DETERMINATION AND MAINTENANCE OF SUBLETHAL RESIDUE LEVELS OF DIELDRIN AND PARATHION IN *COLINUS VIRGINIANUS*

by 6791

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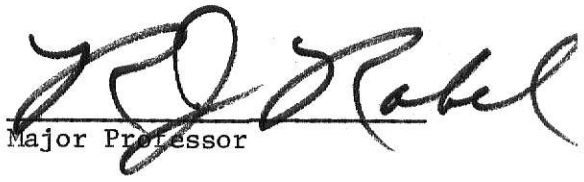

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INTRODUCTION AND REVIEW OF LITERATURE

The use of synthetic organochlorine insecticides began in the mid-forties with the introduction of DDT*. Since that time production and use of pesticides continues to grow to meet the increasing demand.

Farm use of pesticides in 1964 amounted to 457.5 million pounds (active ingredient basis) valued at about $500 million (Pesticide Handbook - Entoma 1969:27). Consumption of DDT is starting to show a general decline from the peak year of 1959 when domestic use reached 79 million pounds, however consumption of cyclodiene insecticides (chlorinated hydrocarbons of the aldrin-dieldrin-toxaphene group) is basically the same as it was in 1965; the year of highest consumption (Pesticide Handbook - Entoma 1969:27).

It is the use of DDT and a number of cyclodienes which, due to their persistent nature, are causing irreversible damage to the world's ecosystems (Carson 1962:24-35). There is an ever growing volume of data to show that many of those frequently used pesticides, especially the cyclodienes, are having a serious effect on our wildlife resources (Carson 1962, Cottam 1960, Cottam 1962, Committee Report-Pesticides and Wildlife 1959 and 1960, Dewitt and George 1960, Dewitt et al. 1962, Hickey 1961, Helley 1961, and Tarzwell 1958).

Organochlorine pesticides last a very long time in the environment, but not necessarily at the site of application (Stickel 1968:1). These pesticides may travel to all parts of the environment by means of wind, rain, surface and underground waters, and animals including man himself (Abbott et al. 1966, Briedenbach 1965, Dustman 1967, Dustman and Stickel 1966, Stickel 1968). Although the ease of movement of organochlorines in

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*See Appendix, Table III for common and scientific names of pesticides mentioned in this thesis.

the environment does not present a problem as great as their persistence, it nevertheless must be considered.

Research in the pesticide field has been primarily conducted to determine the LD_{50} (dosage at which 50 percent of test animals died within a given time period) of various pesticides, survey of pesticides residues in the environment, effects of pesticides on reproductive patterns, analytical procedures and development and testing of compounds.

One of the objectives of this study was to determine if bobwhite quail when fed sublethal levels of diedrin would attain and maintain a storage equilibrium in body and brain tissues. Another objective was to determine whether any correlation existed between pesticide residues which were found in the brain and the remainder of the bird. Dieldrin was selected as the representative chlorinated hydrocarbon to be used in this study since it is widely used by gardeners and agriculturalists alike.

A similar study with an organophosphorous compound was also planned. In this case the objective was to determine whether levels of an organophosphate could be maintained in bobwhite quail and also whether one could quantitate pesticide intake by means of cholinesterase inhibition. The organophosphorous compound chosen to be used in this study was parathion because it is being used in increasing amounts by the agriculturalists. Though parathion was introduced in 1944, it still continues to be one of the most widely used organophosphate (O'Brien 1967:32).

Aldrin

Lindov et al. (1950:175) found the LD_{50} of aldrin ranged from 40 to 50 mg/kg of body weight for albino rats. The LD_{50} of aldrin for mice was found by Borgman et al. (1949) to be 44 mg/kg of body weight. The critical (acute) oral toxicity of aldrin to ring necked pheasants (Phasianus colchius)

was found by Post (1952:496) to be 40 mg/kg of body weight. Tucker and Crabtree (1970:17) found the LD_{50} of aldrin to be 16.8 and 6.59 mg/kg of body weight for 3 to 4 month old pen reared female ring necked pheasants and bobwhite quail (Colinus virginianus) respectively. Three to 4 month old female mallard ducks (Anus platyrhynchos) responded with an LD_{50} of 520 mg/kg of body weight of aldrin (Tucker and Crabtree 1970:17).

The LD_{50} of aldrin, as stated by Dahlen and Haugen (1954:478), for the bobwhite quail and the mourning dove (Zenaidura macoura) is 4.0 to 4.5 mg/kg and 15.0 to 17.0 mg/kg respectively. Dewitt (1956:864) found that diets containing 10 ppm of aldrin produced 100 per cent mortality among adult bobwhite quail within 2 to 8 days after feeding was begun. He further states that when adult birds were fed a maintenance diet of only 1 ppm of aldrin, 100 per cent mortality occurred after 101 days. In Dewitt's (1956) study total toxicant consumed was 9.1 mg/kg of body weight. When Dewitt (1955:673) increased the aldrin dosage to 100 ppm (dietary basis), 100 per cent mortality occurred after 5 days. The same study also demonstrated that if daily feeding levels were increased to 5000 ppm (dietary basis), 100 per cent mortality occurred after 4 days of feeding.

Dieldrin

As with most insecticides, initial studies on dieldrin were concerned with acute toxicities and LD_{50}'s. The oral LD_{50} as reported by Dahlen and Haugen (1954:478) for 8-week-old bobwhite quail is 12-14 mg/kg of body weight and for mourning doves is 44-46 mg/kg of body weight. No discernable difference due to sex was found. Dewitt et al. (1955:675) also reported no difference in susceptibility of male and female bobwhite quail to dieldrin.

Dewitt et al. (1963) reported that consumption of 10 mg/kg of dieldrin killed 50 per cent of young quail in 1 to 10 days when they consumed feed contaminated with 20 ppm of dieldrin (dry weight basis). It was found by Heath et al. (1965) that 40 ppm of dieldrin produced 50 per cent mortality to week-old bobwhite chicks after 5 days on treated food. Dewitt (1955: 783) stated that when adult quail consumed 0.9 and 2.8 mg/kg of dieldrin daily, all died after 6 and 7 days, respectively. However Rudd and Genelly (1956:86,87) reported the LD_{50} of bobwhite quail to be between 12 and 14 mg/kg of body weight.

In another study, Dewitt (1956:863-866) reported that diets which contained 50 ppm of dieldrin produced 100 per cent mortality among adult quail within 2 to 8 days after feeding was begun. He further states that adult quail which were fed 1 ppm of dieldrin in their winter maintenance diet survived a 162 day test period, but suffered heavy mortality (40 percent) when they were subsequently fed high protein (reproduction diets) containing the same level of toxicant.

Genelly and Rudd (1956:5-14) reported on ring necked pheasants which were maintained on various levels of dieldrin. They found that in the fall 6 out of 10 females fed 200 ppm died within 28 days and 5 out of 10 females given 100 ppm died within 38 days. They observed that spring feeding of 50 ppm of dieldrin caused death in all 4 males and 8 out of 20 females.

The LD_{50} reported by Tucker and Crabtree (1970:47) of dieldrin for mallards, ring necked phesants, chukars (Alectoris graeca), and coturnix quail (Coturnix coturnix) was 381.0, 79.0, 23.4, and 69.7 mg/kg of body weight, respectively. McEwen and Brown (1966:604) found that the LD_{50} for wild male sharp-tailed grouse (Pedioecetes phasianellus) which were captured on the breeding grounds, dosed and later released was 6.9 mg/kg of body

weight. Negherbon (1959:224) states that the oral LD_{50} for a 3 to 6 week old domestic chicken to be between 20 and 30 mg/kg of body weight.

Robinson et al. (1967) reported on the pharmocodynamics of pigeons fed a diet of 50 ppm dieldrin for 6 months, then fed a normal diet and killed at various intervals. Bachstom et al. (1965) injected ^{14}C intramuscularly into mice and found the greatest concentration of dieldrin in the fat deposits within 24 hours. Concentrations were also found in liver, intestine, gall bladder, bone marrow and mammary glands. Turtle et al. (1963) analyzed the breast muscle of feral pigeons that had died after oral dosing with dieldrin. The concentrations of dieldrin in the brain and livers of Japanese quail and domestic pigeons (Columba livia) were reported on by Robinson et al. (1967).

Sublethal effect and equilibrium concept

Little work has been done on sublethal effects of dieldrin on birds. Robinson (1967) and Stickel (1968) consider it important to study not only the lethal concentration of an insecticide but also the concentration in living birds that have been dosed but exhibit no ill effects.

Quaife (1967:48) reports that no satisfactory work has been done to show that, following prolonged feeding, equilibrium in animals between intake and storage of dieldrin or aldrin exists. Several studies along this line of study have, however, been done. Ludwig et al. (1964:130) fed aldrin ^{14}C to male rats and an equal intake and output of radioactivity was found to occur after 56 days of feeding. It was demonstrated by Coulson and McCarthy (1963) that a maximum concentration of dieldrin in fat is attained by feeding dieldrin to rats for 16 weeks. Quaife (1967:48) says these studies fail to provide unequivocal evidence of an equilibrium concept.

While not postulating an equilibrium concept, Korte (1965:2017-2016)

states that in mammals dieldrin is either excreted or metabolized and excreted because it does not accumulate above certain levels, even when mammals are fed relatively high daily doses.

Residue content

Stickel et al. (1969:174) state that diagnosis of death of birds and mammals from pesticide poisoning on the basis of residue content of victims is an important and practical problem. Numerous studies have been done in which birds killed by crop spraying were analyzed for the presence of organochlorine insecticides.

Robinson et al. (1967) reported on the concentration of dieldrin in the brain and livers of coturnix quail and domestic pigeons. Turtle et al. (1963) tested for the presence of dieldrin residues in the breast muscle of feral pigeons that had died after oral dosing with dieldrin. However, the publications of Stickel et al. (1969), Robinson et al. (1967), and Turtle et al. (1963) are the only reports of dieldrin residue concentration in organs following known feeding levels of this insecticide. No work at this time has been done with the equilibrium concept in the bobwhite quail.

Organophosphates

It is customary to employ the term organophosphates to cover all toxic compounds containing phosphorous (O'Brien 1967:33). In 1959 this group was reported to contain an estimated 50,000 different compounds (O'Brien 1967). Numerous works have been published concerning the acute toxicities of organophosphates to animals and birds. Tucker and Crabtree (1970:76) state that the LD_{50} for diazinon, another frequently used organophosphate, is 3.45 and 4.33 mg/kg for 3 to 4 month male mallards and ring necked pheasants, respectively. The LD_{50}'s obtained with parathion as reported by

Tucker and Crabtree (1970:92) for 3 to 4 month male mallards, 2 to 3 month male ring necked pheasants, 2 month female Coturnix and 12 to 36 month female sharp-tailed grouse was 2.13, 12.40, 5.95 mg/kg and 4 to 10 mg/kg respectively.

Redeleff (1964) reported the oral LD_{50} of malathion for the adult domestic chicken to be 150 to 200 mg/kg of body weight. Dewitt et al. (1963) reported the acute toxicity of malathion for immature bobwhite quail and ring necked pheasants to be 780 and 550 mg/kg of body weight, respectively. The insecticide in Dewitt's study was administered over a 10-day period instead of as a single dose as is customary with LD_{50} studies. McEwen and Brown (1966:604) state the LD_{50} of malathion for sharp-tailed grouse to be between 200 and 240 mg/kg of body weight.

Parathion, being an organophosphate, metabolizes rapidly (O'Brien 1967). Smith et al. (1960:495) found that two cows fed malathion at the established tolerance level for alfalfa (8 ppm) for 3 weeks never excreted the insecticide in the milk nor was it detectable in the blood, brain, liver, kidney, or rib eye of the one animal that was slaughtered immediately after cessation of the study.

Cassidy et al. (1969:571-575) studied the effect of Supracide, a new organophosphorous compound, on dairy cattle. They found that when ^{14}C labelled Supracide was fed to dairy cows for 5 days, the total amount of radioactivity found in the mild during the 15-day study represented only 0.6 percent of the oral dosage. Fractionalization of the milk indicated extensive metabolism, as did the nature of radioactivity recovered in the urine (24 percent) and feces (34 percent). Total radioactivity determined in tissues indicates that significant storage does not occur. The highest level of radioactivity found in tissues was 0.11 ppm in the liver (Cassidy et al. 1969:571). O'Brien (1960) and Heath (1961) report that the mammalian

liver is the most important source of organophosphate degrading enzymes as shown by physiological, biochemical, and toxicological studies of organophosphorous insecticides.

Kenichi and O'Brien (1968:574) report that in the rat liver, paraoxon, the oxygen analog of parathion, is always degraded more rapidly than parathion by all enzyme fractions. Hollingsworth (1969) also states that most organophosphorous degradation occurs in the liver.

Bunyan et al. (1969:1027) fed several different organophosphorous compounds to pheasants and pigeons. Compounds studied were demeton-methyl, diazinon, dimethoate and phorate. During the 42-day study, high residue levels (260-436 ppm) of demeton-methyl were found in the fat of pheasants during the first 14 days but dropped considerably (0.3 to 5.0 ppm) as feeding reached 42 days in duration. Pigeon tissue showed lower levels; 3.8 ppm at 14 days and 0.7 ppm at 42 days when fed the same amount of demeton-methyl. When fed dizainon, residues in the liver and fat of pigeons were generally higher than those in pheasants over the short feeding periods, but fell off rapidly with time. No residues of diazinon were found in pigeon muscle extracts (Bunyan et al. 1969:1030). In the same study dimethoate was found only in the pair of pheasants fed for 42 days (0.5 and 0.4 ppm in liver and 3.8 and 4.5 ppm in adipose) and in liver of the two pigeons fed for 14 days (0.4 and 1.2 ppm). Bunyan et al. (1969:1030) reported no residues of phorate were found in any tissue examined. It is a general concensus of those who have worked with organophosphates (Bunyan et al. 1969, Heath 1961, Hollingsworth 1969, Kenichi and O'Brien 1968 and O'Brien 1967) that this group of compounds metabolizes very rapidly. Therefore, gas chromatographic analysis of animal tissue is not a wise choice for quantitating most organophosphoric compounds.

The effect of organophosphorous pesticides on avian cholinesterases has been studied by Sherman and Kimatsu (1963), Sherman et al. (1964), Sherman et al. (1968). Rizzoli and Galzinga (1965) reported on the effect of tetraethyl lead on cerebral and blood acetycholinesterase and how they differ from mammalian cholinesterases in their specificity in cleaving choline esters and their reactions with inhibitors (Ferrari 1957, Blaber and Cuthbert 1962, Shellenberger et al. 1966, Lee and Pickering 1967). In comparative toxicological studies with bobwhite quail and Japanese quail bobwhite quail were found to be more sensitive to Guthion (Gough et al. 1967), but less sensitive to Bidrin and Azodrin (Shellenberger 1966).

Bunyan et al. (1968a, 1968b), Lorolev (1965), Bunyan (1966), Bunyan et al. (1969) consider changes in esterase levels the most accurate way to test for the presence of an organophosphate in an animal. Bunyan et al. (1969) state that brain esterase levels, especially cholinesterase, were consistent enough to allow abnormal values to be readily detected. Liver esterase levels are not generally of any use in diagnosing organophosphorous poisoning since levels are too erratic (Bunyan et al. 1969). The same observers further state that esterase inhibition in plasma, even when obtainable from wild birds suspected of being poisoned by organophosphates, is seldom of diagnostic value since it is highly variable and extremely sensitive to inhibition. Shellenberger (1966) and Gough (1967) likewise reported inconsistencies in whole blood cholinesterase levels. Percentage of cholinesterase activity varied between birds and also between sexes. Boyd (1970) states that plasma cholinesterase levels arise from the liver. Bunyan et al. (1969) report that severs plasma cholinesterase inhibition (greater than 80 per cent) occurred in pigeons and pheasants dosed with

organophosphates, but the animals showed no visible symptoms of poisoning. A 90 per cent inhibition of brain cholinesterase was suggested by Bunyan et al. (1969) as the most reliable diagnosis of death due to organophosphate poisoning. Mehrota et al. (1967) support this suggestion.

MATERIALS AND METHODS

Animals

All bobwhite quail used in these investigations were obtained from the Pittsburg Game Farm owned and operated by the Kansas Forestry, Fish and Game Commission. All quail were adult males (160 or more days old).

