SOME EFFECTS OF EXPERIMENTAL FEEDING OF DDT TO *COTURNIX COTURNIX JAPONICA* TEM. & SCHL.

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

David L. Cross

1960

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SOME EFFECTS OF EXPERIMENTAL FEEDING
OF DDT TO *COTURNIX COTURNIX JAPONICA*
TEM. & SCHL.

by

David L. Cross

Submitted to the College of Science and Arts of Michigan
State University of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1960

Approved Ray Hutson

ABSTRACT

Experiments were conducted in 1958 and 1959 to evaluate the effects of DDT-treated food on Japanese quail, *Coturnix coturnix japonica*. This study was conducted on the Michigan State University campus, East Lansing, Michigan.

The following six tests were made: (A) 30-day-old birds were fed concentrations of DDT at 1, 9, 90, 900, and 9000 p.p.m. for a period of 30 days; (B) 50-day-old birds were fed concentrations of 100, 300, 500, and 700 p.p.m. DDT for a period of 30 days; (C) 25-day-old birds were fed concentrations of 100, 300, 500, and 700 p.p.m. DDT for a period of 55 days; (D) 50-day-old second-generation birds from test C were fed acetone-treated food for a period of 20 days; (E and F) feed was withheld from birds remaining from test C as well as from 25-, 35-, and 50-day-old untreated birds.

The Japanese quail proved to be a useful and efficient laboratory animal for this work. DDT did not affect number or weight of eggs laid, nor hatchability. Although the evidence was not statistically significant, there were indications that lower levels of DDT (on the order of 100 p.p.m.) increased the hatchability of eggs. The higher concentrations of DDT (500 and 700 p.p.m.) affected food consumption and resulted in decreased body weight, followed by increased mortality. There is an indication that males were more susceptible to DDT than were females in survival studies. When starved, older birds survived longer than younger ones. There were indications that DDT was stored in the fat at all levels of concentration.

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INTRODUCTION

The insecticidal properties of DDT have led to its widespread use in combating insect populations throughout the world. The increasing use of DDT has aroused much interest in the possible effects of this poison on wildlife. Since World War II, numerous investigators have sought to determine the side effects of insect control with DDT. Previous investigations have shown that varying dosages of DDT have different effects and that environmental factors are important in determining the effects of DDT poisoning.

The main objectives of this study were: (1) to evaluate the use of the Coturnix quail, *Coturnix coturnix japonica*, as a laboratory research animal and (2) to study the effects of given amounts of DDT mixed with food on immature, mature, and second-generation Coturnix quail.

The effects studied were: (1) amount of food consumed per day; (2) body weight gain per day; (3) weight of eggs; (4) number of eggs laid; (5) ratio of fertile to infertile eggs; (6) survival of birds; (7) percent mortality; (8) body weight of birds in relation to sex, age, and number of days on varied DDT diets; and (9) effects of starvation diets on untreated and DDT-treated birds.

REVIEW OF LITERATURE

A review of literature on bird poisoning indicates that many workers have investigated the effects of the insecticide DDT upon adult and nestling birds.

Woodard et al (1945) noted that DDT accumulated in the body fat and in such lipid-rich materials as egg yolk and milk of dogs. Woodard and Ofner (1946), working with rats, found that if exposures were continuous the level of DDT in the body fat tended to reach a steady plateau; balanced by a slow, steady elimination.

Laug and Fitzhugh (1946) reported that prolonged exposures to DDT affected breeding potential of birds even in the absence of immediate effects, and that sub-lethal amounts accumulated in tissues and organs.

In 1946, Hotchkiss and Pough found that a single aerial application of DDT in oil at 1 pound per acre to a forest in Northern Pennsylvania did not disturb bird populations, but that an experimental application of 5 pounds per acre produced a marked reduction of numbers. Similar results were reported by Stewart et al (1946). It was noted that a single application rate of 2 pounds DDT per acre at the Patuxent Research Refuge had little or no effect on the area except for redstarts, Setaphaga ruticilla. It was believed that the DDT collected near the tree tops, probably causing redstarts to receive more than the average dose of DDT. It was not clear from this source whether it was the accumulation of DDT in tree tops or the biology of the redstarts that was important or both. In 1946, Coburn and

Treichler found the acute toxicity of DDT for bob-white quail to be 60 to 85 mg/kg of body weight when administered orally in oil solution and about 300 mg/kg when administered orally in crystalline form. Cottam and Higgins (1946) reported that swallows were unaffected at low rates of application, but were observed to leave areas treated with DDT at 0.8 pounds per acre and higher, probably in response to decreased availability of food. While working at the Patuxent Research Refuge in 1946, Mitchell found that spraying of nests, eggs, and nestlings at 5 pounds DDT per acre produced no appreciable effects.

Nelson and Surber (1947) found no significant changes in total bird populations in areas treated for spruce budworm control with 1.1 pounds or 3 pounds DDT per acre. On the other hand, they report that a dosage of 5 pounds DDT per acre caused a 15 percent decline in total bird populations. Fitzhugh _et al_ (1947) fed rats a diet containing concentrations of 100 to 800 p.p.m. DDT. Toxic effects were observed at all levels of concentrations. This test was conducted for a two-year period with treatments being applied once a year. An increased intake increased toxicity. Concentrations of 400 to 800 p.p.m. in the diet retarded growth in female rats whereas 800 p.p.m. retarded growth in male rats. DDT did not affect food consumption.

Robbins and Stewart (1949) found that when DDT was applied at 5 pounds per acre to five-year-old scrub and sapling growth in Beltsville, Md, the total decrease of the five most common species of birds, which represent 77 percent of the original population, was 65 percent. Adams _et al_ (1949) found no effects on bird populations when 1 pound of DDT per acre was applied, but felt that 5 pounds and 7.5

pounds of DDT per acre may have caused a decrease in bird population. In 1949, Odum and Norris noted that when Georgia pecan orchards were sprayed in late summer with 6.5 pounds of DDT per acre a late summer census showed no real change in the bird populations. The reasons given were that the summer range was outside the grove, the nesting period was over, and few birds were attracted to the DDT-killed insects.

While experimenting with rats in 1950, Laug et al found that accumulation of DDT in the fat occurred at every level of intake, down to and including 1 p.p.m. and that greater storage occurred in the female. There was a progressive rise in storage with age, reaching a maximum at 19 to 23 weeks of age. The age of the animal did not appear to affect the rate at which DDT was stored in the fat. It was found that from 50 to 75 percent of the fat-stored DDT remained one month after a DDT-free diet was resumed and that 25 percent remained after three months. Draper et al (1950) conducted tests on chickens using DDT-treated mash and alfalfa dusted with DDT, ground and included in a mash. Analyses showed that concentrations of DDT in fat tissues of birds fed this mixture was 15, 271, 598, 960 p.p.m. when fed mashes containing 0, 50, 100, 200 p.p.m. DDT respectively and 4, 71, 61, and 146 p.p.m. when fed mashes containing hay dusted with 0, 1, 2, and 4 pounds of DDT per acre respectively. There was no apparent difference in final weight or mortality of the birds except for a higher mortality in the lots fed 200 p.p.m. of DDT in their mash.

Robbins et al (1951) reported that five yearly applications of DDT at 2 pounds per acre caused a 26 percent decrease in breeding bird populations. Benton (1951) while using 2 percent DDT spray in treating Dutch Elm Disease noted a 19.6 percent decrease in the breeding population of birds occurred after spraying. Two weeks later there was a 6 percent increase in the check areas while treated areas showed a 22 percent decrease.

Mitchell et al (1953) reported that in the summers of 1949 and 1950 at the Patuxent Research Refuge, a two-year aerial application of DDT at 3 pounds per acre resulted in a slight decrease in total bird populations. There was no apparent weight difference between second broods in treated or untreated areas. Considerable mortality to young nestlings occurred. Reduction of insect food was uncertain. The amounts of DDT ingested by birds covered a wide range and no average intake was known. It is not clear from this source what actually caused the high mortality to young nestlings as it was not determined whether the young died as a result of DDT or due to the lack of food.

Experimenting with quail, Dewitt (1955) reported feeding diets containing 0.02 percent DDT to breeding quail. Significant decreases in hatchability of eggs and in viability of chicks resulted throughout the breeding season. The 0.02 percent DDT treatment had no apparent effect on quail body weights and numbers of eggs produced. Dewitt et al (1955) ran tissue analyses of DDT-fed quail and pheasants. Indications were that quantities of DDT stored were not proportional to total amounts administered nor to duration of exposure, but were related to the severity of toxic symptoms. Concentrations of DDT in breast muscle of adult quail dying of acute DDT poisoning was approximately 34 µg/gm and in adult pheasants 22 µg/gm.

Barker (1958) reported that analyses of leaves, soil, earthworms, and robins suggested that fewer than 100 earthworms can accumulate 3 mg of DDT. This was approximately the median amount found in dying robins. Hoffman et al (1958) noted that applications of 1 pound of DDT per acre showed DDT was not immediately harmful to "mountain" grouse populations.

Reviews of literature pertaining to insecticides and pesticides have been assembled by Rudd et al (1946) and by Neghebon (1959). The majority of the studies reported in these reviews have been based largely on effects of field spray applications on adult and nestling birds and to a lesser degree on laboratory studies. Comparison of these studies indicates that different rates of application and different environmental conditions have contributed to substantial differences in results.

