THE EFFECTS OF DIELDRIN ON THE BEHAVIOR OF *COLINUS VIRGINIANUS*

by

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INTRODUCTION AND REVIEW OF LITERATURE

Environmental monitoring of insecticides has revealed some unexpected deleterious effects to desirable fauna associated with the use of these toxic chemicals. Normally drugs cannot change the physiological function of tissues, but they can alter the degree of functioning of cellular activity (Thompson and Schuster 1968). Observations of bird populations in agricultural areas throughout the United States indicate high mortalities due to some pesticide applications. However, this is not a new problem. Hickey and Hunt (1960) found robin (Turdis migratorius) mortalities of up to 88 per cent in Wisconsin areas where DDT was applied. Losses of meadowlarks (Sturnella magna), robins (Turdis migratorius), brown thrashers (Toxostoma rufum), starlings, (Sturnus vulgaris), common grackles (Quiscalus quiscula), ring-necked pheasants (Phasianus colchicus), and horned larks (Otocoris alpestris), resulted from a field application of dieldrin in Illinois (Scott et al. 1959). Where DDT was used in Alabama to combat insects, bobwhite quail (Colinus virginianus) populations were considerably lowered, as were populations of insect-eating birds in a toxaphene treated area in Montana (DeWitt and George 1960). Many more such reports can be found in the literature.

Death is not always imminent after exposure to pesticides. An animal's response to a drug depends upon a number of factors including age, sex, genetics, existing environmental conditions, and body weight (Thompson and Schuster 1968). The lethality of organochlorides is normally greater to younger animals. Ingestion of food containing only 1 ppm of dieldrin by sixty

1-day-old bobwhite quail resulted in 100 per cent mortality after 76 days of feeding. (DeWitt 1956;864). By analyzing plant and animal populations on an area that had been treated with aldrin at the rate of 1 lb per acre for 15 years, Korschgen (1970) calculated that ingestion of twenty ground beetles (Poecilus chalcities) by juvenile quail could be fatal. Twenty gound beetles is less than one-third of a juvenile quail's daily food intake. Lethality in adults is associated in part with seasonal stress and fat storage (Stickle 1968). Normal weight cycles in bobwhite quail fluctuate as a result of reproductive occurrences as well as monthly temperature changes throughout the year (Roseberry and Klimstra 1971). Decreased fat storage and fluctuating environmental demands can increase the effect of even low doses of pesticides. Therefore pesticidal effects upon wildlife can only be estimated. Figures from mortality studies are mere suggestions of the fate of chemicals now being sprayed, dusted, and applied to the soil of fields and gardens. Stickel (1968:2) stated that "...most living organisms now contain organochlorine residues." One reason for this is that the poisons do not always reach their purposed destinations, or even if they do, these poisons are subject to movement in the environment via plants, animals, wind, and water (Stickel 1968). This enhances the possibility that the pesticides will contact animals other than the intended victims. Greichus et al. (1968:92) reported an average of 0.5 ppm chlorinated hydrocarbon residues in subcutaneous fat samples from forty-eight pheasants and forty-six sharp-tailed grouse (Pedioecetes phasianellus campestris) collected in South Dakota. Linder and Dahlgren (1970:229) found that dieldrin residues in pheasants from untreated South Dakota areas equalled the residue levels in birds from aldrin-treated areas. Residues in brains of twenty-six hen pheasants analyzed ranged from 0.01 ppm to 0.08 ppm, whereas the range in twenty-two males was 0.01 ppm to 0.10 ppm (Linder and Dahlgren 1970: 228-230). While developing a pesticide minitoring program involving 128 sampling sites across the contiguous United States, Martin (1969) established residue baselines. He found dieldrin in the fat samples of virtually every 10-bird starling sample analyzed. Such reports are common and indicate organochloride pesticides are spreading throughout the environment and are being absorbed and stored in tissues of many wildlife species. It is because of this phenomenon that the present study was undertaken to determine the effects of insecticides upon birds.

Most of the information concerning the effects of sublethal pesticide exposure has centered upon reproduction. The three major topics studied in this area are reproductive success (production of live young), eggshell thinning, and reproductive behavior. The most generalized studies indicate that reproductive success is inhibited by ingestion of pesticides of the aldrin group (Dustman and Stickel 1966, Rosene 1969, DeWitt 1955, Atkins and Linder 1967, and Genelly and Rudd 1956). One explanation for this might be the deposition of dieldrin in the egg yolk prior to its encasement in the shell. Of sixty-seven pheasant eggs collected in South Dakota in May and June, Linder and Dahlgren (1970) found fifty-four contained dieldrin residues. Average residue concentration in the yolk was 0.03 ppm. Graber et al. (1965) studied the red-winged blackbird (Agelaius phoeniceus) population on a wheat field in Illinois which had been sprayed with 0.25 lb per acre of dieldrin just prior to the breeding season. Dieldrin concentrations in fifteen blackbird eggs collected from fifteen nests abandoned on this area averaged 6.0 ppm. Brown et al. (1965) fed 1 ppm of aldrin daily to one chicken and 1 ppm of dieldrin daily to another hen for 700 days. The eggs from these birds were collected daily and analyzed for pesticide residues. The residue level peaked at 300 days. At that point, eggs from the aldrin-fed bird contained 10 ppm dield-fed bird contained 10 ppm dieldrin in their yolk, while the eggs from the dieldrin-fed hen contained an average of 30 ppm dieldrin in their yolk. Mating these hens with untreated cocks produced viable eggs and young which prompted Brown et al. (1965:677) to speculate that chicken eggs could be hatched which contained as much as 25 ppm dieldrin in their yolk. But although the hatchings from his study survived for 16 weeks, no studies were made of their growth rates or behavior.

Eggshell thinning due to pesticides has been blamed for reproductive failure by some researchers. Porter and Wiemeyer (1969: 199) found a significant relationship between eggshell thickness of sparrow hawk (Falco sparverius) eggs and low dosages of dieldrin and DDT was sufficient to induce shell thinning. A decrease in thickness of 0.02 mm occurred in eggs of mallard ducks (Anas platyrhynchos) which had been fed 10 ppm of dieldrin per day for 4 months prior to testing (Lehner and Egbert 1969:1218). Ratcliffe (1970) supported the shell thinning theory stating that organochloride pesticides alter the internal regulation of calcium carbonate metabolism so that the supply to the oviduct is reduced, preventing normal calcification before the egg is extruded. Bitman et al. (1969:44-16) agreed with Ratcliffe's findings and suggested other chlorinated hydrocarbon action which could inhibit normal shell thickness such as the inhibition of medullary bone deposition which is the primary source of calcium during eggshell formation.

Another area of reproduction which has been studied in conjunction with sublethal pesticide exposure is behavior. Breeding failure has been blamed in part upon an alteration of normal reproductive behavior. Of the abnormalities reported, egg destruction was mentioned most frequently. Ratcliffe (1970) observed female peregrines eating their eggs. Egg breakage and disappearance in the nests of golden eagles and sparrowhawks where there was no evidence of predators was also observed (Ratcliffe 1960, Lockie and Ratcliffe 1964, Porter and Wiemeyer 1969). Abnormal behavior in red-winged blackbirds nesting in an area recently sprayed with 0.25 lb per acre dieldrin included a cessation of construction of partially completed nests, a decided lack of responsiveness to human intrusion, and a failure to incubate eggs that had been layed before the pesticide had been sprayed (Graber et al. 1965).

With the evidence suggesting that reproductive behavior is altered by organochloride ingestion, it seems plausible that other forms of behavior might also be affected by the action of this pesticide group. Linder et al. (1970) reported the following symptoms in adult pheasants prior to their death from dieldrin poisoning: tremors, stupor, convulsions, and loss of appetite resulting in emaciation. Symptoms similar to those were also found in chickens with the addition of extreme alternation of dilation and contration of the pupils of the eyes (Scott et al. 1959).

The basis of the insecticidal properties of dieldrin, a cyclodiene derivative manufactured from the oxidation of aldrin, are yet unclear although it is known to disrupt nervous activity (Martin 1969). Koch (1970) has postulated that chlorinated hydrocarbons attack adenosine triphosphate enzymes thus inhibiting electrical potential across nerve membranes. Revzin (1966) found that endrin, a relative of dieldrin, is selectively toxic to the ectostriatum portion of the forebrain in pigeons. This is the visual projection area probably analagous to the mammalian telencephalic visual projection area. Further, he suggests that all chlorinated hydrocarbons have this effect and that the dosage which would produce this deficit will be much lower than the dosage necessary to produce grossly observable behavioral changes.

Very little behavioral research has been conducted involving sublethal pesticidal effects. James and Davis (1965) found that sublethal quantities of DDT impaired the discrimination ability of bobwhite quail. Baxter et al. (1969) reported that visual cliff discrimination was impaired in chicks of pheasant hens fed weekly 8-mg doses of dieldrin. Experiments conducted by Dahlgren et al. (1970) on pheasant chicks whose parents had been fed dieldrin showed that susceptibility to hand capture was significantly greater when both parents had received the insecticide.

It has been suggested that sublethal doses of insecticides affect animal behavior in such a way as to impair feeding efficiency, decrease predator avoidance ability, and hamper the skill required to prepare for environmental pressures (Ratcliffe 1970, Stickel 1968, Revzin 1966, and Dahlgren 1970). The far-reaching implications of such a behavioral effect could be of tremendous importance to wildlife management. But as mentioned earlier, there is a definite lack of experimental work involving testing for behavioral changes due to pesticides. This is due to the difficulty in developing substantial methods for measuring these changes rather than a lack of interest in this area. Therefore, the main objective of this study was the development of laboratory methodology to enable the measurement of behavioral effects of sublethal levels of organochlorides on bobwhite quail. Dieldrin was selected as the representative chlorinated hydrocarbon for this study because of its widespread use in the environment.

METHODS

Behavioral changes can only be measured as deviations from a behavioral norm. Therefore, to test the effect of dieldrin on quail behavior, a standard of laboratory behavior was first established. This was accomplished by subjecting bobwhites to operant conditioning techniques patterned after those of Ferster and Skinner (1957), Cloar and Melvin (1968), Gardner (1969), and Deese and Hulse (1967). Ferster (1963) explained the "...use of free operant refers to any apparatus that generates a response which takes a short time to occur and leaves the animal in the same place ready to respond again." Operant conditioning in this study consisted of training quail to respond to lighted keys to obtain a food reward. Color discrimination, reaction time, and food intake were measured and averaged for each animal. Dieldrin was then administered orally. Its effects upon discrimination, reaction time, and food intake were measured and compared with the predetermined individual norms.

