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TOXICITY OF DICYCLOPENTADIENE AND DIISOPROPYL METHYLPHOSPHONATE TO THE BOBWHITE QUAIL (COLINUS VIRGINIANUS)

by

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ABSTRACT

TOXICITY OF DICYCLOPENTADIENE AND DIISOPROPYL METHYLPHOSPHONATE TO THE BOBWHITE QUAIL (COLINUS VIRGINIANUS)

by

Kathy S. Howell

Three experiments, an LD_{50}, an LC_{50}, and a chronic study, were conducted to determine the toxicity of dicyclopentadiene (DCPD) and diisopropyl methylphosphonate (DIMP) to the Bobwhite quail.

The 14-day LD_{50} values for adult Bobwhites dosed per os with DCPD or DIMP were 1010 $\pm$ 80.0 mg/kg and 1000 $\pm$ 68.2 mg/kg, respectively.

LC_{50} values, based on five day dietary treatment followed by three day withdrawal, could not be determined for either chemical because of insufficient mortality. Predicted zero feed intake levels of the quail were 73028 ppm DCPD and 77959 ppm DIMP.

The chronic study was conducted over a single reproductive period. The dietary levels used were; 400, 1250, and 4000 ppm DCPD plus a control and 1200, 3800, and 12000 ppm DIMP plus a control. The parameters measured included adult mortality, feed consumption, body weight change, egg production, incubation parameters, blood parameters, internal organ weights, and progeny livability. No consistent treatment-effect results were observed for either chemical.

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INTRODUCTION

Statement of the Problem

Increasing pressure from environmental lobbying groups and recently passed environmental legislation, both due to continued interest in the preservation of the balance of ecology, have forced those involved in the use, storage, and/or disposal of potentially hazardous chemicals to exercise more caution in their activities. Army installations, often employed in the production, storage, and disposal of chemicals, are examples of potential ecology offenders working toward environmental clean-up. Recent activities of the U.S. Army have included the identification and toxicological and ecological hazard assessment of potential environmental pollutants discovered in the surface and subsurface water near and around military lands. Two compounds, dicyclopentadiene (DCPD) and diisopropyl methylphosphonate (DIMP), have been identified at the Rocky Mountain Arsenal, but not yet evaluated for toxicological properties.

Background

To reduce potential hazards and incidence of toxicological mishaps allegedly related to the Rocky Mountain Arsenal, toxicological studies are being conducted on several compounds identified at or around the arsenal. Two compounds, DCPD and DIMP, are being investigated to determine their toxicity to both mammals and birds.

DCPD is employed as a starting material for the production of chlorinated insecticides, and is used, as is its monomer,

cyclopentadiene (CPD), in the manufacturing of plastics, elastomers, cycloaliphatic epoxides in resin coatings, rubber hydrocarbons, and other products. Both chemicals have been used at the pesticide facility leased by Shell Chemical Company at Rocky Mountain Arsenal.

DIMP is a by-product produced during the manufacture of GB (Isopropyl methylphosphonofluoridate), a nerve gas. There is no evidence that DIMP is either a metabolite or environmental product of GB. GB was manufactured in a plant at the Rocky Mountain Arsenal.

Several reasons for the investigation of the toxicity of DCPD and DIMP exist. These reasons are: the paucity of toxicological information on either chemical, the presence of both chemicals in sampling wells and surface water both on and off the Rocky Mountain Arsenal, and the occurrence of unexplained mortalities of wild and domesticated animals on or near the arsenal. In addition, the Shell Chemical Company has stated that accidental spillages of chemicals have occurred at the arsenal and the Committee on Military Environmental Research on the Status of Research into Biological Effects of Environmental Contaminants at Rocky Mountain Arsenal has described various improper chemical waste disposal at the arsenal (citation unpublished).

Bobwhite quail were selected as the test subject of this study because they are a representative species at the site of contamination, they are readily available for toxicological testing, they represent an upland game bird, and the Environmental Protection Agency has accepted the Bobwhite as a suitable species for toxicological studies.

LITERATURE REVIEW

Dicyclopentadiene (DCPD) (Appendix A: Chemical Structure and Alternate Names for DCPD and DIMP) is a liquid at room temperature but becomes a waxy solid after cooling to approximately 0°C. DCPD melts at 32°C and has a bulk density of 0.982 g/cc at 20°C (Shashkina, 1965). The calculated distribution coefficient (oil/water) of DCPD is approximately 60,000 and so it is considered to be insoluble in water (Shashkina, 1965). Hart (1976) reported that DCPD had appreciable volatility. Humans can detect a slight odor of DCPD at 0.0004 mg/l, and the odor becomes more unpleasant and can cause headaches and nausea at 0.003 ppm (Shashkina, 1965).

Lethal dose values, both acute and dietary, that have been determined for DCPD are listed in Table 1. Animal species utilized as test subjects include the rat, mouse, rabbit, and Mallard duck. No toxicological information on humans could be found.

In an acute study conducted by Shashkina (1965), reactions of rats exposed to DCPD vapor were categorized as, "at the moment of exposure" and "in the following period". During the exposure period, narcosis, clonic toxicity, motor disturbance, and excitability were observed. During the following period excessive excitability upon contact or noise, spasms, agressiveness, and hemorrhagic discharges from the eyes and nose were reported. Body temperature and blood pressure were lowered by 24 and 6.2 percent, respectively. Pathological changes in the rats, post exposure, were pronounced plethora of the internal

organs, internal hemorrhage in the lungs and brain, emphysema of the lungs, dystrophic changes in the canalicules of the kidney, dystrophy of the liver, lipoid depletion of the adrenal cortex, and hyperfunction of the thyroid gland. All of the observations in the above paragraph were taken from results reported by Shashkina (1965).

Over a six month chronic DCPD inhalation study involving rats, Shashkina (1965) observed a general decrease in the number of leukocytes, no consistent change in the amount of hemoglobin in the blood, a decrease in the number of erythrocytes, and a rise in blood pressure. Observations were made relative to a control group. Pathological changes of the rats at the termination of the study were: proliferative and sclerotic changes in the trachea, bronchial tubes, and lungs plus a wrinkling of the cells in the cerebral cortex and the cerebrum.

Kinkead et al. (1971) conducted several inhalation tests utilizing rats, guinea pigs, rabbits, mice, and dogs to determine the mammalian toxicity of DCPD. In acute studies, Kinkead et al. (1971) reported dogs, guinea pigs, and rabbits more resistant than rats which were more resistant than mice but responses of all species were generally dose related and followed a pattern of eye irritation, lack of normal coordination, convulsions (if death followed), and normal body weight gain. Chronic study results reported by Kinkead et al. (1971) include kidney lesions in male rats inhaling 73.8 and 35.2 ppm DCPD and inconsistent changes in acid and alkaline phosphatase values of dogs inhaling 23.5 ppm DCPD.

Table 1. Toxicity Data on DCPD

Animal	Method	Range[1]	LD_{50}[1]	References
Rat	Oral	262- 478	353	(Kinkead et al., 1971)
Rat	Oral	310- 530	410	(Smyth et al., 1962)
Rat ♂	Oral	420- 645	520	(Hart, 1976)
Rat ♀	Oral	303- 473	378	(Hart, 1976)
Rat	IP[2]		200	(Christensen et al., 1974)
Rat	IP		310	(Kinkead et al., 1971)
Mouse	IP		200	(Christensen et al., 1974)
Mouse ♂	Oral	125- 209	190	(Hart, 1976)
Mouse ♀	Oral	170- 368	250	(Hart, 1976)
Mallard	Oral		>40000	(Jones, 1977)
			LC_{50}[1]	
Rabbit	Dermal	3110- 8290	5080	(Kinkead et al., 1971)
Rabbit	Dermal	2440- 8150	4460	(Smyth et al., 1962)
Rabbit	Dermal	3150-14360	6720	(Smyth et al., 1962)
Rat	Inh[3]	553- 817	660	(Kinkead et al., 1971)
Rat	Inh		359	(Kinkead et al., 1971)
Rat	Inh		385	(Kinkead et al., 1971)
Rat	Inh	1.37-1.69	1.52[4]	(Shashkina, 1965)
Mouse	Inh		145	(Kinkead et al., 1971)
Mouse	Inh	0.69-0.79	0.74[4]	(Shashkina, 1965)
Rabbit	Inh		771	(Kinkead et al., 1971)
Mallard	Oral	Undeterminable		(Jones, 1977)

[1] mg/kg body wt.

[2] IP - Intraperitoneal

[3] Inh - Inhalation

[4] mg/l air

Horace Gerarde, cited by Kinkead et al. (1971), reported that the principal pathological findings in rats dosed with DCPD were generalized congestion, hyperemia, and focal hemorrhage in many tissues including the kidney, intestine, stomach, bladder, and particularly the lungs.

Gage (1970) conducted inhalation studies with rats at levels of 100, 250, 1000, and 2500 ppm DCPD. Gage reported no adverse effects at 100 ppm with 15 6-hour exposures; weight loss, difficulty in breathing, tremors, hypersensitivity, one death and normal blood pressure at 250 ppm with 10 6-hour exposures; eye and nose irritation, dyspnea, incoordination, tremors, hypersensitivity, and no survivors at 1000 ppm with 1 4-hour exposure; and the same symptoms as the 1000 ppm group but with only one death at 2500 ppm with 1 1-hour exposure.

Jones (1977) conducted DCPD toxicity tests with Mallard ducks and reported an undeterminable LD_{50}, an undeterminable LC_{50}, and no adverse effects after feeding 32, 100, and 320 ppm DCPD rations for 22 weeks during a chronic study.

Diisopropyl methylphosphonate (DIMP) (Appendix A: Chemical Structures and Alternative Names for DCPD and DIMP) is an organophosphorus compound, liquid at room temperature with a bulk density of 0.976 g/cc at 25°C (Dacre, 1975) and water soluble at 1-2 g/l (Ringer, 1978). Odor detection limits have not been reported. Thus, it is not known if DIMP could unknowingly be ingested. Lethal dose values that have been determined for DIMP are listed in Table 2.

Table 2. Toxicity Data on DIMP.

Animal	Method	Range	LD_{50} mm^3/kg	References
Rabbit	IV[1]	179- 280	224	(Jacobson, 1953)
Rabbit	PC[2]		>200	(Jacobson, 1953)
Rabbit	SC[3]	>100	<200	(Ford-Moore and Perry, 1948)
Mouse	IP[4]		>250	(Horton, 1948)
Mouse (M)	Oral	903-1201	1041	(Hart, 1976)
Mouse (F)	Oral	1165-1594	1363	(Hart, 1976)
Rat	SC		>200	(Ford-Moore and Perry, 1948)
Rat (M)	Oral		1125	(Hart, 1976)
Rat (F)	Oral	747- 914	826	(Hart, 1976)
Mallard	Oral	1414-1566	1490	(Jones, 1977)

[1] IV - intravenous
[2] PC - percutaneous
[3] SC - subcutaneous
[4] IP - intraperitoneal

Jones (1977) reported an undeterminable LC_{50} value in young Mallard ducks fed DIMP-treated diets. Results of a 24-week chronic study with adult Mallard ducks showed increased feed consumption at 3200 ppm and decreased egg production at 10000 ppm DIMP in their diets.

Jacobson (1953) reported moderate corneal damages in rabbits dosed with 0.25 mm^3/kg DIMP. Hart (1976) found that application of an unspecified amount of DIMP to the eyes of rabbits produced variable results ranging from slight redness to clouding of most or all of the cornea. The symptoms persisted for up to seven days with subsequent complete recovery. Skin applications of 200, 632, and 2000 mg/kg DIMP to rabbits caused some erythema in some rabbits with complete recovery in ten days if the rabbit survived the systemic effects (Hart, 1976). Rabbit mortalities in the skin application studies were 25 and 75 percent at 632 and 2000 mg/kg, respectively.

OBJECTIVES

1. To determine the single oral acute LD_{50} and dose-response curve for DCPD and DIMP to the adult Bobwhite.

2. To determine the eight day subacute dietary LC_{50} for DCPD and DIMP to the young Bobwhite.

3. To determine chronic toxicity, including long-range effects on reproduction parameters, blood parameters, and general body changes, from feeding DCPD or DIMP to adult Bobwhites over their first reproductive cycle.

PROCEDURE

The research consisted of the determination of the lethal dose for 50% of the test subjects (LD_{50}), the determination of the lethal dietary concentration for 50% of the test subjects (LC_{50}), and a long term chronic study. All experiments employed the Bobwhite (Colinus virginianus) as a research subject, and were conducted in a windowless house at the Michigan State University Poultry Science Research and Teaching Center. The Bobwhites were procured from the Poultry Science Department, Michigan State University, East Lansing, MI 48824.

EXPERIMENT 1

This experiment was designed to determine the single, 14-day, oral dose LD_{50} of diisopropyl methylphosphonate (DIMP) and dicyclopentadiene (DCPD) to the Bobwhite. Dosages were reported in mg/kg, a standard unit of measure in toxicological studies. mg/kg is equivalent to ppm of body weight.

Adult Bobwhites, approximately one year of age, in non-laying condition, were utilized. The birds were maintained indoors in cages measuring 85.1 cm (l) x 89 cm (w) x 24.1 cm (h), 20 birds per cage. Cage space per bird was 379 cm^2.

Body weights of all birds were recorded succeeding a one-week holding period. A two-week acclimatization period followed. Body weights were again recorded at the termination of acclimatization to note any significant weight loss before range finding was initiated.

Preliminary range finding was conducted to establish the approximate lethal dose and a geometric scale of dosages was

employed for the test to give a mortality range of 10 to 90 percent.

Testing

Birds used for testing were maintained on quail breeder diet from Michigan State University (Appendix B_1: Analysis of Feed). The feed was free of antibiotics and medication. Feed and water were provided ad libitum throughout the testing period with the exception of a 15-hour minimum fasting period before oral administration of test chemicals. Weekly feed consumption was determined for each group.

The DIMP test and the initial DCPD test utilized twenty birds, ten of each sex, per dose level. The additional DCPD test utilized 10 birds, five of each sex. Weights were recorded immediately preceeding the dosing, and on the third, seventh, and fourteenth days of the succeeding two-week observational period. Post treatment behavior was observed for one hour immediately following dosing, again at 4-5 hours, and daily thereafter for the duration of the observational period.

Administration was by drenching per os from a syringe with a length of polyethylene tubing attached to a needle. The length of tubing corresponded with the distance from the back of the oral cavity to the esophageal opening of the proventriculus. This insured a uniform location for the introduction of the test substances. The syringe was 1 cc, the needle 22 ga, 2.54 cm long, and the tubing measured 0.762 mm ID and 1.29 mm OD.

Necropsies were performed on all birds, including controls, at the time of death or termination of the observational period.

A general gross inspection was performed with special emphasis on the digestive tract, liver, kidneys, heart, and spleen.

Statistical Analysis

The LD_{50} was analyzed by the method of Litchfield and Wilcoxon (1949). Weight changes were analyzed by least squares analysis of covariance with log transformation and the two-sided Dunnett t-test with modification for unequal replication. Feed consumption data were not appropriate for meaningful statistical analysis.

EXPERIMENT 2

This experiment followed the subacute toxicity test protocol as described by Heath and Stickel (1965) and Heath et al. (1972), to determine the minimum repeated oral dosage (mg/kg/day) of DIMP or DCPD that was lethal to Bobwhite chicks.

Range finding pilot studies were conducted with both chemicals to determine the effect or noneffect on mortality, feed consumption, and body weight. Since the mortality that did occur appeared unrelated to the dietary levels of DIMP or DCPD, a geometric scale of dosages was utilized to determine the point of feed refusal instead of 50 percent mortality.

Testing

Randomly selected day old Bobwhite chicks were housed indoors in a Petersime Brood Unit[2] and maintained on a standard quail starter ration (Appendix B_2: Analysis of Feed), free of antibiotics and medication. Feed and water were provided ad libitum. At 14 days of age the chicks were segregated into groups of ten birds of undetermined sex. Each group of birds was randomly assigned to one of twenty dietary treatments (ten treatments per chemical). At the initiation of the experiment one bird from each of the control groups and the low level DIMP group escaped. The experiment was conducted with nine birds in the latter groups. During the eight day test period, treated feed was fed for the first five days and clean feed was fed for the remaining three days. Feed and water were provided ad libitum throughout the test period.

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[2] Petersime Incubator Company, Gettysburg, Ohio 45328.

The test diets were prepared by dissolving the chemical in corn oil, and hand mixing with quail starter to make a premix. The premix was then added to a standard quail ration to yield the appropriate dietary level (Appendix C: Ration Preparation). The DIMP treated diets' chemical-corn oil solution constant was greater than two percent of the diet. The DCPD treated diets' chemical-corn oil solution constant was two percent of the diet. Both control diets consisted of two parts corn oil to 98 parts feed by weight. The ten dietary treatments used for testing each chemical were as follows:

DIMP (ppm): 0, 4000, 8000, 12000, 16000, 20000, 24000, 28000, 32000, and 36000.

DCPD (ppm): 0, 2000, 4000, 6000, 8000, 10000, 12000, 14000, 16000, and 18000.

Body weights were recorded on days zero, five, and eight of the test period. Feed was weighed on days zero and five (treated feed) and days six and eight (clean feed) to provide estimates of average feed consumption. Observations on bird wastage were taken into account in determining the estimated point of zero feed consumption.

Any signs of intoxication or abnormal behavior during the test period were noted. All birds that died during the trial, and those that survived until the termination of the experiment, were necropsied.

Statistical Analysis

Slopes of feed consumption, body weight change, and predicted zero feed consumption were determined by regression analysis.

EXPERIMENT 3

This experiment was designed to determine the effects of continuous long term exposure of DIMP or DCPD to the adult Bobwhite and their progeny over a single reproductive cycle.

Two identical testing rooms were utilized to separate the dietary treatments by chemical. Each chemical test consisted of four dietary treatment groups, three treated levels plus one control. Each treatment group consisted of one female and one male housed in a single cage replicated by fifteen. The birds were allowed a two week acclimatization period before the initiation of the experiment.

Initially dietary treatments were as follows:

DIMP: 0, 1200, 3800, 12000 ppm

DCPD: 0, 400, 1200, 4000 ppm

Subsequent mortality at the 3800 and 12000 ppm DIMP levels caused the reduction to 380 and 0 ppm DIMP, respectively. This decision was reached by general concensus of the principal investigator and the project officer (U.S. Army).

Testing

Test diets were prepared by the addition of a premix to a standard quail breeder ration to attain the appropriate dietary levels. (Appendix C_2: Ration Preparation). The control diets for both DIMP and DCPD consisted of two parts corn oil to 98 parts feed by weight. The diets were fed to the birds for a minimum of ten weeks before the initiation of egg production and a minimum of ten weeks after the attainment of 50 percent egg

production. Feed and water were provided ad libitum throughout the entire experiment.

Feed consumption was measured biweekly for the full duration of the experiment. Body weights were measured at 0, 2, 4, 6, and 8 weeks and at the termination of the study. Body weights were not measured during egg production to avoid any adverse effects that handling may have had on egg production.

During the pre egg production period (Nov. 1 to Jan. 8) both testing rooms were maintained at approximately 18°C with light provided six hours per day. To induce egg production the lighting schedule was increased to 16 hours of light per day. This schedule was maintained throughout the production period (Jan. 8 to May 28). Temperatures of the test rooms during the production period ranged from 15°C to 28°C.

Egg production, mortality, morbidity, and any observable clinical signs of intoxication were recorded daily. All birds, including those that died during the study and those that survived the test, were subjected to gross necropsy, with the following organ weights measured; liver, kidneys, pancreas, proventriculus, gizzard, gonads, heart, and brain. Spleen weights were recorded for the DCPD treated birds but not for the DIMP treated birds, thus, no spleen weights were reported in the results of this experiment. Hemoglobin concentration, packed red cell volume (hematocrit value), and differential counts were determined for all surviving birds at the termination of the experiment.

Egg Collection, Storage, and Incubation

Each day, eggs were marked with the corresponding cage number and date, collected, and stored at 12.8 to 15.6°C until set. The storage time ranged from zero to six days.

Eggs were set at weekly intervals in a Jamesway single stage Model 252 incubator[3]. The incubator was maintained at an average internal temperature of 37.5°C (range 36.9 to 38.1°C) and average relative humidity of 56 percent (range 52 to 65 percent). All eggs were candled on day 0 for shell cracks and again on day 14 to determine fertility and/or early embryonic death. Eggs that were cracked, infertile, or that contained early deads were removed and disposed of properly. Fertile, developing eggs were put in pedigree hatching baskets and were transferred to a hatching unit (Jamesway Model 252) on day 21. The average temperature and relative humidity of the hatcher was 37.2°C (range 36.8 to 38.1°C) and 67 percent (range 65 to 70 percent), respectively. On day 24 the hatched chicks were removed from the hatcher, wing banded, and housed in a Petersime Brood unit for a two week observational period. Clean feed and water were provided ad libitum during the two weeks. Mortality was recorded daily. Survivors were weighed and sacrificed at the termination of the two week observational period and livability calculated.

Eggs that did not hatch were broken open, examined, and placed in one of the following categories; pipped live, pipped dead, live in shell, or dead in shell.

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[3] James Manufacturing Company, Inc. (a subsidiary of Butler Manufacturing Co.), Fort Atkinson, Wisconsin 53538.

One day's egg production was collected at biweekly intervals to be measured for egg shell thickness. The eggs collected for shell measurement were cracked open at the girth, the contents washed out, and the shells were air dried for a minimum of 48 hours. Measurements of the shell plus the membranes were taken at four points around the girth using a micrometer[4] calibrated to 0.01 mm units.

Hematological Preparation

Determinations of differential counts, packed red cell volume, and hemoglobin concentration were completed on all birds that survived until the termination of the experiment.

The dry, fixed smear method was used to determine blood differentials for each bird. This method involved air drying a thin smear of fresh blood on a clean glass slide, then flooding with avian Wright's stain (Appendix D: Preparation of Wright's stain and buffer). After a five minute period a few drops of buffer were added to allow maximum differential coloration of the blood cells. Following an additional five minute period the cells were rinsed with distilled water and blotted dry. Differential counts were determined by hand counting the first 100 white blood cells, repeating the count, then calculating the average of the two counts.

Hematocrits were determined by collecting blood from a puncture of a vein in the wing into two heparinized capillary tubes, sealing one end of each tube and centrifuging at 4500 rpm for 7.5 minutes

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[4] Federal Products Corporation (a subsidiary of Esterline Corp.) 1144 Eddy Street, Providence, Rhode Island 02901.

in an International Microcapillary Centrifuge[5]. The packed red cell volume of each tube was measured using a microcapillary reader[5] after centrifugation.

The cyan-methemoglobin method was used to determine the individual hemoglobin concentrations. In this method five ml of Drabkin's Reagent (Appendix E: Preparation of Drabkin's Reagent) were placed in a clean cuvette. Using a micropipetter[6], 25 microliters of blood were pipetted into the reagent. The micropipetter was rinsed several times in the blood-reagent solution. The cuvette was stoppered and inverted several times to facilitate mixing. A ten minute period followed, allowing for maximum conversion of hemoglobin to cyan-methemoglobin. At the end of ten minutes the cuvette was wiped clean and placed in a Spectronic 20 Calorimeter-Spectrophotometer[6]. The percent absorbance of the sample at 540 nm, was recorded. The hemoglobin concentration was determined by the comparison of the sample percent absorbance against the percent absorbance of standards with human hemoglobin concentration[7] (Appendix F: Determination of hemoglobin concentration).

Statistical Analysis

Data from the chronic study were treated statistically by analysis of variance; sample units with three exceptions, for the variables measured were the individual cages. The exceptions were for body weight change, organ weight, and hemotological parameters where the sample units were the individual birds.