EXPERIMENTAL METHODS AND MATERIALS

Test Animals

The Japanese Quail, *Coturnix coturnix japonica*, Temminck and Schlegel was used as the test animal. Peters (1934) classified the genus *Coturnix* into five species and 14 races. Of the species with which we are concerned, Peters listed eight races, but only seven are recognized by Taka-Tsukasa (Stanford 1957). The validity of the two major races, *Coturnix coturnix coturnix* and *Coturnix coturnix japonica*, the European and the Asiatic races respectively, seems to be questioned by some taxonomists notably Taka-Tsukasa. The feeling has been expressed (Stanford 1957) that the two races may be one.

The Coturnix quail was first introduced into this country in the 1870's and 1880's; also during the period from 1900 to 1925. During this period releases of the European race were made in the Eastern portion of our country, and releases of the Asiatic race were made on the Pacific Coast. In both cases these birds failed to establish themselves and disappeared entirely (Stanford 1957).

More recently the *Coturnix* was introduced into this country from Japan by the Missouri Conservation Commission in order to determine whether it could become established ecologically and thus supplement existing game-bird species. To date, there is little evidence that the *Coturnix* has been established as a wild-game species (Stanford 1957).

Padgett and Ivey (1959) found the *Coturnix* to be very satisfactory as a laboratory research animal, especially to embryologists and physiologists. It was found by the writer to be extremely hardy and easy to raise. It reproduces at 6 weeks of age, is very prolific and does not seem to be susceptible to any of the common diseases of the bob-white quail.

Handling of Test Animals

Breeding. The breeding pen was 10' long, 8' wide, and 6' high. It contained a gravel floor with sides of one-fourth inch mesh hardware cloth and a ceiling composed of cardboard and burlap cloth. Constant lighting was furnished by a 150-watt incandescent light bulb.

Rearing. The rearing pen was 5' long by 2 1/2' wide by 4' high and contained a 4 3/4' long by 2' wide by 1/3' high inner galvanized metal container half filled with gravel. The sides and top of the pen were covered with mosquito netting. The rearing pen was heated by two 150-watt incandescent light bulbs surrounded by a reflector hood. The hood was placed nine inches above the center of the floor area.

Holding. Holding pens consisted of cardboard boxes (3' x 3' x 3') covered with galvanized window screening. The floor area was covered with gravel.

Experimental. Each pen (Plate I-A) consisted of five individual compartments (Plate I-B). Each compartment was one-foot on a side, screened on two sides and the top with one-half inch mesh hardware cloth. The top was covered with removable plastic covers to prevent restlessness and the possibility of wastes being passed downward from one compartment to another. Each compartment was separated from the

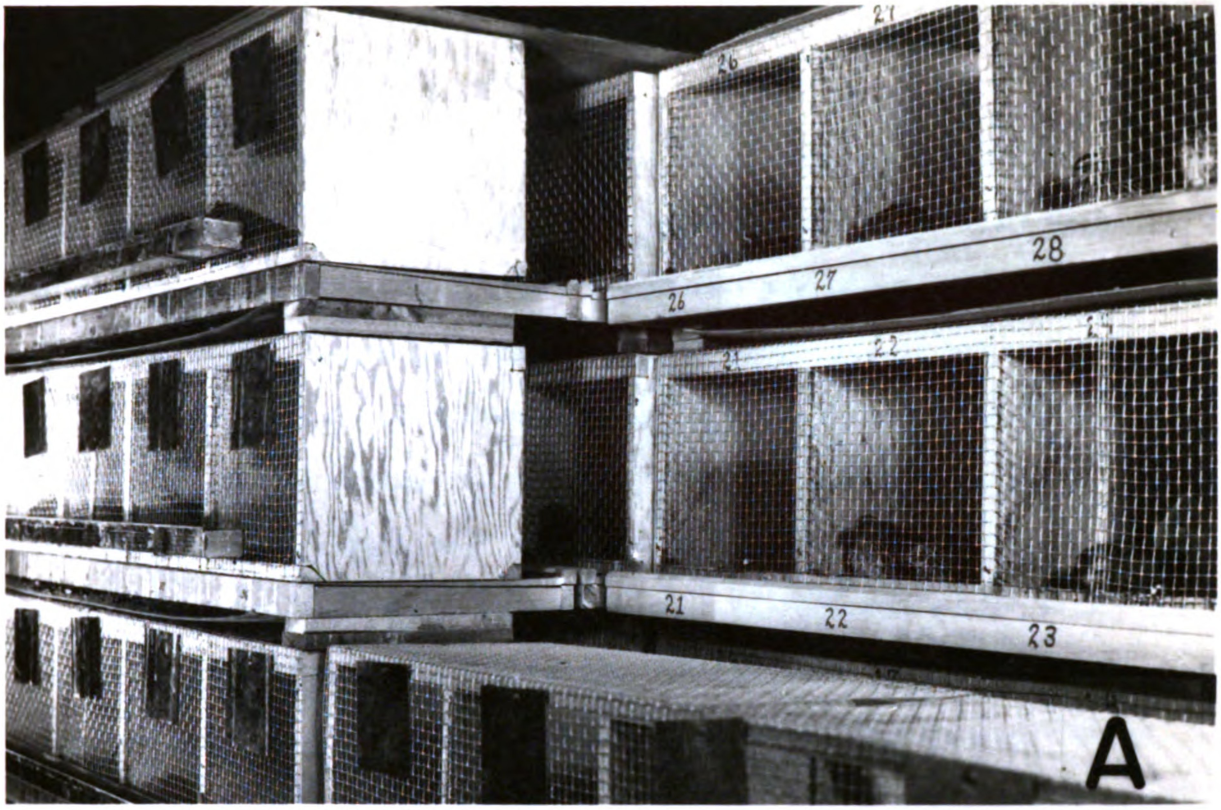

Plate I-A. Bird pens used for experiment.

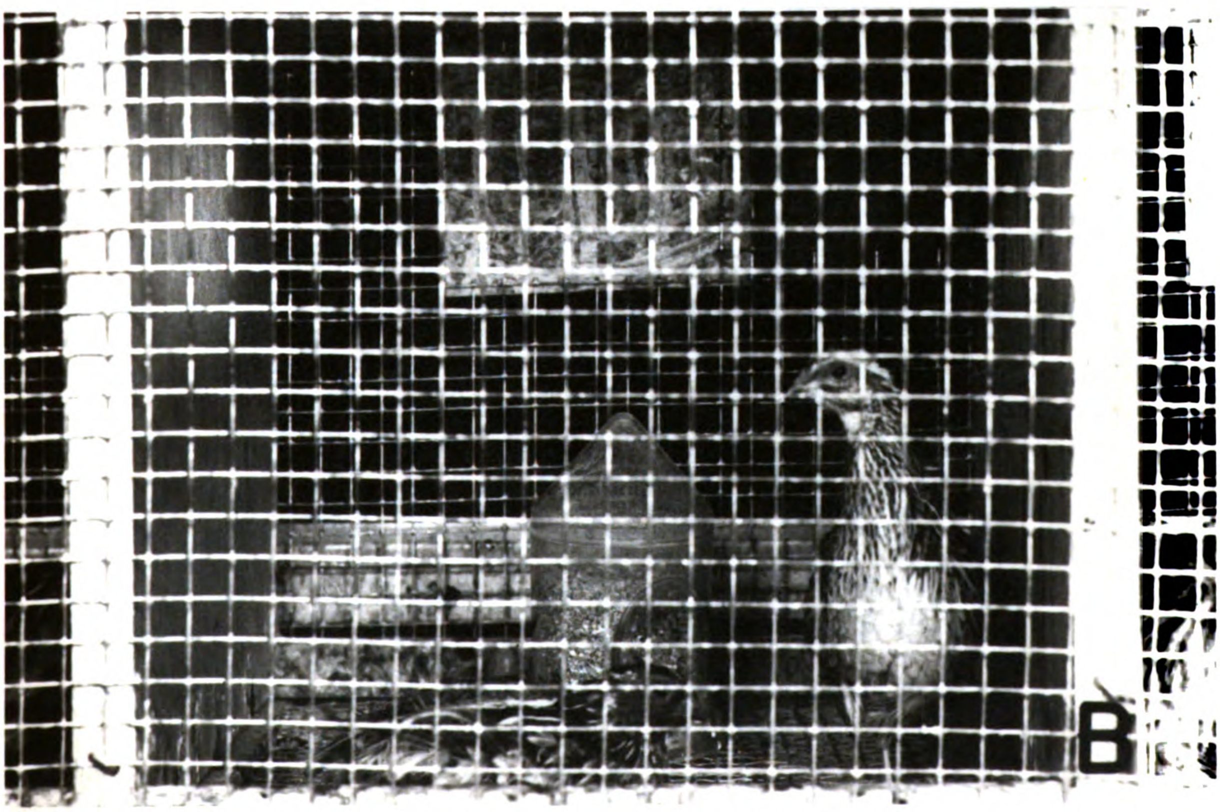

Plate I-B. Individual compartment of pens.

next by a 1/2 inch partition of plywood. All wooden portions of the pens were painted with aluminum paint. The bottoms of the pens were constructed with a half inch slope to prevent egg breakage and to allow wastes to pass through more readily. The bottoms of the pens were covered with 1/4 inch mesh hardware cloth and so placed as to be separate from the pen proper and to allow easy cleaning. A non-porous tray beneath each compartment collected all wastes from the birds. Each compartment housed one pair of quail. One community type water trough of galvanized metal (Plate I-A) 3" high by 3" wide by 50" long supplied water for the five compartments which comprised a pen. These trays were placed outside of the pen proper with individual access for each compartment so that contamination of the common water source by spilled feed or collected wastes was not possible.