Apparatus

Behavioral testing was effected within a wooden experimental chamber, or operant box, measuring 53 x 37 x 31 cm (Fig. 1). In the front end was an aluminum response panel which fit snugly against the walls of the chamber (Fig. 2). Centered on this panel, 8 cm from the bottom of the box, was a 6 x 5 cm feeder opening. A correct response caused a light to go on in the grain dispenser behind this opening. This light, plus the sound of the uplifted metal feeder, signaled the onset of the reinforcement period following correct responses (Fig. 3). Five centimeters above the feeder opening, two

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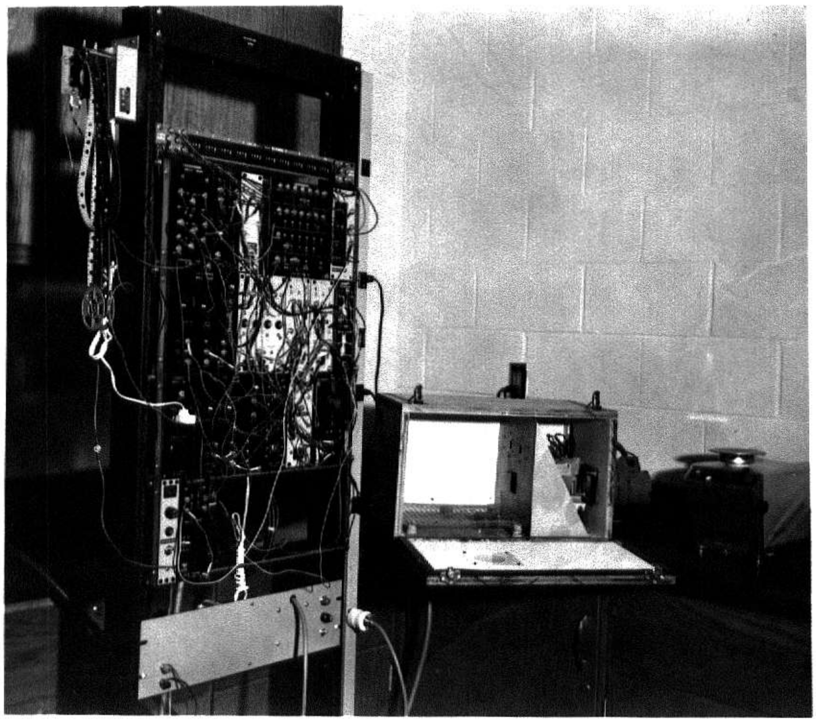

Fig. 1. Wooden experimental chamber and electromechanical scheduling and recording equipment used to measure operant behavior of bobwhite quail receiving dieldrin.

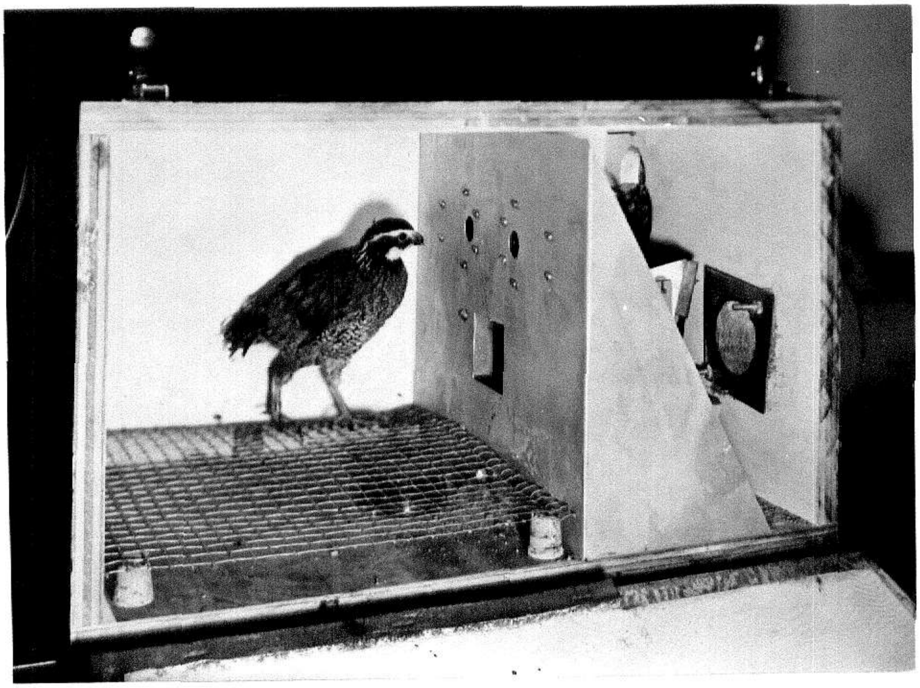

Fig. 2. Bobwhite quail responding to illuminated key on response panel of operant box.

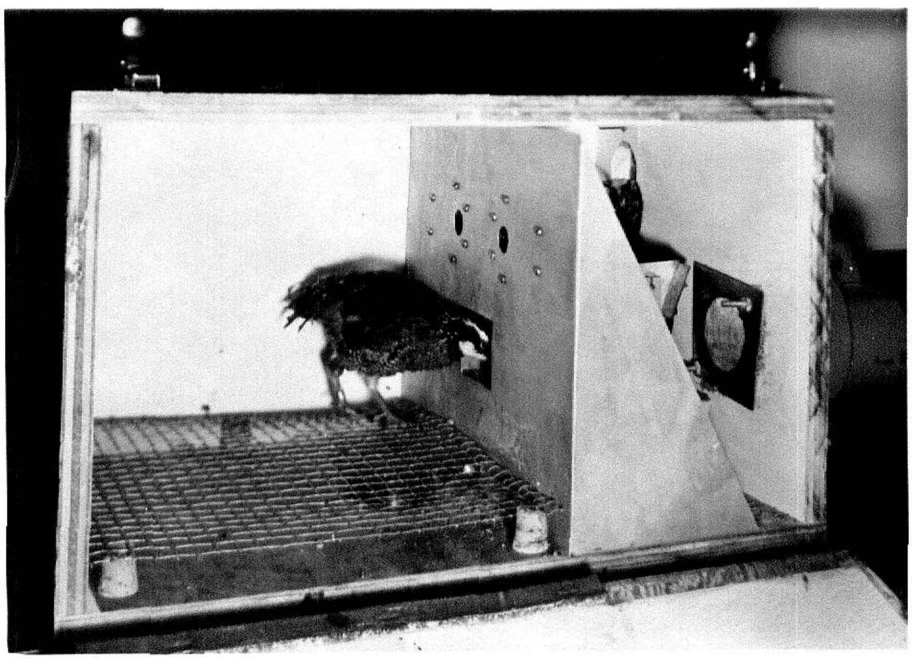

Fig. 3. Bobwhite quail feeding from the grain dispenser of the operant box following a correct response.

translucent keys (1.9 cm in diameter) were aligned 7 cm apart. These keys were lighted simultaneously at the beginning of a trial by individual display cells. Each display cell was capable of producing a green light or a red light. However the pair of display cells were wired to prevent the same color from appearing on both keys during a single trial. The location of the green light for any one trial was randomly determined except that an incorrect response would prevent a color position switch until after a trial in which a correct response had occurred. This prevented the reinforcement of superstitious responses to key position. Any response shut off the key lights immediately and simultaneously to prevent further responses during that same trial. A positive response, a peck on the green light, was reinforced with 6 seconds of feeding. A response to the red light caused a 6-second extension of the intertrial period. This intertrial period, which extended from the end of one trial until the illumination of the key lights for the next trial, ranged from 12 to 20 seconds. A 6-second delay added onto this waiting period was ample punishment for an incorrect response.

A wire floor, 31 x 30 cm, supported on corks 3 cm from the bottom of the operant box separated the quail from fecal material and grain which was thrown from the feeder. A 28-volt houselight centered on the response panel just below the wire floor was illuminated continuously during experimentation. The reflection of this light off the white painted chamber walls illuminated the entire box. An opening in the chamber wall directly opposite the response panel and an exhaust fan behind the response panel enabled fresh air to be pulled through the chamber for adequate ventilation. The noise from the fan served to muffle sounds from outside the chamber which might have disturbed the test animal. An opening 6 cm in diameter on the wall adjacent to the response panel was covered with a piece of clear plexiglass to permit observation of the quail during training. Cardboard was taped over this observation window after the training period to avoid distraction of the test animal. Power for this operant box was supplied by the standard electro-mechanical scheduling and recording equipment shown in Fig. 1. Responses were recorded on a series of counters on this equipment according to the number of seconds elapsed before the test animal responded to the lights. The counter bins were in 2-second intervals up to a maximum response time of 12 seconds. There was one set of counters for positive responses and one for negative responses.

Procedure

There were four procedural stages in this study. These stages, in sequence of application, were the preparatory stage, the training stage, control testing, and dieldrin testing. Data were recorded in the last two stages.

Preparatory stage: Thirty adult male bobwhite quail were obtained from the Kansas State Quail Farm at Pittsburg, Kansas. Females were not involved in the experimentation in order that complications involving the excretion of dieldrin through egg production might be avoided.

To facilitate handling, the flight feathers of both wings were clipped and the birds were banded. Each quail was then individually confined to a numbered, 48 x 25 x 13 cm, white, plastic cage which was equipped with a removable wire floor and top. These cages were aligned in an enviromental room where a temperature of 24°C and a daily 11-hour photo period were maintained.

During a 4-week period of acclimatization, the quail received water and food ad libitum from glass dishes within their cages. The food consisted of a mixture of grain (P-18) which had been finely mashed and then pelleted by the Milling Department of Kansas State University. The following week the birds were weighed daily. This served a twofold purpose. First, a normal body weight was established for each bird. From this weight, individual 60 per cent and 70 per cent levels were calculated for later experimental use. The second purpose of the daily weighing was to acclimate the bobwhites to handling.

In preparation for training, birds were deprived of all food. They did continue to receive water ad libitum in their cages. Daily weighing and handling continued until they reached 70 per cent of their normal body weight. The animals reached their respective 70 per cent levels after varying lengths of deprivation, ranging from 5 to 10 days. Therefore, the birds progressed to the next stage of the experiment individually.

Training stage: During this stage, the electromechanical scheduling equipment was adjusted to allow manual control of events in the operant chamber.

When a quail's weight was at or below 70 per cent of its normal body weight, his hunger suppressed his tendency to "freeze" from fear of a strange environment. Therefore the quail's first day in the operant box occurred when the animal reached this weight level. This first day was his familiarization period. In the box, the houselight was illuminated and the grain dispenser was in an "up" position to give the quail free access to the food. Neither key was lighted, for the purpose of this first day of training was to introduce the animal to the box and the availability of food. The animal was kept in the operant box until he had eaten from the feeder or until 2 hours had elapsed. If the quail did not eat within 2 hours, the familiarization process was attempted the following day. A bird that refused to move from his "freeze" position after 2 days of attempted familiarization was abandoned from the study.

The day after a quail fed in the box, usually day 2 of training, shaping or key-training was begun. At the onset of this session, the left key was permanently illuminated by the green light. The right key was not lighted. The grain dispenser was in the "up" position. Also, three grain pellets were taped to the lighted key.

The quail was placed in the operant box and was allowed to feed from the dispenser for 7 seconds. At that time, the feeder was dropped out of the animal's reach. After an initial fright response, typical behavior included frantic searching, scratching of the floor and panel, and pecking around the feeder opening. Any head or body movement in the direction of the green key was reinforced with 3 to 4 seconds of feeding. When the quail pecked at the grain taped to the light, the grain dispenser was lifted. The dispenser was maintained in this position until the quail fed from it. Although the quail was only allowed a few moments of actual feeding, it was necessary that the dispenser be held up in order that the animal might respond to the feeder light from his position by the green key.

After a repetition of the searching behavior described earlier, the animal began directing his movements toward the green-lighted key. Because the grain dispenser was manually controlled, attempted responses, near misses, and head-bobbing were rewarded with grain. This werved to reinforce the quail's behavior toward the key. After key-pecking commenced, the quail was reinforced with twenty-five 6-second feedings. He was then returned to the environmental chamber.

To insure that the quail was trained to the green color rather than to the key position, the opposite key was continuously illuminated green at the onset of the next training session. As on the previous day, the grain dispenser was up when the bird was first placed in the operant box. Shortly after feeding commenced, the dispenser was dropped, and the quail was forced to peck the key for food. Upon presentation of the tenth reinforcement, the key light was immediately darkened and the red bulb behind the left key was connected. The right key was kept green. At the end of the reinforcement period, both lights were lighted simultaneously. And they were lighted continuously, for the timing mechanism was not yet involved. The quail was allowed three 6-second reinforcements with the light colors in this position.