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[5] International Equipment Company, Boston, Mass.

[6] Bausch and Lomb, Rochester, New York.

[7] Cyanmethemoglobin certified standard, Hycel, Inc., Houston, Texas.

Dunnett's t-test (with modification for unequal replication where applicable) was used to compare all treatment groups against their respective controls for each variable except percent livability of progeny. The latter was analyzed by the split-plot design (Gill, 1977) with arcsin transformation. Body weight change, egg production, and feed consumption were not analyzed by the split-plot design for three reasons: unequal replication, parallelism of the treatment, and control groups' trends and abnormal frequency distribution of the data.

EXPERIMENT 1

Rationale

The common method of expressing acute toxicity is the LD_{50} value; the single, oral dose of a compound that will produce death in 50% of the test subjects. It is this value that is the most rapid and convenient to determine when evaluating the hazard of a chemical, comparing inherent toxicity of chemicals, and providing a basis for the determination of dose levels to be used in subacute and chronic toxicological studies. Caution, however, must be heeded when using the LD_{50} value because the severity of clinical signs, if any, are often excluded, species variation in susceptability can be considerable, and a maximum dose over prolonged exposure may bear no relationship to the LD_{50} figure.

The single oral median lethal dose (LD_{50}) of DIMP and DCPD to the Bobwhite was determined to provide information that was deficient in the literature. Clinical signs noted during the test period, included mortality, morbidity, behavorial changes, and differences in clinical signs between the sexes. The information gathered was used to aid in the determination of the dose levels in the subacute and chronic studies.

Range finding pilot studies were conducted to provide a practical dosage span to be used in the acute test.

DIMP pilot tests began at 200 mg/kg body weight. The dose was repeatedly doubled until a level of 1600 mg/kg body weight was reached with deaths occurring at 800 mg/kg body weight and 1600 mg/kg body weight. Three additional trials were conducted to verify the information gathered from the initial trial. Dose

levels utilized in additional trials were between 200 and 1600 mg/kg body weight. Overall results are shown in Table 3.

DCPD range finding began at 400 mg/kg body weight. The dose was repeatedly doubled until a level of 3200 mg/kg body weight was reached, with deaths occurring at 1600 mg/kg body weight and 3200 mg/kg body weight. As with DIMP range finding, three additional trials were conducted to provide more reliable data to use in the determination of the LD_{50} dose levels. Overall results are shown in Table 3.

The first three range finding trials of each chemical employed one adult bird per dose level. The final range finding trial of each chemical utilized two adult birds per dose level. All birds were provided feed and water ad libitum with the exception of a minimum 15 hour fasting period before per os dosing. A two-week observational period followed dosing. Survivors were sacrificed at the termination of the trial.

Table 3. Results of DIMP and DCPD LD_{50} range finding trials.

Chemical	Level of DIMP (mg/kg body weight)	Number of birds	Mortality %
DIMP	300	2	0
	400	2	0
	600	4	25
	700	1	0
	800	5	20
	900	2	0
	1000	2	50
	1100	2	100
	1200	2	50
	1600	2	100

Chemical	Level of DCPD (mg/kg body weight)	Number of birds	Mortality %
DCPD	400	1	0
	800	1	0
	1000	1	0
	1100	3	67
	1200	3	100
	1300	3	100
	1400	3	100
	1500	3	67
	1600	4	100
	3200	3	100

RESULTS

Experiment 1

Mortality for the quail treated per os with DCPD and DIMP is listed in Tables 4 & 5. Determination of acute oral LD_{50} by the method of Litchfield and Wilcoxon (1949) for the compounds tested was:

Compound	Species	LD_{50} mg/kg
DCPD	Bobwhite	1010 $\pm$ 80.0
DIMP	Bobwhite	1000 $\pm$ 68.2

Mortality curves of DCPD and DIMP for the Bobwhite are plotted in Figures 1 and 2, respectively. The curves may be considered parallel within experimental error ($P < .05$), and the compounds do not differ in potency ($P < .05$). Most deaths occurred within the first 24 hours after dosing with DIMP and from 48 to 96 hours after dosing with DCPD. There was no mortality nor clinical sign differences between the sexes among the treated groups.

Clinical signs of reaction to DIMP per os dosing included an initial comatose state followed by death or recovery. During recovery, staggering, sitting still, and shallow breathing were noted. Recovery was usually complete within 24 hours.

Responses of quail to DCPD dosing were noted after 24 hours when activity decreased and the birds became quiescent. Those that made efforts to walk were unsteady and lacked coordination. Recovery or coma and death followed by 96 hours post-treatment.

During the 14-day post-treatment period, no further signs of intoxication nor significant weight changes of birds in treated groups from those of the control gorups were noted for birds

Table 4. Mortality of adult Bobwhite quail during a 14-day period following a single per os dosing with DCPD.

Treatment level (mg/kg)	Mortality		
	No. died/No. treated		Combined
	male	female	%
0 (control)	0/15	0/15	0
200	0/ 5	2/ 5	20
400	1/ 5	1/ 5*	10
600	1/ 5	0/ 5	10
800	5/15	4/15	30
900	4/10	1/10	25
1000	10/15	9/15	63
1100	2/10	4/10	30
1200	11/15	9/15	61
1400	5/ 5	4/ 5	90
1600	9/10	--	90

* Accidental death

Table 5. Mortality of adult Bobwhite quail during a 14-day period following a single per os dosing with DIMP.

Treatment level (mg/kg)	Mortality No. died/No. treated male	female	Combined %
0 (control)	0/10	0/10	0
800	0/10	2/10	10
900	3/10	4/10	35
1000	7/10	4/10	55
1100	7/10	4/10	55
1200	8/10	9/10	85

Figure 1. Percent mortality of adult Bobwhites, equal numbers of each sex, given a single per os dose of DCPD and observed for 14 days post treatment. In the regression equation x = dose of DCPD in mg/kg of body weight and y = percent mortality.

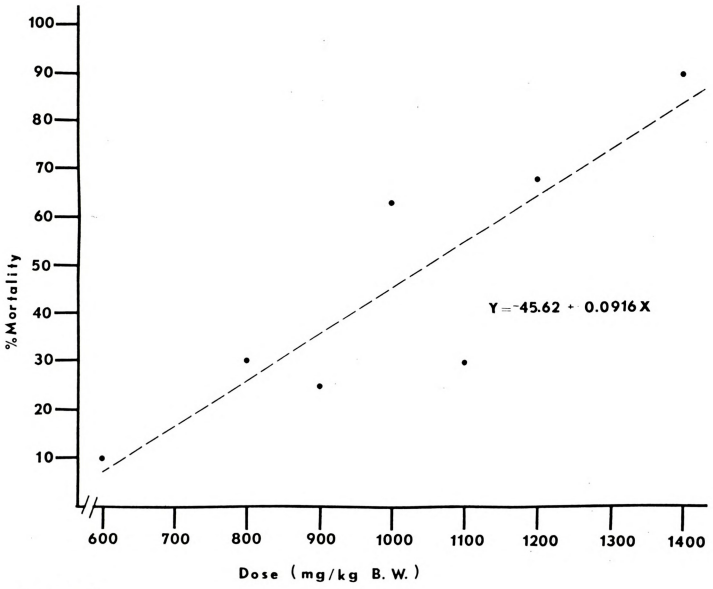
100
90
80
70
60
50
40
30
20
10
% Mortality
Y = -45.62 + 0.0916 X
600
700
800
900
1000
1100
1200
1300
1400
Dose (mg/kg B. W.)

Figure 1.

Figure 2. Percent mortality of adult Bobwhites, equal numbers of each sex, given a single per os dose of DIMP and observed for 14 days post treatment. In the regression equation x = dose of DIMP in mg/kg of body weight and y = percent mortality.

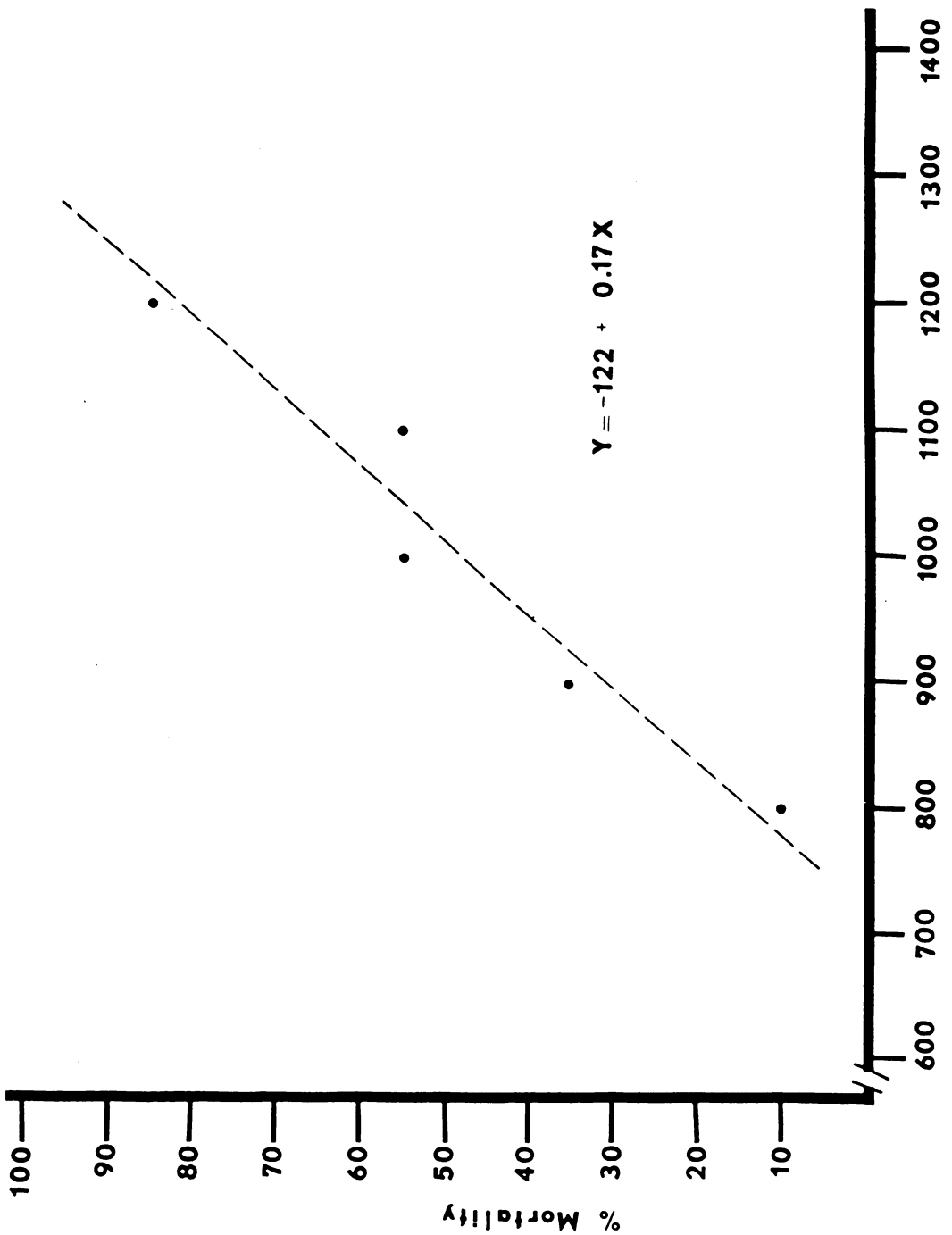
Y = -122 + 0.17X
% Mortality
100
90
80
70
60
50
40
30
20
10
600
700
800
900
1000
1100
1200
1300
1400
Dose (mg/kg B.W.)

Figure 2.

treated with either chemical (Table 6). Necropsies of all birds, i.e., those that died and those that were sacrificed at the end of the post treatment period, showed no gross pathological changes in the quail which had been administered either DIMP of DCPD.

Feed consumption, for the 14-day post-treatment period, is listed in Table 7 for the quail dosed with DIMP or DCPD. With DIMP, feed consumption in the 900 and 1200 mg/kg dosed groups appeared to have been depressed during the first week. At the 100 mg/kg level, feed consumption may have been affected for the entire two-week period.

Quail treated with DCPD at levels higher than 200 and 400 mg/kg showed depressed feed consumption with a marked decrease at the 1400 and 1600 mg/kg levels, during the first week. By the second week feed consumption had improved at all levels.

Table 6. Quail body weight changes during post treatment for LD_{50}.

Level mg/kg	n	Mean body weight (g) Day 0	Mean body weight (g) Day 14	Mean change (g/b/d)
DIMP				
0	20	202.95	207.05	$+.293_a$ [1]
800	18	199.33	195.28	$-.290_a$
900	13	190.23	195.23	$+.357_a$
1000	9	203.22	197.33	$-.421_a$
1100	9	193.67	195.22	$+.111_a$
1200	3	199.67	182.00	-1.262_a
DCPD				
0	19	205.74	198.74	$-.500_b$ [1]
200	8	188.75	196.38	$+.545_b$
400	8	189.00	178.88	$-.742_b$
600	9	186.44	182.11	$-.309_b$
800	21	184.71	193.00	$+.592_b$
900	15	201.80	198.73	$-.219_b$
1000	11	207.45	191.18	-1.162_b
1100	14	188.93	192.29	$+.240_b$
1200	10	197.70	191.80	$-.421_b$
1400	1	194.00	189.00	$-.357_b$
1600	1	205.00	198.00	$-.500_b$

[1] Means having the same subscript are not significantly different from their respective control ($P < .05$).

Table 7. Quail feed consumption (g/b/d) during post-treatment for LD_{50}

Level (mg/kg)	n	First week	Second week
DIMP			
0	20	13.04	14.96
800	18	12.63	13.75
900	13	8.34	15.31
1000	9	10.71	8.76
1100	10	12.43	12.49
1200	3	7.76	14.09
DCPD			
0	19	10.26	13.60
200	8	11.04	11.17
400	9	9.42	9.04
600	9	6.29	11.35
800	21	6.25	10.14
900	15	7.65	13.19
1000	11	8.09	12.57
1100	14	7.65	15.21
1200	10	8.01	14.44
1400	1	3.36	13.57
1600	1	1.86	12.40

EXPERIMENT 2

Rationale

The subacute toxicity test is performed to eliminate error due to atypical results caused by a single oral dose of a chemical, thus providing a more accurate profile of a chemical's toxicity in the field. In this test, a chemical or test substance is incorporated into a standard ration which is subsequently fed ad libitum to the test subjects for a designated duration. Not only does this test better represent the repetitive dose exposure of wildlife to a chemical than the single dose test (LD_{50}), but it also provides additional information by allowing for such variables as daily feed intake, absorption, and metabolism in the test design.

Levels of both chemicals employed for the subacute test were partially determined by the LD_{50} value, the slope of the dosage-mortality curve, the variation within a group's response to the same dose, and the results of the range finding pilot studies. The pilot studies were performed to provide an estimate of the maximum dosage an animal will tolerate on repeated intake of a chemical, and to eliminate unnecessary or inappropriate dosages before initiating further, more costly testing.

The eight-day range finding pilot test utilized six birds for each dietary treatment per chemical. Dietary treatments consisted of 4000, 8000, and 16,000 ppm of DIMP or DCPD. Treated feed was fed for the initial five days of the test and untreated feed for the remaining three days. The three-day clean feed period was included to avoid overestimation of the lethal dosage by calculating mortality before either compound had sufficient time to act.

Body weights were measured at the initiation of the test, the transition between feeding treated and untreated feed, and the termination of the test. Feed consumption was estimated by providing a known amount of treated or clean feed for the birds and weighing the remainder on days five and eight, of the test, respectively. Survivors were sacrificed at the end of the experiment.

Results of the range-finding test were:

Chemical	Level in diet (ppm)	Mean change in body wt. (g/b/d)	Mean feed consumption (g/b/d)	Mortality (%)
DIMP	4000	+3.150	7.055	0
DIMP	8000	+0.185	5.650	33.3
DIMP	16000	+0.340	4.235	0
DCPD	4000	+2.315	7.085	0
DCPD	8000	+0.965	3.740	66.7
DCPD	16000	+1.785	2.400	0

Since there was 33.3 percent mortality at the 8000 ppm DIMP level (the median level) during the range-finding study, the levels for the subacute study included four levels set above the maximum two percent level recommended in the Federal Register (1975). This was to hopefully result in at least 50 percent mortality or to establish a zero feed intake level if the mortality did not reach 50 percent at any level. Since the mortality on DCPD was greater than 50 percent (66.7 percent at the 8000 ppm level) in the range finding study, the subacute levels were set at below two percent of the diet.

Results

DCPD. Compared to the control group, feed consumption of the chicks on diets that contained DCPD increased in six of the treated groups and decreased in three of the treated groups (Figure 3). The differences ranged from a 12.2 percent decline (0.16 g/b/d less than the control) for the birds that received the 10000 ppm diet to a 16.4 percent increase (1.82 g/b/d more than the control) for the birds that received the 4000 ppm diet, with a mean 1.4 percent (0.07 g/b/d) above that of the control. The equation describing the regression line depicting feed consumption is $y = 5.343 - 0.00003x$ (Figure 4), with a correlation between feed consumption and level of DCPD in the diet of -0.4683; the predicted zero feed consumption was calculated from this line to be 73028 ppm DCPD in the diet.

Body weight data (Figure 5) showed that all treatment groups gained weight. Total intake of the chemical ranged from 357.4 to 3051.3 mg/kg/day with the least amount of intake in the three lowest level groups (2000, 4000, and 6000 ppm). The lower level diets (2000 - 8000 ppm) showed a mean gain of 2.86 g/b/d, a 0.1 g/b/d decrease as compared to the control. Birds on higher levels (10000 - 18000 ppm) showed a mean gain of 2.38 g/b/d, a 0.58 g/b/d decrease as compared to the control. The slope of the regression line depicting body weight changes is -0.00004, (Figure 6 shows body weight changes, not body weight) with a correlation between the level of DCPD in the diet and weight gain of -0.6812. Predicted zero body weight gain was calculated to be 80108 ppm DCPD in the diet.

Figure 3. The effect of feeding DCPD at various levels in the diet for five days on feed consumption of 14-day old Bobwhite quail chicks.

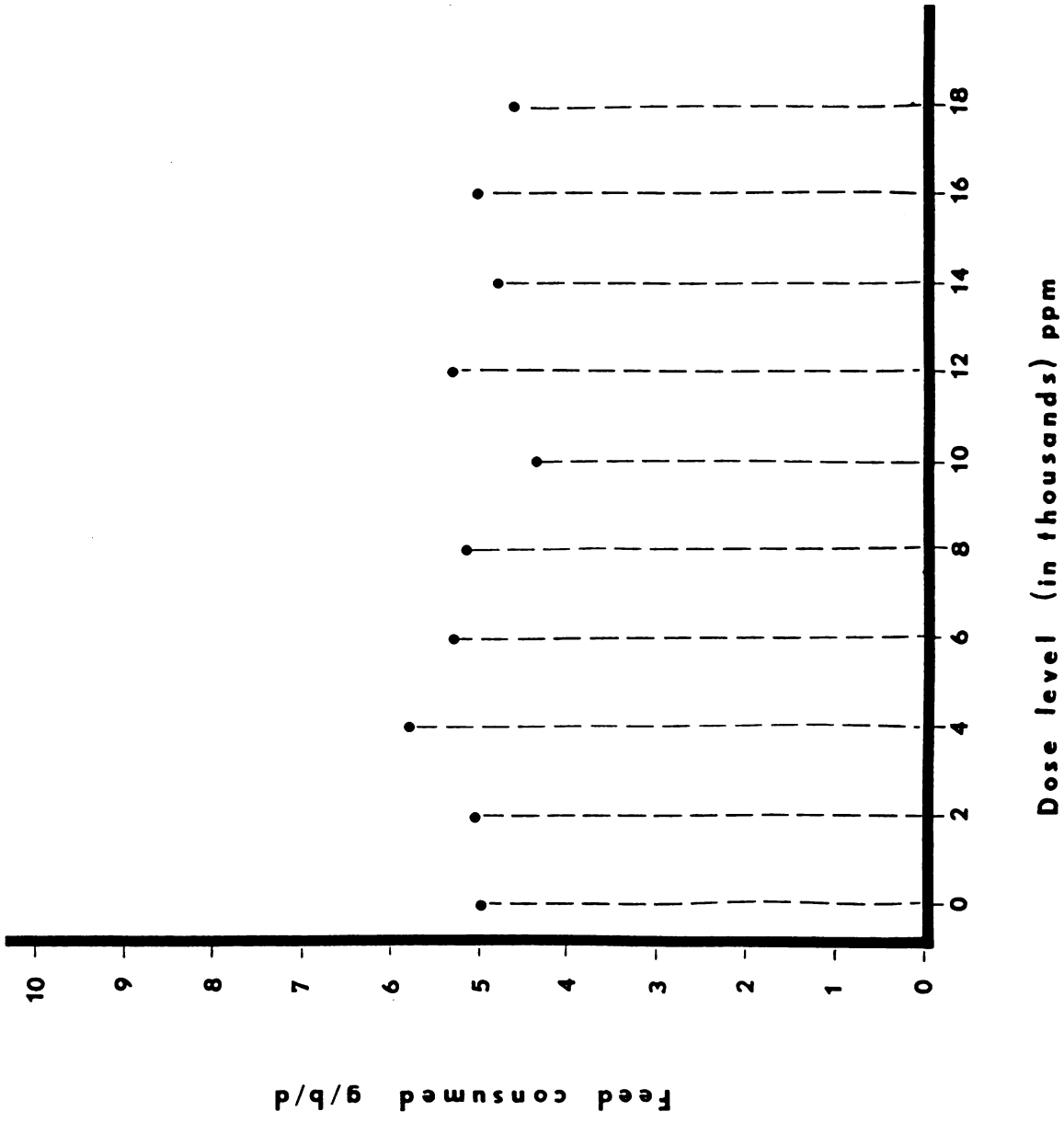
Feed consumed g/b/d
0
1
2
3
4
5
6
7
8
9
10
Dose level (in thousands) ppm
0
2
4
6
8
10
12
14
16
18

Figure 3.

Figure 4. The regression equation of the data shown in Figure 3. In the regression equation x = ppm of DCPD in the diet and y = feed consumption (g/b/d).