Feed

Standard Feeds. The two standard feeds used throughout the experiment were the Game Bird Starter Crumbles (26% protein) and Game Bird Grower (20% protein)[1]. The following feeding schedule was followed throughout this study:

Age of birds	Food
1-14 day-old:	Game Bird Starter Crumbles
15-21 day-old:	1/4 Game Bird Grower, 3/4 Game Bird Starter.
22-28 day-old:	1/2 Game Bird Grower, 1/2 Game Bird Starter.
29-35 day-old:	3/4 Game Bird Grower, 1/4 Game Bird Starter.
36-42 day-old:	All Game Bird Grower.
43-80 day-old:	Game Bird Grower, plus small amounts of scratch grain.

[1]Manufactured by A.E. Staley Manufacturing Company, Decatur, Illinois.

In addition to the standard feeds, all birds three weeks old or older had access to calcium chips and grit.

Experimental Feed. Feed used during the experiment was prepared in 1500-gram lots, mixed with given amounts of technical DDT[1] dissolved in acetone. This material was mixed manually and placed on trays to dry overnight. Batches of the same concentration were then placed in five gallon containers, mixed thoroughly, and again mixed before being added to individual compartment feeders. All check birds received feed mixed with acetone and dried.

Feed Containers. All birds other than experimental pen birds received feed from cafeteria-type trays and water from gravity fountains. Experimental birds received their feed from individual enclosed plastic feeders (Plate II-B). All birds had water and feed available at all times.

Incubation

Eggs were collected daily from breeders and experimental birds. After storage at room temperature (72°F) for a period of seven days they were transferred to incubators at the Poultry Science Department on the Michigan State University campus. All eggs were incubated for a period of 17 days. Hatchability data were based on the total eggs set and the number of birds that did not require help from the shell.

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[1]Manufactured by the Michigan Chemical Co. St. Louis, Michigan.

Plate II-A. Quail eggs (note quarter for size comparison)

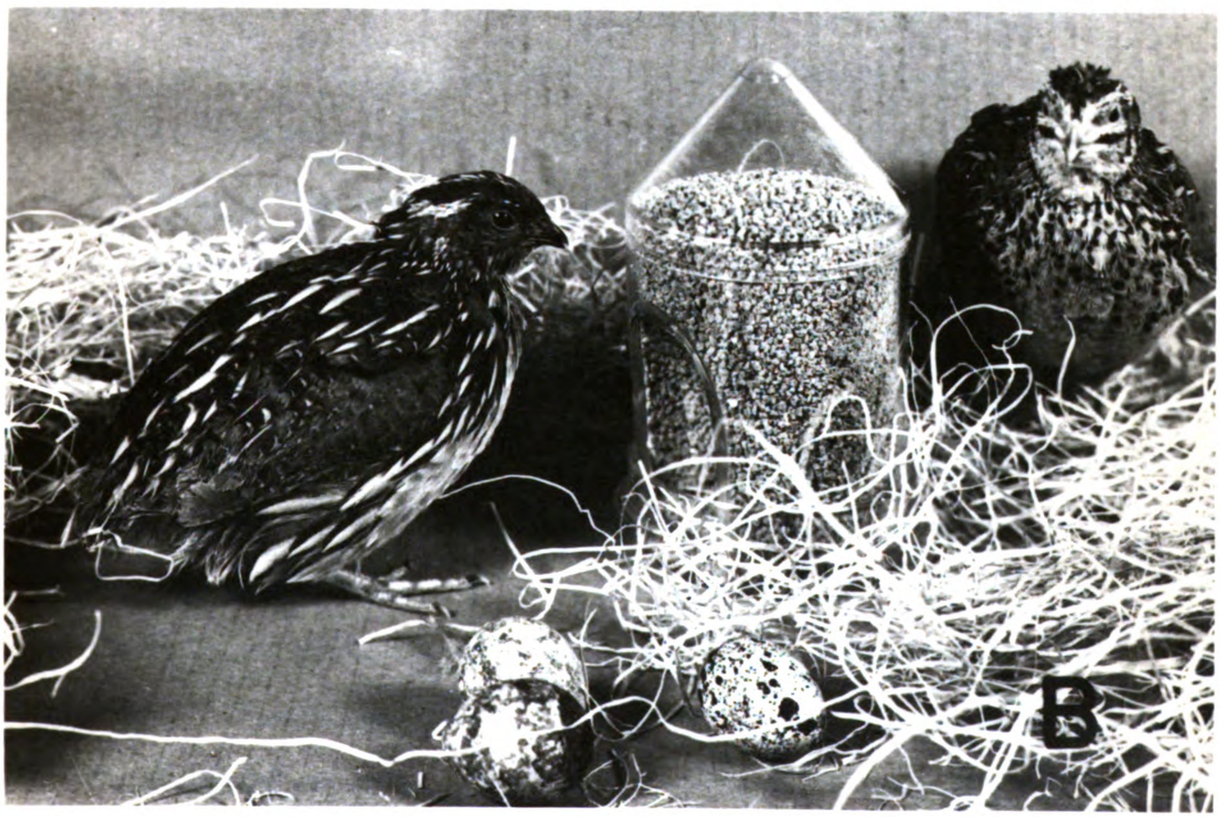

Plate II-B. Adult quail, male on the left, female on the right, shown with feeder and eggs.

Housing

The experiment was conducted in a metallic quonset building located on the Michigan State University campus. This building was steam heated, with the heat being circulated throughout the building by two space blowers located at the opposite end of the building from where the experiment was conducted. The average daily temperature throughout the experiment was 72°F. Variation of daily temperature depended upon the amount of sunshine for any given day. The metal building tended to be warmer during days with continuous sunlight. Constant lighting was furnished by four 150-watt incandescent light bulbs placed near the ceiling of the building. Light intensity varied between 1 to 7 foot candles four feet above the floor with a mean of 1/4 to 4 foot candles within the experimental cages. The light was furnished to prevent restlessness caused by natural migratory instinct. The added light also insured continuous egg laying. Birds became nervous and restless during short daylight periods.

Procedure

All quail used in this experiment came from eggs secured from a commercial game farm. Approximately 150 adult birds of various ages served as breeding stock for the entire experiment.

Progeny hatch from breeder and experimental birds were removed from the incubators and placed in rearing pens for a two-week period. All experimental second-generation birds had colored and numbered plastic leg bands attached for identification and to distinguish them from breeder birds. The birds were then placed in room temperature holding pens. When they reached the age needed for experimental use pairs were selected at random and placed in pen compartments.

Each compartment was checked daily. Food and water consumption, numbers of eggs laid, and symptoms of DDT poisoning were determined each day. Food was added and recorded whenever the supply in the feeder became low. Eggs were collected, weighed, marked with a waterproof, rub-proof marking pencil for future identification (Plate II-A) and recorded. Eggs were stored in a moist, enclosed, wooden container at room temperature prior to being placed in incubators. Eggs were rolled once daily. Criteria for observing effects of DDT poisoning in experimental birds were: slight nervousness; twitching; extreme nervousness; muscle tremors; muscle spasms; and general coordination. The terms mild, medium, and extreme were used when recording these symptoms.

All experimental birds except those on starvation diets were weighed and the weight recorded weekly or at death. Only weights of live birds were analyzed statistically. Also, once a week the wastes were sifted, separating the fecal matter from spilled food. The weight of the spilled food was recorded and the food destroyed.

Experiments

Test A. (Dec. 13, 1958 - Jan. 12, 1959). A preliminary test to find the range of toxicity and to study effects of DDT poisoning was run on 30-day-old birds picked at random. The following dosages of DDT were used in the feed: 1, 9, 90, 900, 9000 parts per million. Each dosage was fed to five pairs of birds.

Test B. (Feb. 17, 1959 - Mar. 21, 1959). The second test run was on 50-day-old birds picked at random and fed concentrations of 100, 300, 500, and 700 p.p.m. DDT. Again 50 birds were selected making each test contain 5 pairs of birds. Five pairs were used as controls. The birds were selected at this age because they were fully mature and had already been laying for a period of one to seven days. These birds continued on this diet until their 80th day of age unless they died before the completion of the test.

Test C. (Feb. 25, 1959 - Apr. 20, 1959). Fifty 25-day-old birds selected at random were fed the same concentrations as in test B. This age group was selected because they were still immature birds and had not started their egg laying cycle. The diet was fed until the 80th day of survival or until death.

Test D. (Apr. 12, 1959 - May 1, 1959). Second generation birds hatched from eggs laid by the survivors of test C were observed from their 50th to 70th day of age as in the previous tests but were fed only acetone-treated feed. Due to the mortality rate of the birds fed 700 p.p.m. in test C there were no eggs produced. The eggs from birds fed 500 p.p.m. in test C failed to hatch. Therefore, the second generation birds consisted of birds hatched from eggs produced by birds from the check, 100, and 300 p.p.m. diets from test C.

Test E. (Apr. 21, 1959 - Apr. 26, 1959). Birds surviving on the 80th day from test C were placed on a starvation diet along with control birds on the same starvation diet. This diet consisted only of fresh water.

Test F. (Apr. 27, 1959 - May 2, 1959). The sixth test was run on five pairs of 25-, 35-, and 50-day-old untreated birds selected at random. The birds were placed on the starvation diet and observed until their death. As in test E, this diet consisted only of fresh water.

PRESENTATION AND DISCUSSION OF RESULTS

The data from test A indicated that the range of dosage resulting in the shortest survival times was found to be between 900 and 9000 p.p.m. Thus the concentration ranges of 100, 300, 500, and 700 p.p.m. were used in succeeding tests to study the effects of DDT. Symptoms of DDT poisoning were observed throughout this experiment and are described in the procedure.