Then the key colors were switched. With two more correct responses, the color positions were again switched. Random switching continued until the quail responded correctly to thirty of these continuously lighted trials. This concluded the shaping procedure.

During shaping, the quail had had an unlimited length of time in which to respond, for the key lights had been continuously illuminated except when color switching occurred. When the lights were switched, all key lights were off and the quail was being reinforced at the grain dispenser. For the measurment of reaction time in this study, it was necessary that the quail respond quickly and consistently to the best of his ability. To encourage this, the timing of the key illumination was controlled. The key lights were turned on simultaneously and shut off simultaneously at the end of 12 seconds and remained off until the next trial began. If the animal failed to respond in the 12-second period, he received no food.

The training session following shaping introduced the quail to the timed trials. At the start of the session, the feeder was in the "up" position and the left key was lighted green. The quail ate and the dispenser was dropped down. After one 6-second reinforcement from a response to the continuously lighted green key, the right key was lighted red. Hesitation in emitting a response was allowed at this time. The key colors were switched randomly until five reinforcements were received. This part of the session insured that the bird was trained to the green light. On the next trial, the lights were on for the 12-second interval only. The trials continued until twenty-five reinforcements were given. The session ended at this time.

For 3 weeks, the quail was placed in the operant box for forty timed trials daily. Running the animal at the same time each day insured that the amount of food deprivation remained fairly constant since no food was provided in the animal's cage. In these 21 days, the animal's weight was raised to approximately 70 per cent of his normal body weight by increasing the daily reinforcement periods to 7 seconds. At the 70 per cent level, the quail responded well in the operant box yet remained in a healthy state for the forthcoming experimentation.

Control testing: This segment was considered the control. Bobwhites with total response times fluctuating less than 20 seconds daily and maintaining weight levels at or near their 70 per cent level were randomly assigned to groups of five. Birds not reaching these requirements were held in the training stage for another week. The animals had learned a pattern of behavior and now a standard of response times had to be determined for each animal. To determine this standard and the effect of future drug administration upon it, the following 14 days were divided into 2-day intervals. On the first day of the interval, each quail was weighed to the nearest gram, placed in the operant box for forty trials, re-weighed, and then force-fed 1/2 cc of Mazola corn oil. The corn oil was to be used as the vehicle for the dieldrin, therefore it was necessary to determine what effects, if any, force-feeding would have upon reaction time in order that they might be discounted during dieldrin testing. Force-feeding was accomplished by holding the bird's head between the thumb and index finger, extending the neck, and tilting the head upward while forcing the bills to separate to allow penetrance of the hypodermic syringe to the crop region. Syringes used for this process were 1 cc Monoject disposable tuberculin syringes (501-TB) from Sherwood Medical Industries.

On the second day of the 2-day interval, each quail was weighed, given forty trials in the operant box, weighed again, and then returned to the environmental room. The animals were not force-fed on day two.

From these 14 days, average response times were calculated for each bird for comparison with data from the next stage of the experiment.

Dieldrin testing: For this portion of the experiment, five quail groups were arbitrarily assigned a dieldrin dosage of either 50, 100, 200, 250, or 300 ug per 0.5 ml corn oil.

Twenty-eight days of testing were conducted in consecutive 2-day intervals comparable to those of control testing. Day 1 consisted of weighing each bird, allowing him forty trials in the operant box, re-weighing, and then force-feeding him the proper dieldrin dosage. Day 2 mimicked day 1 with the exception that the force-feeding was omitted from the procedure. Upon completion of the 28 days of testing, all quail were sacrificed for chemical analysis of their brain. These birds, as well as those that died before completion of this segment, were quick-frozen at -22° C to retard deterioration. Brains were dissected from the body and dieldrin residues were extracted on an individual basis using methods described by Nusz (1971). Brain analysis was accomplished by gas chromatography as described in the Pesticide Analytical Manual Volume I and II (1968).

Disproportionate bird mortality between dosage level groups required the analysis of the data to be done on a within group basis. The design of the experiment was a randomized block experiment (blocks birds) with missing data in some groups. The method employed was outlined by Harvey (1960).

For each dosage level group analysis, the source of variation among days was partitioned into the following:

(1) Control (days 1-14) vs treatment (days 15-40)
(2) Linear change over control days
(3) Quadratic change over control days
(4) Linear change over treatment days
(5) Quadratic change over treatment days

Seven response variables - total pecks, total peck time (sec), time (sec)/peck, weight (gm) before each session, weight (gm) after each session, total food intake (gm), and food intake (gm), and food intake (gm)/peck - were analyzed by the above method using an IBM 360 model 50 computer.

In addition to the analysis of the response variables, a simple one-way analysis of variance was used to test for differences between dosage levels for dieldrin residues in the brain.

RESULTS

Dieldrin Residues

Dieldrin residues were found in the brain of all experimental animals except bird 1 of the 50 ug group (Table I). The sample from bird 1 of the 50 ug group was accidentally lost during laboratory analytical procedures. Analysis of variance followed by Fisher's Least Significant Difference multiple-comparison procedure found that the mean of the 50 ug group (2.58 ppm) was not significantly different (P 0.05) from the mean of the 100 ug group (4.01 ppm). And the residue means from the 200, 250, and 300 ug birds; 11.07 ppm, 11.13 ppm, and 11.82 ppm, respectively, were not significantly different. However, the residue means of the 50 and 100 ug groups were significantly different from those of the 200, 250, and 300 ug groups.

General Behavioral Changes

Twenty-five of the thirty quail obtained for this study survived the training period and learned to discriminate between the key colors sufficiently to be included in the dieldrin testing. Individual data were grouped according to pesticidal dosage, either 50, 100, 200, 250, or 300 ug of dieldrin per 0.5 ml of corn oil. Although testing consisted of a total of 42 days, only the first 40 days were analyzed due to limitations in the program available for computerization of the data. Analysis of the data to detect differences between dosage groups was impossible due to unequal subclasses resulting from lethal dieldrin dosages.

All birds in the 300 ug group died within 13 days after dieldrin was

TABLE 1. Analysis of variance and means of dieldrin residues found in brain of bobwhite quail receiving an assigned dieldrin dosage at 2-day intervals. Brains were analyzed at death for birds receiving lethal dosages, and after 28 days of dieldrin testing for the remaining birds.

Analysis of Variance

Source	d.f.	M.S.
Level	4	90.98
Error	19	12.26

	Bird	Dosage Level 50 ug	100 ug	200 ug	250 ug	300 ug
Individual (ppm)	1	*	7.48	7.83	12.24	3.04
	2	2.94	2.08	14.33	11.95	12.32
	3	1.46	2.81	9.34	7.36	15.47
	4	2.03	1.94	15.38	9.92	10.46
	5	3.87	5.73	8.48	14.19	17.83
Dosage Level Means (ppm)		2.58**	4.01	11.07	11.13	11.82

* Sample accidentally lost through analytical procedure in laboratory.

** Dosage level means above the same horizontal line are not significantly different; those over different lines are with P $\leq$ 0.05).

was first administered. Four of the 250 ug group, and three of the five birds of the 200 ug group died before the end of the 28-day dieldrin testing period (Table 2). Although no birds from the 50 ug nor from the 100 ug bird groups died, some of the members of the 100 ug group exhibited behavioral deviations in the operant box and in their cage comparable to those exhibited by birds that did succumb to the pesticide. One of the most noticeable symptoms was the continuously fluffed feathers and immobile crouching by the birds. Other symptoms included the cessation of normal cage pacing and vocalization. A change of operant behavior occurred in birds succumbing to the dieldrin (Table 3, Fig. 6-8), as well as in the operant behavior of bird 1 and 5 of the 100 ug group and bird 4 of the 200 ug group. Operant responses of birds of the 50 ug group and those of three members of the 100 ug group remained unchanged throughout dieldrin testing (Fig. 4-5). The pattern of change that occurred in the other groups usually began with an increase in the time it took the animal to respond to the green key. This was either a gradual shift, or an abrupt change from a series of sessions of prompt responses to a series composed of various response times. The gradual shift was characteristic of animals receiving 200 ug dosages of dieldrin (Fig. 6), whereas the abrupt shift occurred most often in animals receiving a higher dosage of the organochloride (Fig. 7-8). Slowness in response was also detected in the variables total intake and intake/peck in some cases. The reason for this was that it took longer for the animal to respond to the sound and light changes signaling the onset of the reinforcement period, thus allowing him less time to eat. Intake/peck might also have decreased from a diminished ability to discern individual food pellets or at least to aim pecks at them. A further indication of discernment loss was that the aiming of pecks became progressively less accurate as dieldrin administration continued. This was evidenced by the increase in the number of pecks reaching the response panel around the keys before a key peck was accomplished.

Because less food was obtained per correct response and the desire for food was relatively unaffected during the early days of dieldrin administration, the quail maintained, or in some cases, increased his total daily pecks in an attempt to satiate his desire. Soon, however, the dieldrin and his depleted energy reserves prevented this compensation. His total pecks declined, his response time slowed through lack of energy to an even greater extent, and he received even less food. Finally, he reached the point at which he was losing proportionally large amounts of weight because he no longer possessed the strength to respond enough times to obtain his required daily maintainance calories. At this point, response times became randomized. They often appeared a mere habit, for the quail often failed to reap his reward for a correct response. As he approached the point of starvation, total pecks per day approached zero. Finally, in most cases, the bird stopped pecking entirely for one or several days and then died. At higher dieldrin dosages, this pattern was accelerated and several of the stages became 1-day events. Symptoms preceding death, in addition to those already mentioned, included a loss of appetite (tested by offering food in the cage), drooping wings, a loss of balance accompanied by an inability to regain an upright stance, and severe leg, head, and

Table 2. Responses and weight data for quail succumbing to dieldrin administration given at 2-day intervals originating on day 15.

Bird no.	Last day pecked	No. pecked last day	Average daily pecks	Wt. last measured (gm)	Normal 100 per cent wt. (gm)	Per cent normal wt.	Day of death
				100 ug/0.5 ml			
1	32	8	21	113	185	61	*
				200 ug/0.5 ml			
2	32	10	31	137	177	78	34
5	28	10	34	135	170	79	34
3	36	2	40	147	186	79	38
4	41	2	34	121	206	59	*
				250 ug/0.5 ml			
4	14	7	17	117	206	57	21
1	20	19	28	124	171	72	22
2	19	25	32	121	178	68	22
5	27	25	31	157	206	76	30
				300 ug/0.5 ml			
5	22	18	21	136	192	71	23
1	25	2	34	142	202	70	26
2	25	23	39	126	168	75	26
3	24	6	35	138	195	71	26
4	25	15	38	140	173	81	27

*Bird did not die before end of experiment, but exhibited severe symptoms of dieldrin poisoning.

Table 3. Responses recorded in each of six time bins by a typical bobwhite from each of the five dosage level groups receiving dieldrin at 2-day intervals beginning on day 15 of experimental.

