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TOXICOLOGY STUDY OF DIISOPROPYL METHYLPHOSPHONATE AND DICYCLOPENTADIENE IN MALLARD DUCKS, BOBWHITE QUAIL, AND MINK

FINAL REPORT

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Rocky Mountain Arsenal
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Commerce City, Colorado

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**Poultry Science Department
Michigan State University
East Lansing, Michigan 48824**

Project Officer: CPT Victor W. Robbins, VC
Environmental Protection Research Division
U.S. Army Medical Bioengineering Research and Development Laboratory

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) This study was conducted to determine the toxicity, and tissue residue accumulation, of diisopropyl methylphosphonate (DIMP) and dicyclopentadiene (DCPD) in wildlife. The toxicity was evaluated by acute (LD ₅₀), subacute (LC ₅₀) and chronic tests with Mallard ducks, Bobwhite quail, and mink. Tissue residue analyses for DIMP and DCPD were conducted with Mallard ducks and Bobwhite quail. Based on the results of the LD ₅₀ tests, DIMP was only slightly toxic to		

the test animals. An LD₅₀ of 1490, 1000, and 503 mg/kg of body weight was determined for the Mallard, Bobwhite, and mink, respectively.

An LC₅₀ for DIMP in the Mallard and Bobwhite could not be determined due to lack of mortality, even though the daily consumption of DIMP in these tests exceeded the LD₅₀ values for these species. The 21-day subacute LC₅₀ of DIMP for mink was estimated to be greater than 10000 ppm.

In the chronic test, 3200 ppm dietary DIMP resulted in decreased feed consumption and 10000 ppm caused a reduction in egg production in Mallard ducks. No other consistent adverse effects on reproduction, behavior, feed consumption, growth, hematology, or mortality were observed in the DIMP-fed ducks or quail on the 24-week test. The chronic ingestion of DIMP had no adverse effects on growth or reproductive performance of the mink, although slightly higher mortality occurred in females fed the DIMP-treated diets.

Mallard ducks and Bobwhite quail on the tissue residue study received ¹⁴C-DIMP at 100 mg per kg of diet or were dosed per os at 100 mg per kg of body weight. Plasma, liver, adipose, skin, red blood cells, kidney, brain and muscle samples were obtained from the birds at days 3 and 5 while they were being fed the ¹⁴C-DIMP diet and at days 3 and 5 after withdrawal of the treated diets. Tissue samples of the birds dosed with ¹⁴C-DIMP were obtained at 0, 2, 24, and 48 hours.

The birds fed the diets with radioactive DIMP had ¹⁴C residues averaging less than 1 ppm which declined to less than detection limits, averaging 0.04 ppm, in most tissues, by the 3rd day after withdrawal. All tissues but skin were clear of residue by day 5 off radioactive feed. Skin had 0.05-0.1 ppm at that time.

In the dosing experiment, residues at the second hour were 5.1 to 756 ppm, depending upon tissue and species. The residues, however, decreased rapidly with a biological half-life of 12.7 hours. Most tissues were at, or below, detection limits in 48 hours and clear at 65 hours, based on the half-life value. DIMP was not concentrated in the adipose tissue of either the ducks or quail.

DCPD was found to be relatively non-toxic to Mallards. An LD₅₀ could not be determined, even when levels as high as 40000 mg per kg were administered. For Bobwhites the LD₅₀ for DCPD was 1010 mg per kg. The acute oral toxicity of DCPD for mink was estimated to be greater than 1000 mg per kg of body weight.

An LC₅₀ for the birds could not be determined due to insufficient mortality on diets that contained up to 90000 and 18000 ppm DCPD for the Mallards and Bobwhite, respectively. The 21-day LC₅₀ of DCPD for mink was established as 6800 ppm.

The ingestion of DCPD by the ducks, quail, and mink had no significant effects on any of the parameters (growth, feed consumption, mortality, behavior, reproductive performance, hematology, etc.) measured during the chronic tests.

In the DCPD tissue residue study, Mallards and Bobwhites were fed or dosed with ¹⁴C-DCPD at the same levels and the same tissues collected for analysis as described for the ¹⁴C-DIMP-treated birds.

Both the ducks and quail fed the ¹⁴C-DCPD-treated diets had residues averaging less than 1 ppm which declined to less than detection limits, averaging 0.04 ppm, in most tissues by the 3rd day after withdrawal. All tissues except quail skin and duck liver and kidney were clear of residue by day 5 off the radioactive diets. In the dosing experiment, maximum residues at the second hour were 5.6 to 50.1 ppm, depending upon tissue and species. DCPD-tissue residues, however, decreased rapidly with a biological half-life of 12.7 hours. Most tissues were at or above detection limit in 48 hours. DCPD was not concentrated in adipose tissue of either species.