Food Consumption. Mean food consumption as recorded from tests B, C, and D is compiled in Table 1 and Figure I. In these three tests only one statistically significant difference was noted. In test C, birds receiving 700 p.p.m. DDT consumed significantly less food than the check birds. It will be noted in Table 9, however, that the survival time for these pairs was only 9.2 mean days. The mean quantity of eaten food per day was therefore based on a much shorter period than were the means for the lower concentrations. While the birds lived, however, they consumed less food. Although not statistically significant in test B a trend seems evident: as DDT concentration increased, food consumption decreased (Figure I). The experimental error within tests was large and thus many small differences between treatments could be obscured. This trend suggests that there is a weight loss resulting from increased sub-lethal amounts of DDT. This loss could be due to the effects of the insecticide or to the birds refusal to eat as a result of unpalatable food. However, all birds at the highest level showed the familiar tremors that precede death by DDT

Table 1. Mean amount of food consumed per day, per bird, for birds in tests B and C fed on different DDT diets and in test D (2nd generation birds from test C) fed on untreated diets.

Treatment P.P.M.	No. of Bird Pair Repli-cations	Mean Grams of Food Consumed Per Bird Per Day — Birds' Age and Test Letter: Test B, 25-80 days, 1st Generation	Test C, 50-80 days, 1st Generation	Test D, 50-70 days, 2nd Generation
Check	5	18.8	18.9	18.5 (a)
100	5	17.6	16.5	17.1 (a)
300	5	14.7	16.4	16.4 (a)
500	5	14.2	17.4	-- (b)
700	5	13.3	12.3*	-- (b)

*Differs significantly from all other means in column at 5% level using the studentized multiple range test described by Duncan (1955).

(a) Untreated diets.

(b) There was no hatch to produce second generation birds.

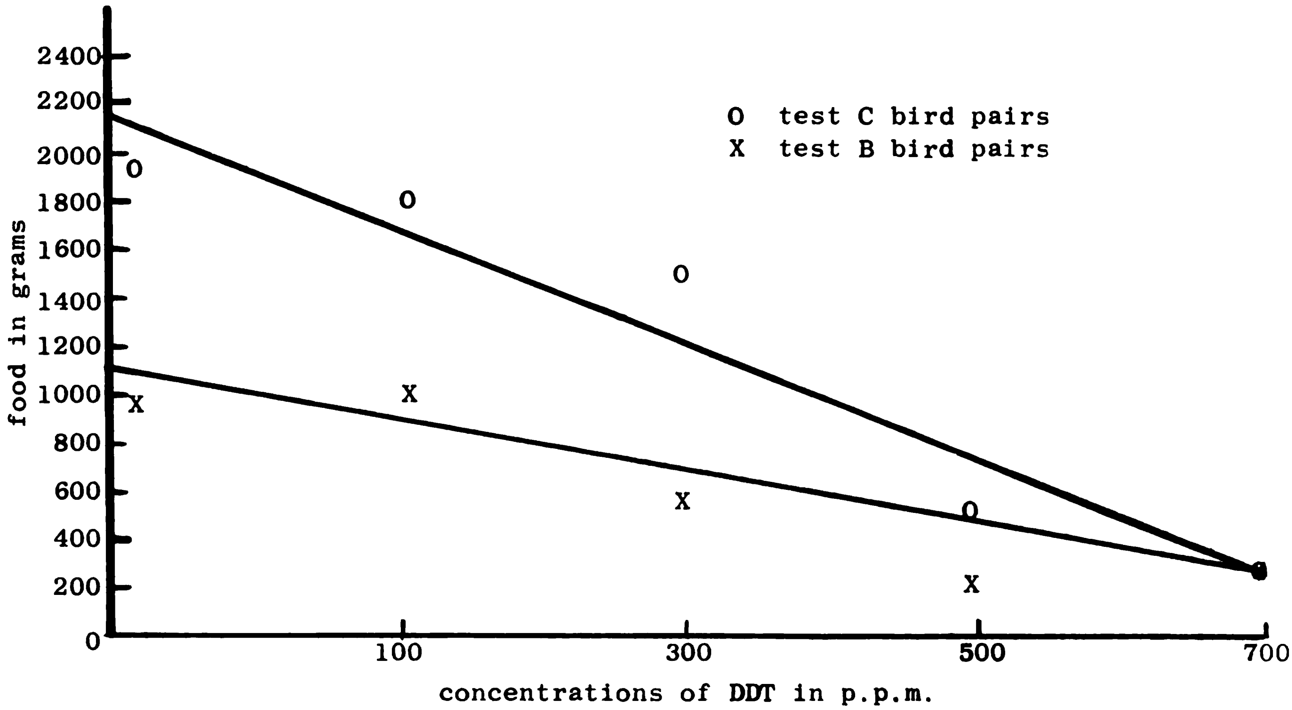

Figure I. Total amount food consumed (entire test) per pair, per day for tests B and C (regression line sight fitted).

poisoning, which indicates that at least some food was consumed. Food was calculated for bird pairs because of the necessity of housing pairs for reproduction purposes and to study subsequent hatchability. It is evident from Tables 2 and 3, that females either gained more weight or lost less than did the males. It cannot be concluded on the basis of these data that food consumption for the female was greater because the feed intake of the individual birds was not measured. Mean food consumed for the entire test was analyzed because the consumption rate for the first half of the test and the last half of the test did not vary greatly for surviving birds from tests B or C.

Mean Egg Weights. Average weight of eggs as recorded from Tests B, C, and D is compiled in Table 4. In these three tests there were no significant differences at any level of treatment which affected the weight of eggs. At the highest concentration, 700 p.p.m., there was no egg production for test C birds and at the 500 p.p.m. treatment there was no hatch to produce second generation birds.

Weight Gain. Mean weight gain of male, female, and paired birds recorded from tests B and C is compiled in Tables 2 and 3 and Figures II and III respectively. In these two tests no significant differences were found, except that in test B birds receiving 700 p.p.m. DDT differed significantly from all other treatments. Test B birds showed that the response of the male to the treatment was significantly different from that of the female. The males lost more weight than the females as the concentrations of DDT increased. Test C birds showed no such sex difference. Weight gain data for second generation birds were not analyzed as there were no obvious trends. Table 5 was constructed

Table 2. Mean weight gain of birds[1] per day for 50-80 day-old males, females, and mated pairs (test B) under different DDT diets.

Treatment P.P.M.	No. of Bird Pair Replications	Mean Weight Gain Per Day in Grams		Weight Gain for Bird Pairs Per Bird in Grams	
		Male	Female	Mean Weight	Class of Significance**
Check	5	.43	.13	.28	II
100	5	.19	.27	.23	II
300	5	-1.65	-.17	-.91	II. I
500	5	-2.06	-1.19	-1.74	II. I
700	5	-5.00	-.23	-3.65*	I

*Differed significantly from check at 5% level using the studentized multiple range test described by Duncan (1955). (D = 2.38).

**If two treatments do not have a common number then the treatments are significantly different at the 5% level, conversely, if two treatments share a common number they are not significantly different. This technique was suggested by Dean L. Haynes.

[1]Mean wt. gain = $\dfrac{\text{beginning wt. - final wt.}}{\text{no. of days in test or until death of bird}}$

Table 3. Mean weight gain of birds[1] per day for 25-80 day old males, females, and mated pairs (test C) under different DDT diets.

Treatment P.P.M.	No. of Bird Pair Replications	Mean Weight Gain Per Day in Grams*		Weight Gain for Bird Pairs Per Bird in Grams	
		Male	Female	Mean Weight	Class of Significance
Check	5	.66	1.01	.84	I
100	5	.64	1.02	.84	I
300	5	.20	.77	.54	I
500	5	.73	1.06	.89	I
700	5	.60	.62	.61	I

*No significant differences between means.

[1]Mean wt. gain = $\frac{\text{beginning wt. - final wt.}}{\text{no. of days in test or until death of bird.}}$

Table 4. Mean weight of eggs for birds in tests B and C fed different concentrations of DDT and in test D (2nd generation birds from test C) fed on untreated diets.

Treatment P.P.M.	No. of Bird Pair Repli-cations	Mean Weight of Eggs in Grams* Birds' Age and Test Letter		
		Test B 50-80 days 1st Generation	Test C 25-80 days 1st Generation	Test D 50-70 days(a) 2nd Generation
Check	5	8.63	8.74	8.86 (c)
100	5	9.04	8.28	8.85 (c)
300	5	7.68	8.76	8.41 (c)
500	5	8.98	8.40	-- (d)
700	5	8.67	0.00 (b)	-- (d)

*No significant difference between means

(a) 2nd generation birds were obtained from 25-80 day old birds and were fed untreated food.

(b) Birds died before egg production.

(c) Untreated diets.

(d) There was no hatch to produce second generation birds.