Pesticides - Reagents - Chemicals

The dieldrin (1,2,3,4,10,10-hexachlore-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1, 4-endo-exo-5, 8-dimethanonaphthalene) and parathion (O,O-diethyl O-p-nitrophenyl phosphorothioate) used in this study were obtained from the Department of Entomology, Kansas State University. Dieldrin was manufactured by the Shell Oil Corporation and was 99.9 per cent pure while the parathion was produced by American Cyanimid and was of 99.7 per cent purity. All analytical reagents used for the dieldrin analysis were doubly distilled in glass and obtained from Burdick and Jackson Laboratories Inc., Muskegon, Michigan. All cholinesterase determinations were done with a Sigma No. 420 diagnostic kit available from Sigma Laboratory, St. Louis, Missouri.

Housing of birds

All quail were confined in 48 X 23 X 14 cm plastic cages having wire bottoms and tops. Cleaning of cages was made considerably easier by placing several paper towels below the wire floor. Plastic paper and aluminum foil was also tried but was unsatisfactory as birds tended to tear and shred these materials.

Birds were maintained under constant temperature and photoperiod. Environmental conditions for the pilot study were 8L·16D (8 hours light, 16 hours dark) and 21°C. Birds in the balance of the studies were held at 10L·14D and 21°C.

Experimental birds were on ad libitum feed, consuming approximately 10 grams of P-18 feed per day. P-18, a balanced mash prepared by the Department of Grain Science and Industry at Kansas State University, contains 20.5 per cent protein, 2.7 per cent fat, and 3.6 per cent crude fiber (Table 1). Food supplies for each bird were replenished between 0900 and 1100 hours each day. No grit was given to quail during the study. Quail had ad libitum access to water.

Preparation of pesticide dosages

Corn oil was used as the carrier solution for both parathion and dieldrin in this study. Dosages of dieldrin were computed on a weight volume basis; i.e. 1 ug of dieldrin dissolved in 1cc of corn oil constituted 1ppm of solution. Dieldrin was weighed to the nearest 0.0001 milligram. In preparing solutions of different concentrations, the solution of lower concentration was obtained by diluting with corn oil the solution of higher concentration. After the solutions were prepared and mixed thoroughly, the mixture was stored in darkness until used.

The parathion dosages were assembled in a similar manner, the only difference being that the parathion was liquid at room temperature whereas dieldrin was chrystalline. Parathion solutions were, as with dieldrin, on a weight-volume basis.

Administration of pesticides

A 1cc Monoject disposable tuberculin syringe (501-TB) of Sherwood Medical Industries was used throughout the investigation to administer dieldrin or parathion directly into the crops of bobwhite quail. Quail were grasped in the left hand with the bird's head between thumb and index

Table 1. Feed formulation of P-18 standard mash.

INGREDIENTS[a]	PERCENT	CUMULATIVE PERCENT
Soybean oil meal (44%)	28.0	28.0
Alfalfa meal (17%)	2.0	30.0
Ground sorghum grain	46.0	76.0
Ground corn	15.0	91.0
Fish meal	1.0	92.0
Meat and bone scraps	1.0	93.0
Distillers dried solubles	2.0	95.0
Ground limestone	0.5	95.5
Dicalcium phosphate	3.0	98.5
Salt	0.5	99.0
Vitamin D_3 (15,000 ICU/g)		
Vitamin A (10,000 IU/g)		
Vitamin B complex (1233)		
Vitamin B_{12} (Proferm 20)	1.0	100.0
Methione		
Baciferm 10		
Histostat 50		
Ground sorghum		

[a]Expressed as percent on a weight basis.

finger. With a pushing motion of these two fingers the neck is elongated and esophagus straightened. The syringe containing the proper dosage of insecticide was held in the right hand and forced into the quail's mouth, esophagus and eventually into the crop (Figs. 1,2). If the syringe was forced about 6.5 cm. down the bird's digestive tract, regurgitation seldom occurred. However if the syringe did not enter the crop, the quail would invariable void the oil solution by shaking its head.

Sacrificing and storage of birds

All birds which were killed by means other than poisoning were done so by squeezing the thoracic cavity until the bird stopped breathing. In this way all blood vessels were kept intact and the bird died a rapid and relatively non-violent death. Storage of all birds was done at -22°C. In many instances organs to be analyzed were dissected immediately after death. In these instances the organs were placed in glass vials, marked as to bird number and dosage level and frozen at -22°C until analysis could be performed.

Preparation of birds for analysis

Quail carcasses were allowed to thaw for 12 hours before any dissection was attempted. Brain and livers which had already been dissected prior to freezing required only several minutes to thaw.

The first step in dissecting the quail was cutting off the head. The quail head was then securly held and three cuts were made into the skull (Fig. 3), peeling off the cranial bones revealed the brain which could be removed in its entirety with a small scalpel. Removal of the brain prior to freezing the bird was found to be easier since the brain became somewhat

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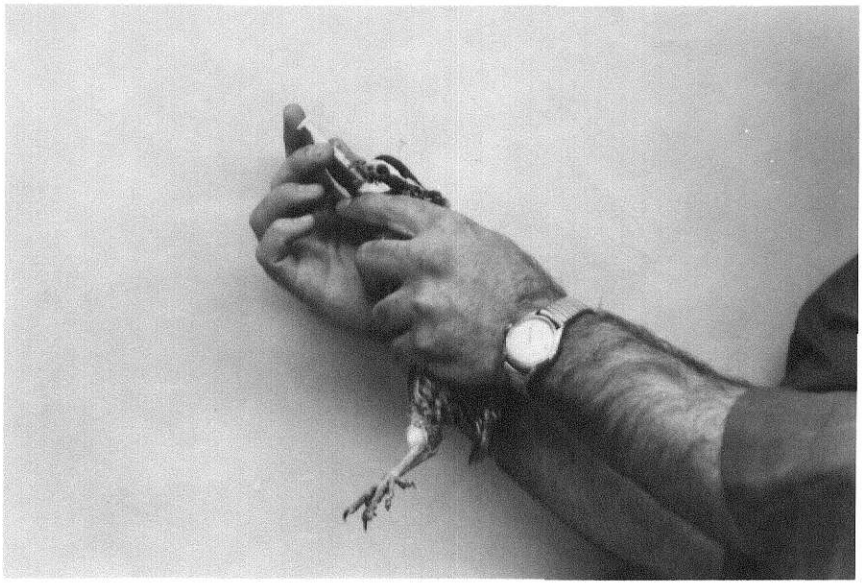

Fig. 1. Technique used to dose quail.

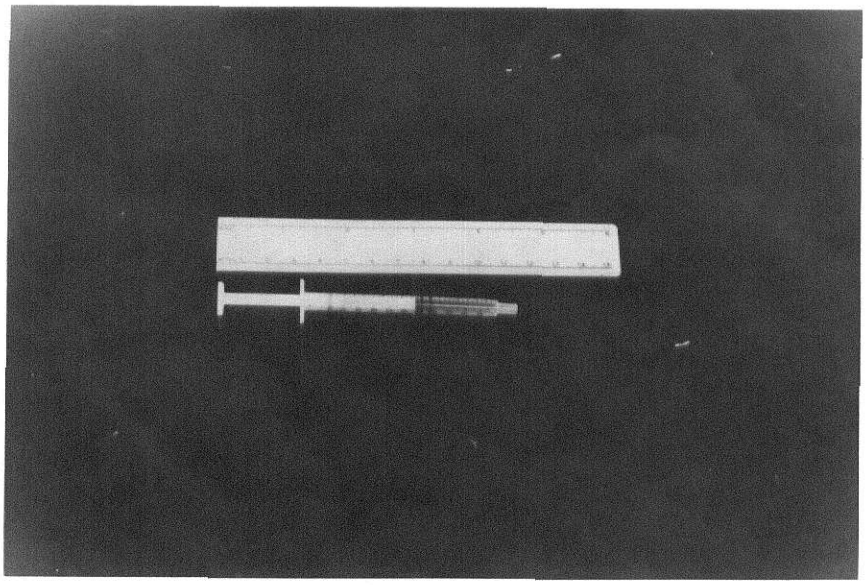

Fig. 2. Monoject syringe used to administer pesticides.

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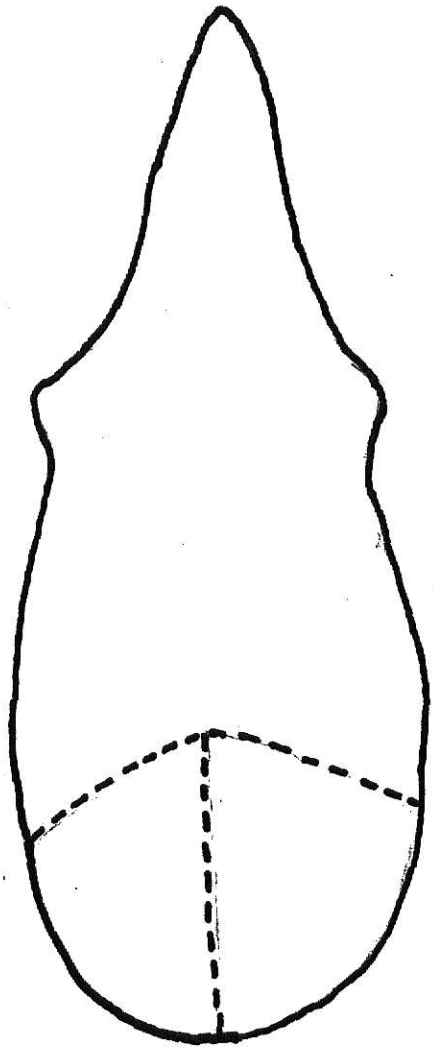


Fig. 3. Location of cuts made into skull to remove brain.
(dotted line represents location of cuts)

viscous after freezing and thawing. Thus, whenever time permitted the brain was dissected from the skull before the bird was frozen. After removing the brain, the wings and legs of the carcass were cut off at the most proximal joint to the body. Skin, feathers, and subcutaneous fat were then removed and discarded as well as viscera (including the complete digestive tract). The livers of only those birds in the pilot study were dissected, retained, marked, placed in glass vials, and quick frozen. The skeleton and its associated musculature minus all viscera, skull, subcutaneous fat, feathers and skin was termed the remainder.

All samples were analyzed on an individual basis, no pooling of tissue was done. In these studies three types of tissue were analyzed for residues of dieldrin, namely liver, brain and remainder.

Extraction, cleanup and analysis of insecticide residues

All tissues analyzed for dieldrin residues were subjected to exactly the same extraction, cleanup and gas chromatographic analysis.

The sample to be analyzed was first weighed to the nearest 0.001 gram. Analysis of brain and liver samples included the entire organ whereas only the right half of the body samples were analyzed.

To the sample tissue was added 150 ml of 30 per cent water and 70 per cent acetonitrile. This combination of tissue and solution was homogenized in a 1 qt Waring explosion-proof, stainless steel blender for a period of 3 minutes. This homogenized solution was vacuum filtered through a single piece of Whatman No. 12 grade filter paper into a 1000 ml erlenmeyer flask. The filtrate was transferred from the flask to a 500 ml separatory funnel, the erlenmeyer flask was rinsed with 5 ml of petroleum ether (Burdick and Jackson: distilled in glass) and the rinsings combined with

the solution in the separatory funnel. The contents of the separatory funnel were then agitated gently for 3 minutes, care being taken to vent off excess pressure. To the separatory funnel 100 ml of distilled water and 20 ml of saturated sodium chloride solution were added. This mixture was then agitated for 30 seconds and the ether and aquaous layers allowed to separate. After separation occurred, usually in 15 to 30 minutes, the bottom layer (aqueous) was drained off into another 500 ml separatory funnel. This solution will henceforth be called the second extraction solution. Fifty ml of petroleum ether was then added to the second extraction solution and the mixture agitated for 3 minutes. Saturated sodium chloride solution was added if emulsion formation seemed likely. This solution was then allowed to separate for 15 to 30 minutes. The bottom or aqueous layer of the second extraction solution was then drained off and discarded. The upper or petroleum ether layer was combined with the petroleum ether layer of the first extraction solution. The separatory funnel of the second extraction solution was rinsed with 5 ml of petroleum ether solution and combined with the two preceding ether solutions. To this solution, 100 ml of distilled water was added, the mixture agitated for 3 minutes and allowed to separate forming an upper ether and lower aqueous layer. The aqueous layer was drained off and discarded. The above step was repeated again. The ether solution is now ready for the Florisil elution column.

For the Florisil cleanup, a 22 mm id by 300 mm chromatographic column that contained 5 inches (after settling) of activated Florisil with 1/2 inch of anhydrous sodium sulfate was used for the cleanup of all dieldrin containing samples. The column was pre-wetted with 50 ml of petroleum ether. A Kuderna Danish concentrator with a 10 ml collection vile was

placed under the column to receive the eluate. The petroleum ether extract from the acetonitrile partitioning step was allowed to pass through the florisil column at a rate of not more than 5 ml/min. The separatory funnel was rinsed with two 5-ml portions of petroleum ether. The walls of the chromatographic column were also rinsed with a 5-ml portion of petroleum ether. The column was then eluted with 200 ml of 6 per cent ethyl ether and 94 per cent petroleum ether (6/94:v/v). Receivers were changed and the column was eluted with 200 ml of 15 percent ethyl ether and 85 per cent petroleum ether (15/85:v/v). Both the 6 per cent and the 15 per cent eluates were concentrated to a 10-ml column in a water bath at a temperature of 55° to 60°C. Evaporation time was approximately 15 minutes.

Since only 3 of the 6 per cent eluates out of 44 analyzed contained dieldrin and then only a trace (less than 0.02 ppm), it was questioned whether efficiency could not be improved by elimination of the 6 per cent eluate in favor of a slightly larger 15 per cent eluate. McCollough (personal communication) recommended a 250 ml volume of 15 per cent eluating solution and dispensing entirely with the 6 per cent solution.

Trying this technique with the remaining half-body samples from the 44 previous analyses in which both 6 per cent and 15 per cent eluates were utilized gave gratifying results. Thereafter only the 15 per cent eluates were used for the 194 remaining samples.

Efficiency of extraction technique

To check the analytical procedure, a known sample of HEOD was obtained from the Food and Drug Administration, Kansas City District. Various pieces of tissue from control birds were then cut up, weighed to the nearest 0.001 gm and placed in the Waring Blender cup. A known amount of pesticide

was then added and the concentration in the sample calculated.

All samples were analyzed exactly the same as outlined previously in the extraction, cleanup and analysis section. An average recovery of 87.7 per cent was experienced (Table 2). These results compare favorably with those presently being attained by the Food and Drug Adminstration (McCollough, personal communication).

Gas chromatography

All gas chromatographic analyses in this study were done according to the Pesticide Analytical Manual, Volume I,II: 1968. Though day-to-day instrument variations occurred, conditions were amintained approximately as follows:

Gas Chromatograph	Barber-Colman Model 5000
Chart Speed	1/2 inch per minute
Detector	Electron Capture
Detector Temperature	220°C
Cell Voltage	40 V
Sensitivity	1×10^{-9} amperes full scale
Column	glass, U-shaped, six feet x four mm id
Liquid Phase	10 per cent DC 200
Support	Chromasorb WHP, 100 mesh
Temperature	200°C
Carrier gas	Nitrogen
Pressure	20 psig.
F H	215°C

Many methods of evaluating gas chromatographic peaks have been developed. The choice of one method over another depends on the type of peak to be

Table 2. Efficiency of analytical technique.

Type of Sample	Weight of Sample[a]	ppm added[b]	ppm found[c]	percent correct
brain	1.011	2.044	1.798	87.9
brain	0.976	0.915	0.771	84.2
remainder	36.971	6.215	5.843	94.0
remainder	41.425	4.321	3.730	86.3
remainder	46.795	1.011	0.794	78.5
remainder	40.621	0.545	0.502	92.1
remainder	33.457	0.418	0.382	91.3
			$\bar{X}$ =	87.7

[a]Grams

[b]Dieldrin added dissolved in petroleum ether

[c]PPM on wet weight basis

measured and the operator's preference (PAM, Vol. I, Sec. 302.41). Both peak height and triangulation methods were used in this study to quantify chromatographic procedure. Triangulation was used when baselines were rather broad. Peak height was the method most often used because most chromatograms were characterized by short baselines and acute peaks (Fig. 4). Standards (known concentrations of dieldrin) were injected after about every three unknowns so that standard and unknown peaks would be similar in size, thus making the peak height method the quickest and most accurate means of interpreting chromatograms (Fig. 4). Throughout this study ppm values are uncorrected for loss. A more complete explanation of evaluation of GLC peaks is presented by Dimbat et al. (1956), Keulman (1960), Dal Nogare and Juvet (1962), and Littlewood (1962).