Dosage Level	Day	Mean Responses per Time Bin (Sec) 0-2	3-4	5-6	7-8	9-10	11-12	Mean Total Pecks
50 ug	1-14	36.0	2.2	0.1	-	-	-	38.3
	15-18	36.5	1.0	-	-	-	-	37.5
	19-22	37.0	1.0	0.5	-	-	-	38.5
	23-26	36.2	0.8	0.2	-	-	-	37.2
	27-30	35.8	1.2	0.5	-	-	-	37.5
	31-34	27.5	1.8	1.0	-	-	-	30.3
	35-38	36.0	1.5	1.0	0.3	-	-	38.8
	39-42	37.0	0.8	-	-	-	-	37.8
100 ug	1-14	27.1	0.9	0.6	0.3	0.5	0.4	29.8
	15-18	20.5	0.5	1.0	0.2	-	0.2	22.4
	19-22	21.3	0.5	0.5	0.5	0.5	0.2	23.5
	23-26	19.8	1.2	1.0	0.8	0.2	0.2	23.2
	27-30	24.8	1.0	-	-	-	-	25.8
	31-34	21.5	1.8	0.2	0.2	-	-	23.7
	35-38	28.5	0.2	1.0	-	-	-	29.7
	39-42	18.2	0.2	0.2	0.2	0.5	0.2	20.1
200 ug	1-14	31.7	2.1	0.7	-	-	-	34.5
	15-18	27.5	3.5	-	-	-	-	31.0
	19-22	26.0	4.2	0.5	-	-	-	30.7
	23-26	33.2	5.0	0.2	-	-	-	38.4
	27-30	30.8	2.5	0.2	-	-	-	33.5
	31-34	24.5	4.0	0.8	-	-	-	29.3
	35-38	30.8	5.2	2.5	-	-	-	38.5
	39-42	10.0	4.5	2.8	0.2	0.2	0.2	17.9
250 ug	1-14	26.4	4.5	1.0	0.8	-	-	28.9
	15-18	20.5	3.2	1.8	1.0	1.2	1.0	27.7
	19-22	6.2	3.0	1.5	-	0.5	0.5	11.7
	23-26	Died on Day 22						
300 ug	1-14	26.5	4.5	2.0	0.2	0.3	0.5	34.0
	15-18	14.5	4.0	3.2	0.2	0.2	0.5	22.6
	19-22	6.0	3.0	1.2	-	-	0.2	10.4
	23-26	-	-	-	-	0.2	-	0.2
	27-30	Died on Day 26						

* Birds considered to typical for each group were: bird 5 in the 50 ug group, bird 2 in the 100 ug group, bird 4 in the 200 ug group, bird 1 in the 250 ug group, bird 1 in the 300 ug group.

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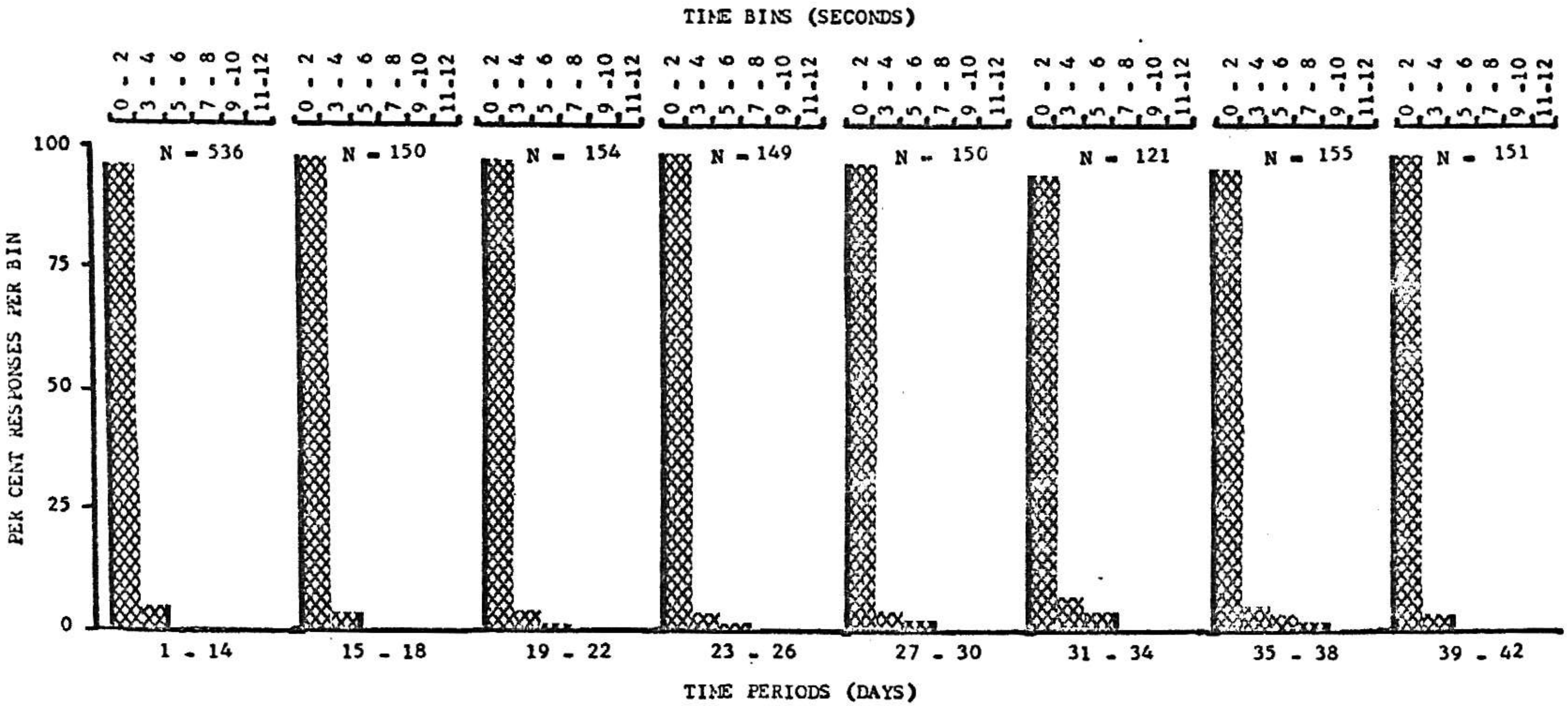

TIME BINS (SECONDS)
0 - 2
3 - 4
5 - 6
7 - 8
9 -10
11-12
100
75
50
25
0
PER CENT RESPONSES PER BIN
N - 536
N - 150
N - 154
N - 149
N - 150
N - 121
N - 155
N - 151
1 - 14
15 - 18
19 - 22
23 - 26
27 - 30
31 - 34
35 - 38
39 - 42
TIME PERIODS (DAYS)

Fig. 4. Percentages of daily responses per time bin for a bird typical of the quail group receiving 50 ug of dieldrin. Total responses for each time period are shown (N) while the number of responses for each time bin for each period in Table 3.

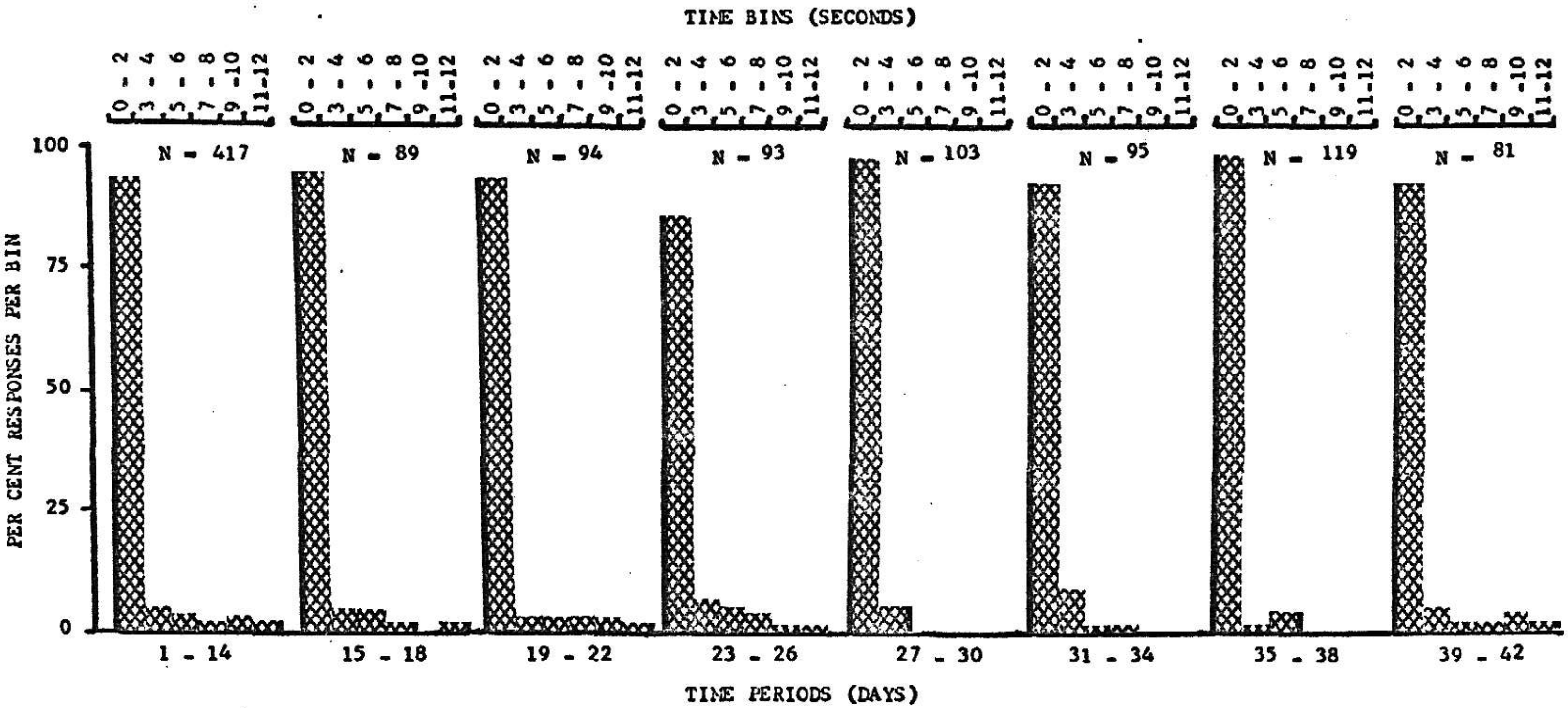
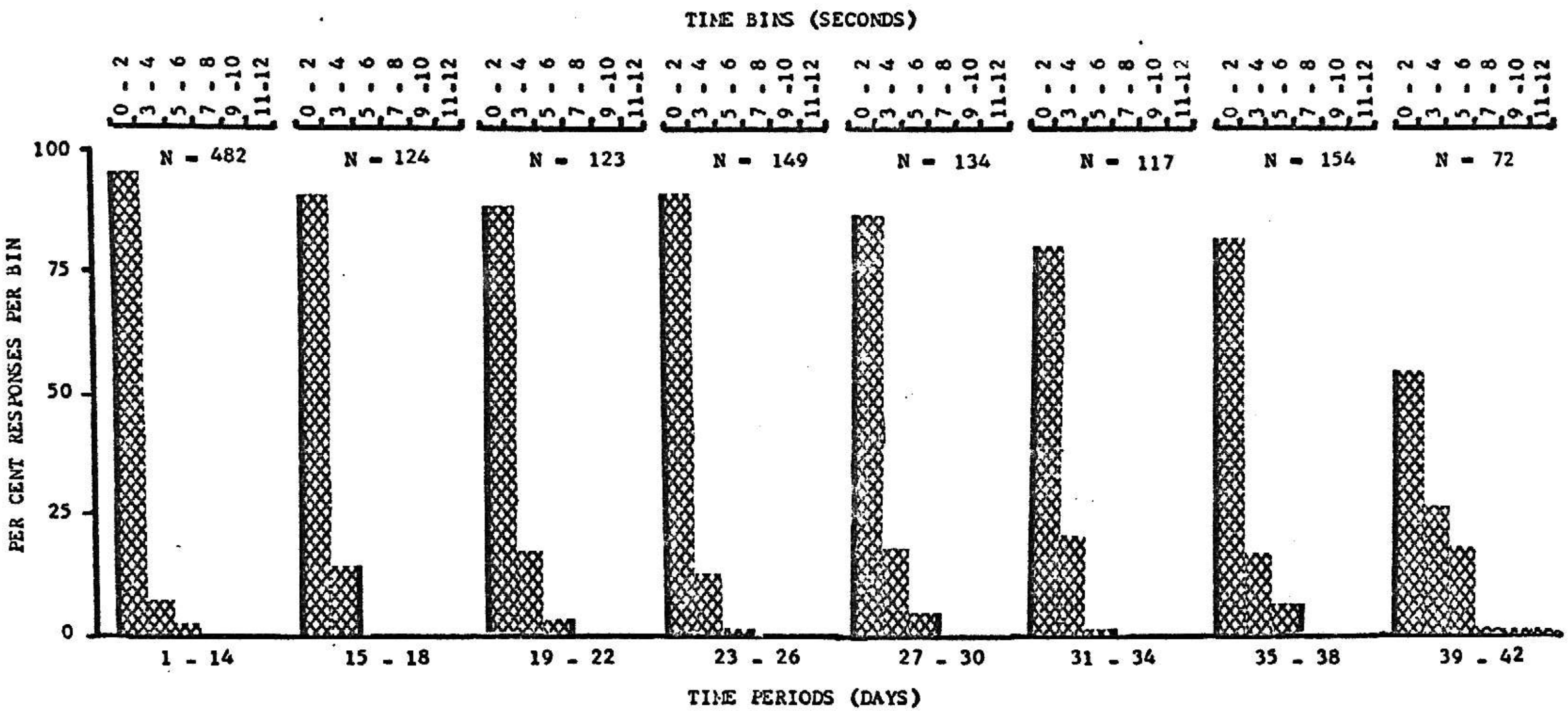