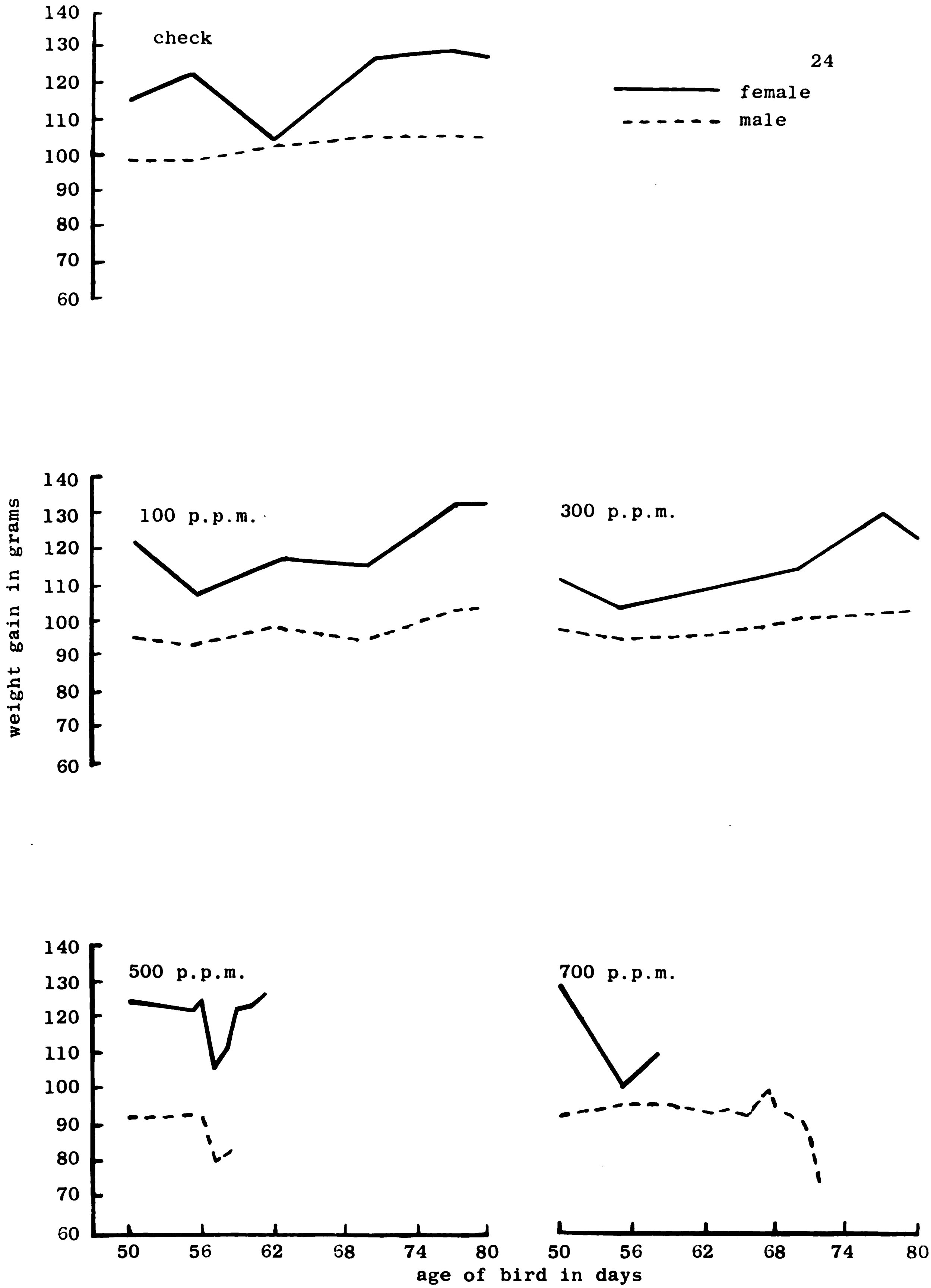

Figure II. Growth curves for test B birds fed different DDT concentration diets starting at an age of 50 days and continuing to the 80th day.

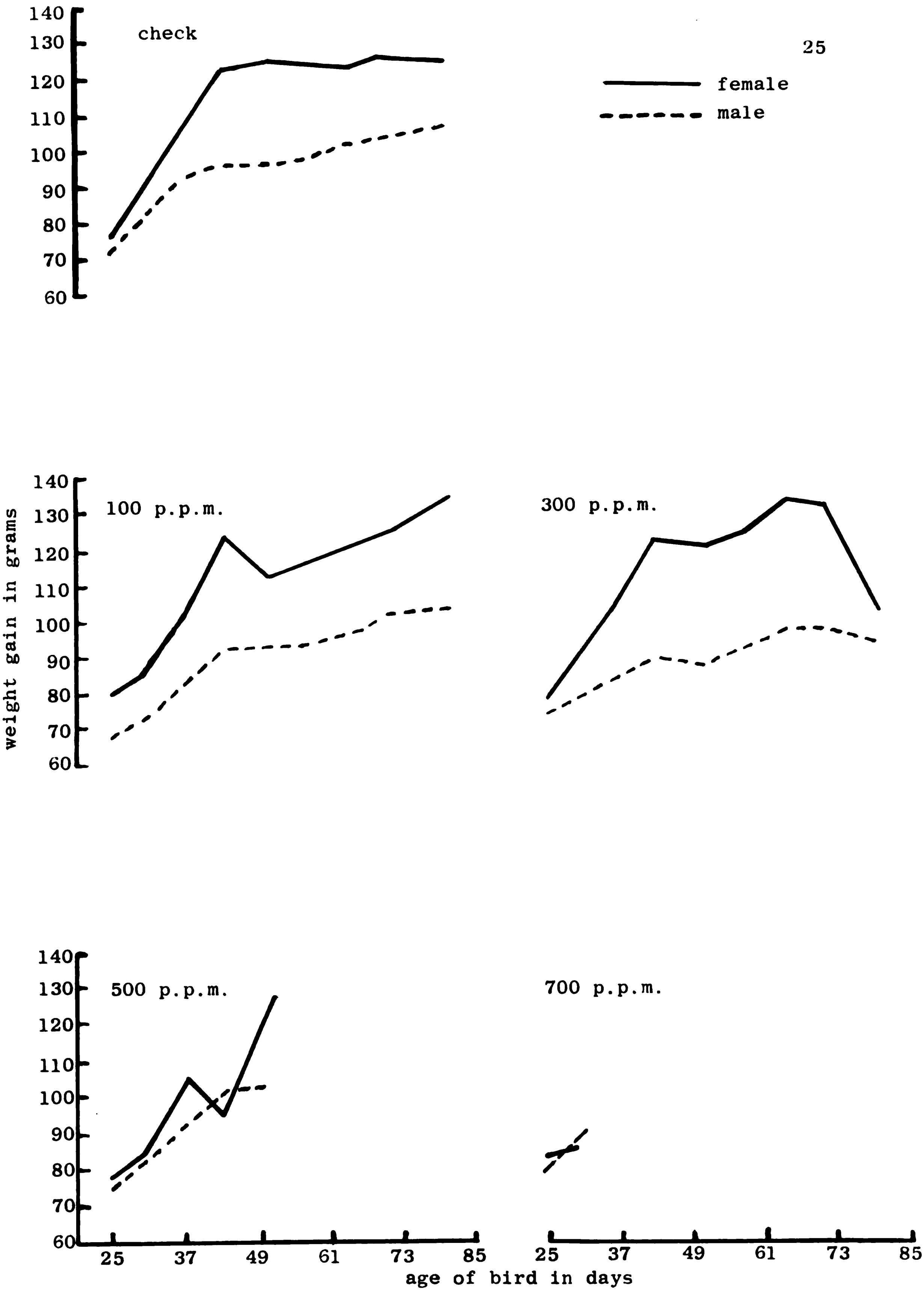

Figure III. Growth curves for test C birds fed different DDT concentration diets starting at an age of 25 days and continuing to the 80th day.

Table 5. Mean body weight of birds in grams according to sex, age, and number of days on varied DDT diets.

Treat-ment P.P.M.	Age of Males and Females at Time of Weighing: 25 Days	50 Days			80 Days	
		On DDT Diet For			On DDT Diet For	
	No DDT	No DDT	25 Days	No DDT	55 Days	30 Days
		mean body weight of male birds in grams				
Check	74.2(a)	96.8(b)	98.4	95.6(a)	107.8	104.2
100			93.2		86.4	102.4
300			67.5(d)		99.7	102.5
500			103.7(d)		92.0	(c)
700			(c)		(c)	(c)
		mean body weight of female birds in grams				
Check	79.3(a)	123.3(b)	126.8	121.6(a)	127.3	127.8
100			113.8		135.7	131.6
300			120.5		138.7	(c)
500			124.7		121.0(c)	(c)
700			(c)		(c)	(c)

(a) Mean weight of 25 birds.

(b) Mean weight of 15 birds.

(c) Birds died before this period in the experiment.

(d) Only one replication remaining.

to show how the body weights of birds at different ages responded to varying lengths of time they were on the DDT diets. It will be noted that no obvious trends appear for birds remaining at the completion of the various tests. As the concentration increases there appear to be greater variations within treatments as shown in Figures II and III. These data were not analyzed statistically due to the lack of replications in higher treatments where heavy mortality occurred.

<u>Number of Eggs Laid and Proportions That Were Fertile</u>. The mean number of eggs laid and proportions of these that were fertile (as recorded from tests B, C, and D) are compiled in Table 6. Table 7 compares the mean number of eggs laid and proportions that were fertile between the 50th and 70th day of the birds life for test B, C, and D. In these three tests no significant differences were found. At the highest concentration, 700 p.p.m., there was no egg production for test C birds and at 500 p.p.m. no hatch occurred, hence there were no second generation birds. The analysis of these data was made by means of an arcsin transformation of the proportions. This technique gives additional weight to the smaller proportions. As Snedecor (1957) points out this technique will bring out smaller differences since proportions are not normally distributed, the smaller proportions having smaller variances. At higher concentrations mortality increased (Figures IV and V) and fewer eggs were laid; therefore, more eggs were compared at the lower than higher concentrations. A comparison between the age classes and second generation birds was not made due to the heavy mortality reducing the number of replications and the fact that the tests were not run for equal periods of time. These variables increased the

Table 6. Mean number of eggs laid per female and fertile eggs from birds on different sub-lethal DDT diets expressed as percentage of mean number of eggs laid.