Dieldrin Studies

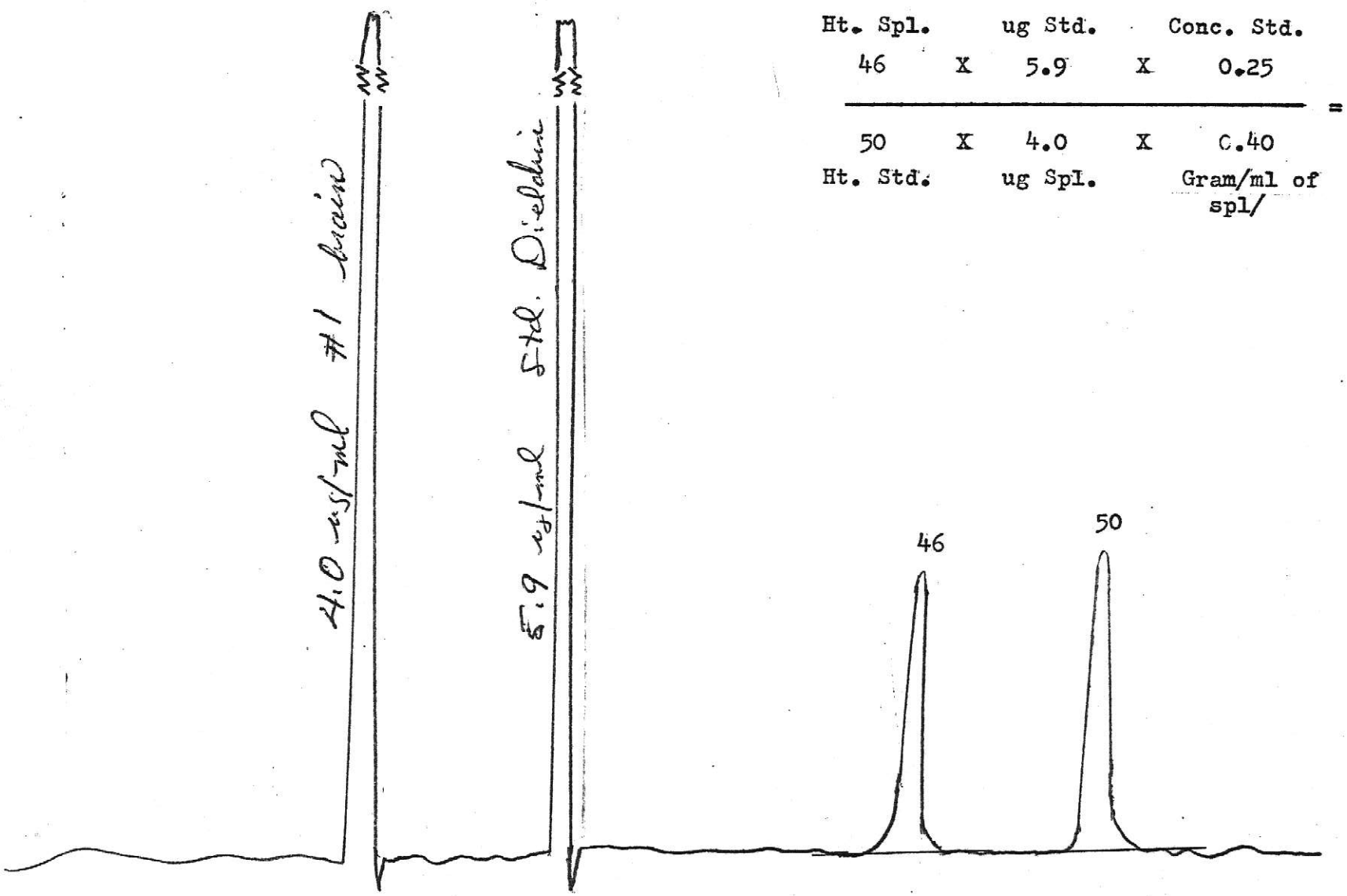
Pilot study

At the iniation of these studies no satisfactory data could be found concerning sublethal pesticide residue accumulation in birds. A pilot experiment was therefore necessary to obtain a general idea of the level of pesticide required to achieve satisfactory levels (1 to 6 ppm in brain tissue) and the length of time required for this accumulation to occur.

The pilot study consisted of two 15-bird groups. One group received 3.25 ug of 99 percent pure dieldrin dissolved in 1 ml of corn oil while the other group was administered a 6.50 ug/ml dosage. Dosages for both groups were administered every other day for a period of 20 days at which time the study was terminated. Intermittant dosing was chosen over daily dosage to minimize stress. Three birds were sacrificed from each group every 4 days and quick frozen at -22°C until residue analysis could be performed. Liver, brain, and remainders were analyzed for residue accumulation.

Fig. 4. Method used to determine amount of dieldrin in tissues.

Ht. Spl.		ug Std.		Conc. Std.	
46	X	5.9	X	0.25	= PPM
50	X	4.0	X	C.40	
Ht. Std.		ug Spl.		Gram/ml of spl/	



6.25 and 12.50 ppm

Following the pilot study, a second experiment was initiated to establish a 1 to 6 ppm residue accumulation in the brain. Two 16-bird groups were confined to individual cages two weeks prior to initiation of treatment to condition birds to cage conditions and to allow for weight stabilization (less than 2 percent weight variation per day).

One group received 6.25 ug of 99 per cent pure dieldrin per ml of corn oil while the other group was given 12.50 ug/ml of dieldrin. Dosages for both groups were administered every 4 days for 66 days. Birds were weighed to 0.1 g every 4 days just prior to dosing. Two birds were sacrificed from each group approximately every 10 days and quick frozen at -22°C until residue analysis could be performed.

Acute and chronic toxicity

As desired residue levels were not yet being attained, an experiment was initiated to determine the lethal and maximal sublethal levels of dieldrin for bobwhite quail under the existing conditions of confinement and dosage technique. Eight birds were used for the acute toxicity and chronic toxicity tests. As in the previous study two weeks were allowed between obtaining and initiation of the experiment.

Two birds each, were dosed with 200, 400, 600, and 800 ug of dieldrin per ml of corn oil. All dosing was done on a daily basis. Bird weights were recorded each day (Appendix, Fig. 5). The study was of 30 days duration, birds which were not killed by direct dieldrin poisoning within that time were sacrificed at the end of the 30 days. Upon death, birds were quick frozen at -22°C until residue analyses could be performed.

50 and 100 ppm

Based on findings from the three previous experiments, it was decided to retain the approximate length of time birds were to be dosed but to decrease the time between doses and also to increase the amount of dieldrin given per dose. An experiment was therefore initiated for a period of 70 days. Two groups, each with 21 birds received the respective 50 and 100 ug/ml dosages of dieldrin every other day. As with the previous studies, birds were caged two weeks prior to iniation of the experiment. All birds were weighed to 0.1 g once every 10 days. Three birds from each group (50 and 100 ppm) were sacrificed every 10 days and quick frozen at -22°C until residue analysis could be performed.

Control Group

The control group consisted of 6 birds. Each bird received 1 cc of pure corn oil every other day for 70 days. Body weight was recorded every 10th day (Appendix, Table 14).

Since no previous information concerning sublethal doses of parathion to bobwhite quail could be found; the first step in this study was to perform an acute toxicity test. Fourteen bobwhite quail were used in this experiment. Two birds each were dosed with 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 mg of parathion per kg of body weight. All dosages were calculated on the hypothetical body weight of 175 grams. Birds were checked hourly for mortality. Dead birds were removed from cages and frozen at -22°C until analyses could be performed.

From the results of the acute toxicity tests it became apparent that the LD_{50} for bobwhite quail was below 5.0 mg/kg. Another study was initiated with 40 birds. These birds were divided into two groups of 20 birds each. One group received 3.0 mg/kg per day while the other group was treated with 1.0 mg/kg per day.

The results of the two previous experiments led to the belief that a dosage level of 0.5 mg/kg per day could be tolerated by bobwhite quail for at least 5 days. Thus ten birds each received 0.5 mg/kg of parathion every day. Two birds were sacrificed every day so that a dose-response curve of cholinesterase inhibition could be constructed.

Cholinesterase determination

Preparation of tissue abstract

The brain of each bobwhite quail to be analyzed was removed and placed in a 7-ml tissue homogenizer. The brain was homogenized in 6 ml of Triton-X-100 solution. Trition-X-100 solution was used for homogenizing brain tissue since organophosphate-sensitive bound esterases are released by this method (Bernshon et al. 1964). The suspensions were centrifuged on a Sorvall Superspeed RC2-B centrifuge at 0° to 3°C, 16,000 g for 45 minutes. The supernatant was collected, care being taken to avoid contaminating solids. All supernatants were stored at 3°C until analysis could be made to reduce the loss of cholinesterase activity (Caraway 1956). All tissue analyzed for cholinesterase inhibition was prepared by the just mentioned procedure.

Quantitating cholinesterase inhibition

All quantative measures of cholinesterase were done with a Sigma No. 420 cholinesterase diagnostic kit following the procedure discussed in the kit. A Baush and Lomb Spectronic 20 at a wavelength of 435 mu was used to measure spectural absorption. The 435 mu wavelength was in the center of the optical density scale, thus increasing the precision of the test. Duplicate analyses were done on eight samples. Repeatibility of this technique was excellent, often with no more than 1 per cent variation between tests.

RESULTS

Residue analysis - 3.25 and 6.50 ug/ml of dieldrin

Birds receiving 3.25 ug/ml of dieldrin daily accumulated highest dieldrin residues in liver tissue. Residues of dieldrin in liver ranged from 0.4216 ppm found in a bird after only 4 days of exposure to 2.8406 ppm in a bird exposed to treatment for 8 days. The mean ($\bar{X}$ of 3 birds) ppm of dieldrin residue in liver tissue was highest after 20 days of exposure; three birds sacrificed on day 20 produced a mean of 1.6409 ppm of dieldrin in liver tissues (Table 3). Liver residues of those birds treated with 6.50 ug/ml were slightly higher than the 3.25 ug/ml group. After 20 days of exposure three birds in the 6.50 group accumulated a mean of 1.8022 ppm of dieldrin in the liver (Table 4). Birds in the 6.50 group showed highest HEOD residues in liver after 16 days while birds which received 3.25 ug/ml accumulated highest residues on the 20th day with second highest mean accumulation occurring on the eighth day. This was, however, due to an abnormally large amount of dieldrin residue found in one bird during the eighth day of exposure. Dieldrin residues (both 3.25 and 6.50 groups) in remainders were generally smaller than those accumulated in liver but larger than that which accumulated in the brain. After 20 days of exposure, remainders of birds dosed with 3.25 ug/ml had a mean residue accumulation of 0.2492 ppm while remainders in the 6.50 ug/ml group contained a mean residue content of 0.4009 ppm (Tables 3 and 4). Birds in the 6.50 group showed a general increase in residue content with time, whereas birds treated with 3.25 ug/ml did not.

Table 3. Concentration of dieldrin residues found in bobwhite quail treated with 3.25 µg/ml of dieldrin for 20 days.

Days of Exposure	Time Interval	Dieldrin Concentration (ppm wet weight)		
		Brain	Liver	Remainder
4	1	0.1138 ± 0.0075[a]	0.0472 ± 0.2770	0.1293 ± 0.0165
8	2	0.1160 ± 0.0049	1.5749 ± 0.6330	0.1519 ± 0.0218
12	3	0.1511 ± 0.0103	1.0401 ± 0.1380	0.2728 ± 0.0746
16	4	0.1567 ± 0.0178	1.4020 ± 0.3835	0.1976 ± 0.0164
20	5	0.1942 ± 0.0123	1.6409 ± 0.1973	0.2492 ± 0.0292

[a]Mean ± one standard error; standard error calculated from three observations.

Table 4. Concentration of dieldrin residues found in bobwhite quail treated with 6.50 µg/ml of dieldrin for 20 days.

Days of Exposure	Time Interval	Dieldrin Concentration (ppm wet weight)		
		Brain	Liver	Remainder
4	1	0.1603 ± 0.0272[a]	1.1779 ± 0.3162	0.1648 ± 0.0469
8	2	0.2256 ± 0.0531	1.3740 ± 0.3492	0.2101 ± 0.0446
12	3	0.2695 ± 0.0405	1.2945 ± 0.3675	0.2748 ± 0.0534
16	4	0.2752 ± 0.0333	2.3695 ± 0.2814	0.3904 ± 0.0672
20	5	0.2990 ± 0.0244	1.8022 ± 0.6190	0.4009 ± 0.0984

[a]Mean ± one standard error; standard errors calculated from 3 observations.

Brain residues in birds dosed with 3.25 µg/ml of dieldrin were somewhat lower than birds treated with 6.50 µg/ml. The highest mean residue accumulation in birds treated with 3.25 µg/ml was 0.1942 ppm which occurred after 20 days of exposure. Similarly, brain residues of birds treated with 6.50 µg/ml exhibited highest mean accumulation (0.2990 ppm) after being treated for 20 days (Tables 3 and 4).

A product moment coefficient of linear correlation was calculated to determine the relationship between brain versus remainder, brain versus liver and liver versus remainder. Birds treated with 3.25 µg/ml showed a significant ($P < 0.05$) correlation between brain versus liver ($r = 0.468$) but did not show any significant ($P > 0.05$) correlation between brain versus remainder ($r = 0.437$) and remainder versus liver ($r = 0.079$). No significant ($P > 0.05$) correlation was found to exist with any comparisons in the 6.50 group (Appendix, Table 5).

Residue analysis - 6.25 and 12.50 µg/ml of dieldrin.

Brain and remainders of 32 bobwhite quail receiving dosages of 6.25 and 12.50 µg/ml every 4 days were analyzed for dieldrin residues. Generally brain residues tended to be larger than residues found in remainders.

Birds treated with 6.25 µg/ml of dieldrin accumulated 0.0593 ppm of HEOD in brain tissue within 10 days. After 66 days of exposure only 0.0947 ppm of HEOD accumulated. Brain residues in birds treated with 12.50 µg/ml accumulated to 0.0648 ppm in 10 days, but increased to 0.3071 ppm after 66 days of exposure (Tables 6 and 7).

Residues found in remainders of birds exposed 10 days to 6.25 and 12.50 µg/ml of dieldrin were 0.0361 and 0.0567 ppm, respectively. After 66 days of exposure residue accumulation had increased to 0.0607 and

Table 6. Concentration of dieldrin in bobwhite quail treated with 6.25 µg/ml of dieldrin every 4 days for 66 days.

Bird No.	Days Exposed	Dieldrin Concentration (ppm wet weight)			
		Brain	$\bar{X} \pm$ S.E. $\frac{b}{x}$	Remainder	$\bar{X} \pm$ S.E. $\frac{}{x}$
250	10	0.0748	0.0593 ± 0.0156	0.0381	0.0361 ± 0.002
251		0.0437		0.0341	
252	18	0.0581	0.0683 ± 0.0102	0.0396	0.0321 ± 0.0075
253		0.0785		0.0246	
254	26	0.0623	0.0572 ± 0.0051	0.0487	0.0366 ± 0.0121
255		0.0521		0.0245	
256	34	0.0502	0.0368 ± 0.0134	0.0346	0.0409 ± 0.0060
257		0.0233		0.0471	
263	42	0.1023	0.0622 ± 0.0402	0.0465	0.0593 ± 0.0128
264		0.0220		0.0721	
262	50	0.1253	0.0935 ± 0.0318	0.0563	0.0541 ± 0.0023
265		0.0617		0.0518	
283	58	0.0910	0.1039 ± 0.0129	0.0522	0.0729 ± 0.0207
284		0.1167		0.0935	
285	66	0.1243	0.0947 ± 0.0296	0.0897	0.0607 ± 0.0291
286		0.0651		0.0316	

[b] Mean ± one standard error calculated from adjacent two observations.

Table 7. Concentration of dieldrin residues in bobwhite quail treated with 12.50 µg/ml of dieldrin every 4 days for 66 days.

Bird No.	Days Exposed	Dieldrin Concentration (ppm wet weight)			
		Brain	$\bar{X} \pm$ S.E.$_{\bar{x}}^{b}$	Remainder	$\bar{X} \pm$ S.E.$_{\bar{x}}$
266 267	10	0.0634 0.0661	0.0648 ± 0.0014	0.0480 0.0653	0.0567 ± 0.0087
268 269	18	0.0866 0.0826	0.0846 ± 0.0020	0.0322 0.0326	0.0324 ± 0.0002
281 282	26	0.0443 0.1035	0.0739 ± 0.0296	0.0762 0.0444	0.0603 ± 0.0159
270 275	34	0.1830 0.1824	0.1827 ± 0.0003	0.1145 0.1025	0.1085 ± 0.0060
272 273	42	0.2143 0.1610	0.1877 ± 0.0267	0.1254 0.0753	0.1004 ± 0.0250
274 277	50	0.2080 0.2823	0.2452 ± 0.0372	0.1731 0.1585	0.1658 ± 0.0073
276 281	58	0.2785 0.2845	0.2815 ± 0.0030	0.0457 0.0845	0.0651 ± 0.0194
283 284	66	0.2956 0.3185	0.3017 ± 0.0115	0.0581 0.1140	0.0861 ± 0.0280

[b]Mean ± one standard error calculated from adjacent two observations.