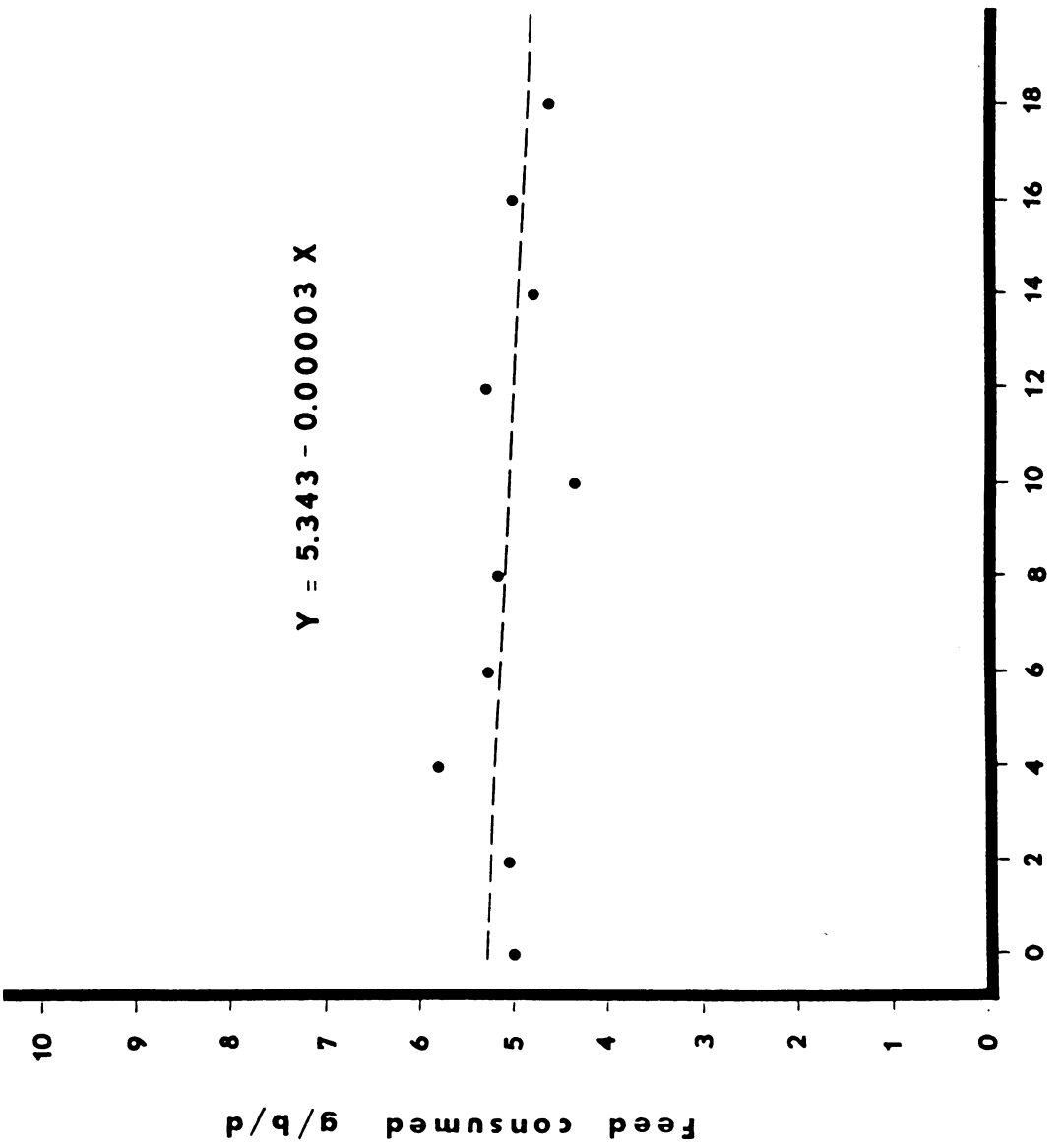
Y = 5.343 - 0.00003 X
Feed consumed g/b/d
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
14
16
18
Dose level (in thousands) ppm

Figure 4.

Figure 5. Effect of feeding various levels of DCPD in the diet for five days on body weight change of 14-day-old Bobwhite chicks.

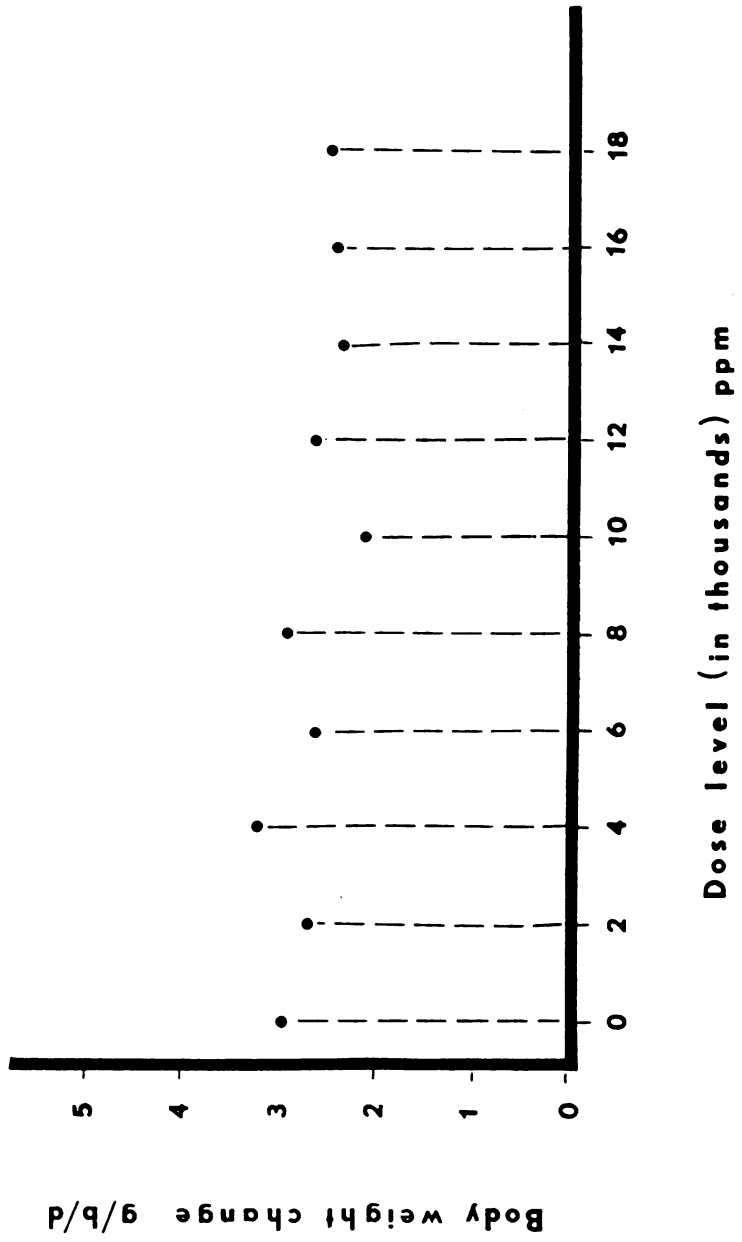
Body weight change g/b/d
5
4
3
2
1
0
Dose level (in thousands) ppm
0
2
4
6
8
10
12
14
16
18

Figure 5.

Figure 6. Regression equation of the data shown in Figure 5. In the regression equation x = ppm DCPD and y = body weight change in g/b/d.

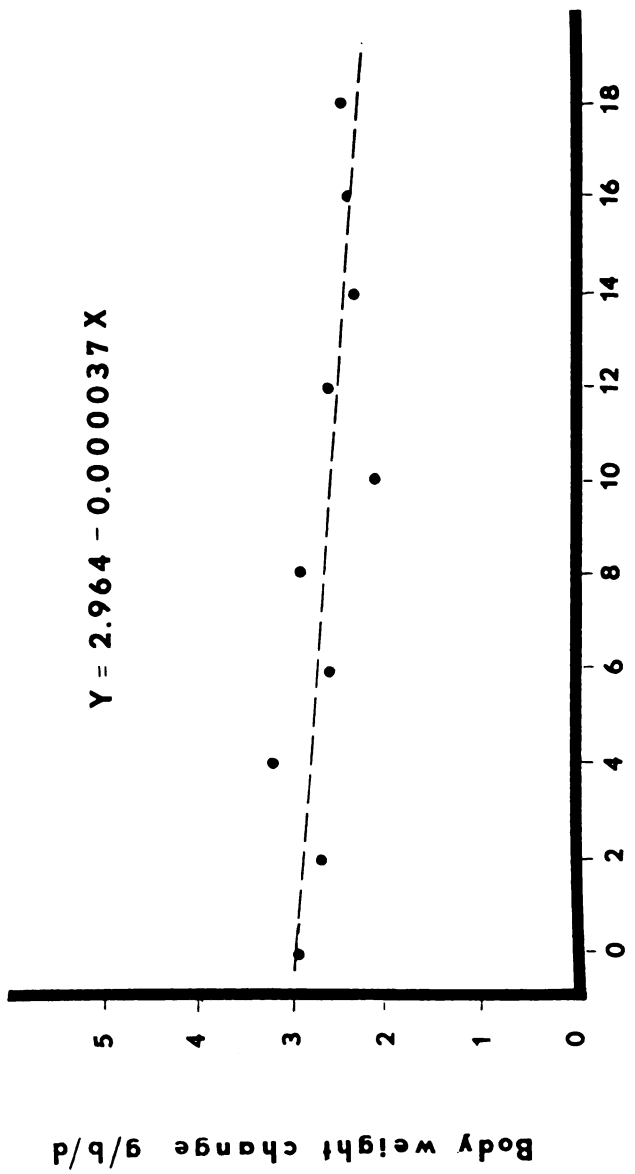
Y = 2.964 - 0.0000037 X
Body weight change g/b/d
0
1
2
3
4
5
0
2
4
6
8
10
12
14
16
18
Dose level (in thousands) ppm

Figure 6.

There were no trends in mortality (Table 8). Both the 2000 and 10000 ppm groups had the highest mortality at 20 percent. The 18000 ppm group, which had the highest intake of DCPD in mg/kg/day, had a mortality of 10 percent. All other groups had no mortality even though levels of 6000 ppm and higher had DCPD intake (in mg/kg/day) levels above the LD_{50} value of 1010 mg/kg body weight. Correlation between mortality and mg DCPD/kg/day ingested is -0.0648.

During the three day post-treatment period, all groups, except the 10000 ppm group, had increased feed consumption as compared to the control (Figure 7). There were no trends in feed consumption since the slope of the regression line is +.00001 and the correlation between the level of chemical in the treated diets and feed consumption is +0.1486. The increases in feed consumption ranged from 3 percent at the 4000 ppm level to 19.94 percent at 8000 ppm level, with a mean increase of 7.74 percent (5.16 g/b/d) as compared to the control.

Body weight changes (Table 9) during the post-treatment period showed no trends. The slope of the regression line is +0.00004, and the correlation between the level of chemical in the treated diets and feed consumption is +0.3730. All groups, with the exception of the 8000 ppm group, showed gains ranging from 4.83 g/b/d at 16000 ppm level to 3.37 g/b/d at 14000 ppm level, with a mean gain of 3.80 g/b/d. This was only 0.718 g/b/d greater than the control. At the 8000 ppm level the body weight gain was 2.6 g/b/d which was 0.44 g/b/d less than the control.

Table 8. Calculated DCPD intake over 5 days and mortality over 8 days for 14-day-old Bobwhite chicks on LC_{50} trial.

Level of DCPD in diet (ppm)	DCPD consumed/ day (mg)	Mean[1] body wt. (gms)	DCPD consumed/ kg bd. wt./ day (mg)	Mortality (%)
0	0	28.3	0	0
2000	10.4	29.1	357.4	20
4000	23.3	30.9	754.0	0
6000	31.8	28.0	1135.7	0
8000	41.3	30.0	1376.7	0
10000	43.9	25.7	1708.2	20
12000	63.8	30.1	2119.6	0
14000	67.2	26.2	2564.9	0
16000	80.6	28.1	2868.3	0
18000	83.3	27.3	3051.3	10

[1] Mean body weight of treatment group for five day interval.

Figure 7. Feed consumption of Bobwhite chicks fed untreated feed during three day post-treatment period after withdrawal of DCPD treated diets.

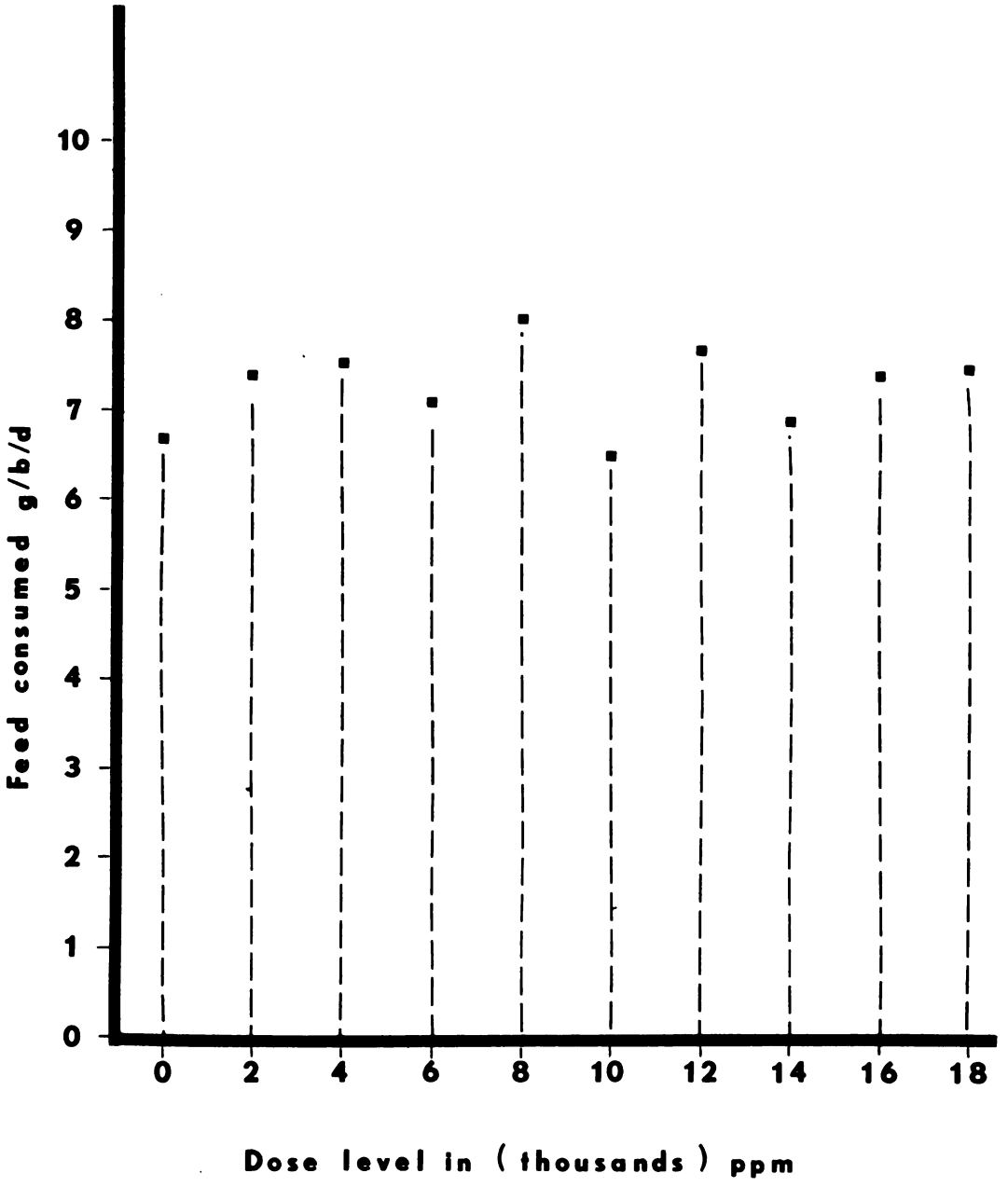
Feed consumed g/b/d
0
1
2
3
4
5
6
7
8
9
10
0
2
4
6
8
10
12
14
16
18
Dose level in (thousands) ppm

Figure 7.

Table 9. Body weight change of Bobwhite chicks during the 3-day period after withdrawal of DCPD-treated diets.

DCPD level in the diet (ppm)	Weight change (g/b/d)	Feed consumed/ weight change
0	3.04	2.19
2000	3.50	2.11
4000	3.70	2.04
6000	3.90	1.82
8000	2.60	3.08
10000	3.96	1.64
12000	3.67	2.09
14000	3.37	2.04
16000	4.83	1.53
18000	3.45	2.16

No gross pathological changes between the DCPD-treated groups and the control were observed during necropsies.

DIMP. Feed consumption (Figure 8) of chicks on the three lowest levels (4000, 8000, and 12000 ppm) was increased compared to the control by 6.72 percent, 8.89 percent, and 7.8 percent, respectively (a mean increase of 7.80 percent or 0.43 g/b/d). Quail fed all other levels of DIMP showed decreased feed consumption as compared to the control, ranging from 15.97 percent at the 28000 ppm level to 43.01 percent at the 26000 ppm level. The slope of the regression line for feed consumption is -0.00008, with a correlation between feed consumption and level of DIMP in the diet of -0.8514 (Figure 9). The predicted zero feed consumption calculated from this line is 77959 ppm. Total intake of the chemical in mg/kg/day ranged from 755.60 at the 4000 ppm level to 4982.90 at the 36000 ppm level. With the exception of the 32000 ppm level, there was a continuous increase in the amount of DIMP ingested as the level of DIMP in the diet increased.

Body weight data showed that all groups gained weight (Figure 10). Birds on lower DIMP levels (4000 to 16000 ppm) showed a mean gain of 2.06 g/b/d; a decrease of 1.03 g/b/d as compared to the control. Birds on the higher levels of DIMP, 20000 to 36000 ppm, showed a mean gain of 1.11 g/b/d; a decrease of 1.98 g/b/d as compared to the control. The slope of the regression line for body weight changes is -.00007 and the correlation between the level of DIMP in the diet and body weight change is -0.8540 (Figure 11). Predicted zero body weight gain is 44547 ppm DIMP in the diet.

Figure 8. Effect of feeding various levels of DIMP in the diet for five days on feed consumption of 14-day-old Bobwhite chicks.

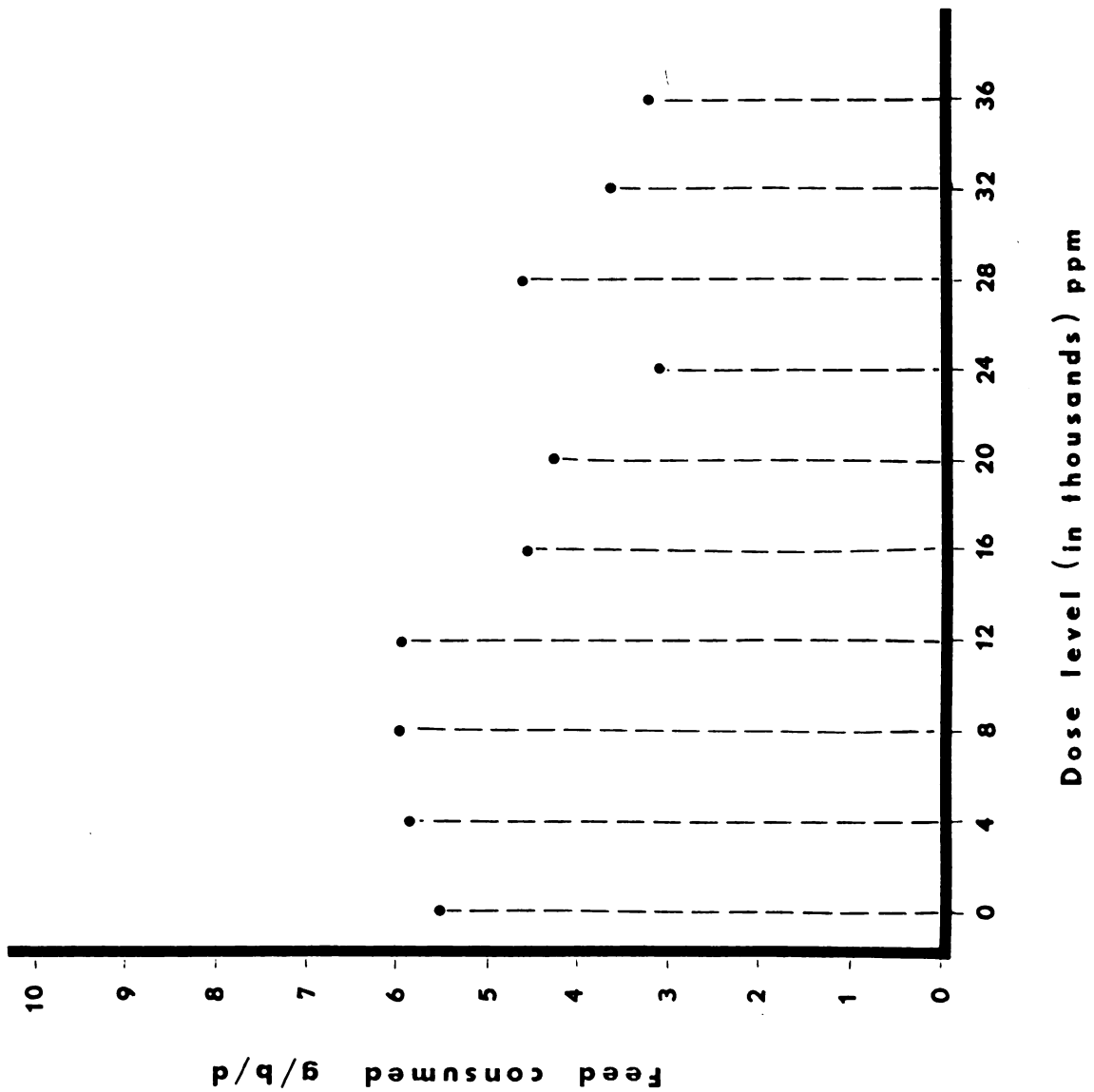
Feed consumed g/b/d
0
1
2
3
4
5
6
7
8
9
10
Dose level (in thousands) ppm
0
4
8
12
16
20
24
28
32
36

Figure 8.

Figure 9. Regression equation of the data shown in Figure 8. In the regression equation x = ppm of DIMP and y = feed consumption in g/b/d.

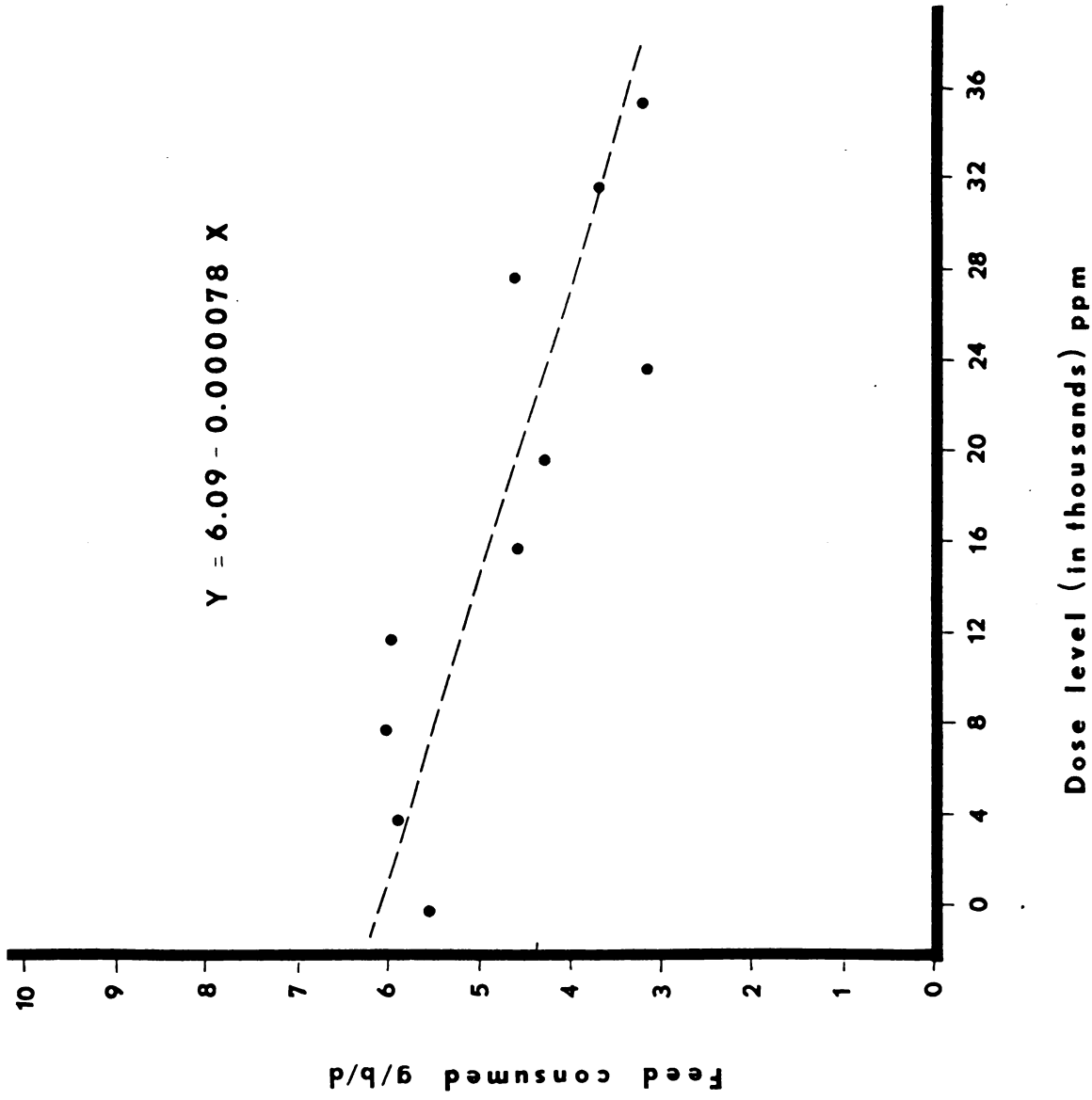
Y = 6.09 - 0.000078 X
Feed consumed g/b/d
0
1
2
3
4
5
6
7
8
9
10
0
4
8
12
16
20
24
28
32
36
Dose level (in thousands) ppm

Figure 9.