Treatment P.P.M.	No. of Repli-cations	Mean Number Eggs Laid (a)	Percent Fertile Eggs	Percent Fertile Eggs Adjusted for Broken Eggs (b)
		Test B 1st generation birds, 50th to 80th day of birds life		
Check	4	22.0	38.4	43.7
100	5	21.8	45.5	48.7
300	1	15.0	47.0	54.0
		Test C 1st generation birds, 25th to 80th day of birds life		
Check	4	33.3	20.1	22.1
100	5	25.3	29.0	31.4
300	1	35.0	40.5	41.5
		Test D 2nd generation birds receiving no DDT(c) 50th to 70th day of birds life		
Check	5	15.4	9.3	11.6
100	2	13.5	30.1	31.1
300	5	14.4	29.1	31.4

(a) No significant differences between means.

(b) No significant differences between proportions using arcsin transformation technique discussed by Snedecor G. W. (1957) p. 316.

(c) 2nd generation birds from test C started at an age of 25 days.

Table 7. Mean number of eggs laid per female and eggs from birds on different sub-lethal DDT diets expressed as percentage of mean number of eggs laid between the age of the 50th and 70th day of birds life.

Treatment P.P.M.	No. of Repli- cations	Mean Number Eggs Laid (a)	Percent Fertile Eggs	Percent Fertile Eggs Adjusted for Broken Eggs (b)
		Test B birds started on diet at beginning of test		
Check	4	13.8	28.8	31.9
100	5	14.2	50.4	53.0
300	1	9.0	44.0	50.0
		Test C birds on diet for 25 days prior to test		
Check	4	18.8	22.6	22.6
100	5	9.8	23.8	25.9
300	3	17.7	24.4	26.2
		Test D 2nd generation birds receiving no DDT(c)		
Check	5	15.4	9.3	11.6
100	2	13.5	30.1	31.1
300	5	14.4	29.1	31.4

(a) No significant differences between means.

(b) No significant differences between proportions using arcsin transformation technique discussed by Snedecor G. W. (1957) p. 316.

(c) 2nd generation birds from test C started at an age of 25 days.

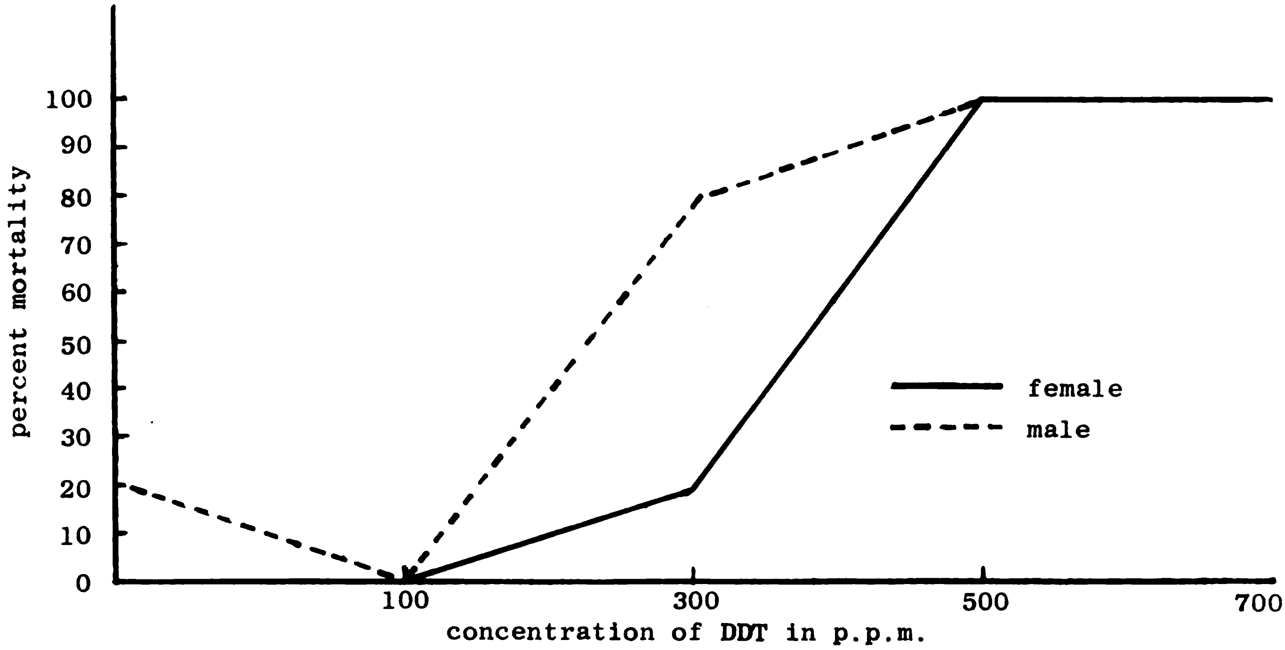

Figure IV. Percent mortality (entire test) per bird for test B.

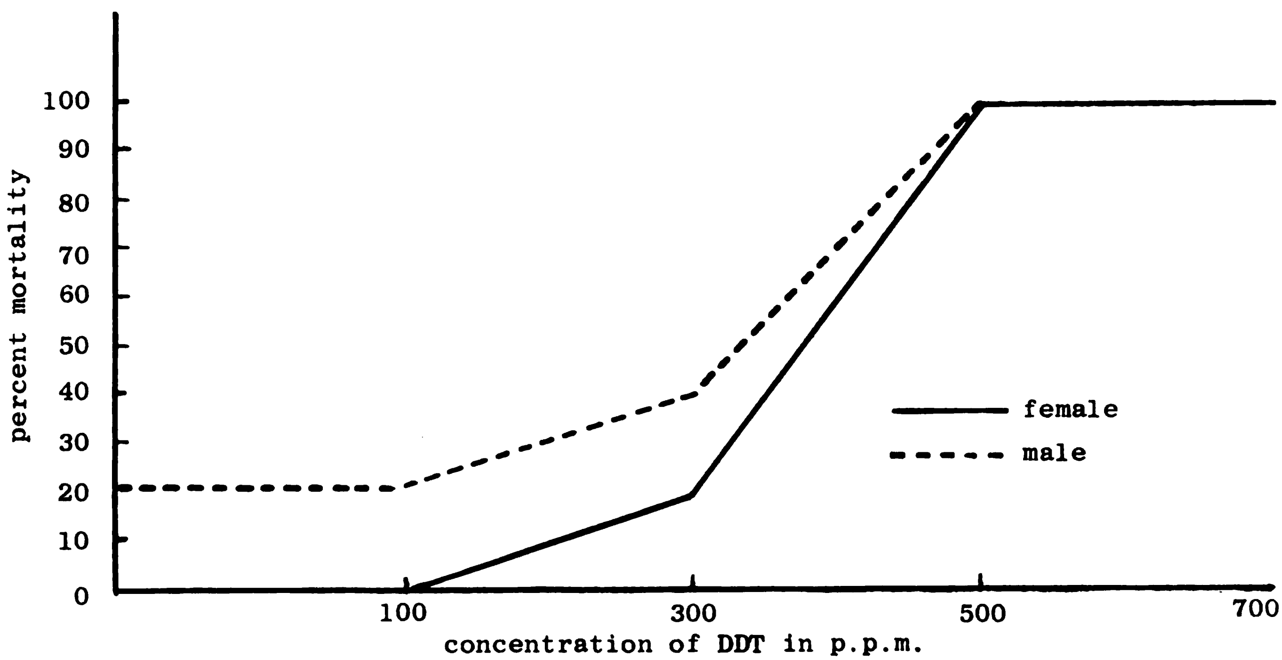

Figure V. Percent mortality (entire test) per bird for test C.

experimental error to such an extent that comparisons between tests would have very little validity. It can be noted that hatchability was rather low; undoubtedly this was due to the fact that eggs were stored at room temperature and due to the fact that it was only possible to roll the eggs once every 24 hours instead of following the recommended practice of once every eight hours. Tables 6 and 7 show that as DDT concentrations increase there appears to be a corresponding increase in hatchability; however, this trend is not significant statistically.

Length of Survival. Mean days of survival as recorded from tests B and C are compiled in Tables 8 and 9 respectively. There were significant differences in survival between the 300, 500, and 700 p.p.m. treatments in test B whereas there were only significant differences between test C birds at the 500 and 700 p.p.m. treatments. The classes of significance column from test B birds shows that the 300 p.p.m. treatment differed significantly from the 500 and 700 p.p.m. treatments but that the 500 p.p.m. treatments did not differ significantly from the 700 p.p.m. treatment. The classes of significance column for test C birds shows that the 500 p.p.m. treatment differed from the 700 p.p.m. treatments. Numbers of second generation birds were not analyzed as there was no mortality. Table 10 was constructed to show a comparison between Tables 8 and 9. It was adjusted for equal amounts of DDT in diets with age being the only variable. The results were the same as for test C birds. These same results were repeated when Table 11 was constructed by pooling the means from Tables 8 and 10.

Mortality. Percent mortality as recorded for total length of test from B and C is compiled in Figures IV and V. The highest concentrations

Table 8. Mean days of survival of test B birds fed on different DDT concentration diets starting at an age of 50 days and continuing to the 80th day.