TIME BINS (SECONDS)
0 - 2
3 - 4
5 - 6
7 - 8
9 -10
11-12
N - 417
N - 89
N - 94
N - 93
N - 103
N - 95
N - 119
N - 81
100
75
50
25
0
PER CENT RESPONSES PER BIN
1 - 14
15 - 18
19 - 22
23 - 26
27 - 30
31 - 34
35 - 38
39 - 42
TIME PERIODS (DAYS)

Fig. 5. Percentages of daily responses per time bin for a bird typical of the quail group received 100 ug of dieldrin. Total responses for each time period are shown (N) while the number of responses for each time bin for each period appear in Table 3.



TIME BINS (SECONDS)
0 - 2
3 - 4
5 - 6
7 - 8
9 -10
11-12
N = 482
N = 124
N = 123
N = 149
N = 134
N = 117
N = 154
N = 72
PER CENT RESPONSES PER BIN
100
75
50
25
0
1 - 14
15 - 18
19 - 22
23 - 26
27 - 30
31 - 34
35 - 38
39 - 42
TIME PERIODS (DAYS)

Fig. 6. Percentages of daily responses per time bin for a bird typical of the quail group receiving 200 ug of dieldrin. Total responses for each time period are shown (N) while the number of responses for each time bin for each period appear in Table 3.

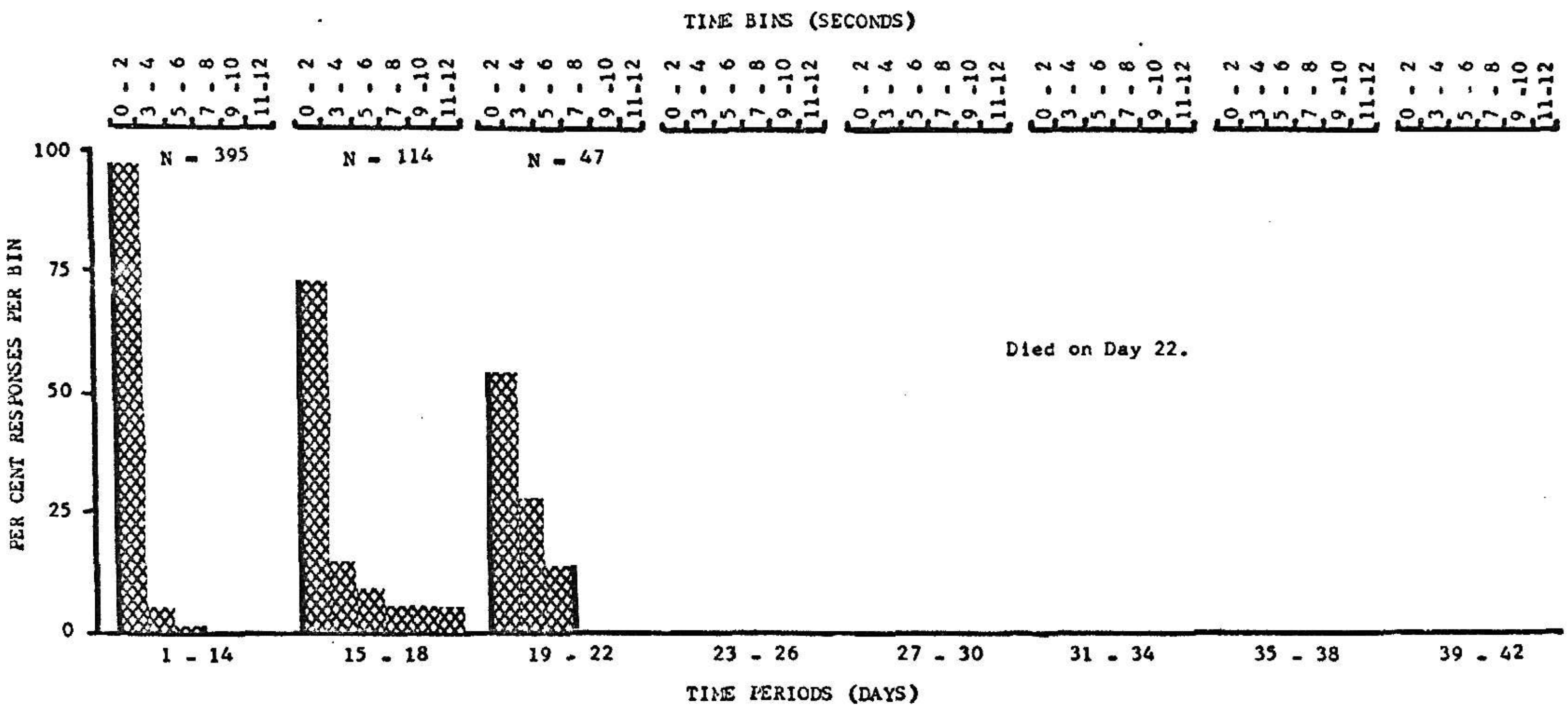

TIME BINS (SECONDS)
0 - 2
3 - 4
5 - 6
7 - 8
9 -10
11-12
100
75
50
25
0
PER CENT RESPONSES PER BIN
N - 395
N - 114
N - 47
Died on Day 22.
1 - 14
15 - 18
19 - 22
23 - 26
27 - 30
31 - 34
35 - 38
39 - 42
TIME PERIODS (DAYS)

Fig. 7. Percentages of daily responses per time bin for a bird typical of the quail group receiving 250 ug of dieldrin. Total responses for each time period are shown (N) while the number of responses for each time bin for each period in Table 3.

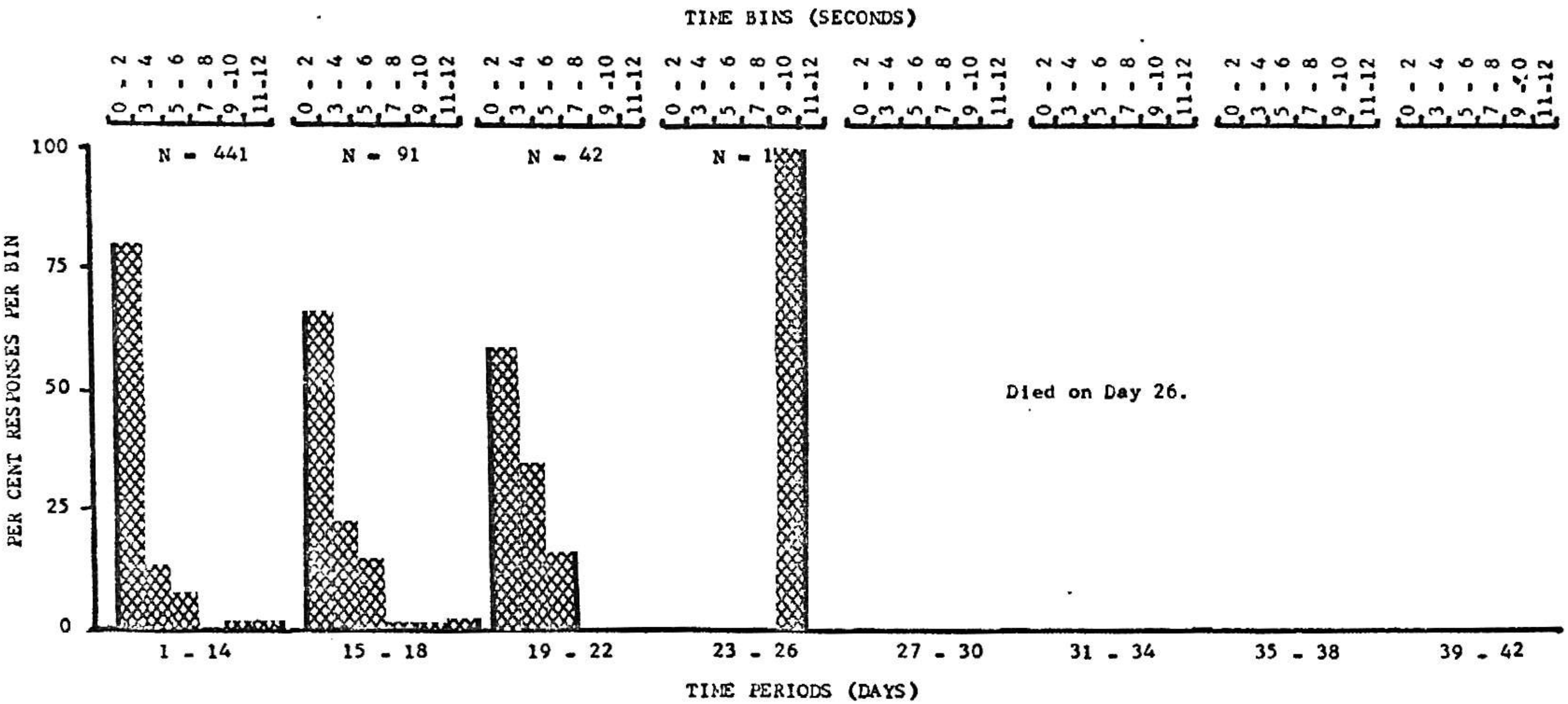

TIME BINS (SECONDS)
0 - 2
3 - 4
5 - 6
7 - 8
9 -10
11-12
N = 441
N = 91
N = 42
PER CENT RESPONSES PER BIN
100
75
50
25
0
Died on Day 26.
1 - 14
15 - 18
19 - 22
23 - 26
27 - 30
31 - 34
35 - 38
39 - 42
TIME PERIODS (DAYS)

Fig. 8. Percentages of daily responses per time bin for a bird typical of the quail group receiving 300 ug dieldrin. Total responses for each time period are shown (N) while the number of responses for each time bin for each period appear in Table 3.

wing tremores.