EXECUTIVE SUMMARY

This study was conducted to determine the toxicity, and tissue residue accumulation, of diisopropyl methylphosphonate (DIMP) and dicyclopentadiene (DCPD) in wildlife.

The toxicity was evaluated by acute (LD₅₀), subacute (LC₅₀), and chronic tests with Mallard ducks, Bobwhite quail, and mink. Tissue residue analyses for DIMP and DCPD were conducted with Mallard ducks and Bobwhite quail.

Based on the results of the LD₅₀ tests, DIMP was only slightly toxic to the test animals. An LD₅₀ of 1490, 1000, and 503 mg/kg of body weight was determined for the Mallard, Bobwhite, and mink, respectively.

An LC₅₀ for DIMP in the Mallard and Bobwhite could not be determined due to lack of mortality, even though the daily consumption of DIMP in these tests exceeded the LD₅₀ values for these species. The 21-day subacute LC₅₀ of DIMP for mink was estimated to be greater than 10000 ppm.

In the chronic test, 3200 ppm dietary DIMP resulted in decreased feed consumption and 10000 ppm caused a reduction in egg production in Mallard ducks. No other consistent adverse effects on reproduction, behavior, feed consumption, growth, hematology, or mortality were observed in the DIMP-fed ducks or quail on the 24-week test. The chronic ingestion of DIMP had no adverse effects on growth or reproductive performance of the mink, although slightly higher mortality occurred in females fed the DIMP-treated diets.

Mallard ducks and Bobwhite quail on the tissue residue study received ¹⁴C-DIMP at 100 mg per kg of diet or were dosed per os at 100 mg per kg of body weight. Plasma, liver, adipose, skin, red blood cells, kidney, brain, and muscle samples were obtained from the birds at days 3 and 5 while they were being fed the ¹⁴C-DIMP diet and at days 3 and 5 after withdrawal of the treated diets. Tissue samples of the birds dosed with ¹⁴C-DIMP were obtained at 0, 2, 24, and 48 hours.

The birds fed the diets with radioactive DIMP had ¹⁴C residues averaging less than 1 ppm which declined to less than detection limits, averaging 0.04 ppm, in most tissues, by the 3rd day after withdrawal. All tissues but skin were clear of residue by day 5 off radioactive feed. Skin had 0.05 - 0.1 ppm at that time.

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An LC₅₀ for the birds could not be determined due to insufficient mortality on diets that contained up to 90000 and 18000 ppm DCPD for the Mallards and Bobwhite, respectively. The 21-day LC₅₀ of DCPD for mink was established as 6800 ppm.

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In the DCPD tissue residue study, Mallards and Bobwhites were fed or dosed with ¹⁴C-DCPD at the same levels and the same tissues collected for analysis as described for the ¹⁴C-DIMP-treated birds.

Both the ducks and quail fed the ¹⁴C-DCPD-treated diets had residues averaging less than 1 ppm which declined to less than detection limits, averaging 0.04 ppm, in most tissues by the 3rd day after withdrawal. All tissues except quail skin and duck liver and kidney were clear of residue by day 5 off the radioactive diets. In the dosing experiment, maximum residues at the second hour were 5.6 to 50.1 ppm, depending upon tissue and species. DCPD-tissue residues however, decreased rapidly with a biological half-life of 12.7 hours. Most tissues were at or above detection limit in 48 hours. DCPD was not concentrated in adipose tissue of either species.

FORWARD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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INTRODUCTION

Statement of the Problem

Army arsenals throughout the territorial United States have stockpiled chemical and biological warfare substances. Some of these substances have been manufactured on the arsenal and others were merely stored there. One such arsenal is the Rocky Mountain Arsenal, Denver, Colorado (RMA). This installation has been used in the production, testing, and disposal of various potentially hazardous chemical and biological substances. Recently, a number of these chemicals (industrial waste materials and by-products) have been recovered from the surface and sub-surface water surrounding the RMA; thus, they are a cause of probable concern for the human, as well as the animal, population. Preventative measures have been and are being taken to minimize the chance of a chemical toxicity incident, but problem areas exist and pose a threat to the environment on the RMA.

Since many chemicals are present at RMA, each must be evaluated for its distribution, concentration, and predictability of toxicity. Thus, compounds that have widespread distribution, substantial amounts released, and an unknown toxicity are high on the testing priority list.

Of the possible contaminants, two, dicyclopentadiene (DCPD) and diisopropyl methylphosphonate (DIMP), were supplied to Michigan State University for toxicological investigation on Mallard ducks, Bobwhite quail, and mink.

Background

In the past, a number of toxicological incidents allegedly related to the RMA and its disposal of waste material have occurred. These incidents may have had environmental consequences such as injury to plants and animals, including wild birds, wild mammals, and domestic livestock. Two compounds which have been detected off post, DIMP and DCPD, are being investigated to determine their toxicity to birds and mammals.