Treatment P.P.M.	No. of Bird Pair Replications	Mean Days of Survival			5%(a) Classes of Significance of Pairs	Percent Influence (b)
		Female	Male	Pair		
Check	5	30.0	26.8	28.4	III	5.3
100	5	30.0	30.0	30.0	III	0.0
300	5	26.8	14.4	20.6*	II	31.3
500	5	8.0	9.4	8.7*	I	71.0
700	5	14.2	4.6	9.4*	I	68.7

*Differs significantly from check at 5% level (D = 7.54) using the studentized multiple range test described by Duncan (1955).

(a) applies only to "pair" mean column.

(b) percent influence = $\frac{\text{length of test (days) - survival time (days)}}{\text{length of test (days)}}$

(Equals number of days lived less than the maximum, expressed as a percentage of the maximum possible days of life in the test).

Table 9. Mean days of survival of test C birds fed on different DDT concentration diets starting at an age of 25 days and continuing to the 80th day.

Treatment P.P.M.	No. of Bird Pair Replications	Mean Days of Survival			5%(a) Classes of Significance of Pairs	Percent Influence (b)
		Female	Male	Pair		
Check	5	55.0	48.6	51.8	III	5.8
100	5	55.0	54.2	54.6	III	0.7
300	5	46.8	48.6	47.7	III	13.3
500	5	26.0	22.4	24.2*	II	56.0
700	5	7.0	11.4	9.2*	I	83.3

*Differs significantly from check at 5% level (D = 15.44) using the studentized multiple range test described by Duncan (1955).

(a) applies only to "pair" mean column.

(b) percent influence = $\frac{\text{length of test (days) - survival time (days)}}{\text{length of test (days)}}$

(Equals number of days lived less than the maximum, expressed as a percentage of the maximum possible days of life in the test).

Table 10. **Mean days of survival of birds fed on different DDT concentration diets from tests B and C. This table was constructed for comparison between Tables 8 and 9 with only age as a variable.**

Treatment P.P.M.	No. of Bird Pair Replications	Mean Days of Survival			5%(a) Classes of Significance of Pairs	Percent Influence (b)
		Female	Male	Pair		
Check	5	30.0	28.6	29.3	III	2.3
100	5	30.0	30.0	30.0	III	0.0
300	5	26.8	30.0	28.4	III	5.3
500	5	21.6	22.2	21.9*	II	27.0
700	5	7.0	10.8	8.9*	I	70.3

*Differs significantly from check at 5% level (D = 4.15) using the studentized multiple range test described by Duncan (1955).

(a) applies only to "pair" mean column.

(b) percent influence = $\frac{\text{length of test (days) - survival time (days)}}{\text{length of test (days)}}$

(Equals number of days lived less than the maximum, expressed as a percentage of the maximum possible days of life in the test).

Table 11. Mean days of survival of birds fed on different DDT concentration diets. This data resulted from pooling the means from Tables 8 and 10.

Treatment P.P.M.	No. of Bird Pair Replications	Mean Days of Survival Female	Male	Pair	5%(a) Classes of Significance of Pairs	Percent Influence (b)
Check	10	30.0	27.7	28.9	III	3.7
100	10	30.0	30.0	30.0	III	0.0
300	10	26.8	22.2	24.5	III	18.3
500	10	14.8	15.8	15.3*	II	49.0
700	10	10.6	7.7	9.2*	I	69.0

*Differs significantly from check at 5% level (D = 5.9) using the studentized multiple range test described by Duncan (1955).

(a) applies only to "pair" mean column.

(b) $\text{percent influence} = \dfrac{\text{length of test (days)} - \text{survival time (days)}}{\text{length of test (days)}}$

(Equals number of days lived less than the maximum, expressed as a percentage of the maximum possible days of life in the test.)

of DDT, (500 and 700 p.p.m.) resulted in 100 percent mortality at the completion of all tests. The other treatments showed varying effects upon the individual paired birds. It appears that the male is more inclined to succumb to the effects of DDT poisoning than the females particularly at the 300 p.p.m. levels of concentration. All birds other than check birds showed typical DDT poisoning prior to death as described previously in the procedure. The duration of the treatments did not appear to affect the rate of growth (Tables 2 and 3, Figures II and III). Second generation birds (test D) were not analyzed as there was no mortality. A trend seems to indicate (Table 12) that females consume more DDT than do males prior to death of the bird. Although this investigation was not to ascertain the specific LD 50 for given age birds, Figures VI, VII, and VIII are presented to consider the topic in a general non-critical manner. Figure VI shows the combined survival rates from tests B and C over a 42.5 day period disregarding sex. This graph indicates that approximately 5.25 milligrams of DDT would have to be ingested per day for 42.5 days to reach 50 per cent mortality. Figures VII and VIII represent mean amounts of DDT ingested in milligrams per bird pairs at different rates. The graphs indicate that there is a difference in the effects of DDT on varied age levels, lengths of feeding, and concentrations of toxicant in diet. Test B birds reached 50 percent mortality at the 300, 500, and 700 p.p.m. levels of concentration whereas test C birds reached this level at only the 500 and 700 p.p.m. treatments.

Comparing the work of Draper _et al_ (1950) with results of this study it would appear that residues normally found in the field would

Table 12. Mean amount of food and DDT consumed by tests B and C males and females in relation to survival time.

Treatment P.P.M.	Mean Days of Survival		Total Mean Amt. of DDT Consumed in mg. Per Bird		Mean Amt. DDT Consumed Per Day in mg. Per Bird
	Male	Female	Male	Female	Male or Female
			Test B		
Check	26.8	30.0	000.0	000.0	0.00
100	30.0	30.0	053.0	053.0	1.77
300	14.4	26.8	064.0	118.0	4.40
500	9.4	8.0	067.0	057.0	7.13
700	4.6	14.2	043.0	132.0	9.30
			Test C		
Check	48.6	55.0	000.0	000.0	0.00
100	54.2	55.0	089.0	091.0	1.65
300	48.6	46.8	239.0	230.0	4.91
500	22.4	26.0	195.0	226.0	8.69
700	11.4	7.0	101.0	062.0	8.86

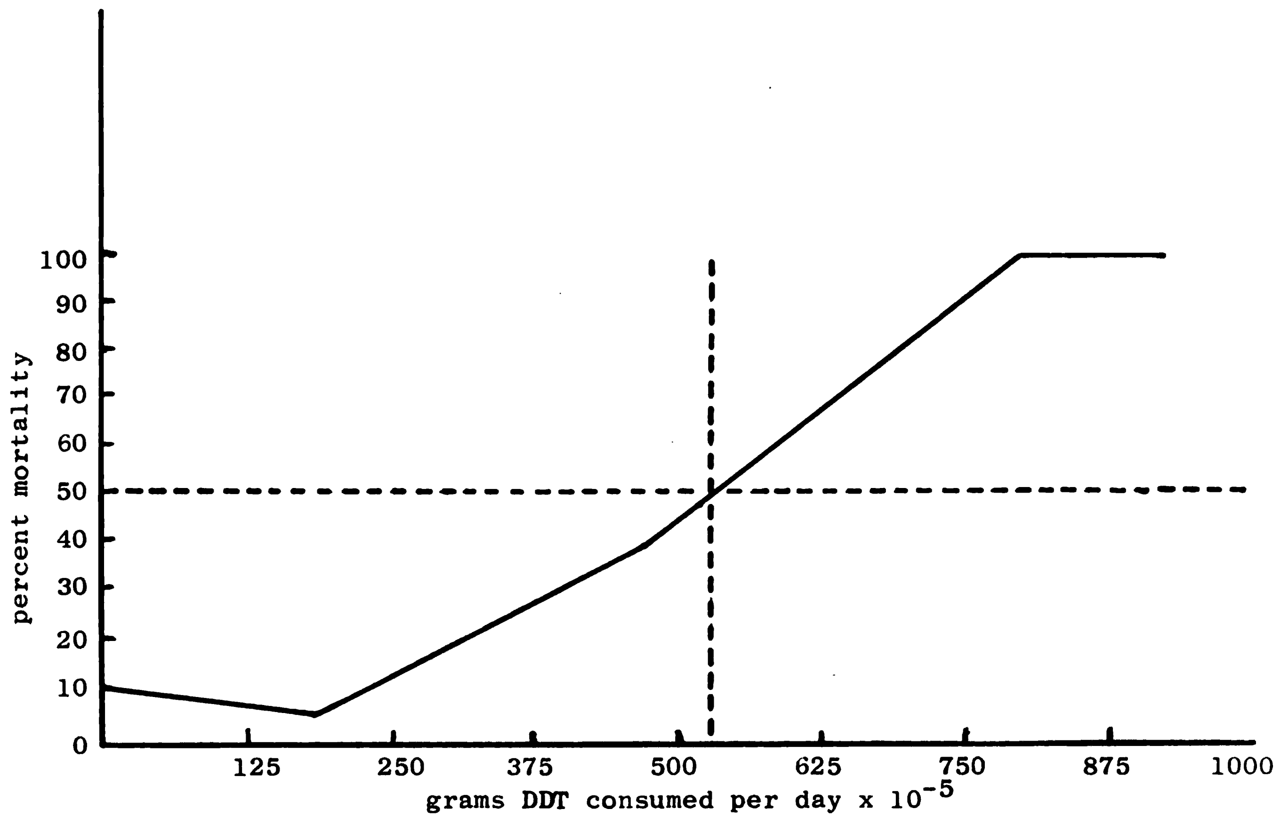

Figure VI. Mortality of birds (entire test) fed DDT for an average of 42.5 days (results from tests B and C disregarding sex).