Figure 10. Effect of feeding various levels of DIMP in the diet for five days on body weight change of 14-day-old Bobwhite chicks.

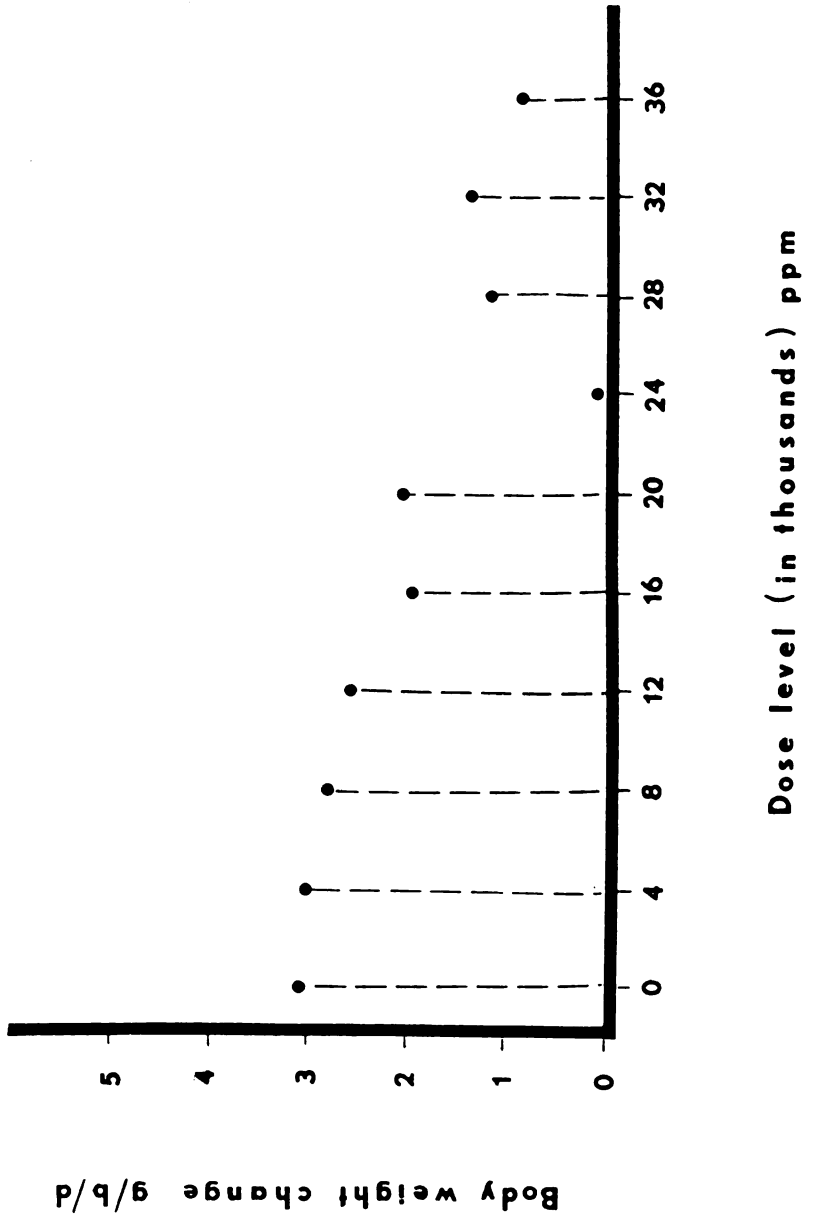
Body weight change g/b/d
0
1
2
3
4
5
0
4
8
12
16
20
24
28
32
36
Dose level (in thousands) ppm

Figure 10.

Figure 11. Regression equation of the data shown in Figure 10. In the regression equation x = ppm of DIMP and y = body weight change in g/b/d.

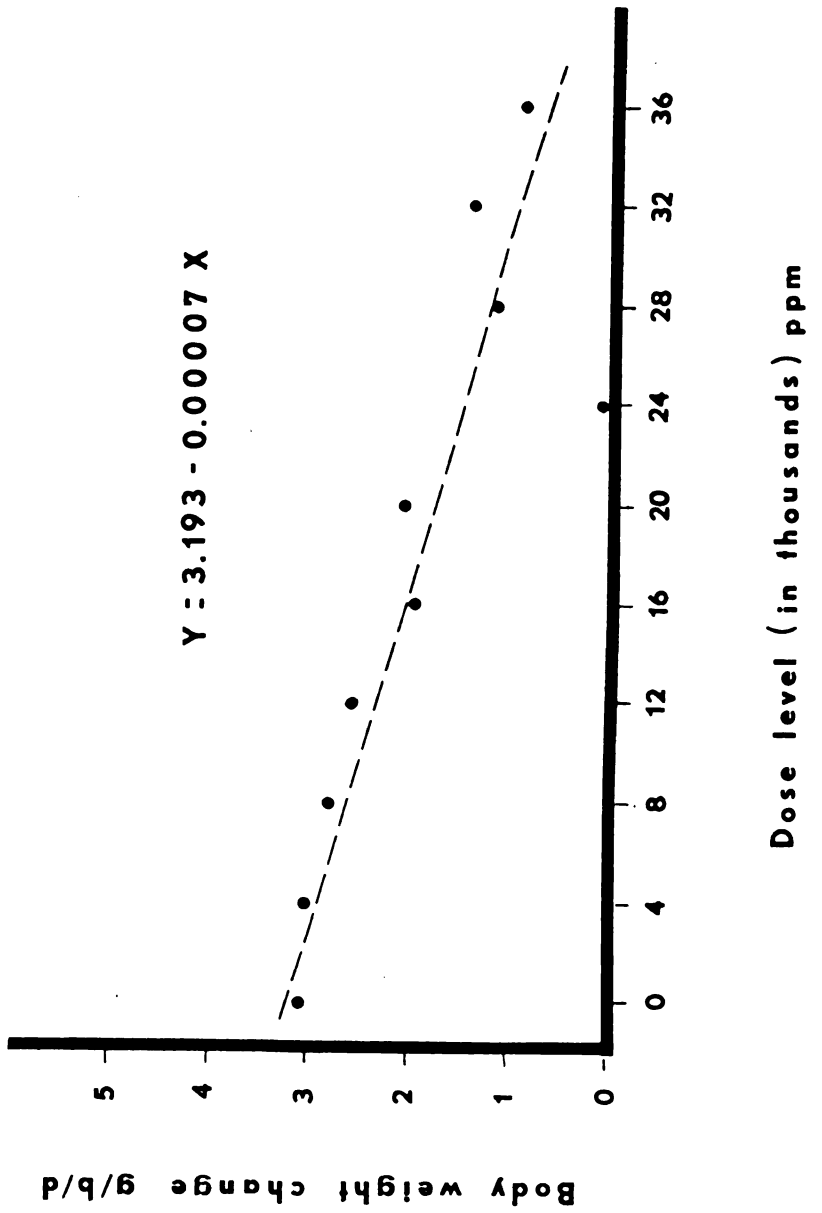
Y = 3.193 - 0.00007 X
Body weight change g/b/d
0
1
2
3
4
5
0
4
8
12
16
20
24
28
32
36
Dose level (in thousands) ppm

Figure 11.

Mortality was limited and showed no trends (Table 10). The 28000 and 24000 ppm groups showed 10 and 60 percent mortality, respectively. The high mortality in the 24000 ppm group was attributed to cannibalism. No other groups showed any mortality even though the amount of DIMP ingested ranged from 755.6 to 4982.9 mg/kg/day which bracketed the LD_{50} value of 1000 mg/kg. Quail in all groups showing no mortality, except the 4000 ppm group, had values of DIMP ingested above the LD_{50} value. The correlation coefficient between mortality and mg DIMP ingested is +0.0905.

During the three-day post-treatment period, feed consumption increased in all DIMP groups including the control, however, the greatest increase generally occurred in those groups that had shown the greatest decrease in feed consumption during the five-day treatment period (Figure 12).

Increases in feed consumption ranged from 105 percent (3.39 g/b/d) at 36000 ppm level to 22 percent (1.33 g/b/d) at 3000 ppm level with a mean of 58.33 percent. The control showed a 41 percent increase (2.27 g/b/d) in feed consumption. In total amount of feed consumed, the 4000 - 20000 ppm groups showed a mean feed consumption of 7.70 g/b/d; 0.08 g/b/d less than the control. Quail in groups fed 24000 - 36000 ppm had a mean feed consumption of 6.24 g/b/d; 1.54 g/b/d less than the control.

Body weight changes, during the post treatment period showed no trends. The correlation between the level of chemical in the previous diet and feed consumption is -0.5222 (Table 11). All groups showed body weight gains ranging from 4.23 g/b/d at 20000 ppm level to 3.01 at 24000 ppm level, with a mean of 3.86 g/b/d. This was 1.47 g/b/d less than the control.

Table 10. Calculated DIMP intake over 5 days and mortality over 8 days for 14-day-old Bobwhite chicks on LC_{50} trial.

Level of DIMP in diet (ppm)	DIMP consumed/ day (mg)	Mean[1] body wt. (g)	DIMP consumed/kg bd. wt./ day (mg)	Mortality (%)
0	0	30.8	0	0
4000	23.5	31.1	755.6	0
8000	48.0	29.2	1643.8	0
12000	71.3	27.9	2555.6	0
16000	73.3	27.3	2685.0	0
20000	86.0	27.5	3127.3	0
24000	75.4	19.6	3846.9	60
28000	129.6	26.4	4909.1	10
32000	116.5	25.1	4641.4	0
36000	116.6	23.4	4982.9	0

[1] Mean body weight of treatment group for five day interval.

Figure 12. Feed consumption of Bobwhite chicks fed untreated feed during three day post-treatment period after withdrawal of DIMP treated diets.

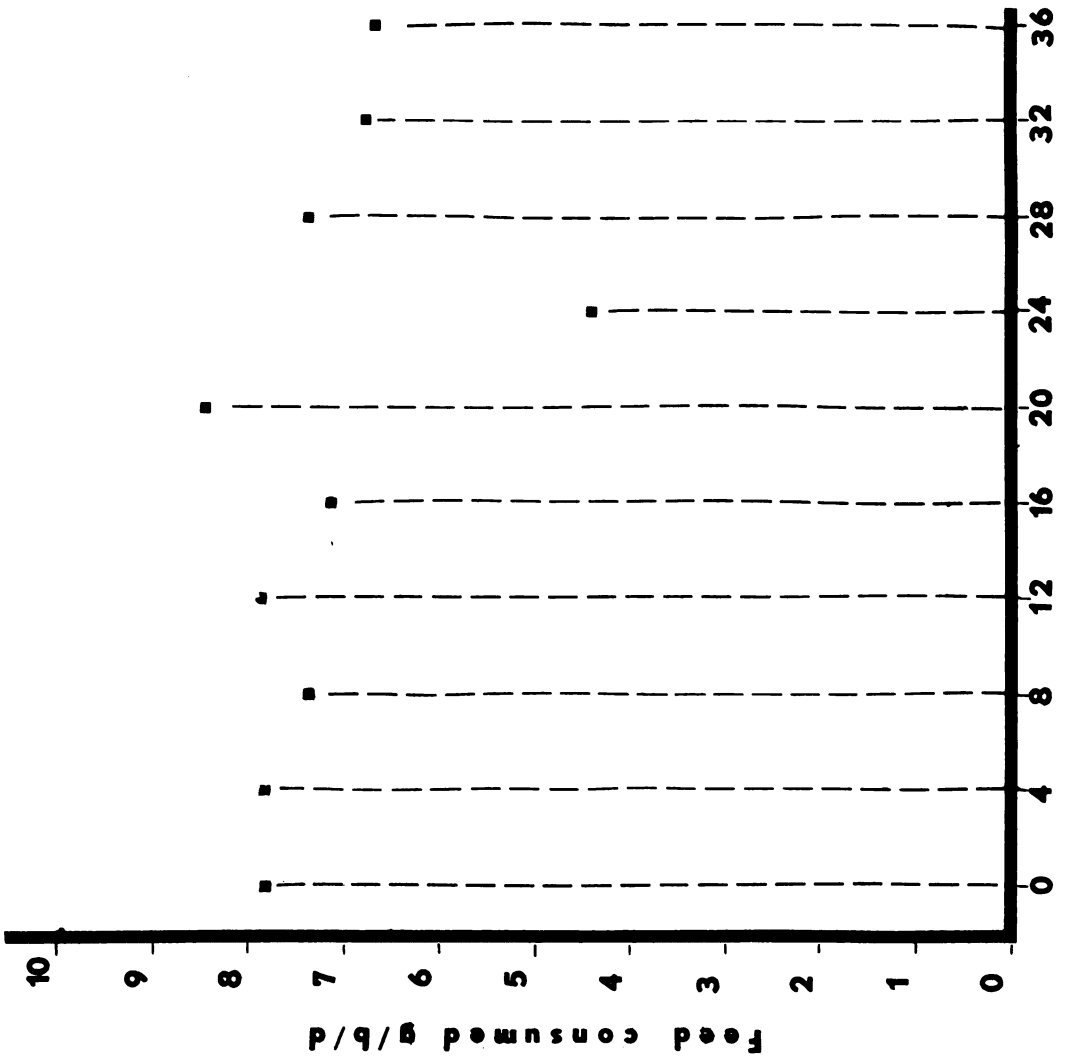
Feed consumed g/b/d
0
1
2
3
4
5
6
7
8
9
10
0
4
8
12
16
20
24
28
32
36
Dose level in (thousands) ppm

Figure 12.

Table 11. Body weight change of Bobwhite chicks during 3-day period after withdrawal of DIMP-treated diet.

DIMP level in the diet (ppm)	Weight change (g/b/d)	Feed consumed/ weight change
0	5.33	1.46
4000	4.07	1.91
8000	3.80	1.93
12000	3.93	2.00
16000	3.97	1.80
20000	4.23	1.99
24000	3.01	1.44
28000	3.93	1.86
32000	3.83	1.75
36000	3.93	1.69

No gross pathological changes between the DIMP-treated groups and the control were observed during necropsies.

Rationale

The reproduction process is a complex phenomenon involving many physiological and behavioral changes governed by the action and interaction of chemicals and hormones. These carefully timed and coordinated changes in both parent and progeny can be very susceptable to a toxicant's effects even when a toxicant is fed continuously at exposure levels that exclude acute mortality and observable morbidity. Such effects in adults may include alteration in spermatogenesis, oogenesis, ovulation, and/or conception in birds, resulting indecreased production or fertility. Toxicants' effects at the progeny level may include embryonic death (embryogenicity) or alteration of embryonic organogenesis resulting in nonlethal abnormalities or malfunctions of the fetus (teratogenicity).

Single oral dose lethality and lethal dietary concentration values provide adequate information when used as an expression of potential hazard or as a comparison of relative toxicities of various substances. However, when compiling a complete data profile to evaluate the safety of a substance, more information of the chemical-biological properties of the substance is necessary. The chronic reproductive test was designed to produce such information through continuous long term exposure extended to reproduction and teratology. The chronic test performed was a single generation reproductive test conducted over a single breeding season instead of a continuous year-long study. This is an accepted practice used by governmental agencies for toxicity studies

utilizing avian species. It is also a test situation applicable to field conditions of avian wildlife populations. The duration of the reproductive study of a representative avian wildlife species also allows for the determination of long term effects (or the absence of) due to the chronic feeding of a suspected toxicant. Such effects could include carcinogenecity or behavioral or physiological reactions previously unobserved.

Dietary levels of the test substances were determined via the results of the LC_{50} experiment and consultations with the Project Officer (United States Army). Decreased feed consumption, as compared to the control, at levels above 12000 ppm DIMP coupled with a decrease in body weight gain, as compared to the control, at levels above 16000 ppm DIMP and absence of mortality at levels lower than 24000 ppm DIMP aided in the decision to place the reproductive study dietary levels of DIMP at 0, 1200, 3800, and 12000 ppm. Similarly, normal feed consumption and body weight gain at 4000 ppm DCPD coupled with mortality at the 10000, 18000 ppm DCPD levels aided in the determination of the reproductive study DCPD dietary levels of 0, 400, 1250, and 4000 ppm[1].

Dietary intake of the test substances was expressed as ppm as opposed to mg/kg/day. Neither method is completely satisfactory. The use of ppm does not allow for reduced or increased feed consumption due to repellant or attractant properties of the test

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[1] The 3800 and 12000 ppm DIMP dietary levels were later reduced to 380 and 0 ppm, respectively. This action was taken after evaluating the mortality percentages occurring at the 3800 and 12000 ppm levels during the first four weeks of the study and consulting with the Project Officer (Unites States Army).

substance; however, the reaction by a laboratory bird to such a property of a test substance would undoubtedly be similar to the reaction of birds in the field. Mg/kg/day can be misleading when the length of time a toxicant is fed to a test subject and the excretion of a toxicant are not reported. The latter method would also require the continuance of biweekly body weight measurements throughout the reproductive period - an action highly undesirable due to possible adverse effects of handling on egg production.

Results

DCPD. Feed consumption data for adult Bobwhites fed DCPD treated diets or control diet are presented in Figure 13. Each point plotted is the mean of 15 cages, each housing one male and one female bird. There was no significant difference in feed consumption between birds fed DCPD diets and birds fed a control diet. A general increase over the entire experimental period in feed consumed on all dietary levels was noted.

Body weight change data of Bobwhites fed DCPD treated or control diets for the initial 10 weeks of the test period are presented in Table 12. During the 10 week period no significant differences in body weight change were found between those birds fed a treated diet and those birds fed control diet.

Body weight change was measured again at the commencement of egg production, and at the termination of the experiment. These data, catagorized according to sex, are given in Table 13. No significant differences between the mean body weight change of treated groups and the control group were found for either sex.

Mortality data of Bobwhites fed DCPD or control diets are presented in Table 14. No diet related trends were noted. The majority of the deaths occurred during a 48-hour period at approximately the 62th day of the experiment. The cause of death of the twelve birds which died during the 48-hour period could not be determined. Other mortality was sporadic.

Egg production data for the DCPD study Bobwhites are plotted in Figure 14. Each point plotted is the mean of 15 cages of one

Figure 13. Effect of feeding various levels of DCPD in the diet for 28 weeks on feed consumption of adult Bobwhites. Each point represents the mean of fifteen cages, each containing one male and one female bird.

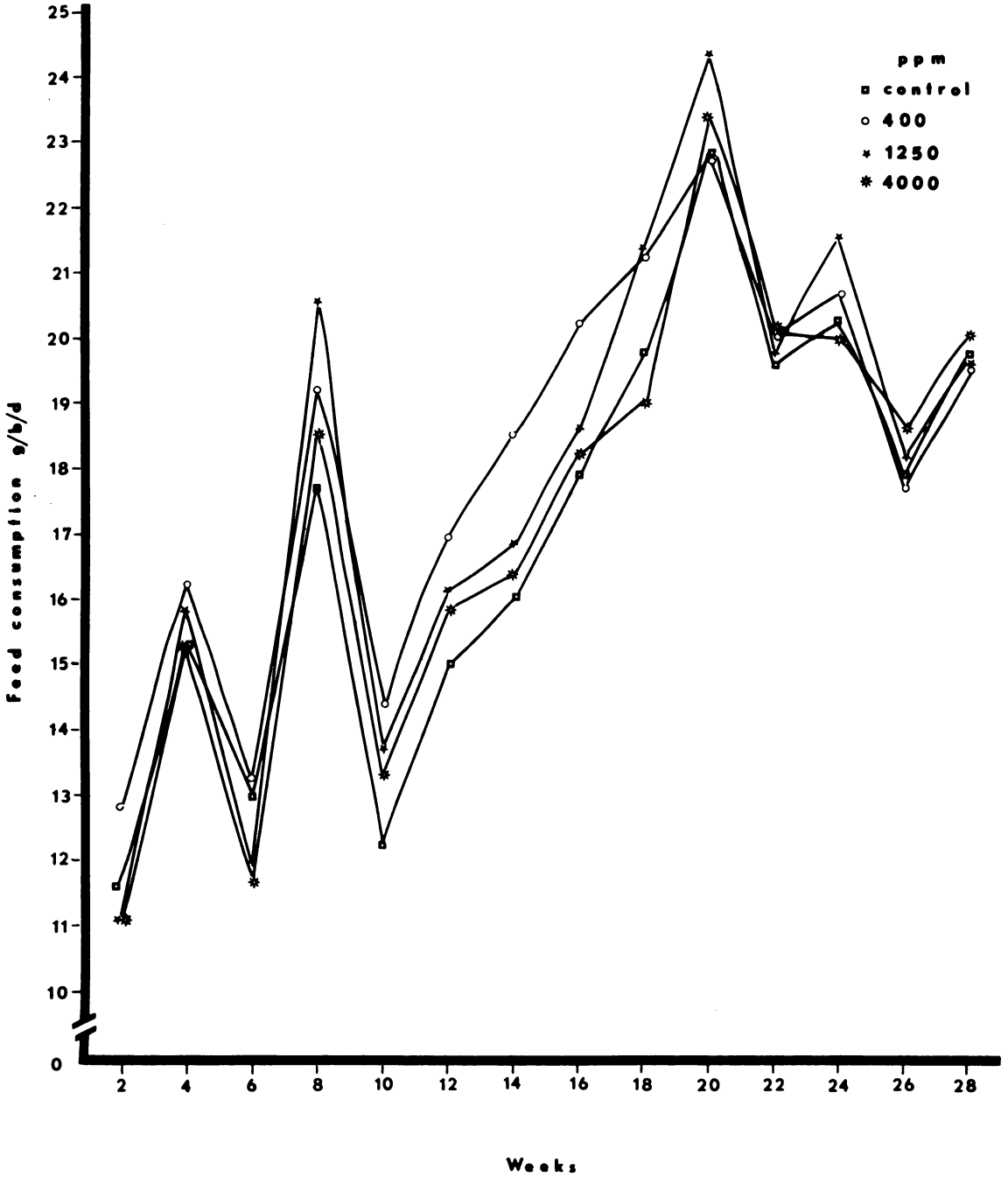

ppm
control
400
1250
4000
Feed consumption g/b/d
Weeks
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28

Figure 13.