Fig. 6. Parts per million of dieldrin found in remainder tissue of bobwhite quail treated with 6.25 and 12.50 µg/ml of dieldrin.

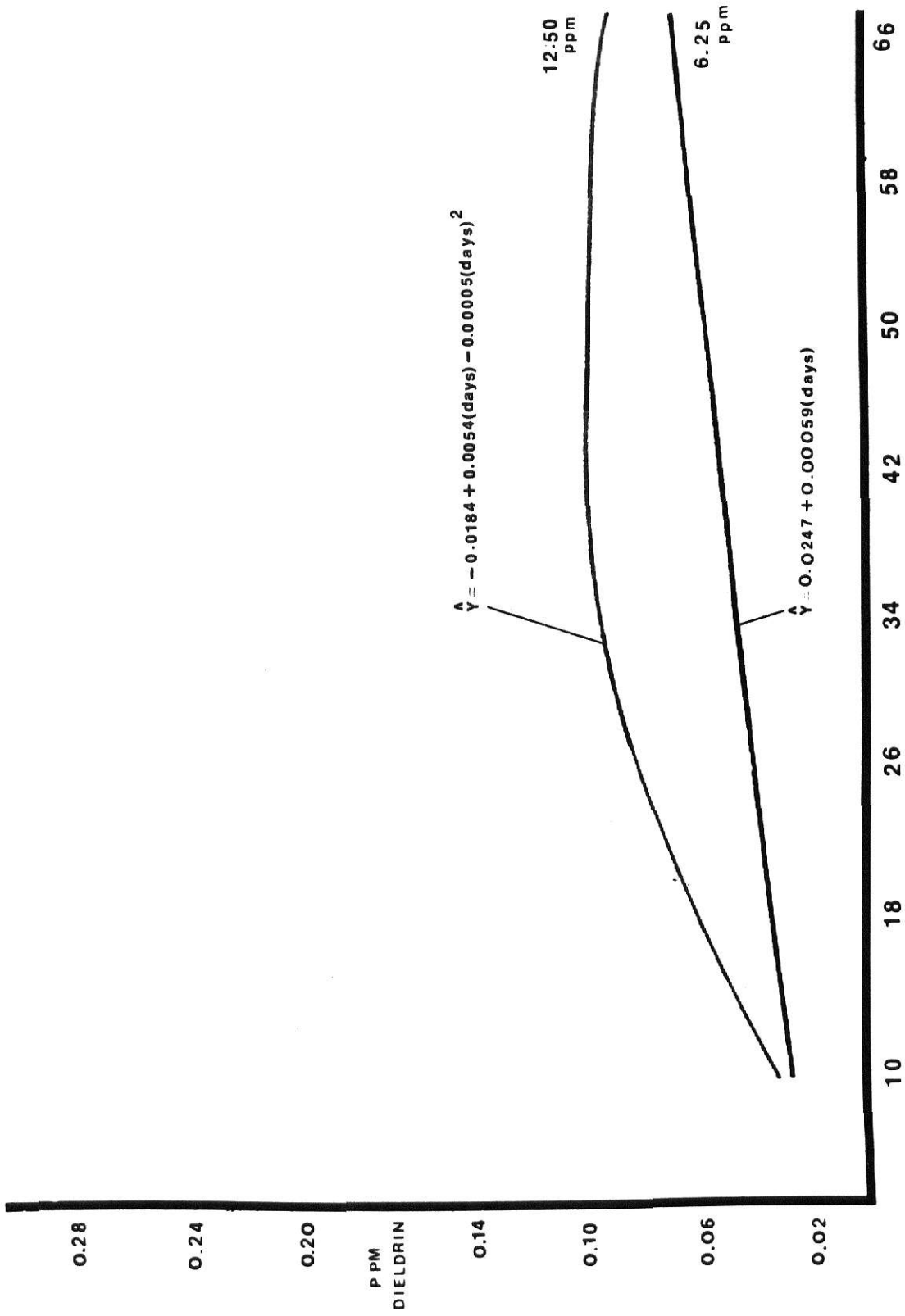
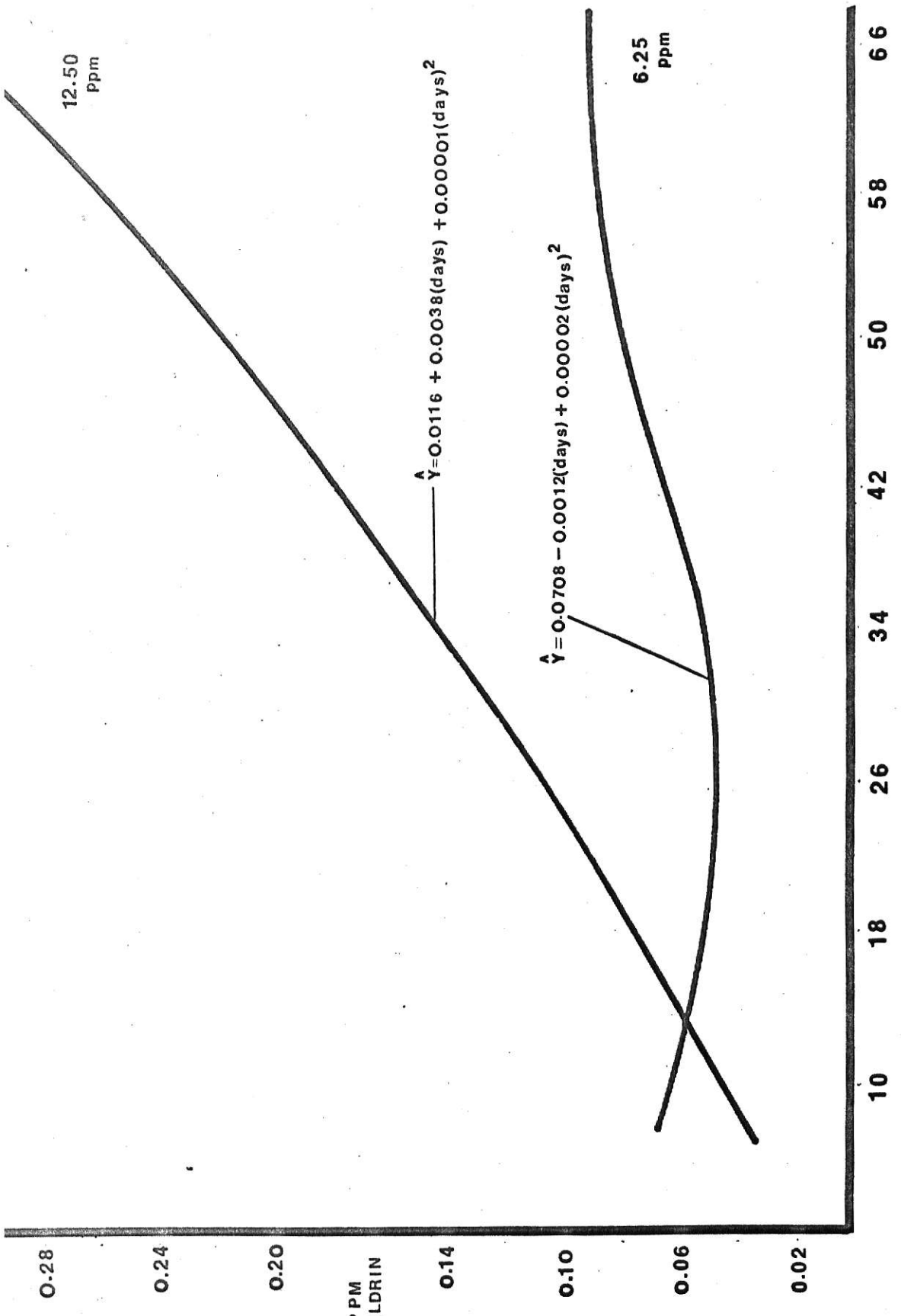

0.28
0.24
0.20
PPM DIELDRIN
0.14
0.10
0.06
0.02
10
18
26
34
42
50
58
66
DAYS OF EXPOSURE
$\hat{Y} = -0.0184 + 0.0054(\text{days}) - 0.00005(\text{days})^2$
$\hat{Y} = 0.0247 + 0.00059(\text{days})$
12.50 ppm
6.25 ppm

Fig. 7. Parts per million of dieldrin found in brain tissue of bobwhite quail treated with 6.25 and 12.50 µg/ml of dieldrin.


12.50 Ppm
$\hat{Y}$=0.0116 + 0.0038(days) +0.000001(days)^2
$\hat{Y}$= 0.0708 – 0.0012(days) + 0.000002(days)^2
6.25 Ppm
P PM DIELDRIN
0.28
0.24
0.20
0.14
0.10
0.06
0.02
10
18
26
34
42
50
58
66
DAYS OF EXPOSURE

0.861 respectively for the 6.25 and 12.50 groups (Tables 6 and 7). The mean concentration versus days exposed to treatment is plotted in Figs. 6 and 7. A two-way analysis of variance was performed to see if residue levels were significantly different between the two dosage levels. It was found that brain levels differed significantly ($P < 0.001$) with treatment as did the concentration of dieldrin in tissues of remainders ($P < 0.001$). Brain residue levels differed significantly with time at the ($P < 0.001$) level at the $P < 0.05$ level. Interaction was also present; it was highly significant ($P < 0.001$) between brain residues but significant at only the ($P < 0.05$) level with body residues (Figures 6 and 7).

A product moment coefficient of linear correlation was calculated between pesticide levels found in brain and body. A non-significant ($P > 0.05$) "r" value of 0.4193 was obtained for the 6.25 µg/ml group while a significant ($P < 0.05$) "r" value of 0.4726 characterized the comparison of brain versus remainder in the 12.50 group.

Residue analysis - 50 and 100 µg/ml

Brain and remainders of 42 bobwhite quail on 50 or 100 µg/ml of dieldrin were analyzed for dieldrin residues. Brain residues of birds treated with 50 µg/ml accumulated to 0.3212 ppm after 10 days exposure. Residues increased to 1.2005 ppm when exposed for 40 days but dropped to 0.9003 ppm after being treated for 70 days (Table 8). Brain residues of birds treated with 100 µg/ml were 0.3453 ppm after 10 days of treatment (Table 9). After 60 and 70 days of exposure residues had accumulated to 2.0125 ppm (highest achieved in 70 days) and 1.6757 ppm, respectively.

Residue accumulation in remainders of birds treated with 50 and 100 µg/ml amounted to 0.0490 and 0.3169 ppm respectively, after 10 days.

Table 8. Concentration of dieldrin residues in bobwhite quail treated with 50 µg/ml of dieldrin every 2 days for 70 days.

Bird No.	Days Exposed	Dieldrin Concentration (ppm wet weight) Brain	$\bar{X} \pm$ S.E.$_{\bar{x}}$[b]	Remainder	$\bar{X} \pm$ S.E.$_{\bar{x}}$
101		0.3859		0.0391	0.0490 ± 0.0067
102	10	0.1329	0.3212 ± 0.0957	0.0622	
103		0.4448		0.0458	
104		0.3151		0.0426	
105	20	0.2719	0.2489 ± 0.0462	0.4115	0.3226 ± 0.1431
106		0.1598		0.5138	
107		0.3216		0.3197	
108	30	0.5248	0.3108 ± 0.1268	0.1233	0.1951 ± 0.0625
109		0.0860		0.1422	
110		0.6564		0.1416	
111	40	1.1639	1.2005 ± 0.3253	0.5260	0.5535 ± 0.2462
112		1.7814		0.9930	
113		0.4184		0.3705	
114	50	1.1903	0.7919 ± 0.2232	1.1130	0.5189 ± 0.3092
115		0.7670		0.0733	
116		1.0580		1.1430	
117	60	0.9875	1.1202 ± 0.0995	0.7750	0.7970 ± 0.1937
118		1.3150		0.4730	
119		0.8270		0.2385	
120	70	0.8630	0.9003 ± 0.0563	1.0328	0.6425 ± 0.2294
121		1.0110		0.6562	

[b] Mean ± one standard error calculated from adjacent three observations.

Table 9. Concentration of dieldrin residues found in bobwhite quail treated with 100 µg/ml of dieldrin every 2 days for 70 days.

Bird No.	Days Exposed	Dieldrin Concentration (ppm wet weight) Brain	$\bar{X} \pm$ S.E.$_{\bar{x}}^{b}$	Remainder	$\bar{X} \pm$ S.E.$_{\bar{x}}$
201		0.8150		0.0707	
202	10	0.1415	0.3453 ± 0.2355	0.0333	0.3169 ± 0.2651
203		0.0793		0.8466	
204		1.1275		0.6415	
205	20	1.3378	1.5588 ± 0.3317	0.2576	0.5938 ± 0.1819
206		2.2110		0.8824	
207		0.4612		0.7953	
208	30	1.7280	0.7623 ± 0.4941	1.1548	0.9728 ± 0.1038
209		0.0977		0.9682	
210		1.0064		1.6510	
211	40	1.4800	1.2156 ± 0.1395	0.9606	1.7102 ± 0.4508
212		1.1605		2.5190	
213		1.6427		1.3000	
214	50	0.5938	0.9537 ± 0.3446	0.2300	0.9043 ± 0.3389
215		0.6245		1.1830	
216		4.0307		2.3290	
217	60	0.9365	2.0125 ± 1.0098	0.6231	1.3111 ± 0.5193
218		1.0703		0.9812	
219		2.1720		1.9376	
220	70	1.3980	1.6757 ± 0.2487	1.1140	1.6818 ± 0.2844
221		1.4570		1.9939	

[b]Mean ± one standard error computed from adjacent three observations.

Fig. 8. Parts per million of dieldrin found in brain tissue of bobwhite quail treated with 50 and 100 µg/ml of dieldrin.

