different at the 0.05 level

[b]C-chlordimeform, 3.2 mg/kg; MP-methyl parathion, 12.5 mg/kg, T-toxaphene, 25 mg/kg

TABLE 37. RETENTION OF ^{36}Cl BY TISSUES OF MALE MICE, ORALLY DOSED WITH ^{36}Cl-TOXAPHENE. SIGNIFICANCE IS IN RELATION TO T ONLY

(ppm ^{36}Cl)

	TISSUE[b]					
Treatment	Brain	Kidney	Lipid	Liver	Muscle	Testes
T[a]	0.19a	0.72a	10.61a	0.54a	1.18a	0.75a
T-C	1.80b	1.26a	11.67a	0.91a	3.81b	4.16b
T-MP	0.54b	0.36b	9.90a	0.32b	0.47a	0.66a
T-MP-C	0.67b	0.29b	10.85a	0.35b	0.49a	0.48a

[a]T-toxaphene, 25 mg/kg; MP-methyl parathion, 12.5 mg/kg; C-chlordimeform, 3.2 mg/kg.

[b]Means followed by the same letter are not significantly different at the the 0.05 level, ANOVA.

TABLE 38. RETENTION OF ^{36}Cl BY TISSUES OF MALE MICE 8 AND 33 DAYS AFTER A REDOSE OF ^{36}Cl TOXAPHENE. SIGNIFICANCE IS IN RELATION TO T OF THAT TISSUE ONLY.
(ppm ^{36}Cl)

	Tissue[a]					
Treatment	Brain	Kidney	Lipid	Liver	Muscle	Testes
8[c]						
T[b]	2.59a	0.32a	16.96a	0.73a	1.96a	2.64a
T-C	2.12a	1.42b	7.91b	2.14a	2.76b	2.27a
T-MP	3.20a	0.54a	16.69a	1.94a	3.73b	2.81a
T-MP-C	2.15a	1.22b	2.79b	0.63a	1.96a	2.68a
33						
T	1.81a	0.49a	3.60a	0.39a	1.48a	2.16a
T-C	1.17a	0.69a	1.96b	0.32a	1.48a	1.64a
T-MP	1.44a	0.39a	3.17a	1.02b	1.38a	2.37a
T-MP-C	2.51a	0.79a	3.17a	1.12b	2.52a	2.41a

[a]Values followed by the same letter are not significantly different at the 0.05 level.

[b]T-toxaphene, 25 mg/kg; MP-methyl parathion, 12.5 mg/kg, C-chlordimeform 3.2 mg/kg.

[c]Days after receiving redose.

TABLE 39. COMPARATIVE RETENTION OF ^{36}Cl BY TISSUES OF MALE AND FEMALE MICE ORALLY DOSED WITH ^{36}Cl-TOXAPHENE. SIGNIFICANCE IS IN RELATION TO EACH DOSE AND WITHIN EACH TISSUE ONLY. (ppm ^{36}Cl)

	TISSUE[a]					
Dose	Brain	Kidney	Lipid	Liver	Muscle	Gonads
Single Dose						
Male	0.19a	0.72a	10.61a	0.54a	1.18a	0.75a
Female	2.17b	1.22a	10.96a	0.40a	3.63b	3.15b
Redose						
Male	2.59a	0.32a	16.96a	0.73a	1.96a	2.64a
Female	3.93a	2.46a	13.74a	0.87a	2.03a	4.58a

[a]Values followed by the same letter are not significantly different at the 0.05 level.

5. Tissue Retention:Chlordimeform

Combining T and/or MP with C resulted in lowering ^{14}C deposition in some tissues (Table 40). Addition of MP led to a drop of ^{14}C levels in the liver, while MP, T, and both together when added to C caused less ^{14}C to be retained in lipid, muscle, and the testes.

Following the redose, C-T led to only one significant change by increasing the level of ^{14}C in the testes. C-MP also increased ^{14}C levels in testes as well as in the liver. When all 3 insecticides were combined, significant increases in ^{14}C levels occurred in the testes, liver, and lipid.