Table 12. Effect of feeding DCPD at various levels in the diet on body weight changes of Bobwhites for the 10 weeks prior to the onset of egg production.

Dietary level (ppm)	n	Biweekly body weight change (%)[1] 2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
0	30	-6.46_a[2] (±3.47)	$+12.63_a$[2] (±4.86)	$+0.56_a$[2] (±3.67)	$+5.16_a$[2] (±2.88)	-2.14_a[2] (±2.13)
400	30	-4.50_a (±5.28)	$+12.29_a$ (±5.93)	-2.78_a (±4.82)	$+8.55_a$ (±6.79)	-1.59_a (±2.17)
1250	30	-8.56_a (±4.25)	$+12.06_a$ (±19.70)	-0.84_a (±3.76)	$+8.75_a$ (±4.08)	-1.69_a (±2.39)
4000	30	-5.51_a (±3.34)	$+7.73_a$ (±18.88)	-2.65_a (±5.49)	$+9.48_a$ (±4.22)	-0.83_a (±2.32)

[1] Data reported as mean ± standard deviation.

[2] Numbers with the same subscript are not significantly different from their respective control ($P > .05$).

Table 13. Effect of feeding DCPD at various levels in the diet for 28 weeks on body weight change of Bobwhites during the 10 week reproductive period.

Sex	Level of DCPD in diet (ppm)	n	Mean body weight (gms) Pre-production (week 18)	Termination (week 28)	Body weight change (%) [1]
Female	0	12	201.58	220.00	+9.21 ± 6.76$_a$ [2]
	400	13	198.00	217.08	+9.64 ± 13.85$_a$
	1250	14	205.43	215.21	+5.83 ± 14.51$_a$
	4000	13	201.38	218.54	+8.69 ± 6.11$_a$
Male	0	13	200.15	201.08	+0.42 ± 6.53$_b$ [2]
	400	12	200.00	198.14	-0.91 ± 2.89$_b$
	1250	13	201.85	222.08	+1.39 ± 5.89$_b$
	4000	12	199.92	195.08	-2.31 ± 5.10$_b$

[1] Data reported as mean ± standard deviation.

[2] Means having the same subscript are not significantly different from their respective controls ($P > .05$).

Table 14. Effect of feeding DCPD on mortality of Bobwhites during the 28 week chronic study.

Dietary levels (ppm)	Mortality days 61-63 of the study	Total mortality	Mortality (%)
0	4	5/30	16.67
400	2	3/30	10.0
1250	2	3/30	10.0
4000	4	5/30	16.67

Figure 14. Effect of feeding various levels of DCPD in the diet for 28 weeks on egg production of adult Bobwhites in their first reproductive cycle. Each point represents the mean of 15 females. Percents calculated from hen-day production.

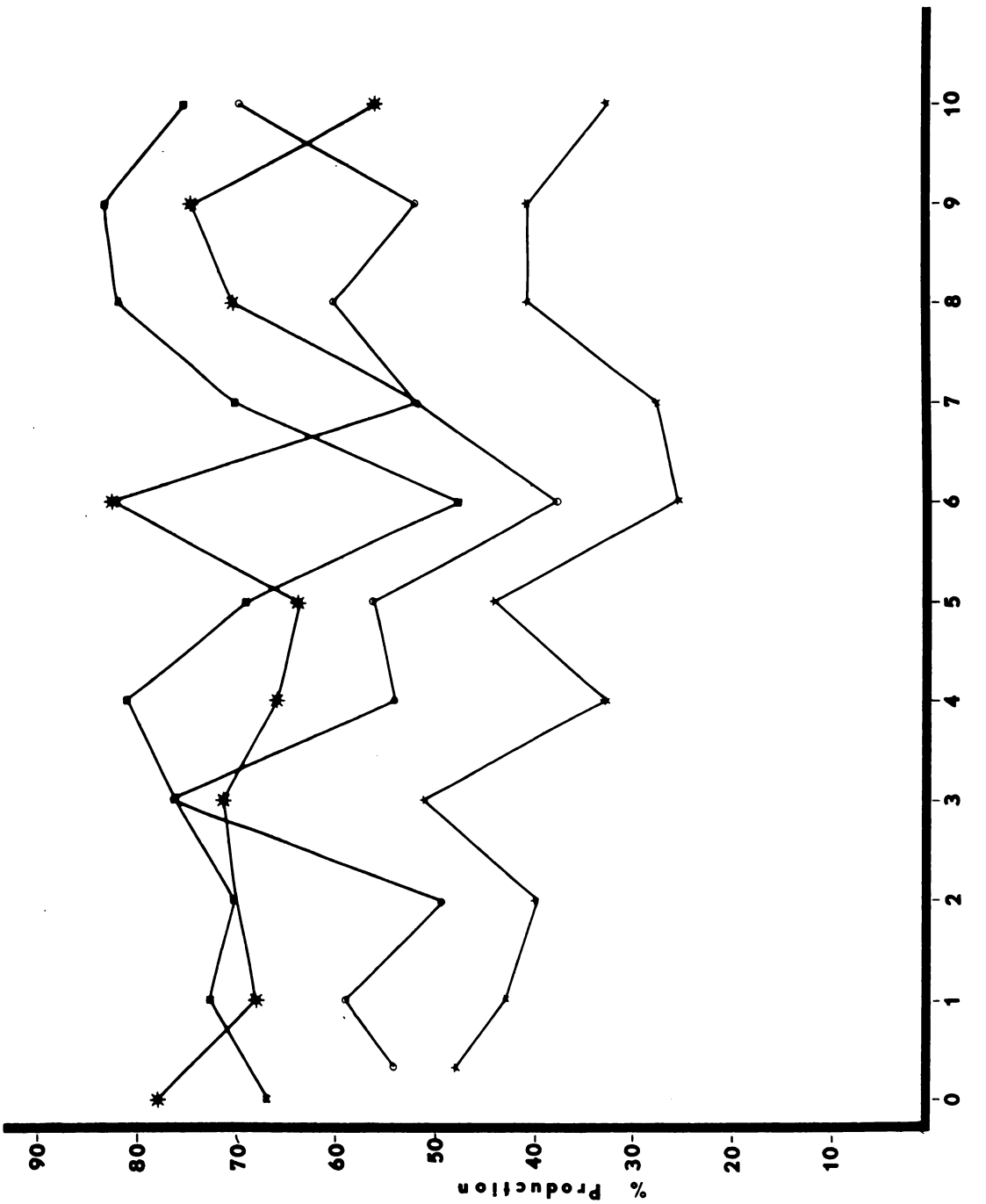

ppm
control
400
1250
4000
% Production
Weeks
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10

Figure 14.

hen each. Percent production was based on hen-day production. Analysis of the data revealed that the egg production of the hens fed 400 ppm DCPD was significantly less than the egg production of the hens fed a control diet. No significant difference between the egg production of hens on 1250 ppm DCPD and 4000 ppm DCPD and the production of the control hens was found. However, production trends of all dietary groups were similar.

Analysis of incubation parameter data of Bobwhites fed DCPD treated diets or the control diet showed no significant difference between any treated group and the control in any category. The percentages of fertile eggs were based on the number of settable eggs (total eggs laid - [cracked eggs + eggs laid by unmated females + eggs used for egg shell thickness measurements]). Percent hatchability, early dead, dead in shell, live in shell, pipped lived, and pipped dead were based on the number of fertile eggs. Incubation parameter data are presented in Table 15.

Eggshell thickness data for Bobwhites on the DCPD study are presented in Table 16. No significant difference was found between eggshell thickness from birds fed DCPD treated diets and birds fed a control diet. All eggs used for shell thickness measurements were included only in the calculations of total egg production percent.

14-day survivability of the progeny of Bobwhites fed DCPD or control diets is plotted in Figure 15. Each point plotted is the 14-day percent livability of all progeny of all Bobwhites in a particular dietary group for a particular hatch. No significant difference between the livability of the progeny of Bobwhites fed treated feed and progeny of Bobwhites fed control feed was found.

Table 15. Effect of feeding DCPD at various levels in the diet for 28 weeks on incubation parameters of Bobwhite quail eggs laid in March, April, and May, 1977.

Parameter	Level in diet (ppm)	Month			Combined[1]
		March	April	May	
		----------	----------	%----------	----------
Cracked	0	5.06	8.21	6.21	6.49 ± 1.59$_a$[2]
	400	4.90	8.85	13.75	9.17 ± 4.43$_a$
	1250	4.14	6.41	5.00	5.18 ± 1.14$_a$
	4000	4.17	10.56	7.14	7.29 ± 3.20$_a$
Fertile	0	91.72	97.21	89.71	92.88 ± 3.88$_b$[2]
	400	72.79	68.93	79.41	73.71 ± 5.30$_b$
	1250	91.88	84.03	66.32	80.74 ± 13.09$_b$
	4000	82.50	91.61	84.44	86.18 ± 4.80$_b$
Hatched	0	80.00	75.29	81.15	78.81 ± 3.10$_c$[2]
	400	79.80	73.24	75.93	76.32 ± 3.30$_c$
	1250	78.23	61.98	84.13	74.79 ± 11.42$_c$
	4000	78.79	65.65	90.79	78.41 ± 12.57$_c$
Early dead	0	1.94	6.32	7.38	5.21 ± 2.88$_d$[2]
	400	6.06	8.45	16.67	10.39 ± 5.57$_d$
	1250	2.72	7.44	3.17	4.44 ± 2.61$_d$
	4000	3.79	9.17	2.63	5.20 ± 3.49$_d$
Dead in shell	0	4.52	3.45	3.28	3.75 ± 0.67$_e$[2]
	400	5.05	7.04	0.00	4.03 ± 3.63$_e$
	1250	4.76	4.96	1.59	3.77 ± 1.89$_e$
	4000	6.82	3.05	0.00	3.29 ± 3.42$_e$
Live in shell	0	0.65	2.30	0.82	1.26 ± 0.91$_f$[2]
	400	0.00	4.26	0.00	1.42 ± 2.46$_f$
	1250	1.36	6.61	0.00	2.66 ± 3.49$_f$
	4000	1.52	2.29	0.00	1.27 ± 1.17$_f$
Pipped live	0	12.26	12.64	7.38	10.76 ± 2.93$_g$[2]
	400	9.09	7.04	7.41	7.85 ± 1.09$_g$
	1250	12.24	18.18	11.11	13.84 ± 3.80$_g$
	4000	7.58	19.85	5.26	10.90 ± 7.84$_g$
Pipped dead	0	0.65	0.00	0.00	0.22 ± 0.37$_h$[2]
	400	0.00	0.00	0.00	0.00 ± 0.00$_h$
	1250	0.68	0.83	0.00	0.50 ± 0.44$_h$
	4000	0.76	0.00	1.32	0.69 ± 0.66$_h$

[1] Data reported as mean ± standard deviation.

[2] Means having the same subscript are not significantly different from their respective controls ($P > .05$).

Table 16. Effect of feeding DCPD at various levels in the feed for 28 weeks on shell thickness values of adult Bobwhite eggs.

Dietary level (ppm)	n	Shell thickness[1] (mm x 10^{-2})
0	41	$21.95 \pm 1.83_a$[2]
400	25	$21.66 \pm 1.82_a$
1250	37	$22.40 \pm 2.06_a$
4000	37	$22.25 \pm 1.69_a$

[1] Data reported as mean $\pm$ standard deviation.

[2] Numbers with the same subscript are not significantly different from their respective control ($P > .05$).

Figure 15. Percent livability of offspring of adult bobwhites fed various levels of DCPD in their diets for 28 weeks.

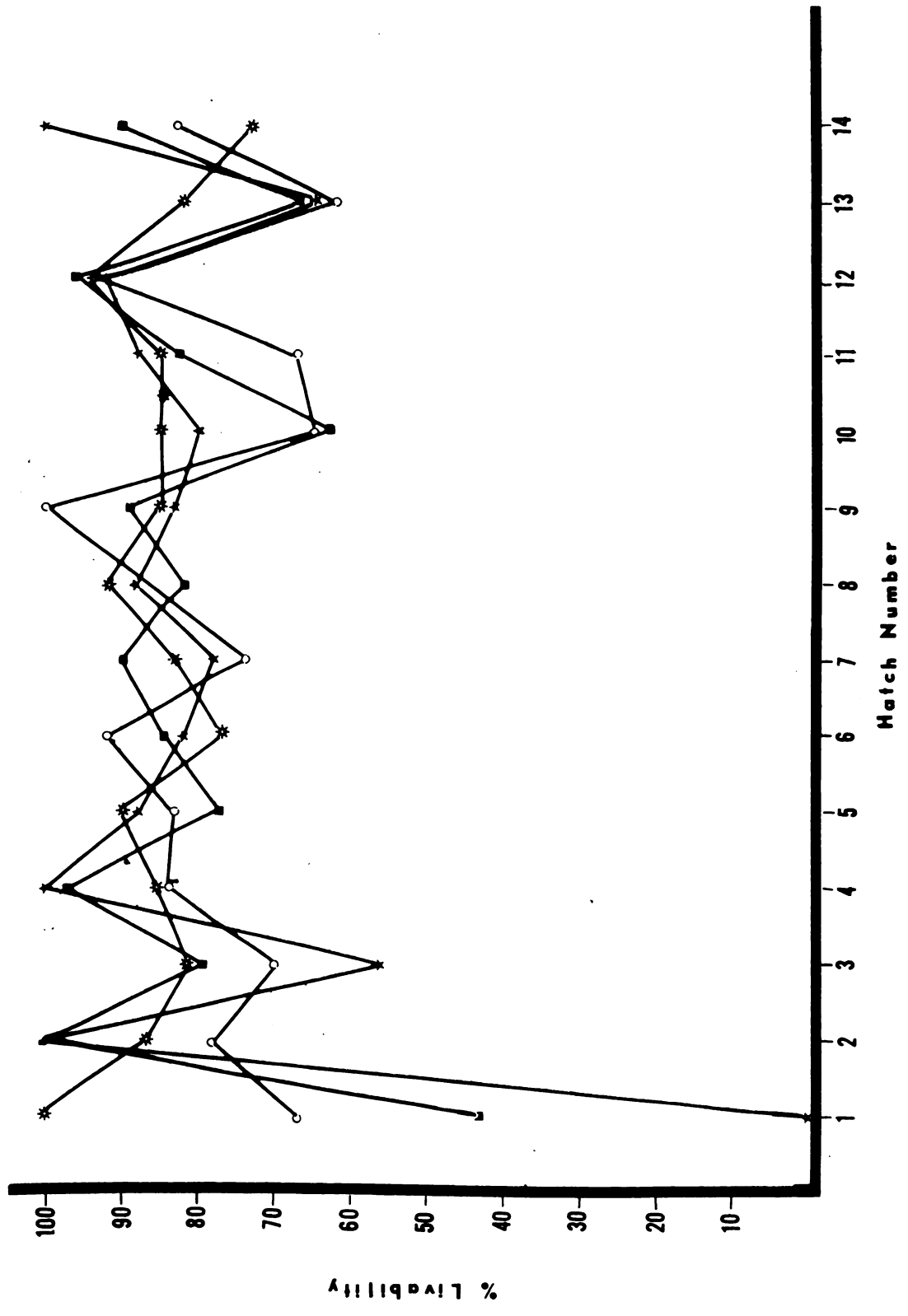

ppm
control
400
1250
4000
% Livability
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Hatch Number

Figure 15.

Organ weight data for DCPD-treated Bobwhites and control Bobwhites are listed in Tables 17 and 18. The liver and gonad(s) weights (absolute) showed differences attributed to sex and so were separated into male and female categories. Liver weights of male Bobwhites fed 4000 ppm DCPD feed were significantly less than liver weights of male Bobwhites fed control feed. No other organ weights of DCPD-fed Bobwhites were significantly different from the respective organ weights of Bobwhites fed control feed.

Hemoglobin values for the DCPD study Bobwhites are presented in Table 19. There were no significant differences found between the hemoglobin values of DCPD-fed males and control males, or the hemoglobin values of DCPD-fed females and control females. However, the mean hemoglobin value of all males was significantly greater than the mean hemoglobin value of all females.

Hematocrit values are presented in Table 19. The results of the analysis of the data showed no significant difference between the hematocrit values of Bobwhites (male or female) fed DCPD diets and Bobwhites (male or female) fed control diets. However, as with the hemoglobin data analysis, the mean hematocrit of all the male Bobwhites was significantly greater than the mean hematocrit of all the female Bobwhites.

The mean corpuscular hemoglobin concentration data of Bobwhites on the DCPD study is shown in Table 20. No significant difference between the mean corpuscular hemoglobin concentrations of male and female birds fed DCPD treated diets and their respective controls was found. Also no significant difference between mean corpuscular hemoglobin concentration of males and females was found.

Table 17. Effect of feeding DCPD at various levels in the diet for 28 weeks on liver and gonad weights of adult Bobwhites.

Sex	Organ	Level of DCPD in diet (ppm)	n	Mean organ wt. (gms)	Organ weight as % of Body wt.	Organ weight as % of Brain wt.[1]
Female	Ovary					
		0	12	6.357	2.84	560.86 ± 174.16$_a$[2]
		400	13	7.602	3.29	655.55 ± 181.52$_a$
		1250	14	6.193	2.79	560.46 ± 142.62$_a$
		4000	12	7.175	3.33	622.77 ± 209.50$_a$
	Liver	0	12	6.746	3.06	605.64 ± 190.72$_b$[2]
		400	13	7.211	2.88	554.35 ± 256.50$_b$
		1250	14	6.689	3.03	607.07 ± 225.88$_b$
		4000	12	6.263	2.71	509.79 ± 98.05$_b$
Male	Testes	0	12	1.35	0.68	118.60 ± 15.20$_c$[2]
		400	14	1.14	0.57	97.74 ± 36.97$_c$
		1250	13	0.98	0.49	85.71 ± 39.57$_c$
		4000	13	1.28	0.65	113.34 ± 54.49$_c$
	Liver	0	13	3.80	1.91	333.50 ± 65.41$_d$[2]
		400	14	4.13	2.09	355.90 ± 63.15$_d$
		1250	13	3.72	1.89	323.50 ± 60.54$_d$
		4000	13	3.40	1.73	263.20 ± 81.81$_e$

[1] Data reported as mean ± standard deviation.

[2] Means with the same subscript are not significantly different from their respective controls ($P > .05$).

Table 18. Effect of feeding DCPD at various levels in the diet for 28 weeks on organ weight in adult Bobwhites.

Organ	Level of DCPD in diet (ppm)	n	Mean organ wt. (gms)	Organ weight as % of Body wt.	Organ weight as % of Brain wt.[1]
Spleen	0	16	.064	0.030	5.61 ± 2.53$_a$[2]
	400	15	.062	0.029	5.46 ± 3.07$_a$
	1250	11	.053	0.025	4.64 ± 2.25$_a$
	4000	20	.403	0.027	5.34 ± 1.65$_a$
Kidneys	0	25	1.49	0.71	133.03 ± 37.60$_b$[2]
	400	27	1.33	3.30	113.61 ± 31.37$_b$
	1250	27	1.50	1.08	132.82 ± 43.71$_b$
	4000	25	1.44	0.70	124.93 ± 31.04$_b$
Pancreas	0	25	.524	0.221	41.06 ± 12.00$_c$[2]
	400	27	.527	0.257	43.37 ± 20.51$_c$
	1250	27	.467	0.224	41.47 ± 11.97$_c$
	4000	25	.485	0.227	40.87 ± 12.54$_c$
Proventriculus	0	25	.912	0.43	81.83 ± 18.76$_d$[2]
	400	27	.930	0.45	71.92 ± 22.55$_d$
	1250	27	.971	0.47	86.37 ± 17.89$_d$
	4000	25	.886	0.44	79.17 ± 16.41$_d$
Gizzard	0	25	4.12	1.97	364.53 ± 61.84$_e$[2]
	400	27	3.99	1.92	342.12 ± 83.44$_e$
	1250	27	4.24	2.06	376.07 ± 68.97$_e$
	4000	25	4.24	2.04	369.55 ± 87.06$_e$
Heart	0	25	0.96	0.49	89.24 ± 14.31$_f$[2]
	400	27	1.02	0.50	87.44 ± 19.27$_f$
	1250	27	1.08	0.52	95.69 ± 22.03$_f$
	4000	25	0.96	0.47	83.55 ± 18.53$_f$
Brain	0	25	1.14 ± 0.09$_g$[2]	---	---
	400	27	1.17 ± 0.11$_g$	---	---
	1250	27	1.13 ± 0.10$_g$	---	---
	4000	25	1.17 ± 0.10$_g$	---	---

[1] Data reported as mean ± standard deviation.

[2] Means with the same subscript are not significantly different from their respective controls ($P > .05$).

Table 19. Effect of feeding DCPD at various levels in the diet for 28 weeks on hemoglobin and hematocrit values of adult Bobwhites.

Sex	Level of DCPD in diet (ppm)	n	Hemoglobin (gm/dl.)[1]	n	Hematocrit (%)[1]
Female	0	8	9.61 ± 1.22$_a$[2]	12	33.2 ± 5.96$_b$[2]
	400	10	10.27 ± 0.78$_a$	13	32.9 ± 4.39$_b$
	1250	11	10.72 ± 0.91$_a$	14	35.8 ± 3.17$_b$
	4000	11	10.55 ± 1.20$_a$	12	37.0 ± 3.39$_b$
Overall		40	10.34 ± 1.073	51	34.75± 4.53
Male	0	10	12.28 ± 0.93$_c$[2]	12	40.8 ± 3.38$_d$[2]
	400	13	12.10 ± 0.96$_c$	14	40.4 ± 3.97$_d$
	1250	11	11.79 ± 0.79$_c$	13	39.1 ± 3.74$_d$
	4000	12	12.33 ± 0.85$_c$	13	43.2 ± 3.72$_d$
Overall		46	12.12 ± 0.881	52	40.87± 3.91

[1] Data reported as mean ± standard deviation.

[2] Means having the same subscript are not significantly different from their respective controls ($P > .05$).

Table 20. Effect of feeding DCPD at various levels in the diet for 28 weeks on mean corpuscular hemoglobin concentration[1] of adult Bobwhites.

Level of DCPD in diet (ppm)	n females	MCHC (%)[2] females	n males	MCHC (%)[2] males	n combined	MCHC (%)[2] combined
0	10	28.93	12	30.05	22	$29.54 \pm 4.28_c$[2]
400	10	29.21	13	29.56	23	$29.41 \pm 2.12_c$
1250	12	29.51	14	28.34	26	$28.89 \pm 2.88_c$
4000	11	28.23	12	28.59	23	$28.42 \pm 1.27_c$

[1] Calculated from data in Table 19.

[2] Data reported as treatment mean ± standard deviation.

[3] Means having the same subscript are not significantly different from their respective controls ($P > .05$).

Enumeration of leukocytes of the DCPD study Bobwhites is presented in Table 21. The number of eosinophils of the Bobwhites fed 1250 ppm DCPD was significantly greater than the number of eosinophils of the control Bobwhites. No other significant difference between leukocyte counts of DCPD-treated Bobwhites and control Bobwhites was found.

Table 21. Effect of feeding DCPD at various levels in the diet for 28 weeks on leukocyte counts of adult Bobwhites.