Behavioral changes observed in the operant box for some members of all pesticide groups except the 50 ug group included increased intertrial pecking (pecking between trials when the key lights were not lighted) and decreased peck accuracy. Decreased accuracy in aiming pecks was revealed by an increase in panel pecking. This panel pecking, missing of the key, normally occurs infrequently once the animal has learned the operant procedure. It was exhibited by many of the birds after dieldrin administration had begun, but not until the end of experimentation in the 100 ug group.

Mean squares for all variables for bird differences were significant (P 0.05) at all dieldrin levels (Tables 4-8). This was to be expected, as individual bird levels of food consumption, weight, and response time were quite different.

Behavioral Changes Within Variables

Total pecks: The first response at the onset of each trial was recorded as the only response for that trial. The total possible daily number of pecks per bird was forty. Pecks made on a key which was lighted red were recorded separately from those of the green key. However, erring pecks, those on a red-lighted key, were infrequent even among birds receiving high dieldrin dosages.

There was a significant difference ($P<0.05$) in the mean squares for total pecks over days in all groups except in the 50 and 250 ug groups (Table 4 and 7). Of these, a significant change occurred in the total number of pecks from days 1 - 14 versus days 15-40 in the 100 and 200 ug groups as well as from days 1-14 versus 15-25 in the 300 ug group (Tables 5-6, 8).

Total time: Total time for each experimental session was calculated by multiplying the total pecks recorded in each peck-time bin by the peck-time seconds of that bin, and summing these products for each bird (Table 10).

The only mean square for time which was found significantly different among days was that of the 200 ug bird group (Table 6). There was no significant change in the total peck time between the control days and the dieldrin testing days in the 50, 100, or 200 ug groups. However, total peck time changed significantly (P 0.05) with time from the control portion of the experiment, days 1-14, to the dieldrin portion in the 250 and 300 ug groups (Tables 7-8).

Time/Peck: Time per peck measured the average in seconds that elapsed before a response was made. This was calculated by dividing the total time recorded for the experimental session by the total number of pecks omitted during that session.

There was a significant (P 0.05) difference in time/peck means between days in the 200 ug and 300 ug groups (Table 6, 8). In the regression analysis, time/peck means were found to be significantly different in days 1-14 versus the dieldrin portion in all groups except the 50 ug group (Tables 5-8). The greatest change found was a linear change in the dieldrin portion of the experiment for the 100, 200, and 300 ug groups.

Weight before and Weight after the session: Each bird was weighed to the nearest gram before and after each experimental session.

Table 4. Mean squares from the response of birds for 40 days receiving 50 ug/0.5 ml dieldrin at 2-day intervals for the last 26 days.

		Mean Squares						
		Time			Intake		Weight	
SOURCE	df	Total Pecks	Total (sec)	Per Peck (sec)	Total (gm)	Per Peck (gm)	Before (gm)	After (gm)
Bird	4	1048.2*	2763.0*	1.38*	50.1*	0.040*	6356.0*	7500.4*
Days	39	40.7	176.8	0.18	5.1*	0.004*	84.3*	78.3*
Day (1-14) vs (15-40) (Control vs Dieldrin)	1	39.8	75.1	0.46	47.11*	0.070*		
Linear (1-14)	1	140.3	167.1	1.46*	2.07	0.014*		
Quadratic (1-14)	1	104.6	221.3	1.36*	0.96	0.008*		
Linear (15-40)	1	54.8	31.4	0.22	4.76	0.002		
Quadratic(15-40)	1	5.2	230.2	0.05	4.26	0.004*		
Error	156	43.4	175.4	0.15	2.10	0.001	37.8	42.9
	B_1:	.1756	-.1916	-.0179	-.0213	-.0018		
	B_2:	.1695	-.2466	-.0193	-.0162	-.0015		
	B_3:	.0433	-.0328	.0028	-.0128	-.0002		
	B_4:	.0079	-.0530	-.0008	.0072	.0002		

(* Significant $P < .05$)

$\hat{B}_1$ = estimated regression of response on days (day 1-14)
$\hat{B}_2$ = estimated regression of response on days squared (day 1-14)
$\hat{B}_3$ = estimated regression of response on days (day 15-40)
$\hat{B}_4$ = estimated regression of response on days squared (days 15-40)

Table 5. Mean squares from the responses of birds for 40 days receiving 100 ug/0.5 ml dieldrin at 2-day intervals for the last 26 days.

		Mean Squares						
		Time			Intake		Weight	
SOURCE	df	Total Pecks	Total (sec)	Per Peck (sec)	Total (gm)	Per Peck (gm)	Before (gm)	After (gm)
Bird	4	685.4*	2991.2*	9.18*	57.8*	0.017*	10151.1*	11718.5*
Days	39	85.2*	735.1	0.91	3.5	0.003	126.2*	119.3*
Day (1-14) vs (15-40)	1 df	466.9*	1209.6	8.77*	1.68	0.00		
Linear (1-14)	1 df	125.6	73.8	0.04	0.17	0.004		
Quadratic (1-14)	1 df	417.7*	99.6	0.35	0.20	0.010*		
Linear (15-40)	1 df	7.2	5947.9*	5.21*	12.53*	0.030*		
Quadratic (15-40)	1 df	215.7	6602.3*	1.69	0.19	0.006		
Error	146	57.8	699.5	0.73	2.9	0.002	48.5	53.9
	$\hat{B}_1$:	-.1662	-.1274	.0030	.0062	.0009		
	$\hat{B}_2$	-.3387	-.1654	.0098	.0074	.0017		
	$\hat{B}_3$	.0157	.4509	.0134	-.0207	-.0011		
	$\hat{B}_4$	.0513	.2839	.0045	.0015	-.0003		

* Significant ($P < 0.05$)
$\hat{B}_1$ estimated regression of response on days (day 1-14)
$\hat{B}_2$ estimated regression of response on days squared (day 1-14)
$\hat{B}_3$ estimated regression of response on days (day 15-40)
$\hat{B}_4$ estimated regression of response on days squared (day 15-40)

Table 6. Mean squares from the responses of birds for 40 days receiving 200 ug/0.5 ml dieldrin at 2-day intervals for the last 26 days.

		Mean Squares						
		Time			Intake		Weight	
SOURCE	df	Total Pecks	Total (Sec)	Per Peck (Sec)	Total (gm)	Per Peck (gm)	Before (gm)	After (gm)
Bird	4	989.5*	20523.8*	19.78*	8.5*	0.011*	4039.3*	4078.6*
Days	39	78.2*	490.1*	0.65*	4.5*	0.002*	134.0*	111.0*
Day (1-14) vs (15-40)	1	542.9*	601.3	5.71*	54.48*	0.020*		
Linear (1-14)	1	0.1	0.9	0.06	0.32	0.000		
Quadratic (1-14)	1	55.0	348.1	0.01	1.22	0.000		
Linear (15-40)	1	17.9	761.8	3.07*	11.53*	0.010*		
Quadratic (15-40)	1	329.4*	274.8	0.86	0.00	0.004*		
Error	132	42.9	311.8	0.35	2.5	0.001	20.1	24.0
	B_1:	-.0035	.0148	-.0037	-.0090	-.0002		
	B_2:	.1310	.3297	-.0012	.0195	-.0002		
	B_3:	-.0264	.1720	.0109	-.0212	-.0006		
	B_4:	.0676	.0617	-.0034	.0001	-.0002		

* Significant (P< 0.5)
$\hat{B}_1$ estimated regression of response on days (day 1-14)
$\hat{B}_2$ estimated regression of response on days squared (day 1-14)
$\hat{B}_3$ estimated regression of response on days (day 15-40)
$\hat{B}_4$ estimated regression of response on days squared (day 15-40)

Table 7. Mean squares from the responses of birds for 40 days receiving 250 ug/0.5 ml dieldrin at 2-day intervals for the last 26 days.

SOURCE	df	Total Pecks	Time Total (Sec)	Time Per Peck (Sec)	Intake Total (gm)	Intake Per Peck (gm)	Weight Before (gm)	Weight After (gm)
Bird	4	568.2*	738.8*	2.25*	36.9*	0.020*	5227.2*	5716.6*
Days	39	62.2	259.1	0.46	5.2	0.004	69.6*	65.8*
Day (1-14) vs (15-40)	1	16.8	2880.8*	4.10*	24.30*	0.020*		
Linear (1-14)	1	26.6	223.7	0.12	1.40	0.010		
Quadratic (1-14)	1	255.9	770.8	0.00	9.40	0.000		
Linear (15-40)	1	523.3*	1812.5*	0.00	0.00	0.020*		
Quadratic (15-40)	1	1.8	1279.5*	1.10	0.01	0.000		
Error	75	66.2	257.9	0.39	4.40	0.003	9.1	11.0
	$\hat{B}_1$:	-.0987	-.2863	-.0066	.0225	.0019		
	$\hat{B}_2$:	-.3423	-.5941	.0015	-.0655	-.0004		
	$\hat{B}_3$:	.1727	.3214	.0007	-.0003	-.0011		
	$\hat{B}_4$:	.0060	.1614	.0047	-.0005	.0001		

* Significant ($P < 0.05$)

$\hat{B}_1$ = estimated regression of response on days (day 1-14)

$\hat{B}_2$ = estimated regression of response on days squared (days 1-14)

$\hat{B}_3$ = estimated regression of response on days (day 15-40)

$\hat{B}_4$ = estimated regression of response on days squared (day 15-40)

Table 8. Mean squares from the responses of birds for 25 days receiving 300 ug/0.05 ml dieldrin at 2-day intervals for the last 11 days.

SOURCE	df	Total Pecks	Time Total (sec)	Time Per Peck (sec)	Intake Total (gm)	Intake Per Peck (gm)	Weight Before (gm)	Weight After (gm)
Bird	4	1215.5*	1317.0*	6.27*	13.7*	0.023*	4659.5*	4863.6*
Days	24	205.6*	688.0	3.75*	13.7*	0.008*	164.9*	145.8*
Day (1-14) vs (15-25)	1	2848.4*	3450.0*	58.30*	174.90*	0.040*		
Linear (1-14)	1	23.5	695.3	1.06	6.95	0.020*		
Quadratic (1-14)	1	55.5	23.9	0.12	0.77	0.007		
Lenear (15-25)	1	2520.9*	42.1	40.30*	168.50*	0.065*		
Quadratic (15-25)	1	366.0*	6278.4*	3.96	9.94	0.010		
Error	87	42.9	548.2	1.03	4.5	0.004	22.4	25.6
	$\hat{B}_1$:	-0.0742	0.4032	0.0157	0.0403	0.0022		
	$\hat{B}_2$:	-0.1273	-.0837	0.0059	-0.0150	0.0014		
	$\hat{B}_3$:	-2.2082	-0.2855	0.2791	-0.5709	-0.0113		
	$\hat{B}_4$:	-0.3013	-1.2578	0.0314	-0.0497	-0.0016		

* Significant ($P < 0.05$)

$\hat{B}_1$ = estimated regression of response on days (day 1-14)

$\hat{B}_2$ = estimated regression of response on days squared (day 1-14)

$\hat{B}_3$ = estimated regression of response on days (day 15-25)

$\hat{B}_4$ = estimated regression of response on days squared (day 15-25)

There was a significant difference ($P < 0.05$) in daily weights for all groups (Tables 4-8).

Total intake: Total intake for each session was determined by subtracting the test animal's weight before the session from that measured after his trials in the operant box.