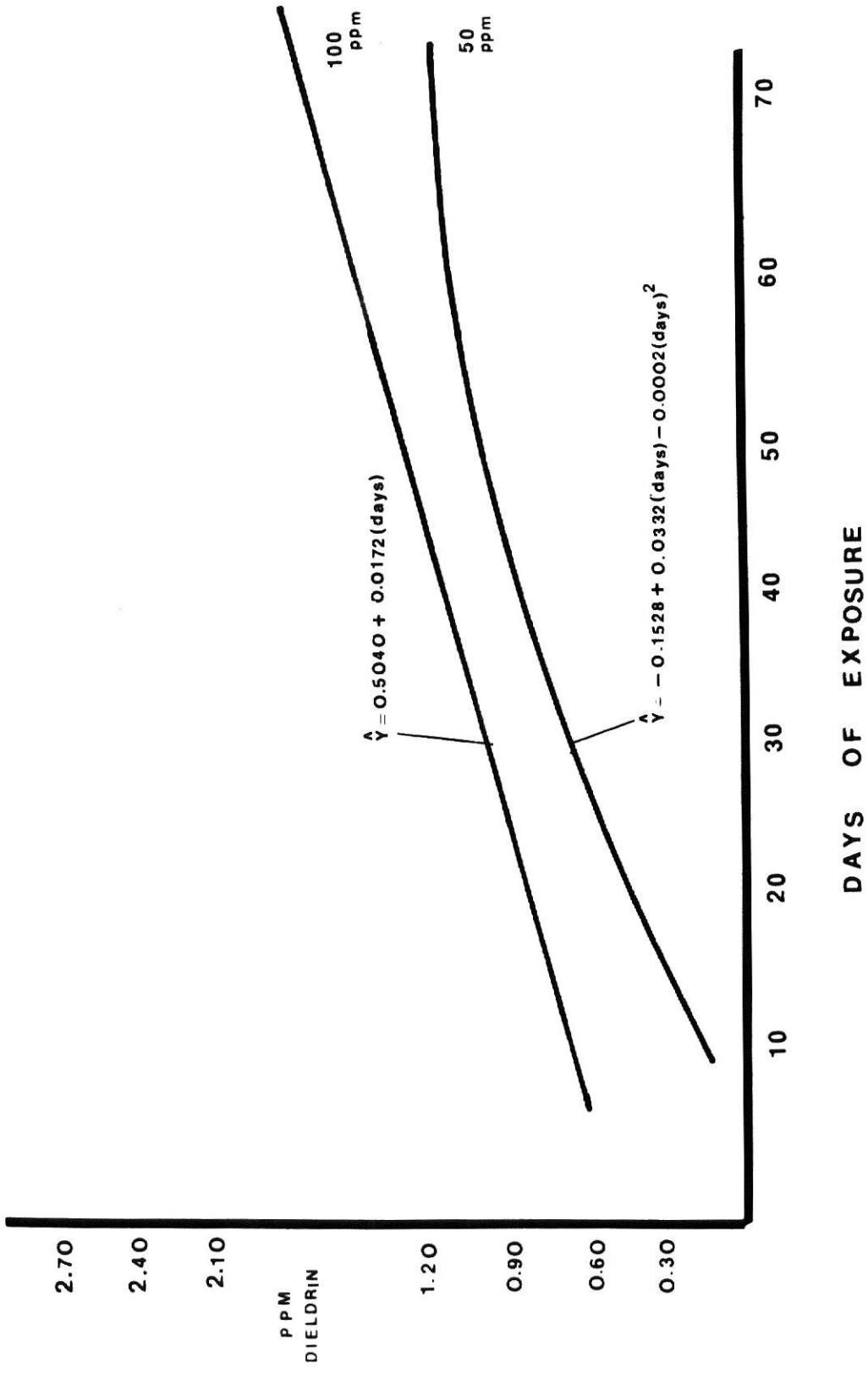
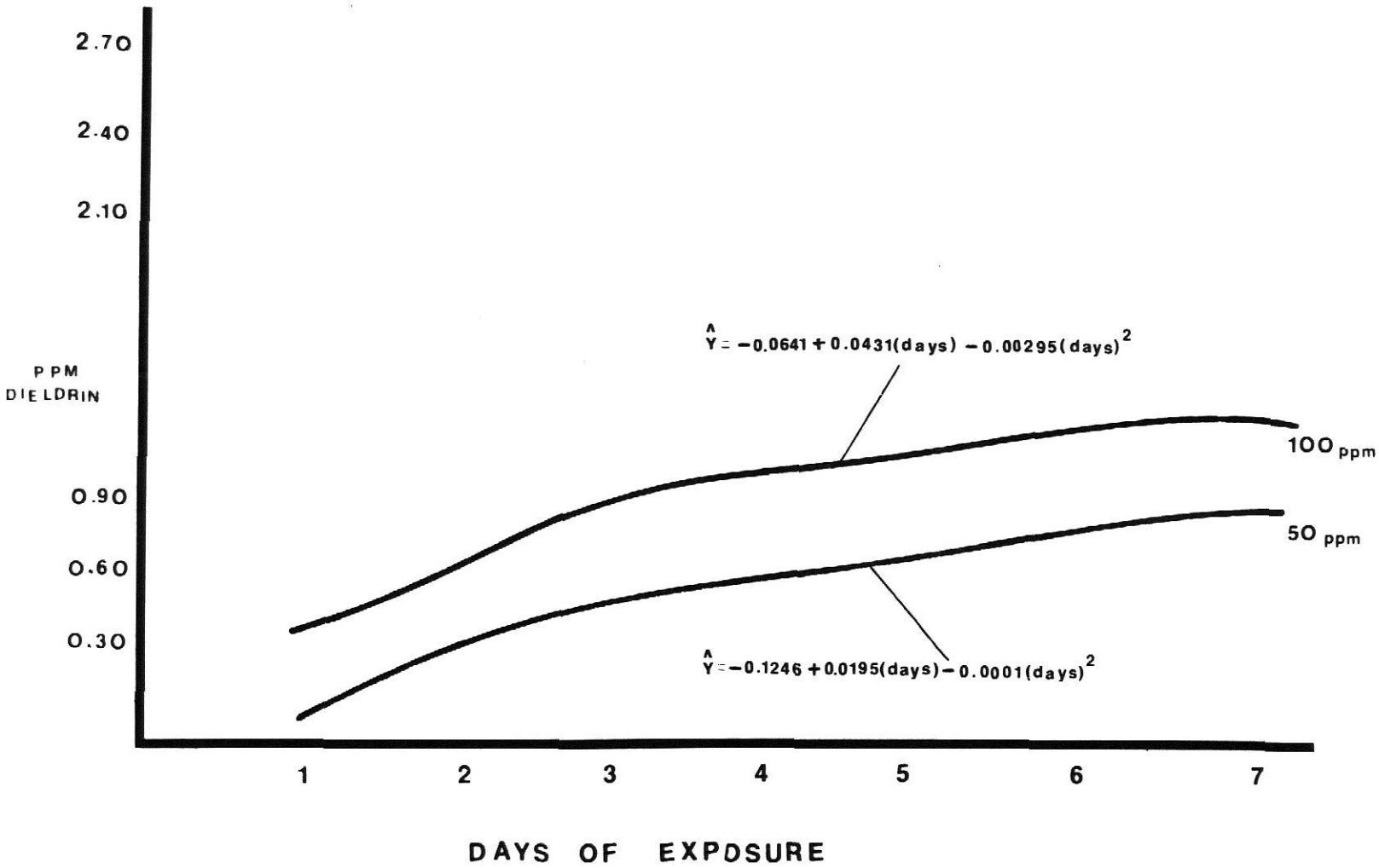

PPM DIELDRIN
2.70
2.40
2.10
1.20
0.90
0.60
0.30
10
20
30
40
50
60
70
DAYS OF EXPOSURE
100 ppm
50 ppm
$\hat{Y} = 0.5040 + 0.0172(\text{days})$
$\hat{Y} = -0.1528 + 0.0332(\text{days}) - 0.0002(\text{days})^2$

Fig. 9. Parts per million of dieldrin found in remainder tissue of bobwhite quail treated with 50 and 100 µg/ml of dieldrin.


2.70
2.40
2.10
PPM
DIELDRIN
0.90
0.60
0.30
$\hat{Y} = -0.0641 + 0.0431(days) - 0.00295(days)^2$
100 ppm
50 ppm
$\hat{Y} = -0.1246 + 0.0195(days) - 0.0001(days)^2$
1
2
3
4
5
6
7
DAYS OF EXPOSURE

After 70 days, remainders of the 50 µg/ml group accumulated 0.6425 ppm whereas remainders of birds treated with 100 µg/ml accumulated 1.6818 ppm of dieldrin residue.

When an analysis of variance was performed, it was found that there was a significant ($P < 0.05$) difference between concentrations of dieldrin found in brain of birds which were dosed with 50 µg/ml and those which received 100 µg/ml. There was a highly significant difference ($P > 0.001$) between concentrations of dieldrin found in remainders which were treated with the two levels of dieldrin.

Concentrations of dieldrin found in remainders differed significantly ($P < 0.01$) with time (Fig. 9), as did that in the brain ($P < 0.05$) (Fig. 8) Interaction was not significant ($P > 0.10$) in either part of this study. A significant correlation of 0.680 ($P < 0.05$) existed between the residues found in the brain and remainder of birds receiving the 50 µg/ml dosage and a significant correlation of 0.562 ($P < 0.05$) was present between residues of brain and remainder of birds treated with 100 µg/ml.

Weight Analysis - 6.25 and 12.50 µg/ml group

The effect of dieldrin on body weight was separated from the combined effects of dieldrin and corn oil by use of body weight data from a 6-bird group fed corn oil, but no insecticide (Appendix, Table 14). The relationship between weight and time was found to be closely represented by $Y = aX^{b}c^{X}$. When linearlized, this relationship becomes $\text{Log } Y = b_o + b_1 \text{log} X + b_2 X$ where:

$$a = \text{anti-log } b_o$$

$$b = b_1$$

$$c = \text{anti-log } b_2$$

Body weight, on a particular day, of pesticide dosed birds was then inserted in this relationship producing the equation Log Wt. = 2.03 + 0.1425 log time - 0.001 time. Time is represented as days of exposure such as 1,2,...etc. Body weight of pesticide dosed birds was then corrected by the following manner:

Weight minus effect of corn oil	= Weight time X	- Weight adjusted	+ Weight initial

Body weight data (Appendix, Table 12) for 4 bobwhite quail treated every 4th day with either 6.25 or 12.50 µg/ml of dieldrin for 56 days were analyzed in a split plot design as described by Cochran and Cox (1957). After 24 days of exposure to dieldrin birds in the 6.25 µg/ml group weighed 176.7 grams while birds in the 12.50 µg/ml group weighed 164.8 grams. Fifty-six days later birds in the 6.25 group weighed 137.6 grams while birds in the 12.50 group weighed only 125.7 grams (Appendix, Fig. 10).

The dose-time effects on body weight were examined to see whether a significant linear or quadratic contribution was present. There were significant ($P < 0.05$) linear effects but no significant ($P > 0.05$) quadratic effects (Appendix, Table 10). There was no significant difference ($P > 0.05$) between weight of birds dosed with 6.25 or 12.50 µg/ml of dieldrin. Since no difference was found to exist between the two groups, a common regression line was calculated ($Y = 210.2 - 1.49X_i$). Generally birds in the 12.50 µg/ml group weighed about 13 grams more than birds in the 6.25 µg/ml group.

Weight analysis - 50 and 100 µg/ml group

Body weights (Appendix, Table 16) for 12 bobwhite quail treated every other day for 60 days with either 50 or 100 µg/ml of dieldrin did not differ significantly ($P > 0.05$). After 10 days of exposure birds subjected to 50 µg/ml weighed 184.9 grams while birds treated with 100 µg/ml

weighed 173.3 grams. After 50 days of treatment birds averaged 133.8 grams and 121.7 grams for the respective 50 and 100 µg/ml groups. Generally it was found that birds dosed with 50 µg/ml of dieldrin weighed about 15 grams more than did birds treated with 100 µg/ml of dieldrin (Appendix, Fig. 11).

The dose-time effects on body weight were examined to determine the degree of linear and quadratic influence on the data. No significant ($P > 0.05$) quadratic effect was present, however a highly significant ($P < 0.05$) linear effect was found to exist. The regression equation ($Y = 190.88 - 1.29$ days) common to the two lines (50 and 100 µg/ml) most adequately describes the effect of 50 and 100 µg/ml of dieldrin on body weight of bobwhite quail.

There was a highly significant ($P < 0.05$) difference between length of time birds were exposed to dieldrin and body weight. However, no significant ($P > 0.05$) interaction between birds and time or dosage and time occurred (Appendix, Table 11).

Acute toxicity of dieldrin to bobwhite quail.

Two bobwhite quail each were dosed with 200, 400, 600, and 800 µg/ml of dieldrin every day until death or until 30 days had elapsed. The first mortality was observed on the fourth day of dosage, a bird receiving 600 µg/ml. On the sixth day one bird receiving 800 µg/ml died. By the 12th day all the birds receiving dosages of 400 mg/kg or more had died. No birds in the 200 µg/ml group succumbed nor exhibited any symptoms during the 30 days of consecutive treatment. Excessive weight loss occurred on the day of death, averaging 12.3 g for the 6 birds which died. The most weight lost by any one bird during the 24 hour period immediately preceding death was 20.5 grams. Birds which succumbed to dieldrin

poisoning exhibited a steady decline in weight. Birds which did not die (200 µg/ml group) acted differently exhibiting no significant change in body weight during the 30-day period.

Both brain and remainders from the eight birds in the acute toxicity study were analyzed for dieldrin residues (Table 13). The product moment coefficient of linear correlation between brain and remainder residue concentration was 0.689 and was significant ($P < 0.05$).

All birds which died from dieldrin poisoning showed similar residue levels of dieldrin in the brain, ranging from 14.7 ppm to 31.1 ppm with a mean of 24.8 ppm. Remainder residues of dead birds exhibited more variation, ranges being 5.5 ppm to 18.1 ppm with a mean of 12.1 ppm.

First observable symptoms of dieldrin poisoning were fluffed feathers and hyperexcitability. Later, as poisoning progressed, birds became very passive and could be easily handled by hand with no means of restraint needed. At this time any type of gait was severely impared, ataxia being very apparent. Death always occurred in the form of wing-beat convulsions with the bird spinning in circles either on its back or side.

Acute toxicity of parathion

Two birds each were given one dose of 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 mg/kg of parathion. Ten minutes after receiving 8.0 mg/kg, one bird in this group died. Three hours later the second bird also treated with 8.0 mg/kg died. During the fifth hour one bird from the 7.5 mg/kg group and one bird from the 7.0 mg/kg group succumbed. The sixth hour of exposure produced the mortalities of one bird each in the 7.5, 7.0 and 6.0 mg/kg groups. During the seventh hour one bird each from the 6.5, 6.0, and 5.0 groups died. During the eighth hour of exposure both birds

Table 13. Concentration of dieldrin residues in tissues of bobwhite quail subjected to acute doses of dieldrin.

Dosage (µg/ml)	Bird No.	Dieldrin Concentration (ppm wet weight) Brain	$\bar{X} \pm$ S.E.$_{\bar{x}}$[a]	Remainder	$\bar{X} \pm$ S.E.$_{\bar{x}}$
200	132	7.245	8.854 ± 1.609	3.919	4.406 ± 0.487
	128	10.464		4.893	
400	110	29.837	26.576 ± 3.270	5.055	8.760 ± 3.705
	123	23.315		12.465	
600	111	14.731	20.178 ± 5.447	5.889	11.550 ± 5.660
	129	25.624		17.210	
800	112	31.145	27.755 ± 3.390	18.127	16.221 ± 1.906
	115	24.365		14.315	

[a]Mean ± one standard error calculated from two adjacent observations.

receiving 5.0 and 5.5 mg/kg died while the other bird treated with 5.0 mg/kg exhibited tremors and convulsions but did not die until the ninth hour.

Each of these birds was analyzed for cholinesterase inhibition (Table 15). It was found that percent cholinesterase inhibition ranged from 75.5 per cent to 42.2 per cent with a mean inhibition of 59.1 per cent. There was no significant ($P > 0.05$) difference in percent cholinesterase inhibition between acute dosage levels (Appendix, Tables 17 and 18).

Birds dying from parathion exhibited symptoms characteristic of most organophosphorous poisoning. Tremors and convulsions accompanied by fluffing of the feathers, upward and downward movements of the head and a lengthening and shortening of the neck were the most noticeable symptoms. Lacrimation usually occurred as did excessive salivation and repeated defecation in the form of diarrhea. Carcasses typically were characterized by the head being folded acutely to one side or the other, wings pressed tightly against the body, with legs and feed rigid and extended.

Parathion - dosage 3.0 mg/kg and 1.0 mg/kg

All birds treated with 3.0 mg/kg of parathion died within 24 hours after receiving a single oral dose. Thirteen birds out of 20 died after receiving a single dose of 1.0 mg/kg. Of the 7 birds remaining, only 1 appeared to act normally. The remaining exhibited symptoms such as sluggishness, ruffling of the feathers, etc. A second dose of 1.0 mg/kg was administered 24 hours later. On the following day, the 6 birds which showed symptoms were found dead. The remaining bird still appeared healthy. Three more daily doses of 1.0 mg/kg were given this bird (#17) before it was sacrificed on the fifth day of treatment. At no time did this bird

Table 15. Rappaport Units of cholinesterase found in brain tissue of bobwhite quail dosed with parathion.[a]

Bird No.	Dosage (mg/kg)	R. U.	% Inhibition
1	5.0	33	42.2
2		21	63.8
3	5.5	24	58.0
4		29	51.0
5	6.0	22	62.8
6		16	61.5
7	6.5	29	49.2
8		19	66.8
9	7.0	12	79.0
10		18	68.5
11	7.5	33	42.2
12		29	49.2
13	8.0	24	58.0
14		14	75.5
	CONTROL		
C1	none	72	
C2	"	77	
C3	"	20	
C4	"	60	
C5	"	83	$\bar{X} = 57.09 \pm 5.13$
C6	"	45	
C7	"	54	
C8	"	53	
C9	"	50	
C10	"	57	
C11	"	57	

[a]One Rappaport Unit of cholinesterase will hydrolyze one μMole of Acetycholine in 30 minutes at 25°C and pH of 7.8.

[b]Percentage inhibition from control mean.

show any symptoms of parathion poisoning. Of all birds which were dosed with 1.0 mg/kg, bird #17 showed the least percent of cholinesterase inhibition (Table 19).

Parathion - dosage 0.50 mg/kg

No birds died directly from parathion until the 4th day at which time two quail succumbed to the 0.5 mg/kg dosage. The amount of cholinesterase in the brain of these birds was typical of that found in other birds which had died from parathion poisoning. There was no significant difference ($P > 0.05$) between the Rappaport Units of cholinesterase in the brain of those birds that died receiving 0.5 mg/kg and those that died receiving 3.0 mg/kg and 1.0 mg/kg (Appendix, Table 18).