Cell	Level DCPD in diet (ppm)	n	Mean[1]	Range
Basophil	0	25	2.60 ± 2.04$_a$[2]	0- 8
	400	27	2.30 ± 1.64$_a$	0- 5
	1250	26	2.81 ± 1.77$_a$	0- 7
	4000	23	3.57 ± 2.82$_a$	0-12
Eosinophil	0	25	2.76 ± 2.57$_b$[2]	0-10
	400	27	3.33 ± 2.25$_b$	0-11
	1250	26	4.96 ± 4.50$_c$	1-14
	4000	23	3.70 ± 2.98$_b$	0-12
Heterophil	0	25	30.32 ± 14.85$_d$[2]	0-62
	400	27	27.67 ± 16.24$_d$	2-69
	1250	26	24.92 ± 15.75$_d$	5-67
	4000	23	25.83 ± 14.36$_d$	6-50
Lymphocyte	0	25	56.52 ± 18.14$_e$[1]	19-88
	400	27	57.85 ± 16.60$_e$	20-89
	1250	26	58.69 ± 20.27$_e$	2-88
	4000	23	58.91 ± 15.10$_e$	30-85
Monocyte	0	25	7.32 ± 4.68$_f$[1]	1-17
	400	27	7.52 ± 3.69$_f$	1-15
	1250	26	9.50 ± 4.60$_f$	2-17
	4000	23	8.00 ± 4.17$_f$	0-15

[1] Data reported as mean ± standard deviation.

[2] Means having the same subscript are not significantly different from their respective controls ($P > .05$).

EXPERIMENT 3

Results

DIMP. Feed consumption data for adult Bobwhites fed DIMP treated rations or control rations are presented in Figure 16. Each point plotted is the mean of 15 cages, each housing one male and one female bird. The dietary levels of DIMP for the initial time interval plotted were: 0, 1200, 3800, and 12000 ppm. Due to considerable mortality occurring in the 3800 and 12000 ppm groups, the dietary levels were reduced to 380 and 0 ppm, respectively. Thus, the first time interval of the feed consumption data was analyzed separately from the remaining time intervals. The feed consumption of the birds receiving the 12000 ppm diet was found to be significantly less than the feed consumption of the control birds. No other significant differences were found between the feed consumption of the birds fed treated diets and the control birds.

The body weight change data of Bobwhites fed DIMP treated diets or control feed for the initial ten weeks, are presented in Table 22. No significant differences in body weight change of treated birds and control birds were found. For reasons stated in the above paragraph, the initial time interval was analyzed separately from the remaining time intervals.

Body weight change data over a ten-week reproductive period are given in Table 23. The data were analyzed separately for males and females. No significant differences between body weight changes of treated quail and control, of either sex, were found.

During the initial four weeks of the DIMP study, considerable mortality occurred in the 3800 and 12000 ppm diet groups (Table 24).

Figure 16. Effect of feeding various levels of DIMP in the diet for 29 weeks on feed consumption of adult Bobwhites. Each point represents the mean feed consumption of fifteen cages, each containing one male and one female bird.

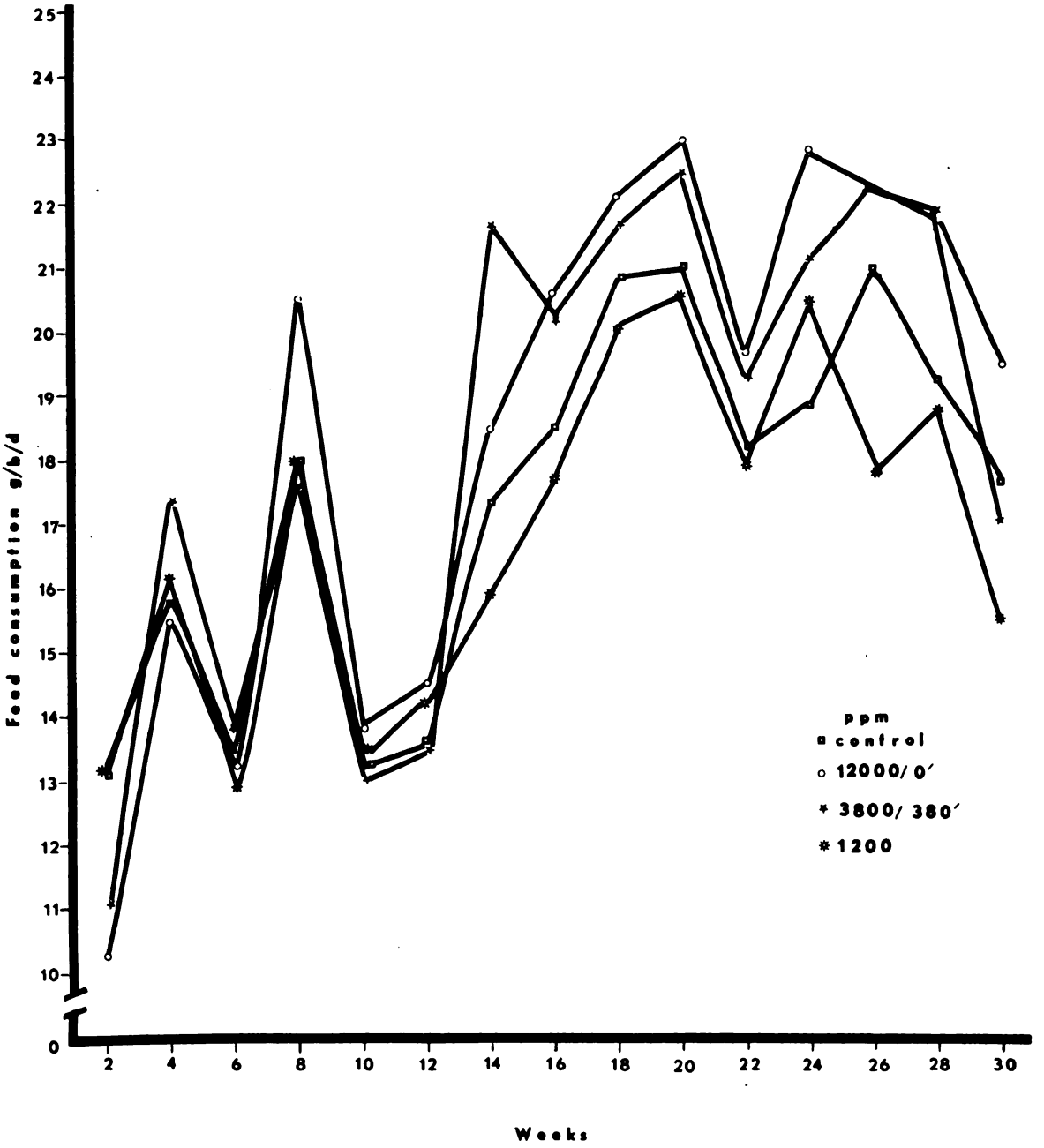

Food consumption g/b/d
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Weeks
ppm
control
12000/0'
3800/380'
1200

Figure 16.

Table 22. Effect of feeding DIMP at various levels in the diet on body weight changes of Bobwhites for the 10 weeks prior to the onset of egg production.

Dietaty level (ppm)	n	Biweekly body weight change (%)[1] 2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
0	30	-8.09_a[2] ($\pm$3.94)	$+7.04_b$[2] ($\pm$13.63)	-0.16_c[2] ($\pm$4.77)	$+5.00_d$[2] ($\pm$4.74)	-2.24_e[2] ($\pm$2.92)
1200	30	-6.17_a ($\pm$3.64)	$+7.15_b$ ($\pm$3.76)	$+0.52_c$ ($\pm$3.34)	$+5.58_d$ ($\pm$3.41)	-3.90_e ($\pm$2.46)
3800/380[3]	26	-8.64_a ($\pm$6.69)	$+10.72_b$ ($\pm$7.16)	$+0.77_c$ ($\pm$3.81)	$+4.41_d$ ($\pm$3.91)	-3.87_e ($\pm$2.80)
12000/0[4]	26	-11.43_a ($\pm$8.89)	$+12.64_b$ ($\pm$13.85)	-2.51_c ($\pm$6.92)	$+11.64_d$ ($\pm$9.08)	-3.17_e ($\pm$2.40)

[1] Data reported as mean $\pm$ standard deviation.

[2] Numbers with the same subscript are not significantly different from their respective control ($P > .05$).

[3] 3800 ppm reduced to 380 ppm after 26 days.

[4] 12000 ppm reduced to 0 ppm after 18 days.

Table 23. Effect of feeding DIMP at various levels in the diet for 29 weeks on body weight change of Bobwhites during the 10 week reproductive period.

Sex	Level of DIMP in diet (ppm)	n	Mean body weight (gms) Pre-production	Mean body weight (gms) Termination	Body weight change (%)[1]
Female	0	13	192.33	199.38	+3.29 ± 8.69_a[2]
	1200	14	196.80	196.30	-0.91 ± 9.69_a
	3800/380	13	197.15	207.15	+5.09 ± 10.10_a
	12000/0	13	194.62	207.85	+6.88 ± 7.94_a
Male	0	13	199.50	200.54	+0.53 ± 5.79_b[2]
	1200	14	196.27	191.50	-2.06 ± 8.43_b
	3800/380	12	193.85	202.17	+3.94 ± 5.15_b
	12000/0	13	201.15	201.46	+0.17 ± 4.79_b

[1] Data reported as mean ± standard deviation.

[2] Means having the same subscript are not significantly different from their respective controls ($P > .05$).

Table 24. Effects of feeding DIMP on mortality of Bobwhites during the 29 week chronic study.

Dietary level (ppm)	Mortality wks. 1-4	Total mortality	Mortality (%)
0	0	4/30	13.33
1200	0	2/30	6.67
3800/380[1]	4	5/30	16.67
12000/0[2]	4	4/30	13.33

[1] 3800 ppm reduced to 380 ppm after 26 days.

[2] 12000 ppm reduced to 0 ppm after 18 days.

The cause of the deaths was attributed to dietary levels of DIMP but could not be verified by gross necropsy. Mortality, other than that previously mentioned, was sporadic with no diet related trends.

Egg production data for the DIMP-study Bobwhites are plotted in Figure 17. Each point plotted is the mean of approximately 15 cages of one hen each. Percent production was based on hen-day production. Analysis of the data revealed that the egg production of hens fed 1200 ppm was significantly less than the production of hens fed control feed. No other significant difference in egg production between treated hens and control hens was found. Production trends of all dietary groups and the control were similar.

Analysis of data on incubation parameters of Bobwhites fed DIMP treated diets or control diets showed no significant differences between any treated group and the control group in any category. The percentages of fertile eggs were based on the number of settable eggs (total eggs laid - [cracked eggs + eggs laid by unmated females + eggs used for eggshell thickness measurements]). Percent hatchability, early dead, dead in shell, live in shell, pipped live, and pipped dead were based on the number of fertile eggs. Table 25 contains the incubation parameter data.

Eggshell thickness data for the Bobwhites on the DIMP study are given in Table 26. No significant difference was found between the shell thickness of eggs from hens fed DIMP treated diets and the hens fed a control diet. All eggs used for shell thickness measurements were only included in the percent egg production calculations.

Fourteen-day survivability of the progeny of DIMP-study Bobwhites is plotted in Figure 18. Each point plotted is the 14-day

Figure 17. Effect of feeding various levels of DIMP in the diet for 29 weeks on egg production of adult Bobwhites in their first reproductive cycle. Each point represents the mean egg production of fifteen females. Percents calculated from hen-day production.

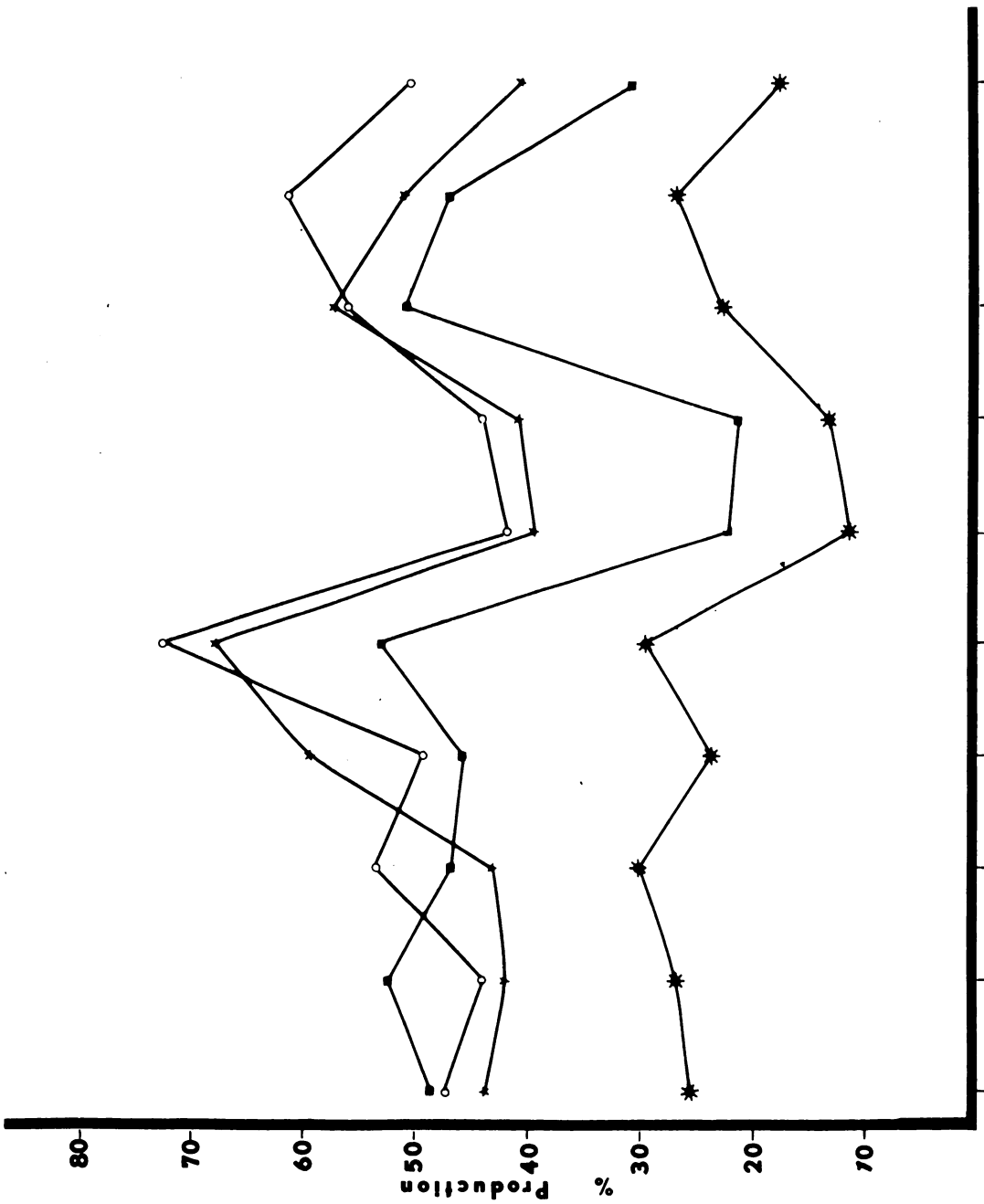

ppm
control
12000/0
3800/380
1200
% Production
Weeks
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10

Figure 17.

Table 25. Effect of feeding DIMP at various levels in the diet for 29 weeks on incubation parameters of Bobwhite quail eggs laid in March, April, and May, 1977.

Parameter	Level in diet (ppm)	Month			Combined[1]
		March	April	May	
		----------	----------	%----------	----------
Cracked	0	16.55	11.11	1.68	9.78 ± 7.52$_a$[2]
	1200	11.48	8.96	0.00	6.81 ± 6.03$_a$
	3800/380	5.62	5.48	10.48	7.19 ± 2.85$_a$
	12000/0	5.66	3.47	7.79	5.64 ± 2.16$_a$
Fertile	0	89.66	90.44	87.07	89.06 ± 1.76$_b$[2]
	1200	62.26	83.61	56.86	67.58 ± 14.14$_b$
	3800/380	82.93	94.78	93.26	90.32 ± 6.45$_b$
	12000/0	76.00	76.05	68.09	73.38 ± 4.58$_b$
Hatched	0	83.65	73.98	78.22	78.62 ± 4.85$_c$[2]
	1200	69.70	64.71	72.41	68.94 ± 3.91$_c$
	3800/380	79.41	70.87	83.13	77.80 ± 6.29$_c$
	12000/0	85.53	69.29	83.33	79.38 ± 8.81$_c$
Early dead	0	5.77	4.88	6.93	5.86 ± 1.03$_d$[2]
	1200	9.09	13.73	3.45	8.76 ± 5.15$_d$
	3800/380	5.88	5.51	3.61	5.00 ± 1.22$_d$
	12000/0	2.63	5.51	8.33	5.49 ± 2.85$_d$
Dead in shell	0	3.85	2.44	2.97	3.09 ± 0.71$_e$[2]
	1200	3.03	7.84	13.79	8.22 ± 5.39$_e$
	3800/380	0.00	5.51	6.02	3.84 ± 3.34$_e$
	12000/0	5.26	5.51	3.13	4.63 ± 1.31$_e$
Live in shell	0	0.96	4.07	3.96	3.00 ± 1.76$_f$[2]
	1200	0.00	7.84	3.45	3.76 ± 3.93$_f$
	3800/380	0.00	3.15	2.41	1.85 ± 1.65$_f$
	12000/0	0.00	6.30	4.17	3.49 ± 3.20$_f$
Pipped live	0	5.77	13.82	7.92	9.17 ± 4.17$_g$[2]
	1200	15.15	5.88	3.45	8.16 ± 6.17$_g$
	3800/380	13.24	13.39	3.61	10.08 ± 5.60$_g$
	12000/0	2.63	13.39	0.00	5.34 ± 7.09$_g$
Pipped dead	0	0.00	0.81	0.00	0.27 ± 0.47$_h$[2]
	1200	3.03	0.00	3.45	2.16 ± 1.88$_h$
	3800/380	1.47	1.57	0.00	1.01 ± 0.88$_h$
	12000/0	3.95	0.00	1.04	1.66 ± 2.03$_h$

[1] Data reported as mean ± standard deviation.

[2] Means having the same subscript are not significantly different from their respective controls ($P > .05$).

Table 26. Effect of feeding DIMP at various levels in the feed for 29 weeks on shell thickness values of Bobwhite eggs.

Dietary level (ppm)	n	Shell thickness[1] (mm x 10^{-2})
0	38	$21.17 \pm 2.08_a$[2]
1200	18	$21.83 \pm 2.19_a$
3800/380[3]	35	$22.13 \pm 2.11_a$
12000/0[4]	26	$22.17 \pm 1.38_a$

[1] Data reported as mean $\pm$ standard deviation.

[2] Numbers with the same subscript are not significantly different from their respective control ($P > .05$).

[3] 3800 ppm reduced to 380 ppm after 26 days.

[4] 12000 ppm reduced to 0 ppm after 18 days.

Figure 18. Percent livability of offspring of adult Bobwhites fed various levels of DIMP in their diet for 29 weeks.

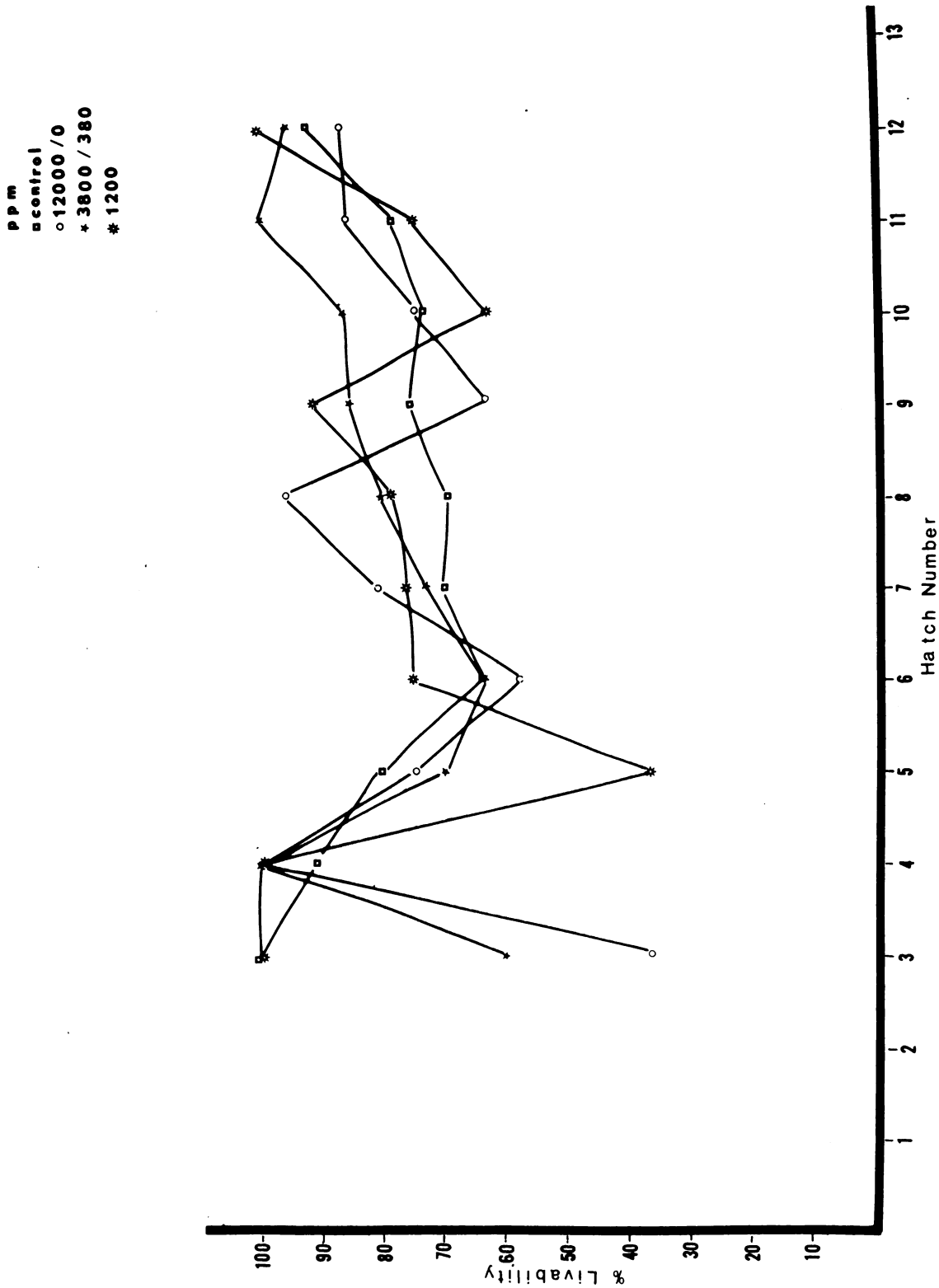

ppm
control
12000/0
3800/380
1200
% Livability
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
Hatch Number

Figure 18.

livability of the progeny of Bobwhites fed a particular diet for a particular hatch. Treated birds' progeny-percent-livability was not significantly different from control birds' progeny-percent-livability.

Mean organ weight data are presented in Tables 27 and 28. Due to weight differences attributed to sex differences, the liver and gonad(s) weights were categorized according to sex (Table 27). Liver and gonad weights of females were further separated into "producing" and "non-producing" categories. Males were not differentiated by reproductive capacities. Significant increases in mean proventriculus and gizzard weights, were found in the 12000 ppm DIMP group, as compared to the controls. No other significant differences between organ weights of DIMP-treated birds and their respective controls were found.

Hemoglobin values for DIMP-treated or control Bobwhites are given in Table 29. There was no significant difference found between the hemoglobin values of DIMP-fed Bobwhites and control Bobwhites of either sex. However, the hemoglobin values of the males were significantly greater than the hemoglobin values of the females.

Hematocrit values for Bobwhites on the DIMP study are presented in Table 29. The results of the analysis of the data showed no significant difference between the hematocrit values of DIMP-fed females and control females, and no significant difference between DIMP-fed males and control males. The hematocrits of the male birds were significantly higher than the hematocrits of the female birds.