TABLE 40. RETENTION OF ^{14}C BY TISSUES OF MALE MICE ORALLY DOSED WITH ^{14}C-CHLORDIMEFORM. SIGNIFICANCE IS IN RELATION TO C, WITHIN EACH TISSUE, AND WITHIN EACH DOSE ONLY
(ppm ^{14}C)

Treatment	TISSUE[a]					
	Brain	Kidney	Lipid	Liver	Muscle	Testes
Initial Dose						
C[b]	0.56a	0.75a	0.25a	0.56a	0.36a	0.60a
C-T	0.40a	0.81a	0.08b	0.52a	0.19b	0.32b
C-MP	0.38a	0.51a	0.09b	0.33b	0.21b	0.19b
C-T-MP	0.48a	0.72a	0.12b	0.44a	0.19b	0.17b
Redose						
C	0.17a	0.42a	0.16a	0.65a	0.23a	0.10a
C-T	0.49a	0.29a	0.14a	0.60a	0.11a	0.22b
C-MP	0.39a	0.57a	0.32a	1.03b	0.27a	0.36b
C-T-MP	0.42a	0.47a	0.38b	1.15b	0.33a	0.40b

[a]Values followed by the same letter are not significantly different at the 0.05 level.

[b]C-chlordimeform, 3.2 mg/kg; MP-methyl parathion, 12.5 mg/kg; T-toxaphene, 25 mg/kg.

SECTION VI

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SECTION VII

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SECTION VIII

GLOSSARY

AB - Abbreviation of abdominal, refers to abdominal section of central nervous system.

Active Transport - Movement of ions and molecules across cell membranes by expending energy.

Adenyl Cyclase - Enzyme which converts ATP to cAMP.

Aldrin - 1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 8, 8a-hexahydro-endo-exo-1, 4:5, 8 - dimethanonaphthalene.

Asymptomatic - Not displaying symptoms.

ATPase - Adenosine triphosphatase enzymes.

Axonal - Associated with axons (extensions of nerve cells which transmit impulses).

BR - Abbreviation of brain, refers to brain section of central nervous system.

C - Abbreviation of chlordimeform.

$^{45}Ca^{++}$ - Symbol for radioisotope of calcium.

cAMP - Cyclic adenosine 3',5'-monophosphate.

Cercal Nerve - Nerve leading from the cerci (sensory structures on the posterior of insects) of the cockroach into the ventral nerve cord.

cGMP - Cyclic guanosine 3',5'-monophosphate.

Chlordimeform - N' - (4-chloro-o-tolyl)-N, N-dimethylformamidine.

$^{36}Cl^{-}$ - Symbol for radioisotope of chloride ion.

C-MP - Abbreviation of chlordimeform, methyl parathion mixture.

CNS - Abbreviation of central nervous system.

Cyclic Nucleotides - High energy phosphate compounds, i.e. cAMP, cGMP.

Cyclodiene Insecticide - Any one of a group of compounds, derived by the Diels-Alder reaction in which hexachlorocyclopentadiene is one of the reactants.

DDT - 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane.

D-Vac® - Apparatus used to collect insects by vacuum action.

Dieldrin - 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-exo-1,4:5,8-dimethanonaphthalene.

Efflux - Diffusion outward.

Extraneuronal - Outside or surrounding the nervous system.

Fat Body - Diffuse "tissue" in insects below the epidermis and around the gut, which serves as a store of glycogen, protein, and plays an active part in metabolism.

Ganglia - Small solid masses of nervous tissue containing numerous cell bodies.

Gastric Caeca - Tubular extensions of the fore part of the midgut of insects.

Hemipteran - Refers to insects of the order Hemiptera (true bugs).

In Vitro - Experimentation on a tissue or organ removed from the animal.

In Vivo - Experimentation on the whole living animal.

Ion - Atom or group of atoms that carries a positive or negative charge.

Ionic Flux - Movement of ions across a membrane.

$^{42}K^{+}$ - Symbol for radioisotope of potassium.

Larvae - (sing. larva) Immature stages of insects displaying complete metamorphosis.

LC-50 - Lethal concentration, median (lethal for 50% of inoculated group), dose per animal (mg/animal)

LD-50 - Lethal dose, median (lethal for 50% of inoculated group), dose per unit weight (mg/g).

Lepidopterous - Refers to insects of the order Lepidoptera (moths and butterflies).

Malphighian Tubules - Tubular glands, excretory in function.

Methyl-parathion - 0,0-Dimethyl 0-p-nitrophenyl phosphorothioate.