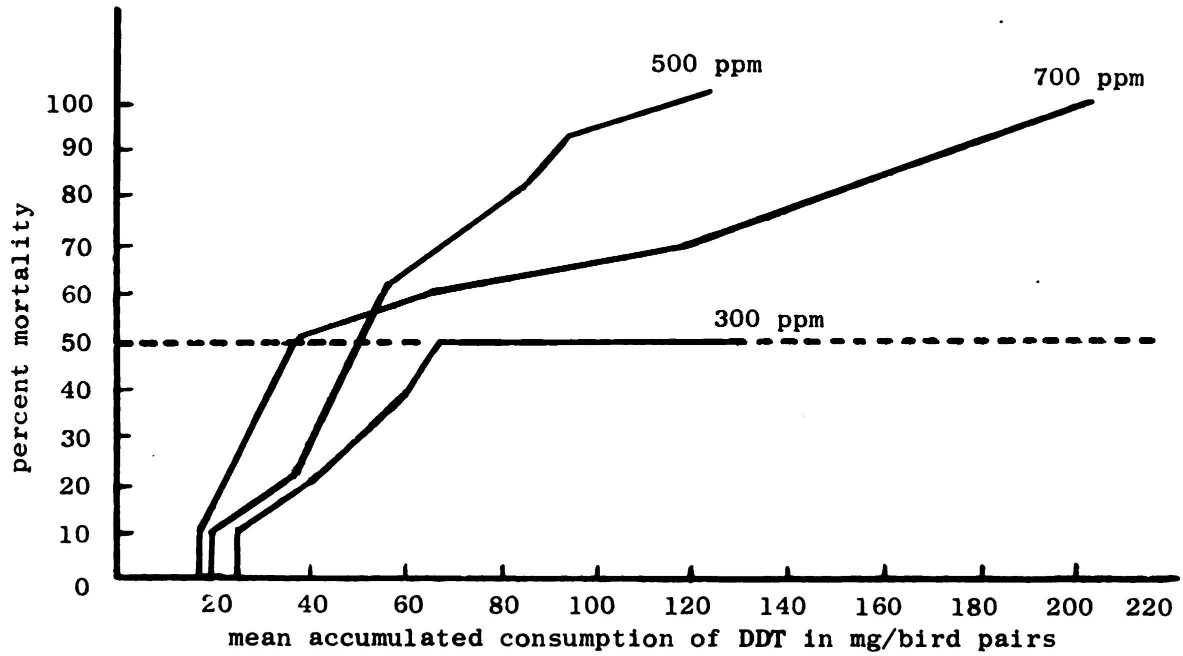

Figure VII. Mortality and mean amount of DDT ingested in milligrams for test B bird pairs.

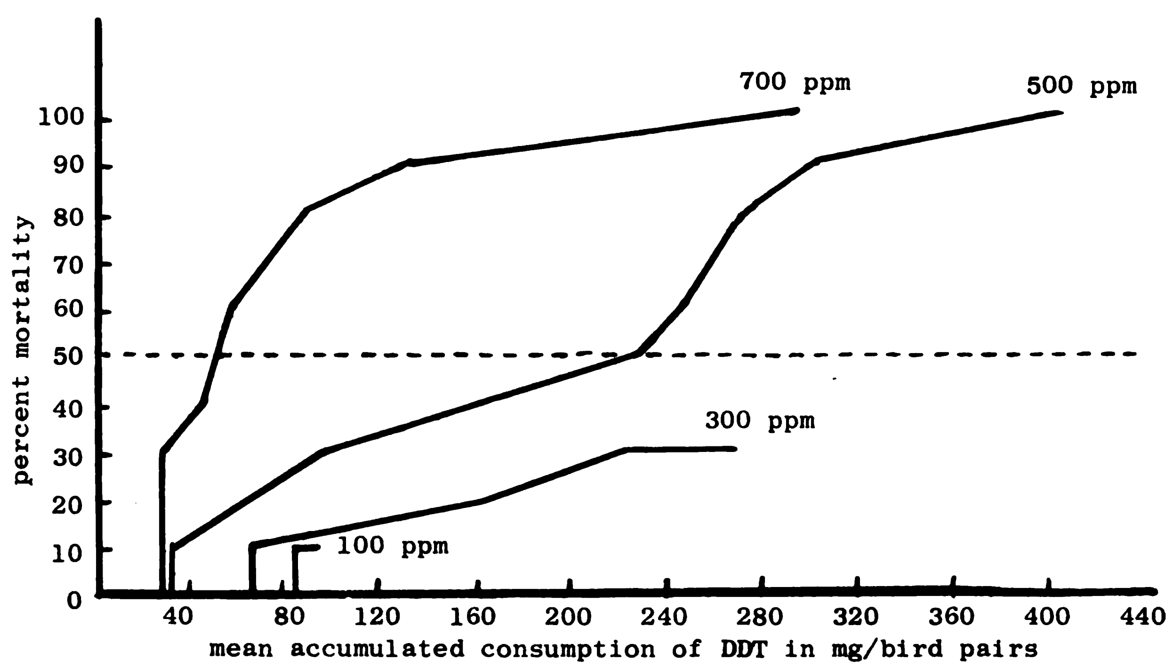

Figure VIII. Mortality and mean amount of DDT ingested in milligrams for test C bird pairs.

not approach the point where mortality would result. The higher concentrations which caused significant differences in this study would be comparable to applying a treatment of DDT of more than 1000 pounds per acre. Coburn and Treichler (1946) found that DDT administered in oil solutions was approximately 3 1/2 times more toxic than when administered in crystalline form. This would indicate that the higher concentrations which caused significant differences in this study would be comparable to the use of DDT-oil solutions at the rate of about 330 pounds of DDT per acre. This amount would be far above the standard practices employed.

Starvation of DDT-Treated Birds. Effects of starvation on DDT-treated birds (test E) are compiled in Table 13. Birds remaining from test C were placed on starvation diets. They were supplied only with fresh water. All birds from DDT-treated diets showed definite symptoms of DDT poisoning when the bird was forced to rely on stored body foods. No significant difference between males in the check, 100, and 300 p.p.m. treatments were noted. The DDT-treated females differed significantly from the check birds but did not differ from each other. This evidence supports the known fact that DDT at all concentrations is stored in the body fat and can later become toxic. This has already been noted in the literature review. All check birds alive at the completion of 6 days were taken off the starvation diet since all DDT-treated birds were dead.

Starvation Diets on Untreated Birds. Results of starvation diets on untreated birds (test F) are compiled in Table 14. Untreated 25-, 35-, and 50-day-old birds were placed on a starvation diet. In all age

Table 13. Mean days survival of test E birds on starvation diets. Birds remaining at the completion of test C which were fed a DDT diet for a period of 55 days were used for this study.

Treatment P.P.M.	No. of Replications		Mean Days of Survival	Class of Significance	Mean Days of Survival	Class of Significance
	Male	Female	Males		Females	
Check (a)	5	4	5.6	I	6.0	II
100 (b)	5	4	5.2	I	2.0*	I*
300 (b)	3	3	4.7	I	3.3*	I*

*Differs significantly from the check at 5% level (D = 1.63) using the multiple range test described by Duncan (1955), but the 300 p.p.m. did not differ from the 100 p.p.m. diet.

(a) Taken off starvation diet at the end of 6 days.

(b) All birds showed symptoms of DDT poisoning.

Table 14. Mean days survival of 25-, 35-, and 50-day-old untreated test F birds placed on starvation diets.

Days of Age at Start of Starvation	No. of Replications Male	No. of Replications Female	Males: Mean Days of Survival	Males: Class of Significance	Females: Mean Days of Survival	Females: Class of Significance
25	5	5	1.8	III	2.0	II
35	5	5	3.0	II*	2.6	I*
50	5	5	4.2	I*	4.4	I*

*Differs significantly from the check at 5% level (male D = .52, female D = .89) using the multiple range test described by Duncan (1955).

groups there were significant differences in sexes with the males living longer than the females. Again as in test E, birds were supplied only with fresh water. It can therefore be concluded that the age of the bird and its sex influences the survival under starvation conditions. This suggests that in areas where the natural food has been destroyed by a chemical treatment birds have either a choice of leaving or starving and younger birds would be expected to succumb earlier.

SUMMARY

Technical DDT was dissolved in acetone and mixed in feed at various concentrations and fed at different intervals to Japanese quail, *Coturnix coturnix japonica*. This experiment was conducted on the Michigan State University campus during 1958 and 1959.

Data were obtained on: (1) mean amounts of food consumed per day; (2) mean body weight gained per day; (3) mean weights of eggs; (4) mean number of eggs laid; (5) proportion of eggs laid that were fertile; (6) mean days of survival of birds; (7) mortality of birds; (8) mean body weight of birds according to sex, age, and number of days on varied DDT diets; and (9) effects of starvation on untreated and DDT-treated birds supplied only with fresh water.

This investigation was designed to evaluate progressive feeding of established rates of DDT and not to ascertain a specific LD-50 for given age birds.

Experimental results indicate that:

1. The Japanese Quail served as a very useful and efficient laboratory animal in this investigation.

2. There was a trend towards decreased food consumption following an increase in the concentration of DDT in food.

3. Levels of DDT up to 700 p.p.m. had no effect on egg weights, number of eggs laid, or hatchability.

4. In one test, increased concentrations of DDT in the food decreased body weight.

5. There was a general increase in mortality as the concentration of DDT increased in the diet.

6. The minimum lethal dosage was estimated to be between 300 and 500 p.p.m. of DDT.

7. Male birds were more susceptible to effects of DDT than were females.

8. Treated birds appeared to store DDT in the reserve food supply at all levels of DDT feeding.

9. The age of the bird influenced its survival under starvation conditions.

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