Table 27. Effects of feeding DIMP at various levels in the diet for 29 weeks on gonad and liver weights of adult Bobwhites.

Sex	Organ	Level of DIMP in diet (ppm)	n	Mean organ wt. (gms)	Organ weight as % of Body wt.	Organ weight as % of Brain wt.[1]
Female[2]	Ovary	0	7	1.51	0.74	125.98 ± 11.00$_a$[4]
		1200	3	2.33	1.07	202.99 ± 13.43$_a$
		3800/308	7	2.93	1.33	269.68 ± 24.51$_a$
		12000/0	9	2.59	1.21	229.80 ± 14.54$_a$
	Liver	0	7	7.44	3.58	628.96 ± 21.10$_c$[4]
		1200	3	6.59	3.02	560.65 ± 11.77$_c$
		3800/380	7	8.05	3.74	736.60 ± 21.91$_c$
		12000/0	10	7.67	3.88	682.02 ± 20.49$_c$
Female[3]	Ovary	0	5	0.46	0.353	23.28 ± 3.19$_b$[4]
		1200	10	0.38	0.194	33.09 ± 1.79$_b$
		3800/380	5	0.38	0.281	32.63 ± 2.75$_b$
		12000/0	3	0.67	0.353	23.28 ± 3.19$_b$
	Liver	0	5	5.11	2.58	464.24 ± 10.30$_d$[4]
		1200	10	4.27	2.28	379.85 ± 9.50$_d$
		3800/380	5	5.89	2.99	532.02 ± 11.55$_d$
		12000/0	3	5.96	3.13	542.50 ± 364.16$_d$
Male	Testes	0	14	1.05	0.51	87.01 ± 15.89$_e$[4]
		1200	14	0.86	0.44	80.29 ± 20.82$_e$
		3800/380	13	1.08	0.54	96.72 ± 12.91$_e$
		12000/0	13	0.97	0.48	81.25 ± 13.08$_e$
	Liver	0	14	3.81	2.58	320.01 ± 21.67$_f$[4]
		1200	14	3.40	2.28	320.14 ± 14.42$_f$
		3800/380	13	4.03	2.99	362.99 ± 10.30$_f$
		12000/0	13	4.42	3.13	367.65 ± 14.07$_f$

[1] Data reported as mean ± standard deviation.

[2] Females producing eggs.

[3] Females not producing eggs.

[4] Means with the same subscript are not significantly different from their respective controls ($P > .05$).

Table 28. Effect of feeding DIMP at various levels in the diet for 29 weeks on organ weight of adult Bobwhites.

Organ	Level of DIMP in diet (ppm)	n	Mean organ wt. (gms)	Organ weight as % of Body wt.	Organ weight as % of Brain wt.[1]
Kidneys	0	26	1.47	0.75	125.74 ± 2.26$_a$[2]
	1200	28	1.34	0.70	120.74 ± 2.60$_a$
	3800/380	25	1.37	0.63	127.95 ± 3.10$_a$
	12000/0	26	1.49	0.73	129.04 ± 3.94$_a$
Pancreas	0	26	0.52	0.27	44.31 ± 1.88$_b$[2]
	1200	28	0.51	0.26	44.45 ± 1.26$_b$
	3800/380	25	0.47	0.23	42.61 ± 1.22$_b$
	12000/0	26	0.50	0.24	46.23 ± 1.93$_b$
Proventriculus	0	26	0.92	0.46	78.98 ± 1.45$_c$[2]
	1200	28	1.11	0.57	98.39 ± 3.64$_d$
	3800/380	25	0.96	0.47	87.64 ± 1.98$_c$
	12000/0	26	0.96	0.47	83.25 ± 1.92$_c$
Gizzard	0	26	4.21	2.15	360.51 ± 6.04$_e$[2]
	1200	28	4.93	2.49	448.70 ± 15.79$_f$
	3800/380	25	4.48	2.23	410.75 ± 10.49$_e$
	12000/0	26	4.48	2.20	389.15 ± 6.09$_e$
Heart	0	26	0.99	0.51	81.74 ± 2.27$_g$[2]
	1200	28	0.93	0.48	83.70 ± 3.19$_g$
	3800/380	25	1.01	0.50	91.62 ± 1.75$_g$
	12000/0	26	1.03	0.50	89.55 ± 2.40$_g$
Brain	0	25	1.17 ± 0.01$_h$[2]	--	---
	1200	28	1.15 ± 0.03$_h$	--	---
	3800/380	25	1.11 ± 0.02$_h$	--	---
	12000/0	26	1.16 ± 0.01$_h$	--	---

[1] Data reported as mean ± standard deviation.

[2] Means having the same subscript are not significantly different from their respective controls ($P > .05$).

Table 29. Effect of feeding DIMP at various levels in the diet for 29 weeks on hemoglobin and hematocrit values of adult Bobwhites.

Sex	Level of DIMP in diet (ppm)	n	Hemoglobin (gm/dl.)[1]	n	Hematocrit (%)
Female	0	10	10.0 ± 1.37	12	$37.4 \pm 4.40_b$[2]
	1200	14	10.0 ± 1.45	14	$36.4 \pm 4.76_b$
	3800/380	11	10.6 ± 0.57	12	$37.2 \pm 2.77_b$
	12000/0	10	10.2 ± 0.71	13	$37.2 \pm 3.52_b$
	Overall[1]	45	10.2 ± 1.12	51	37.0 ± 3.87
Male	0	13	11.3 ± 0.94	14	$41.5 \pm 3.35_d$[2]
	1200	14	10.9 ± 1.00	14	$40.7 \pm 2.79_d$
	3800/380	12	11.7 ± 1.02	13	$42.3 \pm 4.46_d$
	12000/0	12	11.2 ± 1.19	13	$40.9 \pm 3.91_d$
	Overall[1]	51	11.3 ± 1.05	54	41.4 ± 3.61

[1] Data reported as mean ± standard deviation.

[2] Means having the same subscript are not significantly different from their respective controls ($P > .05$).

Table 30 lists mean corpuscular hemoglobin concentration data for the DIMP-study Bobwhites. No significant difference was found between the mean corpuscular hemoglobin concentrations of the treated birds and the mean corpuscular hemoglobin concentrations of the control birds. Also, no significant difference was found between the mean corpuscular hemoglobin concentration of the males and the females.

Enumeration of leukocytes of Bobwhites on the DIMP study is given in Table 31. No significant difference in leukocyte counts was found between the treated birds and their respective control.

Table 30. Effects of feeding DIMP at various levels in the diet for 29 weeks on mean corpuscular hemoglobin concentration[1] of adult Bobwhites.

Level of DCPD in diet (ppm)	n females	MCHC (%)[2] females	n males	MCHC (%)[2] males	n combined	MCHC (%)[2] combined
0	10	26.91	13	27.40	23	$27.18 \pm 1.38_a$[3]
1200	14	27.36	14	26.50	28	$27.39 \pm 2.20_a$
3800/380	11	28.69	12	27.84	23	$28.25 \pm 1.83_a$
12000/0	10	27.48	12	27.24	22	$27.35 \pm 1.95_a$

[1] Calculated from data in Table 29.

[2] Data reported as mean ± standard deviation.

[3] Means having the same subscript are not significantly different from their respective controls ($P > .05$).

Table 31. Effect of feeding DIMP at various levels in the diet for 29 weeks on leukocyte counts of adult Bobwhites.

Cell	Level of DCPD in diet (ppm)	n	Mean[1]	Range
Basophil	0	26	2.57 ± 1.28$_a$[2]	0- 6
	1200	28	2.63 ± 1.78$_a$	0- 8
	3800/380	25	2.76 ± 2.09$_a$	1-10
	12000/0	26	3.65 ± 2.47$_a$	0- 9
Eosinophil	0	26	4.27 ± 2.63$_b$[2]	0- 9
	1200	28	2.85 ± 2.91$_b$	0-10
	3800/380	25	3.44 ± 2.26$_b$	0-11
	12000/0	26	2.73 ± 2.34$_b$	0- 9
Heterophil	0	26	19.23 ± 10.47$_c$[2]	4-46
	1200	28	23.04 ± 12.95$_c$	2-56
	3800/380	25	21.04 ± 14.36$_c$	1-63
	12000/0	26	22.19 ± 11.26$_c$	2-43
Lymphocyte	0	26	65.77 ± 13.01$_d$[2]	39-93
	1200	28	62.56 ± 14.85$_d$	40-84
	3800/380	25	63.24 ± 18.55$_d$	14-89
	12000/0	26	61.88 ± 15.45$_d$	32-88
Monocyte	0	26	7.69 ± 4.24$_e$[2]	1-21
	1200	28	9.00 ± 4.08$_e$	1-20
	3800/380	25	9.40 ± 5.63$_e$	4-24
	12000/0	26	9.50 ± 5.62$_e$	2-21

[1] Data reported as mean ± standard deviation.

[2] Means having the same subscript are not significantly different from their respective controls ($P > .05$).

DISCUSSION

Experiment 1

The LD_{50} is the most common expression of acute toxicity. Though other values such as the LD_5 or LD_{95} can be calculated, the dosage required to cause death of one half of the test subjects (LD_{50}) is the easiest to determine and more precise statistically. This median lethal dosage is also used as a standard of comparison of the relative toxicities of compounds to various species of animals.

DCPD. The LD_{50} of DCPD for rats and mice (Hart, 1975) were smaller than the LD_{50} of DCPD for Bobwhites. Quail were roughly twice as resistant to DCPD as were rats and approximately five times as resistant to DCPD as were mice. Bobwhites were, however, less resistant to DCPD than were Mallard ducks (Jones, 1977) and mink (Kavanagh, personal communication). LD_{50} values for the Mallard could not be determined because no deaths occurred at dosages up to and including 40000 mg/kg bwt (Jones, 1977). Mink suffered no effects other than regurgitation and/or suffocation due to the per os treatment with liquid DCPD at dosages up to and including 960 mg/kg bwt (Kavanagh, personal communication). Table 32 lists several compounds and their LD_{50} values of DCPD for the Bobwhite (Tucker and Crabtree, 1970). The LD_{50} value of DCPD for the Bobwhite is included in the table for comparison of relative toxicities. Table 1 lists the LD_{50} of DCPD for several species. Based upon the following chart (Loomis, 1968), DCPD is slightly toxic to the Bobwhite.

Table 32. The LD_{50} values of thirteen compounds for the Bobwhite quail at various ages.

Compound	Primary use	Sex	Age	LD_{50} mg/kg (95% conf. limits)
Azodrin	I[1]	Male	1-2 yr.	0.944 (0.749-1.19)
Furadan	I	Female	3 mo.	5.040 (3.64-6.99)
Aldrin	I	Female	3-4 mo.	6.59
Dieldrin	I	Both	2-3 mo.	12-14
Accothion®	I	Male	2-3 mo.	27.4 (19.0-37.1)
DDT oil sol.	I	Both	----	60-85
Toxaphene	I	Male	3 mo.	85.4 (59.2-123)
Lindane	I	Male	2-3 mo.	120-130
DDT crystalline	I	Both	----	300
SD 15418	H[2]	Female	3-5 mo.	400-500
DCPD	-[3]	Both	Adult	1010 (930-1090)
DIMP	-[3]	Both	Adult	1000 (931.8-1068.2)
Ceresan L	F[4]	Male	2-3 mo.	1060 (841-1330)

[1] I = insecticide
[2] H = herbicide
[3] See Literature Review
[4] F = fungicide

1.	Extremely Toxic	(1 mg/kg or less)
2.	Highly Toxic	(1 to 50 mg/kg)
3.	Moderately Toxic	(50 to 500 mg/kg)
4.	Slightly Toxic	(0.5 to 5 g/kg)
5.	Practically Nontoxic	(5 to 15 g/kg)
6.	Relatively Harmless	(more than 15 g/kg)

Two trials were conducted to determine the LD_{50} of DCPD for the Bobwhite. The results of the first trial were considered inconsistent and thus a second trial was performed. The additional test included three dosages of DCPD from the initial trial plus five other dosages. The results of the second trial were similar to the first trial. Coburn and Treichler (1946) in determining the acute toxicity of DDT to the Bobwhite reported the inconsistent data they collected as indicating the toxic action of DDT to be irregular due to low and variable absorption.

The slope of the dose-response curve is an estimate of the margin of safety of a compound (the magnitude of the range of doses, and thus responses, between a no effect dose and a lethal dose). A steep curve limits the range of doses. A flat curve encompasses a large range of doses. The dose-response curve of DCPD for the Bobwhite is somewhat flat (Slope = .096) indicating variable response to the compound.

When not lethal, DCPD did not produce lasting effects on feed consumption and/or body weight during the 14-day observation period. The feed consumption of Bobwhites post DCPD treatment in this study followed the same feed consumption pattern as Mallard ducks dosed with DCPD (Jones, 1977). Generally, a decrease in feed consumption the first week post-treatment was succeeded by an increase in feed consumption, up to control levels, the second

week post-treatment. Reduced feed consumption post chemical treatment is not an uncommon result in the reported literature. Coburn and Treichler (1946), and Dehlen and Haugen (1954) reported reduced feed consumption of the Bobwhite following treatment with DDT, aldrin, dieldrin, toxaphene, and lindane, respectively.

During the 14-day post-treatment period, the body weight change of treated birds showed no significant difference from the body weight change of the control birds; a maximum of 0.5 percent change. Results from other investigators varied. Dahlen and Haugen (1954) reported an average weight loss of 15 to 25 percent in Bobwhites dosed with aldrin, dieldrin, toxaphene, or lindane. Bergstrand and Klimstra (1962) reported a mean weight gain of 3.5 percent in Bobwhites dosed with fenuron. Kinkead et al. (1971) conducted mammalian studies with DCPD and found normal weight gains in the treated animals. Jones (1977) reported a six percent mean increase in the body weight of Mallard ducks dosed with DCPD.

The similarity in response to DCPD administration of male and female Bobwhites is consistent with findings on other compounds by Coburn and Treichler (1946); Dahlen and Haugen (1954); and Tucker and Haegele (1971). Hart (1975) reported no difference in response, attributable to sex, in either rats or mice dosed with DCPD.

The majority of the deaths of Bobwhites dosed with DCPD occurred within 48 hours of the treatment. Of that majority, half occurred on day 1 and the other half occurred on day 2. The remaining deaths occurred sporadically. One to two day mortality time was reported by Coburn and Treichler (1954) after dosing quail with DDT.

DIMP. The LD_{50} of DIMP for Bobwhites is similar to the LD_{50} of DIMP for rats and mice reported by Hart (1976), but approximately 30 percent less than the LD_{50} of DIMP for Mallard ducks reported by Jones (1977). Based on the chart in the DCPD discussion, DIMP is slightly toxic to the Bobwhite. Table 32 lists the LD_{50}'s of several compounds for the Bobwhite. The DIMP LD_{50} for the Bobwhite is included for comparison purposes. Table 2 lists the LD_{50}'s of DIMP for several species.

The slope of the dose-response curve of DIMP for Bobwhites can be considered flat. As mentioned in the DCPD discussion, a steep curve limits the range of dosages between the no-effect dose and the lethal dose. A flat curve provides more variability of dosages (thus responses) between the no-effect dose and the lethal dose.

Male and female Bobwhites responded similarly to DIMP administration. Lack of response difference due to sex is not uncommon. Hart (1976) and Jones (1977) found no difference in response to treatment with DIMP by sex for mice and Mallard ducks, respectively. Tucker and Haegele (1971) reported no difference in response by sex to 108 different compounds in 22 species of birds.

Feed consumption of Bobwhites post DIMP treatment did not show a typical dose-response relationship. The 900 mg/kg and 1200 mg/kg groups showed reduced feed consumption compared to the control group during the first week with an increase to control levels the second week post-treatment. The 1000 mg/kg group showed a reduction in feed consumption the second week post-treatment. Other dietary levels showed little change during the observation

period. Jones (1977) reported similar inconsistent data with Mallard ducks dosed with DIMP.

Body weight change of DIMP treated birds showed no significant difference from the control birds during the 14-day observation period. This is consistent with findings by Jones (1977) who found no significant difference in body weight change of Mallard ducks dosed with DIMP with the exception of the ducks treated with the highest level of DIMP. Dahlen and Haugen (1954) reported weight losses in Bobwhites treated with either of four insecticides. Bergstrand and Klimstra (1962) found the percent weight gain of birds treated with fenuron greater than the weight gain of control birds.

The vast majority of deaths of Bobwhites treated with DIMP occurred between one and three hours post-treatment. The lethal effects of per os dosing with DIMP appeared much more rapidly for Bobwhites than for rats, mice, or Mallard ducks. Jones (1977) reported Mallard duck deaths occurring within 24 hours after dosing with DIMP. Hart (1975) reported most deaths occurring during the first 24 hours and no deaths occurring more than 48 hours after administration of DIMP to rats and/or mice.

EXPERIMENT 2

Toxicity can be expressed not only as a single lethal dose but also as a lethal dietary concentration. Bein (1963), Worden and Harper (1963), and Stickel et al. (1965) have reported that the toxicity of the compound administered via gavage can be considerably different from the toxicity resulting from incorporation of the compound into the diet. The results of this study exemplify such difference in toxicity due to method of administration.

LC_{50} values of DCPD or DIMP could not be determined for the Bobwhite due to insufficient mortality, even though the average mg of compound consumed per bird per day of either chemical was greater than the respective LD_{50} value. The mortality occurring in the DCPD fed birds reached a maximum of only 20% and was not dose related. The mortality of the DIMP fed birds occurred in only one dietary level group and was attributed to cannibalism. The predicted points of zero feed consumption of both chemicals were above 70000 ppm. These results are in agreement with Jones (1977) who reported undeterminable LC_{50} values for the Mallard duck fed DCPD or DIMP treated diets and predicted points of zero feed consumption of 77,300 and 23,222 ppm of DCPD and DIMP, respectively.

Several values taken from extensive LC_{50} determinations (Heath et al., 1972) of 89 pesticidal chemicals are listed in Table 33. The LC_{50} value of DDT in Table 33 was taken from results by Heath and Stickel (1965).

In general, comparison of the susceptability of the Bobwhite to various compounds by Heath et al. (1972) has shown polychlorinated

Table 33. Median lethal concentrations (LC_{50}'s) of various pesticidal chemicals for Bobwhite quail chicks two to three weeks of age, listed alphabetically.

Chemical	Primary use	LC_{50} (ppm)	95% confidence limits
Aldrin	I[1]	37	33- 41
Aroclor 1254 (PCB)	Id[2]	604	410- 840
Ceresan M	F[3]	57	42- 74
Chlordane	I	331	197- 479
DDE	Dp[4]	825	697- 976
DDT	I	611	514- 724
Dieldrin	I	39	37- 41
Diquat	H[5]	2932	1811-5256
Endrin	I	14	11- 24
Fenitrothion	I	157	135- 183
Fenuron	H	5000	no mortality
Heptachlor	I	92	76- 113
Lindane	I,A[6]	882	755-1041
Malathion	I	3497	2959-4117
Paraquat dichl.	H	981	784-1213
Toxaphene	I	828	619-1102

[1] I = insecticide

[2] Id= industrial

[3] F = fungicide

[4] Dp= degradation product

[5] H = herbicide

[6] A = acaricide

biphenyls (PCB) to be the most toxic, organochlorines the least toxic, and organophosphates as intermediately toxic. Heath et al. (1972) also reported aldrin and dieldrin to be more toxic than 20 of the 23 organophosphates tested. It was suggested in this same study that gradual exposure to a compound via the diet can allow the test subject's detoxification system sufficient time to degrade the relatively unstable compounds, such as the organophosphates but insufficient time to degrade the relatively stable compounds such as organochlorines. Obviously this was not the case for the Bobwhite in this investigation with the compounds under consideration.

Results of studies by Hill (1971) and Heath et al. (1972) utilizing several species of aves have shown considerable variation in species sensitivity to various compounds. Hill (1971) suggested that avian sensitivities to DDT and organophosphates was associated with body size and species type, i.e., wild or farm raised. Hill found true wild Bobwhites were approximately 1.4 times as sensitive as farm raised Bobwhites. Heath et al. (1972) compared the sensitivity of the Bobwhite, Japanese quail, Mallard duck, and pheasant to various compounds. The conclusions of that comparison study are as follows:

1. Bobwhites, Japanese quail, and pheasants are similar in sensitivity to organochlorines.

2. Bobwhites and Japanese quail are similar in sensitivity to all the compounds tested with the exception of polychlorinated biphenyl; the Bobwhites were the most sensitive and the Japanese quail the least sensitive of the four species.

3. Bobwhites and Japanese quail are more sensitive to organophosphates than pheasants or Mallard ducks.

4. Mallard ducks were generally the least sensitive of the four species tested.

In general, the feeding of DCPD treated diets to the Bobwhite had little effect on their feed consumption, though small decreases in feed consumption at the higher DCPD dietary levels were noted. Diets containing 16000 ppm or more of DIMP caused a pronounced reduction of feed intake with the least feed intake at the highest DIMP dietary level. Both of the observations on feed consumption were made during the first five days of the eight day test period. This reduction of feed intake of birds fed either chemical was undoubtedly due to a repellant effect of the compounds rather than a toxic effect since the birds increased their feed consumption when fed clean feed during the three day post-treatment period. Voluntary feed restriction of treated diets is not uncommon; Nestler and Bailey (1941) observed discriminatory feeding habits of quail given a choice of toxic Crotalaria seeds or clean feed. Ernst (1966) reported that quail voluntarily restricted their feed intake when sufficient levels of some pesticides were added to their diets. Frings and Boyd (1952) reported olfactory discrimination by the Bobwhite. Jones (1977) reported that incorporation of DCPD into the diet greatly reduced feed consumption in twelve-day-old Mallard ducklings but had little effect on the feed consumption of 23-week-old Mallard ducks. In this same investigation Jones reported a great decrease in feed intake of Mallard ducks fed DIMP treated diets at levels of 12000 ppm and above. Linduska and Springer (1951) reported various feed consumption results when feeding six different insecticides to Bobwhites for 32 days.

Body weight gains were generally reduced in Bobwhites fed DCPD or DIMP diets; the least weight gains occurring in birds fed

the highest dietary levels of the compounds. However, while the reduced body weight gain of the birds fed DIMP diets was fairly consistent with the decrease in feed consumption, the decreased weight gain of the birds fed DCPD treated diets was more pronounced than the reduction in feed consumption. Jones (1977) suggested an "unknown mechanism causing decreased uptake of nutrients" as explanation of body weight loss coupled with unchanging feed consumption when feeding DCPD treated diets to 23-week-old Mallard ducks. Another possible explanation of noncorresponding body weight and feed consumption data is a low estimate of feed waste. Jones (1977) also reported decreased body weight gain for Mallard ducks fed DIMP treated diets.

Feed efficiency of Bobwhites fed DCPD or DIMP treated diets during the three day post treatment period showed no trends.

All dietary concentrations of DCPD or DIMP were greater than their respective LD_{50} values and all birds consumed a greater amount of chemical (mg/kg/day) than the respective LD_{50} values, with the exceptions of the birds fed the lowest dietary levels. Fitshugh and Schouboe (1965) reported that animals tolerating an amount of chemical in their diet that was greater than the LD_{50} value was uncommon. A possible explanation of the phenomena investigated by Fitshugh and Schouboe (1965) is an observation by Stickel et al. (1965) who reported that absorption of some compounds through the gastrointestinal wall can be more efficient if the compound is incorporated into the diet than when given as a single dose.

EXPERIMENT 3

The chronic test is conducted to determine the effects of feeding a chemical in the diet over an extended period of time to a test subject. This test differs from the subacute test in duration and parameters measured.