Mg^{++} - Symbol for magnesium.

Mitochondria - Microscopic bodies occurring in the cytoplasm of a cell, and responsible for energy production.

Mode of Action - Method by which a compound exerts its effects.

MP - Abbreviation of methyl parathion.

mV - Millivolt, 10^{-3} volts.

μCi - Microcurie; unit of radioactivity containing 10^{-6} curies or 37,000 disintegrations per second.

$^{24}Na^{+}$ - Symbol for radioisotope of sodium.

Neonatal - New born.

Non-Target Insects - Insects not intended to be killed by insecticides.

Ouabain - Cardiac glycoside obtained from seeds of *Strophanthus gratus*, specifically inhibits Na^{+}-K^{+} ATPase, $C_{29}H_{44}O_{14}$.

Partitioned - Seperation of substances between solvents of different polarities.

Parturition - birth

Perinatal - About the time of birth, in this instance, prior to birth.

Phosphodiesterase - Enzyme responsible for the breakdown of cyclic nucleotides.

Pi - Symbol for inorganic phosphate.

Plasma Membrane Vesicles - Small spheres of cell membrane formed when the cell membrane is fractured.

POPOP - Phosphor used in liquid scintillation cocktails, p-bis-2-(5-phenyloxazolyl)-benzene.

Postnatal - Following birth.

Postpartum - Following birth.

ppm - parts per million.

PPO - Phosphor used in liquid scintillation cocktails, 2-5-diphenyloxazole.

Radiolabelled - Compound in which one or more of the elements of it's structure are radioactive.

Scintillation - Method of quantifying radioactivity by counting the flashes of light given off by a phosphor excited by ionizing radiation.

Scintillation Cocktail - Solution of phosphors used to evaluate radioactivity.

Site of Action - Location in a tissue or organ where a compound exerts it's effect.

Spp - Species.

Suboesophageal Ganglion - Ganglion located at the base of the arthropod head, lying below the esophagus.

Supernate - Fluid portion of material which has been separated by centrifugation.

Symptomatic - Displaying symptoms.

T - Abbreviation of toxaphene.

Target Insects - Insects which are intended to be killed by insecticides.

T-C(C-T) - Abbreviation of toxaphene, chlordimeform combination.

TH - Abbreviation of thoracic, refers to thoracic section of the central nervous system.

T-MP(MP-T) - Abbreviation of toxaphene, methyl parathion combination.

T-MP-C(C-T-MP) - Abbreviation of toxaphene, methyl parathion, chlordimeform combination.

Topical Dose - A dose which is applied externally.

Trachea - Cuticle lined tube conveying air from the spiracles to the tissues in insects and other arthropods.

Triton X-100 - Emulsifier; alkyl phenoxy polyethoxy ethanol.

Uptake - Incorporate, or absorb.

VNC - Abbreviation of ventral nerve cord.

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(Please read Instructions on the reverse before completing)

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15. SUPPLEMENTARY NOTES

16. ABSTRACT

A study was made concerning the mode of action, excretion, metabolism, and behavioral effects of toxaphene and combinations of toxaphene, methyl parathion, and/or chlordimeform in various insects, the mouse and rat. Toxaphene (1) altered ionic movements within the central nervous system of the cockroach, Periplaneta americana, (2) increased levels of cAMP and cGMP in tissues of the cockroach, Leucophaea maderae, and the mouse, and (3) inhibited ATPase enzymes in tissues of P. americana and the mouse. Rat pups perinatally exposed to sublethal doses of toxaphene and methyl parathion showed few significant changes in motor skills, behavior, or learning ability. Postnatal exposure to toxaphene did lead to an impairment of learning ability in adult rats as measured by a simple T-maze. Combining chlordimeform and methyl parathion with ^{36}Cl recovered in feces and the amount deposited in tissues of orally-dosed mice. Combining toxaphene with methyl parathion did not potentiate the toxicity of methyl parathion under laboratory conditions to various hemipteran predators and lepidopterous pests.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Toxicology Poisons Insecticides Pesticides Hazardous Materials Physiological Effects Chlorine organic compounds	Toxaphene Cyclodiene Chlorinated camphene organophosphate methyl parathion formamidine chlordimeform	06, A,M,T

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