When an analysis of variance was performed on the data (Rappaport Units), it was found that birds receiving 0.5 mg/kg of parathion showed significant ($P < 0.05$) brain cholinesterase inhibition compared to control birds (Table 17 and 18). Rappaport Units of cholinesterase of the 0.5 mg/kg group also differed significantly ($P < 0.05$) from the groups receiving the acute toxicity doses, 3.0 mg/kg, and 1.0 mg/kg. As birds received more insecticide, the amount of cholinesterase in the brain was decreased (Fig. 12).

Fig. 12. Rappaport Units of cholinesterase found in bob-white quail treated with 0.5 mg/kg of parathion.

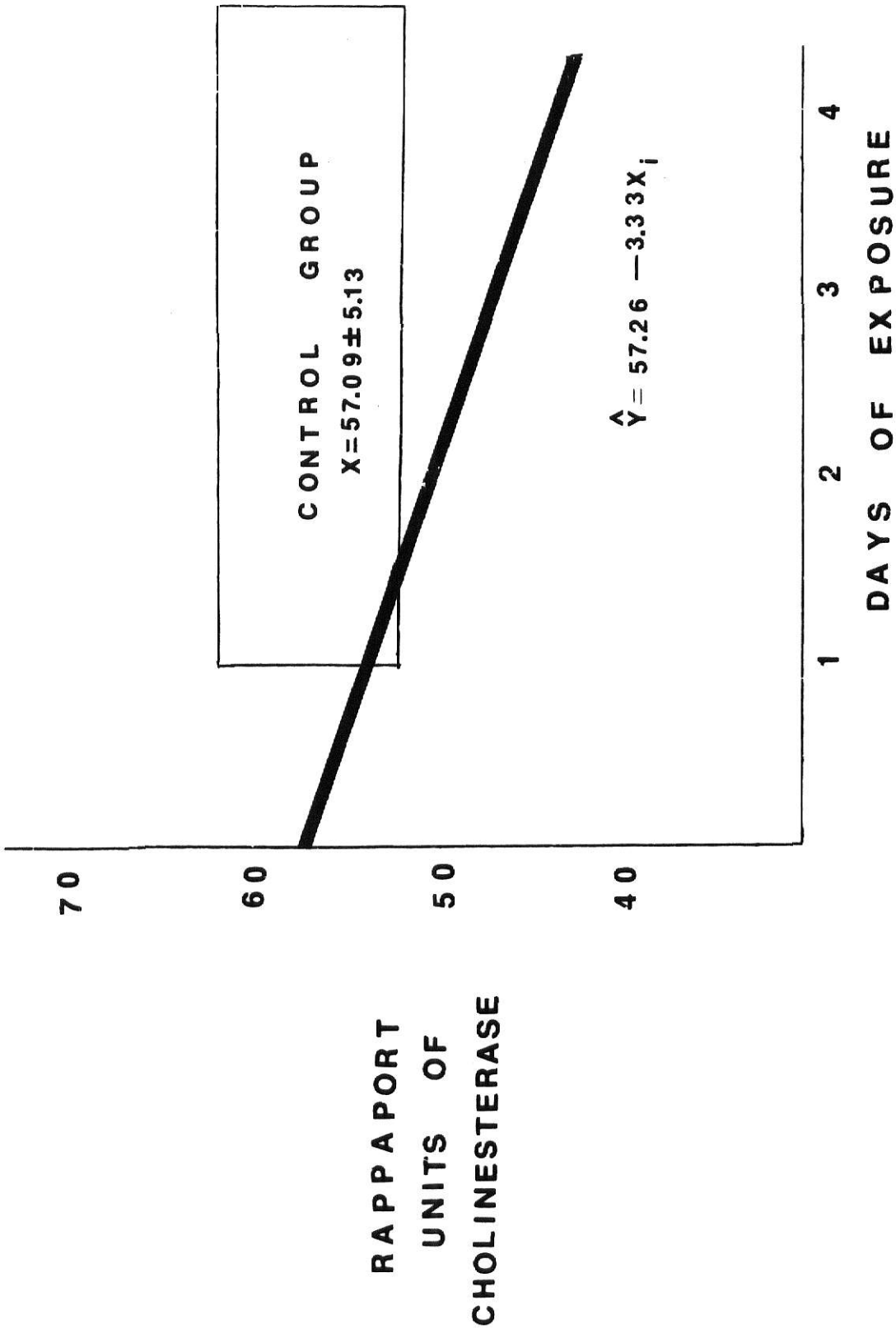

CONTROL GROUP
X=57.09±5.13
$\hat{Y}$= 57.26 —3.33X_i
RAPPAPORT UNITS OF CHOLINESTERASE
70
60
50
40
1
2
3
4
DAYS OF EXPOSURE

Table 19. Rappaport Units (RU) of cholinesterase found in brain tissue of bobwhite quail treated with parathion.[a]

Dosage	1 mg/kg			3 mg/kg		
	Bird No.	R.U.	% inhib.[b]	Bird No.	R.U.	% inhib.[b]
	1)	10	82.5	21)	31	45.7
	2)	36	37.0	22)	20	65.0
	3)	28	51.0	23)	15	73.8
	4)	35	38.7	24)	15	73.8
	5)	20	65.0	25)	25	56.3
	6)	38	33.5	26)	25	56.3
	7)	24	58.0	27)	24	58.0
	8)	7	87.8	28)	27	52.8
	9)	20	65.0	29)	31	45.7
	10)	20	65.0	30)	21	63.8
	11)	30	47.5	31)	35	38.7
	12)	28	51.0	32)	29	49.2
	13)	15	73.8	33)	20	65.0
	14)	15	73.8	34)	20	65.0
	15)	27	52.8	35)	28	51.0
	16)	34	40.5	36)	20	65.0
	17)	46	19.5[c]	37)	30	47.5
	18)	35	38.7	38)	25	56.3
	19)	40	30.0	39)	60	
	20)	20	65.0	40)	15	73.8
		$\bar{X} = 26.40 \pm 2.34$	$\bar{X} = 55.61 \pm 3.89$		$\bar{X} = 25.80 \pm 2.21$	$\bar{X} = 57.7 \pm 2.29$

[a]One Rappaport Unit of cholinesterase will hydrolyze one µMole of acetylcholine in 30 minutes at 25 centigrade and pH of 7.8.

[b]Percentage inhibition from control mean.

[c]Bird sacrificed after 5 days of treatment - value excluded from computation of $\bar{X}$.

DISCUSSION

Residue Accumulation

The concentrations of dieldrin found in the brains and remainders of birds that died as a result of poisoning were considerably greater than those in birds which received sublethal doses. The average concentration of dieldrin found in brains of acutely poisoned birds was 24.509 ppm (wet weight) while the average concentration found in remainder was 12.177 ppm (wet weight). This information compares favorably to that reported by Stickel (1969) in which brain levels of chronically poisoned Coturnix averaged 21.7 ppm and 14.8 ppm for females and males, respectively. Stickel (1969) states that concentrations of dieldrin in remainders of acutely poisoned birds averaged 17.2 ppm. The concentration of dieldrin in the brains and remainder of quail at time of death is independent of the dosage level as long as that level caused death. Concentration in brains of dead birds did not differ significantly with time of death. Stickel (1969) also reports no correlation between brain residue and time of death. Robinson et al. (1967) further substantiates this conclusion. One should not rely heavily on the ppm of dieldrin in remainders as being indicative of death by dieldrin poisoning. Birds vary tremendously in the amount of body fat and body weight, thus great error is introduced in residue analysis of this tissue. Inflated ppm values can be obtained if all body fat is not removed from analysis. Metabolism varies greatly between individual animals further complicating interpretation of remainder residues.

Robinson et al. (1967) state that when the concentration of dieldrin reaches a particular level (threshold concentration) a sequence of events that leads to death is initiated, a redistribution of dieldrin among the

tissues during the period before death may also occur. They postulate that concentration of HEOD in a tissue at death, particularly that in the brain, is a function of the threshold concentration. From information gathered in this study I tend to agree with Robinson and his co-workers concerning the brain residue dynamics of acutely poisoned birds.

Field application of this must be dealt with carefully because field conditions differ drastically from those encountered in a laboratory. Robinson et al. (1967) state that it is important to appreciate that there may be differences between the residues found in birds dying a sudden death, either accidental or by shooting, and those dying from disease, starvation, or directly from poisoning. The concentration of dieldrin in the latter will probably be higher than those in the former even though the body burden of dieldrin has no casual relationship to death. However, this investigator considers it safe to say that if bobwhite quail are found dead or dying on or near dieldrin treated fields and the concentration of dieldrin in the brain approaches 14.0 ppm or greater that it is extremely likely the bird died of dieldrin poisoning rather than a natural death.

The lethal doses reported in this study are somewhat smaller than those reported by Dahlen (1952). Dahlen reported bobwhite quail died when fed 10 µg/ml of dieldrin. He reports an LD_{50} of 12 - 14 mg/kg of dieldrin. My 800, 600, 400, and 200 µg/ml doses corresponds approximately to 4.57, 3.43, 2.29 and 1.14 mg/kg. The discrepancy is resolved when feeding procedures are compared. Dahlen dosed birds by placing a weighed amount of dieldrin in a gelatin capsule and then forcing the capsule down the throat of the bird. I weighed specific quantities of dieldrin, dissolved them in the appropriate volume of corn oil and administered this solution with a syringe. Jeffries and Davis (1968) report that the rate of absorption

of a pesticide in the gut is altered depending on what the pesticide is in or on. Cottam (1946) reported that DDT in oil was much more toxic than the crystalline form due to a higher absorption rate.

Storage of dieldrin in relation to dosage was studied with birds receiving 3.25, 6.25, 6.50, 12.50, 50.0, and 100 µg/ml of dieldrin. Significant differences between amount of residues stored in brain tissue of birds treated with 6.25 and 12.50 µg/ml existed. This was also true between residues of carcass remainders. Hayes and Dale (1964) reports that in several species, storage of DDT is less efficient at higher dosage levels. Lehman (1948) showed that in varying degrees, the same is true of dieldrin. If this is in fact true, it would help to account for the rather small residue accumulation in relation to dosage level experienced with the 50 and 100 µg/ml studies. Also of consequence is the fact that birds in the 50 and 100 µg/ml studies were treated on an every other day basis, thus allowing the animals metabolic devices two full days to detoxify and excrete the insecticide or its resultant metabolites. Hayes and Dale (1964) reports DDT concentrations in organs reached a maximum in 2 to 5 hours after a single large oral dose. Redistribution is gradual, leading to a higher concentration in fat than in other tissues. Hayes and Dale (1964) further states that the distribution in favor of fat is more marked following repeated doses than after a single dose. Taking this into account and reviewing the treatment and analytical procedures followed in this study, much of the first observed discrepancies can be resolved. This line of thought helps explain the fact that residue contents of brain and remainder tissue of birds treated with daily doses of 3.25 µg/ml of dieldrin were constantly larger than tissue residues of birds treated every other day with 12.50 µg/ml of dieldrin.

Body weight analysis

The ultimate problem concerning the biologist in studying the effects of pesticides on wildlife are residue buildup and how lethal the pesticide is to the species in question. However, the sublethal effect of a pesticide on a species may be of more importance than the lethal effect. McEwen and Brown (1966) demonstrated the increased susceptability to predation of sharp-tailed grouse which had been dosed with malathion. Observations by Koeman and van Genderen (1965) further support the idea of increased susceptability to predation.

Due to the fact that the "condition" of an animal is extremely important to its well being; weight fluctuation which may be pesticide induced are extremely important.

From my control bird study it was found that the pure corn oil carrier produced a weight gain. After some preliminary statistical investigation it was found that body weight versus time of exposure for only corn oil fed birds could be expressed by the equation $\text{Log } Y = b_o + b_1 \log X + b_2 X$ or more simply $Y = aX^b c^S$ where X is days of exposure and Y is body weight in grams. R^2 for this equation was 0.89. When the value obtained by the foregoing equation was incorporated into the relationship of $\text{Weight}_{\text{time X}} - \text{Weight}_{\text{adjusted}} + \text{Weight}_{\text{initial}}$, the body weight of each bird was thus corrected for the additative effects of corn oil. When this was done it was found that even a low dose (6.25 µg/ml) of dieldrin administered every 4 days produced weight loss in bobwhite quail. The magnitude of weight loss was very similar with the two dosage levels, the 12.50 µg/ml group losing slightly more weight than the 6.25 µg/ml group. Dieldrin at the higher dosage levels (50 and 100 µg/ml) caused individual bird weights to

fluctuate more drastically than those of birds treated with 6.25 and 12.50 µg/ml. Birds dosed with 50 µg/ml did not lose as much weight as those treated with 100 µg/ml. Although regression equations describing the effect of dieldrin on bobwhite quail appear to be relatively similar for the 6.25-12.50 and 50-100 µg/ml groups, care should be used when comparing these two groups as they did not contain the same number of birds nor was the dosing schedule the same.

When dissecting birds it was observed that many were in poor flesh and with little or no body fat. Control birds were generally more plump and in better flesh than pesticide dosed birds. Jeffries and Davis (1968) state there appeared to be a loss of storage and organ fat in song thrushes poisoned by dieldrin. They consider this fat loss to be dieldrin induced. From my study, it must be concluded that pesticide levels which seem relatively harmless have an adverse effect on quail in the form of a weight loss and possibly decrease in condition. The field implications of this are many and of much importance.

At the present time very little information is available concerning sublethal pesticide effects on wildlife under field conditions. Research in this area is much needed.

Equilibrium concept

There have been many studies concerned with the equilibrium concept of pesticides. Ludwig et al. (1964) reported that after about eight weeks, aldrin ^{14}-C in the rat, reaches a steady storage state. The studies of Clayborn et al. (1960), Coulson and McCarthy (1963), seem to show that following prolonged feeding, equilibrium in animals between intake and storage of aldrin and dieldrin exists. Quaife et al. (1966) state that no conclusive data are available which show that saturation levels of dieldrin

in the fat are reached and maintained. A relationship existed between time and residue accumulation in brain tissue of quail dosed with 6.25 and 12.50 µg/ml of dieldrin. Residue accumulation remained relatively stable for the first 26 days, then uptake increased sharply in the 12.50 µg/ml group until the termination of the experiment. In the 6.25 µg/ml group, a similar uptake occurred but began on the 34th day instead of the 26th day. As with the 12.50 µg/ml group, this uptake proceeded until the end of the study. Graphic observation allows one to infer that uptake is greater when birds are dosed with 12.50 µg/ml than when dosed with 6.25. It is difficult however to postulate that the small increase in residue accumulation in the 6.25 µg/ml group is due to a dynamic equilibrium being achieved. To do this adequately would require analysis of excreta, metabolic CO_2, etc.

No equilibrium was achieved in brain or body tissues of birds dosed with 50 and 100 µg/ml of dieldrin. The one important factor in this study was the pattern of residue accumulation. The pattern of residue accumulation in birds dosed with 50 µg/ml mirror that of birds dosed with 100 µg/ml with the exception the 100 µg/ml residues are of a greater magnitude. One may infer from this that accumulation of dieldrin occurred in a similar fashion with the two dosage levels. The physiological mechanisms causing this were not investigated.

Hayes (1964) says that there is a great variation in the accuracy of the different estimates of time necessary to reach a steady state of storage. There are few satisfactory measurements. Hayes (1964) reports that some investigators found a steady state already achieved when their earliest measurements were made, others more or less took the highest observed storage as being a steady state. If the equilibrium concept is to be explained, long range studies covering the entire life cycle of the

animal must be planned and different dosage levels should be used if satisfactory results are to be obtained. In this particular study it is the opinion of the investigator that the length of time birds were exposed to dieldrin was the main reason that equilibrium curves could not be obtained. However there is the possibility that equilibrium may be obtained by altering the dosage technique such as only dosing birds every third or fourth day. From information obtained in this study when birds were dosed with 6.25 and 12.50 µg/ml the foregoing idea seemed to substantiate such a hypothesis.

Parathion Poisoning

Effect of parathion on bobwhite quail was measured by cholinesterase activity since its inhibition is the classical effect produced by organophosphate compounds (Bunyan et al. 1968b). Blaber and Cuthbert (1962) state that some anticholinesterases have markedly different potencies in the fowl and the mammal. They further state the domestic fowl does not possess a typical butyrocholinesterase, plasma contains a cholinesterase intermediate in properties between those of fowl acetylcholinesterase and mammalian butycholinesterase. Brain and skeletal muscle contain acetylcholinesterase and a small portion of a second cholinesterase which may be identical with the "intermediate cholinesterase" mentioned earlier. Sigma Tech. Bulletin No. 420 state that their 420 kit, which was used in this study measures the total activity of numerous identified and unidentified "esterases" which cleave acetycholine. Thus care should be taken when comparing the actual amount of cholinesterase found in this study with that found by other workers.