Feed consumption was unaffected in Bobwhites fed either DCPD or DIMP-treated diets with one exception: the 12000 ppm DIMP birds showed a reduced feed intake for the first two-week period of the experiment. As noted previously the 12000 ppm DIMP ration was reduced to 0 ppm at approximately the fourth week of the experiment.

During the ten-week pre-production period all groups of birds fed either chemical followed the same feed consumption pattern. During production, feed consumption of all groups of birds again followed a general pattern, with feed consumption steadily increasing to a peak followed by a gradual decline. The feed consumption pattern just described is typical of normal, untreated birds during their reproductive period. Scott et al. (1969) reported that feed intake increases to accomodate for the increased energy expenditure of egg production then decreases as egg production declines. The chronic study feed consumption results are in agreement with the findings by Jones (1977) who reported that feed consumption patterns of Mallard ducks fed DCPD (32,100 or 320 ppm) or DIMP (1000, 32000, or 10000) ppm) treated diets remained unchanged. Uneffected feed consumption of several other avian species fed various "treated" diets has been reported. Genelly and Rudd (1965) reported that 300 ppm toxaphene and 25 and 50 ppm dieldrin reduced the feed

intake of laying pheasants while 100 and 400 ppm DDT did not. Lillie et al. (1975) reported unaltered feed consumption in producing Leghorn hens after feeding various levels of PCB and PBB in the diets for eight weeks. Polin and Ringer (1978) reported 126, 625, and 3125 ppm PBB reduced feed intake of producing Leghorn hens.

Pre-production body weight change of Bobwhites fed either DCPD or DIMP treated diets coincided with their respective feed consumption results and showed no treatment effects. These results are consistent with results reported by Jones (1977). Jones found no treatment effect on the body weight changes of pre-productive Mallard ducks fed either DCPD or DIMP diets (for dietary levels see preceding paragraph). In other studies conducted, Japanese quail fed Dybar (fenuron) or DDT gained more weight than their respective controls but consumed less feed than the controls (Bergstrand and Klimstra, 1962; Ernst, 1966), respectively.

During the reproductive period, both weight change of Bobwhites fed DCPD or DIMP-treated diets showed no treatment effects. Female birds did show a greater weight gain than male birds in all groups. This type of weight difference between the Bobwhite sexes is consistent with the findings of many other investigators; Stoddard (1931), Aldrich (1946), Nestler (1949), Baldini (1951), Ripley (1960), Mahmoud (1966), and Georgis (1970). Again, Jones (1977) reported similar body weight change results in producing Mallard ducks fed DCPD or DIMP diets. Lillie et al. (1975) reported no effect on body weight changes due to the levels of PCB or PBB in diets fed to Leghorn laying hens.

The egg production of the DCPD fed Bobwhites showed no treatment-egg production relationship. Percent productions of birds fed the higher levels of DCPD (1250 and 4000 ppm) or control feed were near the standard value of 68.2 percent reported by Coleman (1930) cited from Hutt (1949). Percent production of the low level group (400 ppm) was reduced but generally the egg production pattern followed the same pattern as that of the control birds.

Egg production of DIMP fed Bobwhites was reduced in the 1200 ppm group, the highest level of DIMP fed. However, the egg production pattern was similar to the egg production pattern of the control birds except that it was consistently lower. Overall, egg production of the control birds, the 12000/0 ppm birds and the 3800/380 ppm birds was less than the standard reported by Colemen (1930) cited from Hutt (1949) but well within the ranges reported by Nestler (1943), Nestler et al. (1944), DeWitt et al. (1949), Baldini et al. (1952, 1954), Kirkpatrick (1964), and Wilson et al. 1973).

No effect on the incubation parameters measured occurred in either the DCPD or the DIMP experiments. The percentages of cracked eggs for each of the dietary groups and the control groups were above the normal range reported in the Federal Register (1975) but no percentages differed significantly from their respective control. The percent fertility and percent hatchability of eggs produced by birds of each dietary group, including the control group, were well within the normal range reported in the Federal Register (1975). Three week embryo viability was not measured for the following reasons: the difficulty of its determination, the possible adverse effects on the developing embryos from removing

the eggs from the incubator for parameter measurement, and the difficulty in stabilizing the temperature and the humidity of the incubators during parameter measurement.

Jones (1977) reported normal values for egg production fertility, hatchability, and number of cracked eggs of Mallard ducks fed either DCPD or DIMP treated diets, results which are in agreement with the results of this quail study. Many investigators have found adverse effects on egg production of various avian species treated with different compounds. Genelly and Rudd (1965) found unaffected egg production, fertility, and hatchability of pheasants fed two levels of DDT but reported depressed egg production in pheasants fed toxaphene (300 ppm) or dieldrin (25 and 50 ppm). In this same study, the investigators reported reduced hatchability at 300 ppm toxaphene and reduced fertility at 50 ppm dieldrin. Ernst (1966) reported variable egg production results and unaffected fertility and hatchability of Japanese quail fed DDT (100, 300, and 500 ppm), Zectran® (100 and 300 ppm), and Zytron® (500, 700, 2000, and 4000 ppm). Ernst (1966) also reported that 300 ppm Zectran® reduced egg production. Polin and Ringer (1978) reported reduced egg production of chickens and reduced hatchability of their progeny, when the chickens were fed PBB at 45 ppm and 30 to 45 ppm, respectively. Dewitt (1955, 1956), Shellenberger and Newell (1965), and Walker et al. (1969) have reported adverse effects on egg production, fertility, and hatchability of quail fed various levels of dieldrin. The levels of dieldrin necessary to cause the adverse effects just mentioned are not in agreement among the workers cited. Cross et al. (1962) and Gish and Chura (1970) found reduced egg production in quail fed DDT treated diets. Sauter

and Steele (1972) reported reduced egg production of laying hens fed various levels of malathion, DDT, lindane, or diazinon. Schom et al. (1972) found severely reduced egg production in quail fed azodrin (25 ppm).

Two week livability of Bobwhite hatchlings from parents treated with DCPD or DIMP showed no treatment effect. Percentages of chick survival of all groups including the control group, were within the normal range reported in the Federal Register (1975). These results are in agreement with Jones (1977) who reported no effect on two week livability of progeny of Mallard ducks fed DCPD or DIMP treated diets. Progeny livability of several other avian toxicological studies have shown no effect and/or adverse effects. Ernst (1966) found a reduction in the fourteen day livability of progeny of quail fed DDT-treated diets. Genelly and Rudd (1956) reported unaffected fourteen day progeny survival of pheasants fed various levels of DDT, toxaphene, or dieldrin. Jones and Summers (1968) reported 200 ppm DDT fed to Japanese quail reduced their progeny survival.

Eggshell thickenss data from either DCPD or DIMP treated Bobwhites were consistent with normal values reported in the Federal Register (1975). These results were not unexpected since the Bobwhite is not susceptible to eggshell thinning (U.S. Army, 1975). Jones (1977) reported no effect on shell thickness of eggs produced by Mallard ducks fed either DCPD or DIMP-treated diets.

In general, the feeding of DCPD or DIMP-treated diets to Bobwhites had no effect on the appearance and/or weights of their various internal organs. Exceptions to the preceding generality

were the proventriculus and gizzard weights of the Bobwhites fed 1200 ppm DIMP and the liver weights of the male Bobwhites fed 4000 ppm DCPD. The proventriculi and gizzards of Bobwhites fed 1200 ppm DIMP weighed more than did the same organs of the control birds. The livers of male Bobwhites, fed 4000 ppm DCPD, weighed less than the livers of the control birds but no lesions were observed. Jones (1977); however, reported unaffected organ weights of Mallard ducks fed DCPD or DIMP treated diets. Other various organ aberrations have been reported in avian toxicity studies. Gish and Chura (1970) found the number of males with testes exceeding 1000 mg (full spermatogenic activity) was inversely proportional to the amount of DDT in the diet. Burlington and Lindeman (1950) observed a decrease in the development and size of the testes of Leghorn cockerels injected with DDT. Genelly and Rudd (1955, 1956) reported liver degeneration in pheasants poisoned with DDT.

Two blood parameters, hemoglobin concentration and hematocrit (packed cell volume), plus the calculated mean corpuscular hemoglobin concentration, were measured in the DCPD or DIMP treated Bobwhites and found to be unaffected by treatment. Male Bobwhites, in all groups fed either chemical, had both a higher level of hemoglobin and higher number of erythocytes than the respective female Bobwhites. There was no difference in the mean corpuscular hemoglobin concentration between male and female birds. The greater hemoglobin concentration and hematocrit values of the males compared to the females is consistent with findings by numerous investigators. The relationship of a greater level of hemoglobin and maleness is correlated with the increased numbers of erythrocytes in the male due to testosterone. Androgen increases the

numbers of erythrocytes whereas estrogen tends to decrease the number of red cells (Sturkie, 1976). Nirmalin and Robinson (1972) reported that estrogen decreases erythrocyte numbers in Japanese quail by initially decreasing erythropoietic activity. The mean hemoglobin values determined for Japanese quail by Nirmalin and Robinson (1972) were slightly higher than the hemoglobin values determined for the Bobwhite in this study.

The mean hematocrit values, male or female, were within the normal ranges (Spiers, personal communication) and very near the values reported by Bond and Gilbert (1958) and Ernst et al. (1971). Lucas and Jamroz (1961) reported a five percent variation in hematocrit values in chickens as normal. Ernst et al. (1971) reported no significant variation in the mean packed cell volume by sex, which was not in agreement with results of this study. Nice et al. (1935) reported a mean crythocyte count of 3.5 mill./ mm^3 for three female Bobwhites.

In agreement with the unaffected blood parameters of quail in this study, Jones (1977) reported normal hemoglobin concentrations, hematocrit, and mean corpuscular hemoglobin concentration values of Mallard ducks fed DCPD or DIMP-treated diets. Different effects on blood parameters of other avian species treated with various compounds have been reported. Rehfeld et al. (1972) found decreased hemoglobin and hematocrit values in chicks treated with PCB's. Ernst (1966) studied the effects of several pesticides on Japanese quail and found that DDT, Zeetran, and Zytron when incorporated into the diets of male birds caused a decrease in packed cell volume but not the mean corpuscular volume "indicating normocytic and normoblastic anemia". Burlington and Lindeman (1950) observed a

reduction in erythocytes coincident with normal hemoglobin concentrations in male chickens chronically injected with DDT. Van Thiel (1977) reported a decrease in hematocrit and hemoglobin concentration in Leghorn cockerels fed 150 ppm PBB.

Leukocyte differentials of Bobwhites fed DCPD or DIMP were generally unaffected. Only the birds fed 1250 ppm DCPD showed any aberration. In the 1250 ppm DCPD group the mean number of eosinophils was significantly greater than the mean number of eosinophils of the control birds. However, the control birds of the DIMP experiment had a similar mean number of eosinophils. Sturkie (1976) and Lucas and Jamroz (1961) have reported a great variability in differential counts due to the methods used for counting, age of the subject, sex of the subject, physical condition of the subject, environment, diet, hormones, drugs, and other factors. Lucas and Denington (unpublished data cited in Lucas and Jamroz, 1961) found that "if the coefficient of variability for the 95 percent fiducial interval were to be held to some fixed values, for example, $\pm$ 10 percent of the group mean, then the following number of birds would be needed for a study on each of the components of the blood:

Blood component	Number of chickens
Lymphocyte/mm^3	130
Monocyte/mm^3	352
Heterophil/mm^3	240
Eosinophil/mm^3	3615
Basophil/mm^3	551

The normal leucocyte differentials of Bobwhites are in agreement with Jones (1977). In Jones' investigation, he reported no

significant differences between the leucocyte differentials of Mallard ducks fed DCPD or DIMP treated diets and their respective controls. Again different results were obtained in studies utilizing other avian species and compounds. Ernst (1966) reported that Japanese quail fed DDT or Zectran showed no consistent changes in leukocyte differentials. In this same study, Ernst reported that the feeding of Zytron to Japanese quail caused a consistent increase in the lymphocyte percentage of blood of the male quail coincident with a decrease in the percentage of heterophils of the blood. A decrease in the blood percentage of heterophils of female quail was observed by Ernst (1966). Ernst (1971) reported the following leucocyte values for the Bobwhite:

Cell type	% of blood* Male	Female
Lymphocyte	63	50
Monocyte	2	2
Heterophil	30	42
Eosinophil	3	3
Basophil	2	2

* Values estimated from graphs

During the chronic study the Bobwhites suffered two periods of high mortality. The initial period occurred during the first four weeks of the experiment when four birds from each of the highest levels of DIMP (3800 and 12000 ppm) died. The cause of death could not be determined by gross examination of the birds at necropsy. Upon consultation with the Project Officer (U.S. Army) the decision was made to reduce the DIMP dietary levels of 3800 and 12000 ppm to 380 and 0 ppm, respectively. The second period of high mortality occurred during a three day span in the eleventh week of the experiment. In these three days, mortality was independent of DCPD dietary level. At necropsy, hemorrhagic

lungs were observed in all the birds that expired during the three day period. No other abnormalities were observed. The cause of death could not be determined.

Mortality other than the two cases of high mortality mentioned was sporadic and not treatment related.

CONCLUSIONS

LD_{50}: Observations on mortality, feed consumption, and body weight change of Bobwhite quail show both DCPD and DIMP to be slightly toxic to that species.

LC_{50}: Generally the lack of treatment effect on feed consumption, mortality, and body weight gain of young Bobwhites fed DCPD treated diets suggests that the quail could not consume enough chemical to produce sufficient mortality to calculate a LC_{50} value. Bobwhites fed DIMP treated rations showed decreasing feed consumption coincident with decreasing body weight gain as dietary level of DIMP increased. These results coupled with no mortality trends suggest that the quail voluntarily restricted their feed intake and thus did not consume a sufficient amount of chemical to cause enough deaths to calculate a LC_{50} value.

Chronic: Of the various parameters measured very few of those of the treated quail were significantly different from the respective parameters of the control quail. The parameters that did show significant aberrations showed no consistent treatment-effect results. Thus, the results suggest that DCPD and DIMP have little effect on Bobwhite survival and reproduction at the levels tested. However, before the 3800 and 12000 ppm DIMP dietary levels were reduced, unexpected mortality occurred at those levels. Therefore, additional studies should be conducted, perhaps using different criterias of toxicity, different animal species, and/or different parameters to provide a better toxicity profile of DIMP.

APPENDICES

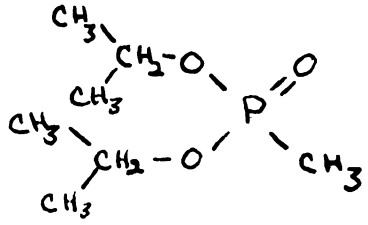
APPENDIX A

CHEMICAL STRUCTURES AND ALTERNATE NAMES FOR DIMP AND DCPD

DIMP

Structural formula

$((CH_3)_2CHO)_2(CH_3)P = O$

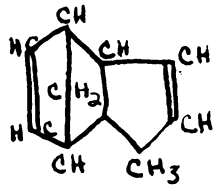


Alternative names

diisopropyl methylphosphonate; DIMP; phosphonic acid, bis-(1-methylethyl) ester (Chem. Abstr. after 1971); phosphonic acid, methyl-, diispropyl ester (1947-1971); methanephosphonic acid, diisopropyl ester.

DCPD

Structural formula



Alternative names

dicyclopentadiene; bicyclopentadiene; biscyclopentadiene; 3a,4,7,7a-tetrahydro-4, 7-methanoindene.

APPENDIX B

ANALYSIS OF FEED

B_1. Quail Breeder QB 72

Ingredients	kg
Corn	408.41
Soybean meal, 49%	296.65
Meat scrap, 50%	45.36
Alfalfa meal, dehy.	40.82
Animal fat, stabl.	51.71
Limestone	45.36
Dicalcium phosphate	6.35
Choline chloride, 50%	2.72
Methionine hydroxy analogue	0.91
Salt, iodized	3.45
Mineral mix A	2.72
Vitamin mix A	2.72
Antioxidant	0.11
	907.29

B_2. Quail Starter QS 72

Ingredients	kg
Corn	348.64
Soybean meal, 49%	384.55
Fish meal	28.18
Meat scraps, 50%	31.82
Alfalfa meal, dehy.	40.91
Animal fat, stabl.	53.64
Dicalcium phosphate	11.82
Choline chloride, 50%	2.73
Methionine hydroxy analogue	.91
Salt, iodized	3.18
Vitamin mix A	2.73
Mineral mix A	2.73
Antioxidant ethoxyquin/or BHT	0.11
	911.95

APPENDIX C

DIET PREPARATION

C_1: LC_{50} diets.

A premix of DCPD or DIMP was prepared by adding the pure chemical to corn oil and mixing by hand with quail starter diet. The final individual diets were prepared in one kilogram quantities by combining a quantity of premix with quail starter diet (Table C_1). All final diet mixing was completed by tumbling the mixture for 15 minutes in a seven kilogram capacity feed can in a Paul G. Abbe mixer[1]. The total amount of chemical-corn oil solution was not more than two percent of the diets containing DCPD.

Table C_1.

Premix

Chemical	Amount of chemical (gms)	Amount of feed (gms)	Total (gms)	ppm
DCPD	90	4410	4500	20,000
DIMP	180	4320	4500	40,000

[1] Paul G. Abbe, Inc., Little Falls, New Jersey 07424.

Table C_1 con't.

Diets

Chemical	Amount of premix (gms)	Amount of feed (gms)	Total (gms)	ppm
DCPD	100	900	1000	2000
	200	800	1000	4000
	300	700	1000	6000
	400	600	1000	8000
	500	500	1000	10000
	600	400	1000	12000
	700	300	1000	14000
	800	200	1000	16000
	900	100	1000	18000
DIMP	100	900	1000	4000
	200	800	1000	8000
	300	700	1000	12000
	400	600	1000	16000
	500	500	1000	20000
	600	400	1000	24000
	700	300	1000	28000
	800	200	1000	32000
	900	100	1000	36000

C_2: Chronic diets

A premix of DCPD or DIMP was prepared by the same method as employed in the premix preparation of the LC_{50} experiment. Final individual diets were prepared by the addition of an appropriate amount of premix to quail breeder diet. All mixing was completed by handmixing for ten minutes.

Table C_2.

Premix

Amount of oil (gms)	Amount of chemical (gms)	Amount of feed (gms)	Total (gms)	ppm
DCPD				
60	120.0	2820.0	3000	40000
60	37.5	2902.5	3000	12500
60	12.0	2928.0	3000	4000
DIMP				
60	360	2580	3000	120000
60	114	2826	3000	38000
60	36	2904	3000	12000
60	11.4	2928.6	3000	3800

Diets

Premix (gms)	Feed (gms)	Total (gms)	ppm
DCPD			
100	900	1000	4000
100	900	1000	1250
100	900	1000	400
DIMP			
100	900	1000	12000
100	900	1000	3800
100	900	1000	1200
100	900	1000	380

APPENDIX D

PREPARATION OF WRIGHT'S STAIN AND BUFFER

Wright's Stain

3.3 grams Wright's powder is added to 500 cc fresh, pure methyl alcohol. The stain is ripened for several months to room temperature in a stoppered brown bottle.

Buffer

3.80 gm Na_2HPO_4

5.47 gm KH_2PO_4

Dissolve in 500 ml distilled water and bring total volume to 1000 ml. Set pH at 6.4.

APPENDIX E

PREPARATION OF DRABKIN'S REAGENT

1000 mg Sodium Bicarbonate $NaHCO_3$

50 mg Potassium Cyanide KCN

200 mg Potassium Ferricyanide $K_3Fe(CN)_6$

1250 mg

Mix to dissolve and dilute to 1 liter.

The solution should be stored in a sealed amber bottle and kept refrigerated.

APPENDIX F

DETERMINATION OF HEMOGLOBIN CONCENTRATION

Figure F1. Sample hemoglobin concentration calculation. The line is constructed by plotting the % absorbance of each standard against its known hemoglobin concentration.

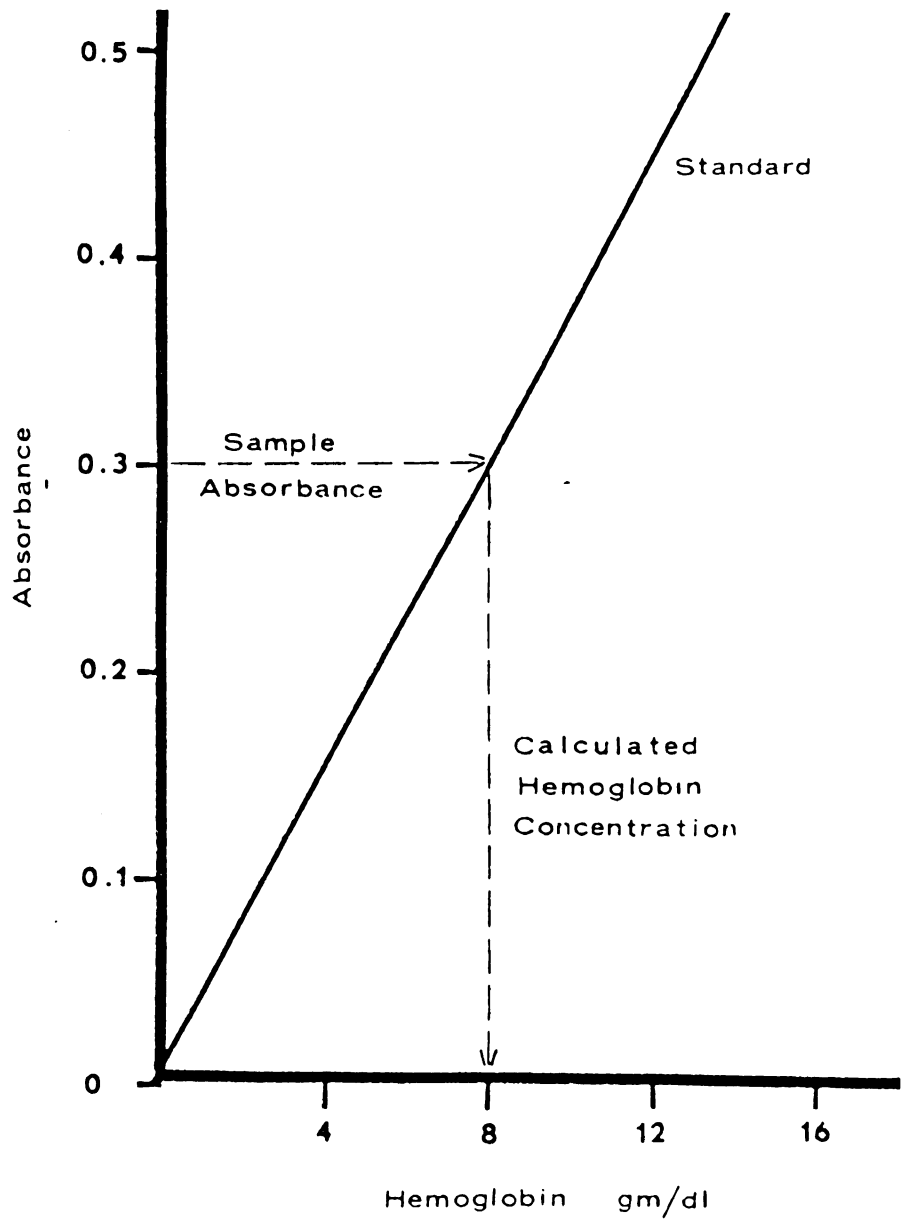

0.5
0.4
0.3
0.2
0.1
0
Absorbance
Standard
Sample
Absorbance
Calculated
Hemoglobin
Concentration
4
8
12
16
Hemoglobin gm/dl

Figure F1.

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