Mean squares between days for this variable were significant for the 50, 200, and 300 ug birds (Tables 4, 6, 8). There was a significant difference (P 0.05) in food intake between the control days and the test days in birds of the 50, 200, 250, and 300 ug groups (Tables 4, 6, 8). This change was linear and revealed a decrease in intake during the dieldrin portion for the 100, 200, and 300 ug groups.

Intake/Peck

This variable represented the average amount of food eaten per 6-second reinforcement period. It was calculated by dividing the difference between the bird's weight before and after the session by the total pecks emitted during that session.

Mean square differences for days occurred in the feeding responses of the 50, 200, and 300 ug bird groups (Tables 4, 6, 8). Not only did the intake/peck means change significantly in the control versus dieldrin testing analysis for the 200, 250, and 300 ug groups; but also, they decreased in a linear fashion during the dieldrin testing for all three bird groups (Tables 6-8).

DISCUSSION

Weight changes

Statistical significance of the variables weight before and weight after data was to be expected for all bird groups. The birds had been starved to almost 60 per cent of their normal body weight during training. After they learned the procedure for obtaining food and adjusted to the sounds and light flashes characteristic of the box, their pecking and eating efficiency increased. Pecking efficiency increased as the birds learned that one peck brought a reward. In the beginning, all birds tended to peck several times at the key. This was a waste of energy and it shortened their feeding time. Feeding efficiency increased when the birds overcame their fear of the sound of the feeder as it dropped down at the end of the reinforcement period. The second reason for the significant weight gain over days was the administration of corn oil. These added calories dod not cause a decrease in operant responses because the corn oil was given to the test animal after his session in the operant box. The corn oil was digested before the next day's test session, and did not create a decline in the animal's desire for food. On the contrary, the regression mean square for total intake for days 1-14 vs 15-40 indicated an increased desire for food for all groups except the 100 ug group.

Behavioral Effects 50 ug bird group: The data analysis from the responses of the five quail in this group revealed that a dieldrin dosage of 50 ug/0.5 ml had no measurable adverse effect upon behavior. Significance of the variable time/ peck for days 1-14 merely indicated that as a group, these quail decreased their response time with the practice of the additional sessions. And it further substantiated the belief that the corn oil administration did not cause a decline in hunger.

<u>100 ug bird group:</u> Results of the regression analyses for this group indicated dieldrin had altered behavior. However, for the mean squares analysis for days, only the responses to the variable total pecks changed with time. Thus it was necessary to evaluate factors other than dieldrin which might have influenced behavioral changes. The underlying cause was found to be the decreased body weights of birds in this group. This, coupled with dieldrin administration, resulted in overreaction to the drug, and thus group analysis did not necessarily represent the average reaction to this dosage. Birds 1 and 5 began the control session with body weights below 70 per cent of their normal weight, as did birds 2 and 3 of the group. But the latter birds were able to increase their weights enough to maintain an average weight of 72 per cent of their normal weight. This was accomplished either by pecking more times per session, or by ingesting more grain per reinforcement period, or by a combination thereof (Table 9). Although bird 1 ate quite a bit per reinforcement, his average total daily pecks were not enough to provide him with a food intake sufficient to meet his daily energy requirement. Thus he actually lost weight during the experiment. Bird 5 had such a low intake per peck, that even a reasonable number of total pecks was insufficient to provide him with the necessary daily food intake. Those weight deficits in bird 1 and 5 probably accounted for the animals' disproportionate metabolism and storage of dieldrin in their brain. The other

Table 9. Individual response means for bobwhite quail receiving 100 ug/0.5 ml dieldrin every second day during the final 28 days of a 42 day experiment.

Bird	Pecks	Time (sec)	Time/peck (Sec)	Intake (gm)	Intake/peck (gm)
1	19.4 ± 1.9	44.5 ± 4.9	2.21 ± 0.16	3.46 ± 0.32	0.18 ± 0.01
2	26.2 ± 1.2	37.2 ± 4.2	1.46 ± 0.14	4.50 ± 0.27	0.17 ± 0.01
3	25.9 ± 1.2	59.1 ± 4.2	2.39 ± 0.14	4.40 ± 0.27	0.17 ± 0.01
4	32.0 ± 1.2	38.9 ± 4.2	1.23 ± 0.14	6.67 ± 0.27	0.21 ± 0.01
5	25.1 ± 1.2	44.4 ± 4.2	1.72 ± 0.14	3.88 ± 0.27	0.15 ± 0.01

three birds in this group averaged 2.28 ppm dieldrin residues in their brain tissue, whereas the dieldrin level in the brain of bird 1 was 7.48 and that of bird 5 was 5.73.

Interpretation of the results for the 100 ug group are difficult for it can be argued that a severe nutrient deficiency alone could begin a circle of events which would affect quail behavior to the extent of blatant abnormality. However, the effects of the pesticide cannot be altogether dismissed with brain residues over 5 ppm. Observations of birds 1 and 5 revealed outward symtoms comparable to those displayed by fatally dosed birds of other groups. Both animals crouched immovably in their cages with the characteristic fluffed feathers. Bird 1 stopped pecking altogether on day 32 of the experiment as did many birds receiving higher doses (Table 2). At that point, he had lost 7 gm from his weight at the beginning of the experiment. Nusz (1971) found that a weight loss occurred in quail that were fed dieldrin in corn oil, but not in quail that received corn oil only. Weight loss, immobility, and the cessation of operant responding, observed collectively, pointed more toward dieldrin as their cause than merely to an energy deficiency.

The normal peck time behavior of bird 5 was altered. Characteristically, this quail responded within 2 seconds after the onset of a trail responded within 2 seconds after the onset of a trail with only an occasional exception. His total pecks ranged from 18 to 40, but his responses were consistently recorded in the first bin of the electrical counters. Within 15 days after dieldrin administration began, his response time began to slow noticeably. One-fifth of his daily responses now occurred after 2 seconds had elapsed. During the last 8 days of the experiment, his response time became erratic. Half of his responses would occur within 2 seconds and half would occur randomly between 3 and 12 seconds after the onset of the key lights. The behavior of this bird, with brain residues of 5.73, was comparable to that of bird 1 of the 250 ug group which had dieldrin residues of over 12 ppm in his brain tissue (Fig. 7). Also, bird 5 experienced a decrease in total daily pecks. This decrease was not a result of a lack of hunger since he did attempt responses. Several times the animal responded just after a trial ended. Thus the response was not recorded in with his total pecks, and the bird did not receive reinforcement for his effort. Instead, the pecks were recorded by the experimenter as intertrial pecking.

Dieldrin affected two of the birds of this group. Since these two out of five birds in the group stored high levels of dieldrin in their brain and experienced definite behavioral changes, variables measured in terms of group action would be expected to show a statistical significance ($P < 0.05$) when measured for a change with time.

<u>200 ug bird group</u>

That dieldrin affects quail behavior is quite apparant at this dosage level. Three of the five group members died before completion of the 42-day test period (Table 2). Of the two remaining birds, both exhibited an altered peck time (Fig. 6). Originally, 81 per cent of the responses of both birds occurred within the first 2 seconds of each trial. After 24 days of dieldrin testing, only 58 per cent of their responses occurred during the first 2 seconds of timing. Total pecks fluctuated little, but response times were slowing.

Daily fluctuations became extreme. One day, only 58 per cent of the responses were within the first 2-second interval. The next day, 85 per cent of the responses occurred before 2 seconds elapsed. By day 42, both birds exhibited definite operant behavioral changes due to the dieldrin. Only 27 per cent of the responses of bird 1 occurred before 2 seconds elapsed, and bird 4 made no response whatsoever on day 42. During dieldrin testing, all the quail of the 200 ug group adopted the frozen, fluffed feather posture and ceased normal vocalization. The onset of this behavioral change was individually staggered, but once the modification of behavior had begun, the process never reversed. Although bird 4 did not die, on day 42, he was unable to suppress the body tremors which were characteristic of the birds that died. These tremors, a wavering stance, and a weight level below 59 per cent of his normal body weight, made death appear imminent.

All variables analyzed for the 200 ug group showed a significant change ($P < 0.05$) with time except for the regression analysis of peck time. This appeared inaccurate, therefore the voluminous pages of daily records, inclusion of which in this work would be impractical, were reviewed. An alteration in the pattern of the individual daily peck times recorded for the quail of this group was apparent: The peck time pattern for all quail of this group changed as a result of the dieldrin. In general, the pattern of change was comparable to that of the two members described previously in this section.

However, significance did not appear in the regression analysis for this group. This was because of the manner in which the data was handled for computerization. Through this handling, it was possible for the peck time for one session in which all pecks were emitted within the first 2 seconds after the onset of the lights to appear equal to that in which response times had occurred after 7 seconds had elapsed (Table 10). In such an instance, the time/peck calculation must determine the existence of a behavioral change. Such was the case in the results for the 200 ug bird group. There was a significant change in the time/peck mean over days, and a significant linear change with time in the group's time/peck variable in the regression analysis.

<u>250 ug bird group</u>

A significant difference ($P < 0.05$) was detected in all variables from day 1-14 as compared to those of days 15-40; with the exception of total pecks. Contrary to expectation, none of the mean squares analysis revealed a significant difference between days for any of the variables except weight. Yet dieldrin did affect the quail in this group. Symptoms of the poisoning were apparent, and four of the birds died with dieldrin residues of over 9.00 ppm in their brain. Behavioral changes in the normal responses of birds 2, 4, and 5 followed the general pattern shown in Fig. 7. Simple computer analyses were misleading. The problem of detecting changes in the variables arose because of the rapidity and degree of behavioral alteration that occurred. Responses were collected daily from all five birds for calculating group means of the variables for days 1-14. But from day 15 until the completion of the experiment, progressively fewer birds contributed their responses to

Table 10. Three response patterns of bobwhite quail resulting in the same total peck time.

	Response times (sec)							
Bird	0-2	3-4	5-6	7-8	9-10	11-12	Total	Total peck time
1	37	3	-	-	-	-	40	37(1.0) + 3(3.5) = 47.5
2	2	5	2	1	1	-	11	2(1.0) + 5(3.5) + 2(5.5) + 1(7.5) + 1(9.5) = 47
3	-	-	-	2	1	2	5	2(7.5) + 1(9.5) + 2(11.5) = 47.5

the daily group means. As of day 28, the group mean for all variables was determined solely by the responses of one bird. The rest of the group had either stopped pecking or died. Therefore, the mean squares analyses and the regression analyses for days 15-40 were not necessarily representative of the group. Also, since the means of the variables for the control were determined by the responses of five birds, it was not possible for the computer to reveal a behavioral change in an individual bird unless the mean of his normal responses coincided with that of the group. Thus the mean squares analyses for days revealed no change.

300 ug bird group.

This dosage was so strong that it promoted immediate behavioral changes and a rapid death for all animals in the group. Because of this, regression analysis involved 25 days rather than 40 days. The first 14 days were analyzed in the usual manner. Because of these adjustments, it was possible to detect behavioral changes which occurred as a result of dieldrin administration. The mean squares for changes with time were significant (P 0.05) for all variables except total time. The inability to detect any change in total time by the one-way analysis of variance was due to the abruptness of change in the animals' behavior. The pesticide dosage, 300 ug, was so strong that the response patterns shifted from normal to zero responses within 11 days after dieldrin was first administered. Death occurred by day 27 in all birds of this group.

Curiously, the brain analysis for one bird of this group revealed 3.04

ppm dieldrin as compared with a mean residue of 14.02 for the other four quail in the group. This level was comparable to residues in the brains of birds from the 50 and 100 ug groups. Although the per cent of normal body weight of this animal was higher than that of his comrades at the onset of the experiment, and metabolic rates differ, it is unlikely that the range in deposition would be so large for a group of animals receiving the same dosage and subject to the same environmental stress. The symptoms exhibited by this bird, the change in his operant behavior, and his early death suggested an error in residue analysis.