Sources of Error in Determination of Cholinesterase

Several sources of error in the cholinesterase assay are common to this and most other procedures. First, the liquid in which the tissue is homogenized must always be of the same volume. If it is not, inaccuracy of results in the form of dilution and concentration will occur thus biasing the colorimetric tests. Secondly, incubation temperatures and time must be held constant if results are to be repeatable.

Another source of difficulty in the determination of cholinesterase is the fact that the cholinesterases are inhibited by tertiary and quarternary ammonium compounds. Many of the common detergents used for cleaning laboratory equipment are cationic compounds having a quarternary ammonium structure (Witter 1963).

Bunyan et al. (1968b) state that esterase activity was not significantly altered after leaving tissue at ambient temperatures for up to 12 days post mortem before dissection. In routine laboratory analysis Bunyan (1968b) as well as Witter (1963) and Sigma Tech. Bulletin No. 420 state that samples should be stored at 0° to 5°C. Stability at this temperature is several weeks.

In this particular study, centrifugation techniques proved to be of utmost importance. Removal of the supernatant from centrifuge tubes must be done as soon as the instrument stops. If this is not done, molecular activity causes solid particles to distribute themselves in the supernatant causing serious error in colorimetric readings.

Effect on birds

Quail were found to be extremely sensitive to parathion. Death occurred in one bird 10 minutes after receiving a 8.0 mg/kg dose of

parathion. Time of death in other groups ranged up to 9 hours after treatment depending on dosage level. There appeared to be a casual relationship between time of death and dosage level. Further work should be done in this area. The average cholinesterase inhibition for 34 acutely poisoned birds was found to be approximately 57 per cent. Bunyan et al. (1968a, 1969) reports brain cholinesterase inhibition in pheasants fed chlorfenviphos, demeton-methyl, dimethoate and Guthion are in excess of 90 per cent of the normal mean value. They further report that diazinon is a major exception; where one bird examined did not show any significant inhibition and another bird showed inhibition similar to most sublethally poisoned birds. Bunyan et al. (1969) report that differences in cholinesterase inhibition is greater between species than those noted between pesticides. Pigeon brain cholinesterase appear to be more sensitive (Bunyan et al. 1969) and give more consistent results than do pheasant brain esterases (Bunyan et al. 1968b). Lee and Pickering (1967) likewise found differences in brain cholinesterase activity between ducks, geese and hens.

Armbrecht and Dewitt (1963) have suggested that Japanese quail (Coturnix), may be intermediate in its sensitivity to pesticides when compared with the bobwhite and ring-necked pheasants. Shellenberger et al. (1966) state that brain cholinesterase was not inhibited in male or female Coturnix fed Azodrin or Bidrin at a level of 0.5 ppm for 3 weeks. At the 5.0 ppm level of Azodrin, brain enzyme activities of males and females were 69 and 83 per cent of controls, respectively. In birds fed Bidrin at 5.0 ppm, the values were 78 percent of the control for males and 80 per cent for females. When a lethal dose of Bidrin (50 ppm) was administered brain cholinesterase activity dropped to 38 per cent (62 per cent inhibition).

This compares favorably with results obtained in my studies on parathion. Gough et al. (1967) compared the biological reactions of Coturnix and Colinus to Guthion. They found bobwhite quail to be much more sensitive to Guthion than the Japanese quail. Bobwhite quail exhibited an 80 per cent inhibition of cholinesterase activity when fed a lethal dose of Guthion. Gough et al. (1967) explained the sensitivity difference between the two species by suggesting that desulfuration of P = S to P = O proceeds more rapidly in the bobwhite than in Coturnix. O'brien (1967) states that paraoxon is much more toxic than parathion (parathion is connected to paraoxon in vivo).

When parathion (0.5 mg/kg) was fed to bobwhite quail in hopes that a dose-response curve could be obtained, results were hampered by death of birds before the end of the 5 day study period. However enough information was collected to form a tentative hypothesis. When mean Rappaport Units of cholinesterase present in birds which survived the 0.5 mg/kg dosage were compared (LSD, alpha = 0.05) with Rappaport Units of control birds acutely poisoned, it was found that there was a significant difference between the 0.5 group and the control group. There was also a significant ($P < 0.05$) difference between the 0.5 group and the acutely poisoned group. As one can see from Fig.12 there is a trend for percentage inhibition to increase as treatment time increases. Whether this response is linear or not requires more investigation. This investigator does not feel it safe to say at the present time that one can predict intake of parathion from percentage cholinesterase inhibition but does not rule out the fact that it is possible to do so. Gough et al. (1967) report a marginal inhibition of brain cholinesterase at sublethal levels while Bunyan et al. (1968b) states that in most sublethally poisoned birds, cholinesterase levels are

relatively unaffected. In none of the last two previously mentioned studies do the investigators state any real, positive relationship between dosage and percentage cholinesterase inhibition.

Bunyan et al. (1968b) report that brain esterase levels are extremely consistent even when analyses were performed on birds which were dead for 12 days. The inhibition detected in brain esterase levels, even in old tissue from field samples allow reliable conclusions to be drawn. Analysis of plasma esterases from birds killed in the field is almost impossible due to difficulty in obtaining enough material with which to work. Liver esterases especially alpha-naphthyl acetate esterase may well be useful in diagnosis of poisoning while triacetin esterase appears to be very similar to cholinesterase. Low esterase levels in kidneys rule them out as suitable tools for diagnosis of organophosphorous poisonings.

It is this investigator's conclusion that death can be contributed to parathion poisoning when brain cholinesterase inhibition exceeds 55 percent of the normal mean value and diagnosis of sublethal poisoning can be made where brain inhibition differs significantly from the normal mean.

SUMMARY

In the fall of 1969 a study was initiated at Kansas State University to determine the sublethal effects of dieldrin and parathion on bobwhite quail. Inherent with this study, it was hoped that an equilibrium between intake and uptake could be achieved within 70 days and correlation between brain and carcass remainder residues would occur. A pilot study was initiated with 30 birds each. One group received 3.25 µg/ml of dieldrin while the other received 6.50 µg/ml. Dosing was on a daily basis. Gas chromatography was the method used to quantitate residues. In this particular study, three tissues were analyzed for residues. A significant correlation was found to exist between brain and remainder and liver and remainder of only those birds dosed with 3.25 µg/ml. No equilibrium between intake and uptake was present.

Another study was initiated in which 32 bobwhite quail were divided into two groups of 16 birds; the groups being treated with 6.25 and 12.50 µg/ml of dieldrin. Dosing took place every four days. Residues were somewhat smaller than those of birds which were treated with daily doses of 3.25 µg/ml. Correlation between brain versus remainder (6.25 group) was 0.4193 and was not significant ($P < 0.05$). Correlation between brain and remainder tissues of birds dosed with 12.50 µg/ml was significant ($P < 0.05$). Graphically it appears an equilibrium between intake and uptake of dieldrin may have been achieved with the 6.25 µg/ml treatment. The 12.50 µg/ml dosed birds tended to show a gradual increase in residue uptake until termination of the study.

Forty-two bobwhite quail were then divided equally into two groups, one receiving 50 µg/ml, the other receiving 100 µg/ml of dieldrin on an every other day basis. No equilibrium was found to occur in this study

which lasted 70 days. Brain and remainders were found to be significantly correlated ($P < 0.05$) in both treatment groups. Residue values were smaller than expected probably due to increased metabolism of dieldrin and storage of it in adipose tissue.

Results of the acute toxicity tests produced values which were smaller than those generally reported in the literature. I found that the LD_{50} of bobwhite quail dosed with dieldrin in corn oil to be between 200 and 400 µg/ml. This is approximately 1.14 and 2.29 mg/kg of body weight. The reason for my obtaining mortality at low dosage levels lie in the enhanced absorption of dieldrin due to the corn oil.

A study was also performed on parathion to see whether any suitable method was available to quantify the amount of parathion in a bird's system. Since parathion degrades rapidly in the animal's system an indirect measurement such as percentage of cholinesterase inhibition was deemed feasible. An acute toxicity test was executed with bobwhite quail receiving 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 mg/kg of parathion dissolved in corn oil. All these levels proved fatal to quail within 24 hours, the larger concentration causing death in ten minutes. Another study was initiated in which birds received 3.0 and 1.0 mg/kg of parathion. From this study it was determined that the LD_{50} of parathion in corn oil was 1.0 mg/kg of body weight. Death usually resulted in about 24 per cent inhibition of cholinesterase. Cholinesterase inhibition seems to be a very useful tool for analysis of birds which are believed victim of parathion poisoning. A study was initiated in hopes that a dose response curve could be produced with cholinesterase inhibition. Research on this aspects is not as lengthy as it should be but from what was done it is believed that percentage

cholinesterase inhibition is inversely proportional to dosage of parathion. Whether this relationship is linear or not requires extensive study. If this would be done it would prove to be a valuable tool in analysis of birds thought to be poisoned by parathion.

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APPENDIX

Table 3. Common and scientific name of commonly mentioned pesticides

Common Name	Scientific Name
Aldrin	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a - hexahydro - 1,4,-endo, exo-5, 8-dimethanonaphthalene
Bidrin	dimethylphosphate 3-hydroxy N, N-dimethyl-eis-crotonimide
Chlordane	1,2,4,5,6,7,8-8-Octachloro-2,3,3a,4 7,7a-hexahydro-4,7-methanoindane
Diazinon	0,0-Diethyl 0-[2-isopropyl-4-methyl-pyrimidyl(b)] thiophosphate
Dieldrin	1,2,3,4,10,10-herachloro-6,7-epoxy-1,4,4a, 5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonapthalene
Dimethoate	0,0-Dimethyl s-(methylcarbamo-OYl methyl) phospharodithioate
Demeton-methyl	0,0-Dimethyl P (and S) -2-(methylthio) methyl phospharothioate
DDT	Dichlaro diphenyl trichloroethane
Guthion	0,0-dimethyl S-[4-oxo-1,2,3-benzotriazin-3 (4H)-ylmethyl] phosphoiodithioate
Lindane	1,2,3,4,5,6-Hexachlorocyclohexane
Malathion	0,0-Dimethyl dithiophosphate

Table 3 (cont.)

Common Name	Scientific Name
Parathion	0,0-Diethyl 0-p-nitrophenyl phospharothioate
Phorate	0,0-Diethyl S(ethylthiomethyl) phosphoro-dithioate
Toxaphene	a chlorinated camphene

Table 5. Correlation exhibited between various tissue analyzed for dieldrin.

	Brain[a]	Liver	Remainder
Brain[b]		0.011	0.115
Liver	0.468		0.162
Remainder	0.437	0.079	

[a]Lower diagonal - 3.25 µg/ml group

[b]Upper diagonal - 6.50 µg/ml group

Table 10. Analysis of variance of weight data - split plot design (6.25-12.50 group).

Source	D. F.	Mean Square	F
Dose	1	3,974.47	2.08
Bird/Dose (error a)	10	1,909.66	
Time	6	9,288.70	
linear	1	55,634.02	1090
quadratic	1	90.11	
remainder	4	2.01	
Dose/Time	6	16.92	<1
(error b)	60	46.08	

Table 11. Analysis of variance of weight data - split plot design (50-100 group).

Source	D. F.	Mean Square	F
Dose	1	4,304.02	<1
Bird/Dose (error a)	6	8,577.82	
Time	13	5,132.14	
linear	1	60,215.33	1400
quadratic	1	825.55	
remainder	11	607.00	
Dose/Time	13	3.27	<1
Error (error b)	78	4.30	

Table 12. Unadjusted body weights of bobwhite quail treated every 4 days with 6.25 or 12.50 µg/ml of dieldrin.

	Days of Exposure (6.25 µg/ml group)													
	4	8	12	16	20	24	28	32	36	40	44	48	52	56
Bird 1	176.7[a]	176.3	177.1	177.8	179.3	178.9	179.6	181.5	181.9	181.7	184.3	184.0	183.1	183.0
2	164.9	163.7	163.9	164.4	164.0	164.0	164.6	164.8	163.7	163.4	164.3	165.6	164.9	165.4
3	178.5	178.8	179.1	178.7	178.0	176.3	177.2	177.5	177.4	178.6	178.3	179.9	179.4	178.2
4	187.8	186.3	186.5	187.3	188.4	188.0	187.4	186.5	183.2	184.1	183.0	183.3	184.6	184.3

	Days of Exposure (12.50 µg/ml group)													
	4	8	12	16	20	24	28	32	36	40	44	48	52	56
1	156.1	156.0	155.3	155.2	156.2	163.0	161.4	156.8	156.3	158.1	156.9	158.6	158.4	158.0
2	188.1	186.3	186.4	188.2	186.4	187.8	188.4	188.8	189.5	189.3	189.4	190.0	189.9	189.3
3	159.4	158.5	161.2	160.1	161.0	161.4	166.4	158.7	161.3	161.2	161.6	163.8	159.2	160.8
4	176.6	177.8	178.6	178.7	176.6	175.5	176.4	177.1	182.8	185.1	183.4	183.9	184.8	182.3

[a]All weights expressed in grams.

Table 14. Body weight of bobwhite quail treated with 1cc of corn oil every other day for 70 days.

	Days of Exposure							
	0	10	20	30	40	50	60	70
1	145.1	162.9	165.1	165.4	165.9	168.5	171.7	172.3
2	117.8	147.2	149.7	152.2	155.4	158.2	163.3	163.7
3	163.5	168.6	164.8	166.8	165.0	169.6	169.2	171.5
4	160.7	168.0	164.0	165.1	164.7	164.2	162.7	165.9
5	129.1	143.7	149.4	157.5	155.5	152.8	156.3	158.3
6	153.7	166.5	171.7	170.0	168.1	175.7	175.6	174.3

[a]All weights expressed in grams

Table 15. Rappaport Units of cholinesterase found in bobwhite quail treated daily with 0.5 mg/kg of parathion.

Days of Exposure			
1	2	3	4
53.01	56.31	54.34	45.22
49.25	50.12	50.77	35.82

[a]All values given in Rappaport Units of cholinesterane

Table 16. Unadjusted body weights of bobwhite quail treated every other day with 50 or 100 µg/ml of dieldrin.

	Days of Exposure (50 µg/ml group)						
	1	10	20	30	40	50	60
1	172.2[a]	173.3	172.4	169.7	171.3	169.9	168.8
2	180.2	177.3	177.6	183.6	185.3	186.5	189.8
Bird 3	161.1	161.7	164.8	169.3	171.8	166.1	167.3
4	162.1	159.9	166.0	171.8	168.8	165.3	162.0
5	180.2	173.2	173.4	174.3	175.7	176.6	170.3
6	170.5	163.7	167.5	167.5	165.1	166.3	164.6

	Days of Exposure (100 µg/ml group)						
	1	10	20	30	40	50	60
1	152.0	158.3	155.5	165.1	164.2	155.7	146.6
2	173.5	189.1	182.3	183.1	189.1	186.1	187.0
Bird 3	167.7	171.6	175.2	177.5	172.1	168.8	175.7
4	157.2	165.3	165.9	166.7	169.3	163.6	168.9
5	162.5	165.6	167.7	171.0	166.9	151.5	151.6
6	159.1	151.2	157.6	156.5	157.6	150.4	152.4

[a]All weight expressed in grams.

Table 17. Analysis of variance on data from cholinesterase study.[a]

Source of Variation	D.F.	Sum of Squares	Mean Square	F	Decision $H_o: \mu_1 = \mu_2$
Between	4	9697.357	2424.339	20.255	Reject
Within	70	8378.295	119.689		
Total	74	18,075.652			

[a]Analysis of variance calculations performed on Rappaport Units of cholinesterase.

Table 18. Mean Rappaport Units of cholinesterase from five dosage levels and indication of significant differences ($P<0.05$) among the means.[a,b]

	Acute Tox.[c]	3 mg/kg	1 mg/kg	0.5 mg/kg	Control
$\bar{X}$	23.07	25.80	26.40	44.30	57.09
N	14	20	20	10	11

[a]The X_i's lying above the same horizontal line are not significantly different.

[b]All calculations according to Fryer 1966.

[c]Values from different dosage levels were pooled and mean taken from the pooled sum.

Fig. 11. Body weight of bobwhite quail during 50 and 100 µg/ml study.

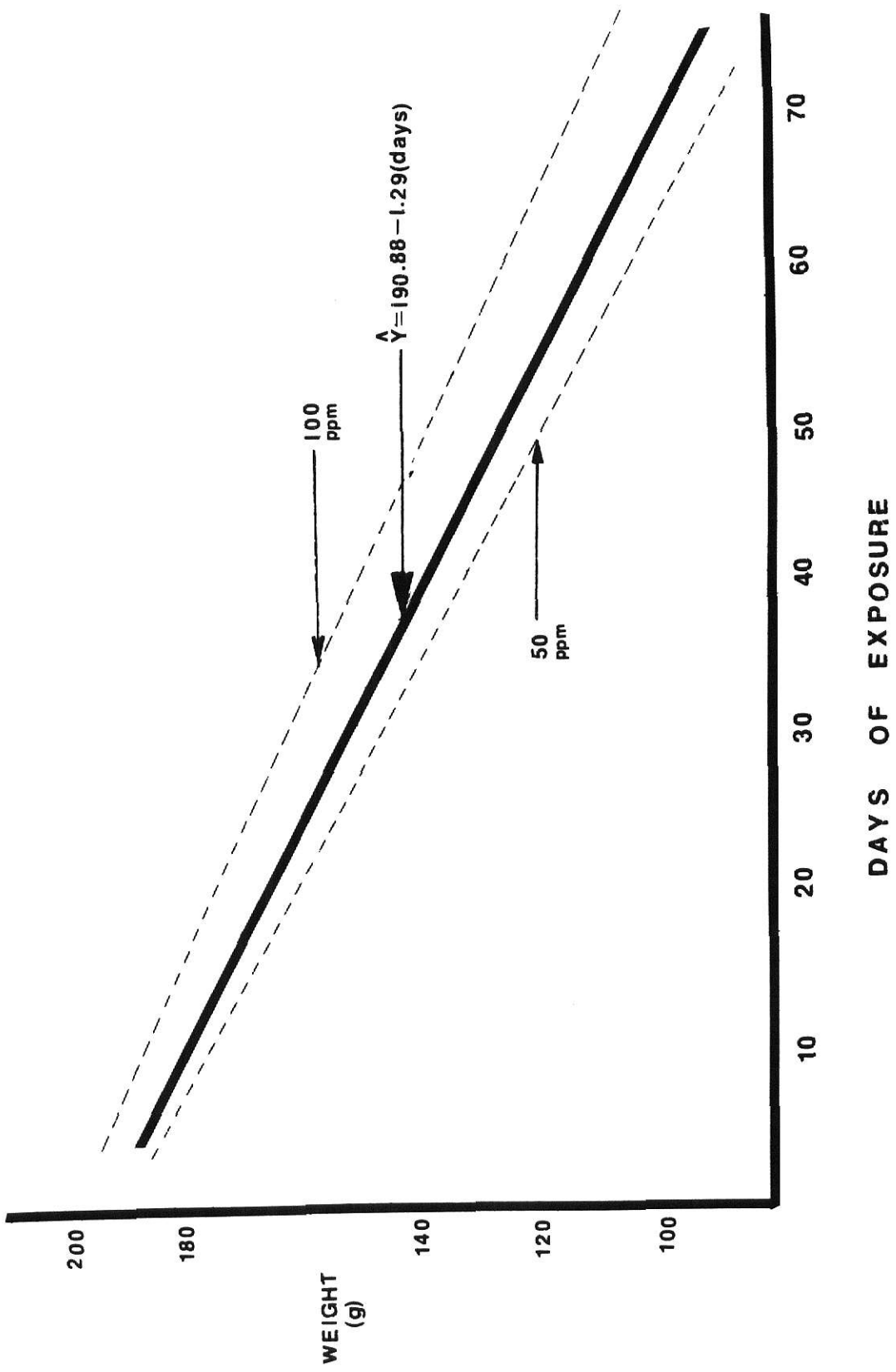

WEIGHT (g)
200
180
140
120
100
10
20
30
40
50
60
70
DAYS OF EXPOSURE
100 ppm
50 ppm
$\hat{Y}$=190.88−1.29(days)

Fig. 10. Body weight of quail during 6.25 and 12.50 µg/ml study.

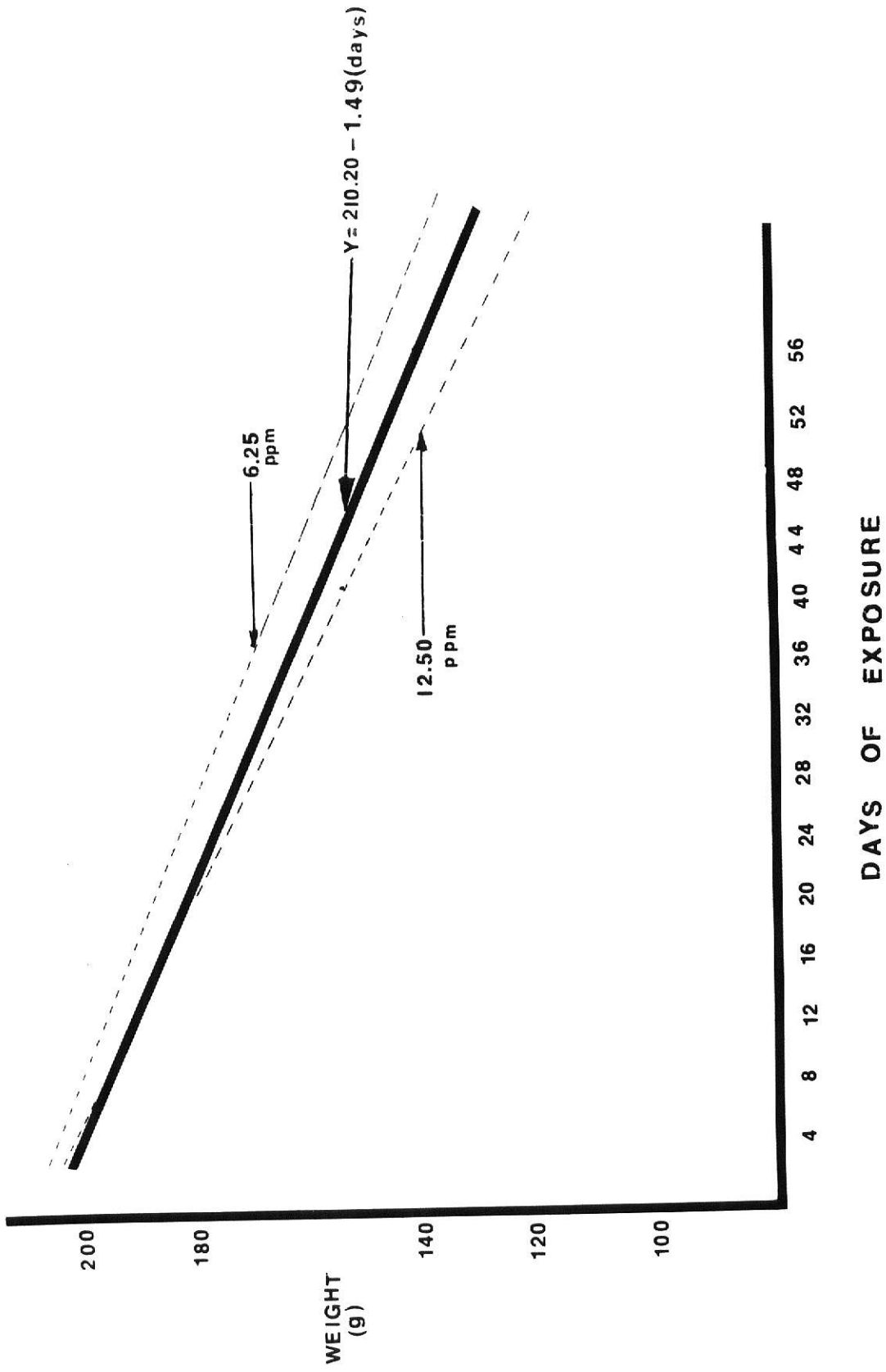
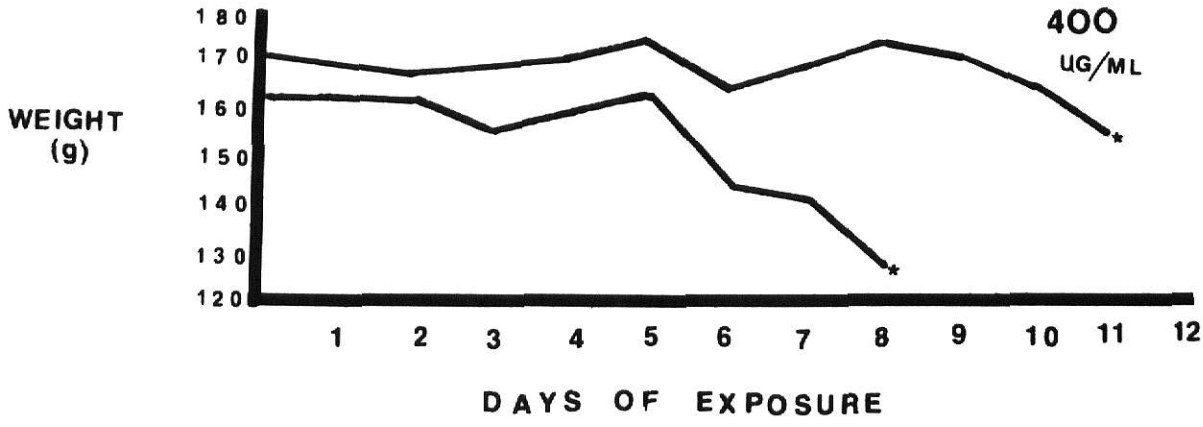
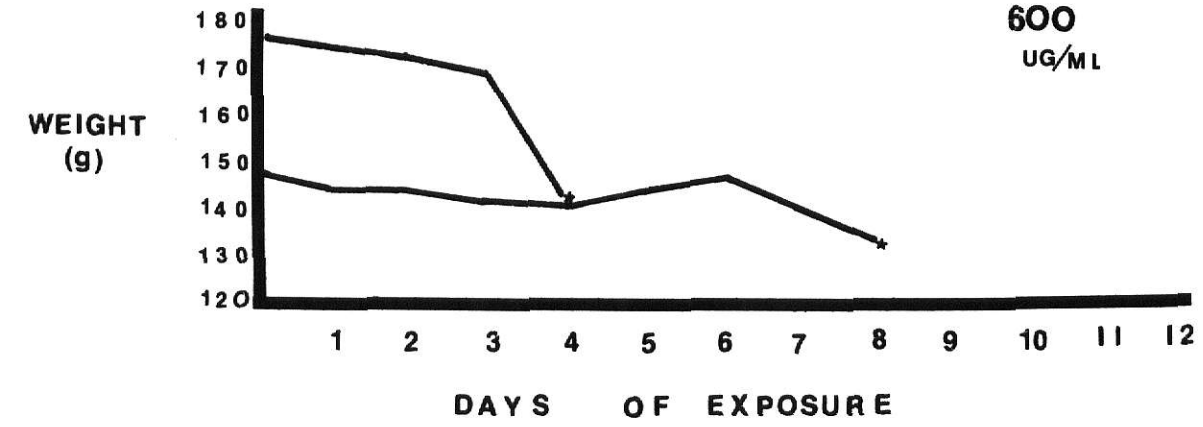
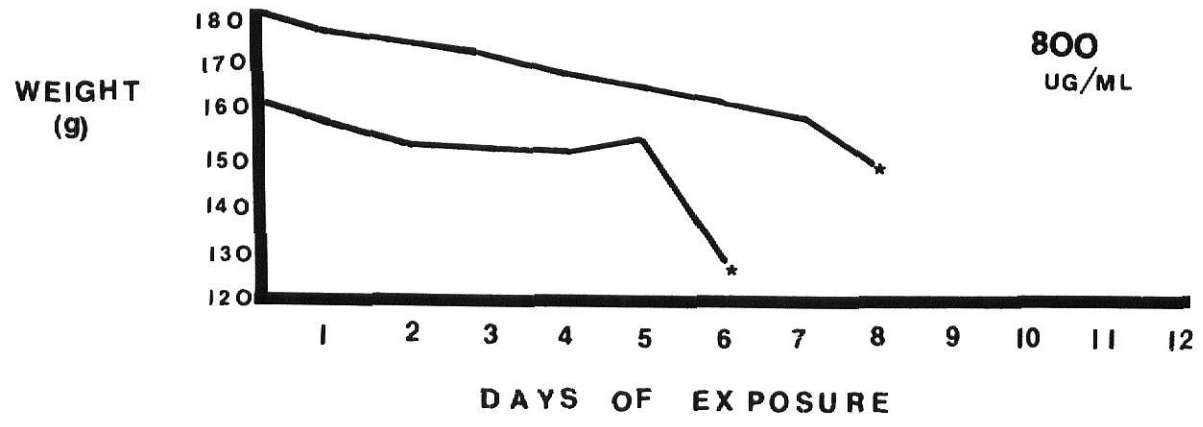

Y= 210.20 – 1.49(days)
6.25 ppm
12.50 ppm
WEIGHT (g)
200
180
140
120
100
4
8
12
16
20
24
28
32
36
40
44
48
52
56
DAYS OF EXPOSURE

Fig. 5. Weight of birds subjected to acute doses of dieldrin.


400
UG/ML
WEIGHT
(g)
180
170
160
150
140
130
120
1 2 3 4 5 6 7 8 9 10 11 12
DAYS OF EXPOSURE


600
UG/ML
WEIGHT
(g)
180
170
160
150
140
130
120
1 2 3 4 5 6 7 8 9 10 11 12
DAYS OF EXPOSURE


800
UG/ML
WEIGHT
(g)
180
170
160
150
140
130
120
1 2 3 4 5 6 7 8 9 10 11 12
DAYS OF EXPOSURE

DETERMINATION AND MAINTENANCE OF SUBLETHAL RESIDUE LEVELS OF DIELDRIN AND PARATHION IN *COLINUS VIRGINIANUS*

by

WILLIAM ALAN NUSZ

B. S., Kansas State University, 1969

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Biology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1971

An interrelated study of pesticide, bioenergetics, behavioral and parasite effects on bobwhite quail (Colinus virginianus) has been in progress since 1966. In the spring of 1969, a study was initiated at Kansas State University to determine the effects of dieldrin and parathion on bobwhite quail.

Four separate studies were conducted each with dieldrin and parathion. Dosing of birds was accomplished by dissolving the pesticide in corn oil and administering the solution orally with a syringe.

Residue analysis for dieldrin was done according to the Pesticide Analytical Manual Volume II, Methods for Individual Pesticide Residues. Effects of parathion was determined by means of cholinesterase inhibition and was measured with the aid of a Sigma Chemical Kit #425. In all dieldrin studies both brain and remainders were analyzed for residue concentration. In the parathion studies, only brain cholinesterase levels were monitored.

Birds receiving 3.25 µg/ml of dieldrin on an every other day basis achieved brain and remainder residues of 0.1942 and 0.2492 ppm (wet weight basis) respectively after 20 days of dosing. Quail receiving 6.50 µg/ml (every other day) for 20 days achieved levels of 0.2990, 0.4009 ppm in respective brain and remainder tissues. No equilibrium between dieldrin intake and residue accumulation could be found. Quail receiving 6.25 µg/ml of dieldrin every 4th day for 66 days accumulated brain and remainder residues of 0.0947 and 0.0607 ppm respectively while birds being dosed with 12.50 µg/ml accumulated brain and remainder residues of 0.3017 and 0.861 ppm respectively. An equilibrium between intake and residue concentration seemed to be present. Quail dosed with 50 µg/ml of dieldrin every other day for 70 days produced brain and remainder residues of 0.9003 and 0.6425 ppm while birds treated with 100 µg/ml achieved residue levels of 1.6757 and

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1.6818 ppm in brain and remainders. No equilibrium between dieldrin intake and residue concentration was found to exist. When quail were fed acute doses of dieldrin, residue levels of approximately 25 ppm in brain tissue were found to exist.

When quail were fed doses of parathion ranging from 5.5 to 8.0 mg/kg of body weight brain cholinesterase inhibition was approximately 59 percent. Death occurred anywhere between ten minutes and eight hours after administration of the pesticide. Birds dosed with 3.0 mg/kg of parathion all died within 24 hours. Brain cholinesterase inhibition was found to be 57 percent. Birds dosed daily with 1.0 mg/kg were all dead within 5 days. Percentage cholinesterase inhibition was found to be 55 percent. When birds were treated with daily doses of 0.5 mg/kg and sacrificed daily a dose - response curve was found to exist between amount of parathion administered and percentage cholinesterase inhibited. Two birds out of 10 which received 0.5 mg/kg died directly from parathion on the 3rd day of treatment.