Management aspects

Pesticide residues have been found in tissues of birds sampled throughout the United States (Hickey and Hunt 1960, Scott et al. 1959, Stickel 1968, Greichus et al. 1968, Linder and Dahlgren 1970, Martin 1969, Reichel et al. 1969, Mulhern 1970). In many cases, dieldrin residues in the brain of these animals averaged less than 1.00 ppm. This level is quite low and is well below that found in the brain of most of the test animals in this study. However, several factors should be considered before dismissing the field residues as insignificant. In most of the field studies conducted on agricultural sites, sampling has occurred several months to 2 years after the pesticide application (Martin 1969, Linder and Dahlgren 1970). This delay could bias the results, since the birds receiving higher pesticide dosages could have succumbed to the dosage or to natural mortality, or may have excreted much of the dieldrin before sampling occurred. Even so, reports of high dieldrin levels in the brain of some birds are not uncommon. Reichel et al. (1969) found dieldrin residues of up to 8.0 ppm in the brain of bald eagles (Halieutus leucocephalus) in 1964. And Mulhern et al. (1970) reported dieldrin residues in the bald eagle's brain of up to 9.5 ppm in 1967.

An analysis of the feeding habits of adult bobwhites reveals that the birds are exposed to levels in the field which are comparable to the dosages in this study. Rosene (1969: 107) found that the animal matter in the diet of adult quail in the summer totaled up to 30 per cent of the daily intake. Case (1971) reported a dry weight consumption of approximately 16 grams per day when the ambient temperature is 25° C. Thus an adult quail would be eating approximately 4.8 grams of animal matter per day in the summer. Korschgen (1970) reported from a study of Missouri cornfields that 1 gram of Poecilus beetles, a common ground beetle composing 72 per cent of a juvenile quail's diet, contained 90.51 ppm dry weight of dieldrin and aldrin residues. A total of 17 beetles weighed 1 gram. An adult quail feeding on this area could consume as much as 4 grams of Poecilus beetles, thus ingesting a total of 362 ug of dieldrin-aldrin residues in his daily feeding. The highest dosage in my study was 300 ug of dieldrin per 2 days. Four grams of beetles might be an overestimate of consumption, but 1 gram of this beetle per day is reasonable. If such is the case, the adult quail on these fields would receive 181 ug of the pesticides every 2 days. Thus behavioral changes could be expected in these birds comparable to those observed of the birds in the 50, 100, and 200 ug groups in my study.

Another factor to consider is that birds respond differently to exposure to drugs. Age, sex, genetics, existing environmental conditions, and body weight are factors which combine to determine an animal's response to a drug (Thompson and Schuster 1968). Therefore one bird might suffer only minor effects from a small dosage of dieldrin that proved fatal to another bird.

Behavioral changes which I observed in bobwhite quail as a result of dieldrin administration included a cessation of feeding and normal vocalization, an increase in response time, and a loss of accuracy in pecking. All of these could contribute to increased mortality in animal populations due to increased vulnerability to predation, injury, and disease, and through inappropriate behavior which would cause a decline in breeding success.

Behavioral patterns that are genetically determined depend upon post-hatching stimuli for their development (Collias 1952: 127). Avoidance behavior is not present at hatching in Co turnix quail (Schaller and Emlen 1962: 372, Rubel 1970: 427), rather it developes within 61-70 hours (Schaller and Emlen 1962: 373). Before its development, a chick will accept the presence of objects that are noisy, large, and animated. After avoidance behavior developes, the chick will exhibit fear responses toward every object and will continue to do so until a positive reinforcement can be connected with the object. Strangeness must be overcome by familiarity. The possibility does exist that dieldrin could affect this development in young quail in the field (Rosene 1969, Revzin 1966). Perhaps a bird could develop this familiarization toward improper objects. A loss of discrimination could be the cause.

He may not experience the degree of fear necessary for him to adopt the behavior that would protect him from predation. The usual alarm response of the bobwhite when encountering a strange object is to approach the situation with head held forward and erect, moving it from side to side to better discern the object (Stokes 1967). At this time the quail's discrimination ability, interpretation, and response time determine whether this bird will survive as will other covey members dependent upon his signal. The importance of reaction time and discrimination cannot be overemphasized. Dieldrin alters these skills, thus it could increase the quail's vulnerability to natural mortality.

Birds normally expend some portion of their available energy pecking at inanimate objects. However, a bird whose discrimination is impaired by dieldrin might waste large amounts of energy upon objects which appear familiar and might actually ingest large amounts of non-digestible materials. Selection of a suitable winter range might even be hindered by dieldrin ingestion. In winter, quail must have an area which combines protective cover with exposed ground for feeding (Rosene 1969). Poor selection of this range could result in the death of the entire covey.

Mating failure could result from dieldrin ingestion (Dustman and Stickel 1966, Dewitt 1955, Atkins and Linder 1967, Genelly and Rudd 1956). Visual signals are important in the lives of many birds. Broodiness developes in chickens and prevents the hens from attacking her chicks (Collias 1952). Dominance hierarchies, mate selection, and group and mate recognition are all developed through early visual experiences (Smith 1967, Guhl 1956, Hess 1958, and Collias 1952). In this development, the animal must learn to discriminate between members of his group, and between group members and outsiders. Also, he must learn the proper responses expected of him. If he is unable to learn these correctly or if his normal behavior is altered, he may not be accepted as a mate and he might not be accepted in a covey. One interesting point observed by Stokes (1967: 8) was that a male quail challenges his mate with a frontal display before accepting her. If the male's discernment were impaired, he might ward her off his territory believing her to be an intruder. On the other hand, failure to recognize a challenger could cause a loss of a mate or territory.

With the possibility of such implications to game management, it is apparent that more pesticide research is needed. The direction of this research is of utmost importance. Environmental monitoring of pesticides should continue, but now more emphasis should be directed toward determining the effects of these residues on wild animals. More substantial biochemical studies of the chlorinated hydrocarbons must be completed. Although it is generally agreed that these chemicals disrupt nervous activity, exact properties are not known. To what extent these drugs disrupt the nervous system, the length of this disruption, and the extent of disruption of normal behavioral activity, are topics for which there must be data before the continued use of these chlorinated hydrocarbons can be justified.

Further expansion of knowledge, that of determining the effects on normal behavior in the environment, must occur. Fatality studies are numerous, but what of the animals receiving sublethal doses of these pesticides? Long term experiments need to be designed to answer this question. Groups of game animals, pen-reared on game farms, could be maintained on pesticide-dosed food until reaching maturity. At this time, the groups could be banded and released on selected test areas. Interrelationships in the groups, territoriality, feeding habits, mating and nesting behavior, and mortality could all be studied. If the observer were someone who had a knowledge of the behaviour of "normal" populations of this species, fine points, as well as extreme differences might be recorded. Of course such long-term projects would be costly and time-consuming, but they are necessary and must be completed if we are to fully comprehend the role of pesticides in the environment. And there are equally important studies to be done on a smaller scale. Laboratory studies of egg-laying, egg viability, and broodiness of female birds receiving chlorinated hydrocarbons need to be refined and/or re-tested using wild species. More operant tests which require finer discrimination abilities, such as movement of the test object, or a series of pattern choices, need to be developed. Disease susceptibility in conjunction with pesticide exposure is another area to be further investigated. And there are yet numerous other areas to be considered and researched before the environmental impact of the chlorinated hydrocarbons can be determined.

SUMMARY AND CONCLUSIONS

This project was initiated in September of 1969 to study the effects of organochloride pesticides on the behavior of bobwhite quail. Equipment was developed to test discrimination ability, reaction time, and feeding behavior. After a period of training, 25 quail were tested for 2 weeks to gather individual baseline data for the variables. Five 5-bird groups were then randomly assigned a dieldrin dosage of either 50, 100, 200, 250, or 300 ug to be administered at 2-day intervals for the next 28 days of behavioral testing. Upon completion of this testing, all quail were sacrificed and their brain was removed for pesticide residue analysis by gas chromatography.

Analysis of data collected during this study supports the following conclusions.

1. Operant testing methods are suitable for measuring behavioral changes in bobwhite quail.

2. Individual uptake and storage of a pesticide differs among birds when the age, sex, genetic strain, and environment are held constant.

3. Dieldrin ingestion causes a loss of body weight, and the body weight at the time of ingestion largely determines the effect the dieldrin will have upon the bird.

4. Behavioral changes were not detected in birds receiving 50 ug of dieldrin.

5. Reaction time slowed significantly ($P < 0.05$) with the ingestion of 100 ug or more of dieldrin.

6. Feeding efficiency and appetite declined with the ingestion of 100 ug or more of dieldrin.

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APPENDIX

Table II. Normal weight, weight at onset of experimentation, and average weight throughout experimentation of 25 bobwhite quail involved in dieldrin experiment.

	Bird no.	Normal Weight (gm)	Day 1 Weight (gm)	Per cent Normal	Average wt. Maintained (gm)	Per cent Normal
50 ug	1	160	128	80	139.6	87
	2	152	130	86	142.7	94
	3	162	119	73	119.0	73
	4	172	114	66	116.8	68
	5	151	130	86	140.0	93
100 ug	1	185	120	65	118.8	64
	2	177	109	61	128.1	72
	3	178	121	68	128.9	72
	4	192	140	73	161.3	84
	5	180	123	68	127.2	71
200 ug	1	166	110	66	126.5	76
	2	177	130	73	142.7	81
	3	186	118	63	143.2	77
	4	206	137	67	154.6	75
	5	170	134	79	138.8	82
250 ug	1	171	113	66	126.4	74
	2	178	111	62	126.4	70
	3	186	125	67	140.0	75
	4	206	118	53	126.3	61
	5	206	146	71	162.5	79
300 ug	1	202	149	72	159.8	79
	2	168	107	64	119.9	71
	3	195	135	69	143.4	74
	4	173	123	71	137.3	79
	5	192	128	67	134.4	70

THE EFFECTS OF DIELDRIN ON THE BEHAVIOR OF *COLINUS VIRGINIANUS*

by

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A study was initiated in September of 1969 at Kansas State University to determine the effects of dieldrin on bobwhite quail (*Colinus virginianus*) behavior. Behavior to be tested included color discrimination, reaction time, and feeding.

Quail were taught to respond to operant conditioning techniques. Then baselines of behavioral responses were determined on an individual basis. The animals were randomly assigned to dosage groups of either 50, 100, 200, 250, 250, or 300 ug of dieldrin. Daily behavioral testing continued with dieldrin administration occurring at 2-day intervals for the following 28 days. At the completion of the experiment, the brain tissue of each bird was analyzed by gas chromatography for pesticide residues.

Quail that received 50 ug of dieldrin had average brain residues of 2.58 ppm. Residues in the 100 ug group averaged 4.01 ppm. Residues of 11.07, 11.13, and 11.82 ppm were found in the brain tissue of the 200, 250, and 300 ug dieldrin groups, respectively.

Behavioral changes were not detected in the 50 ug group. Red-green color discrimination was not affected by the dieldrin levels used in this study, however, response time (the time it took a bird to select the correct key color) slowed with the ingestion of 100 ug or more of dieldrin. Quail receiving 100 ug or more of dieldrin experienced decreased feeding efficiency, decline of appetite, and weight loss. Deaths occurred in bird groups receiving 200, 250, and 300 ug dieldrin dosages.