Dicyclopentadiene (DCPD)

DCPD is used as a starting material for organochlorine insecticide production. DCPD and cyclopentadiene (CPD) are also used in the manufacture of elastomers, cycloaliphatic epoxides in resin coatings, rubber hydrocarbons, plastics, and other materials. CPD spontaneously converts to DCPD on standing and, thus, testing for its toxicity is not necessary. DCPD has been found in sampling wells and in surface water inside and outside RMA. Shell Chemical Company, which has an organochlorine insecticide manufacturing plant on RMA land, has stated that accidental spillage of pesticides and other chemicals has occurred at times. The chemicals have gotten into a stream and have, thus, been transported to a nearby lake.

At the lake, semiannual kills of migrating waterfowl feeding on snails and other foodstuff have prompted investigation of this compound. Since DCPD has very limited water solubility and very low odor threshold, it is unlikely that this pollutant could be unknowingly ingested.

Diisopropyl methylphosphonate (DIMP)

DIMP is a by-product produced during the manufacture of isopropyl methylphosphonofluoridate (GB), a nerve gas, but is not a metabolite nor environmental product of GB. DIMP is usually found at 2-3 percent in isopropyl methylphosphonate (IMP) waste and has been discovered in sampling wells both on and off the RMA. Since DIMP is a liquid at room temperature and is slightly soluble in water, there is a fairly high chance of ingestion by animals.

Mallard ducks were selected for this study because they are representative of species at the site of contamination, are readily available for toxicological testing and represent an aquatic form of avian wildlife. Bobwhite quail were selected because they, too, are a representative species at the contamination site, are readily available for testing and represent a ground dwelling form of avian wildlife. Mink were selected because as a carnivore they are at the top of the food chain, they are indigenous to the region and are one of the few wild mammals which reproduce readily in captivity and about which a large amount of base data has been accumulated.

Toxicity of DIMP to Mallard Ducks

The research was divided into three tests. Test 1 was concerned with the lethal dose for 50 percent of the animals (LD50); test 2 dealt with the lethal chronic level (LC50), and test 3 was a long term chronic study. Mallard ducks¹ (Anas platyrhynchos) were used in all three tests. The Mallards were procured from two locations:

1. Max McGraw Wildlife Foundation, Dundee, IL 60118
2. Frost Game Farm, Coloma, WI 54930

All tests were conducted in a windowless house at the Michigan State University Poultry Science Research and Teaching Center.

TEST 1 - ACUTE (LD50)

Procedure

This test was designed to determine the single oral dose LD50 of diisopropyl methylphosphonate (DIMP) to the Mallard.

Adult Mallards, approximately one year of age in non-laying condition, were utilized. The birds were held indoors in batteries. The batteries measured 122 cm (l) X 78.7 cm (w) X 35.6 cm (h) and there were ten ducks per battery for 960 cm² floor space/bird. The birds were held for one week and then body weights were taken. A two week acclimatization period followed. Birds were reweighed at the termination of the two weeks to note if any significant weight loss occurred before range finding began.

Preliminary range finding was done to establish the approximate lethal dose and a series of dosages was employed for the test to give mortality ranging from 10 to 90 percent.

Testing

Birds used for testing were maintained on duck breeder developer (Appendix A: Analysis of Feed). This feed was free of antibiotics and medication. Feed and water were provided ad libitum throughout the testing period. Food consumption was determined weekly for all groups. Before oral administration of chemicals, a fasting period of at least 15 hours was utilized.

Twenty birds were used per dose level, ten of each sex; the control groups consisted of ten birds of each sex dosed with water. All birds were weighed before dosing and on days 3, 7, and 14 after dosing. Administration was by drenching per os from a syringe with a length of tubing attached to the needle. The length of tubing used corresponded with the distance from the back of the oral cavity to the esophageal opening of the proventriculus. This insured a uniform location for introduction of the chemical. The syringe was either 3 cc or 5 cc, the needle was 20 ga, 3.81 cm long, and the tubing measured 1.143 mm ID and 1.575 mm OD. The total volume of chemical had a constant volume to body weight factor per animal.

¹ Phenotypically indistinguishable from wild Mallards.

Minimum observation-time for each animal was: during the first hour after dosing, four to five hours after dosing, and daily thereafter.

Necropsies were performed on all birds, including controls, at the time of death or at termination of the 14 days of observation. A general gross inspection was performed with special emphasis on the digestive tract, liver, kidneys, heart, and spleen.

Statistical Analysis

The LD₅₀ was analyzed by the method of Litchfield and Wilcoxon (1949). Feed consumption was analyzed by ordinary t-test, and approximate t-test. Weight changes were analyzed by one-way analysis of variance with Dunnett t-test.

Results

Mortality for the ducks treated per os with DIMP is listed in Table 1. Determination of acute oral LD₅₀ by the method of Litchfield and Wilcoxon (1949) was 1490 mg/kg. The 95% confidence interval was 1416.1-1567.7. Mortality for DIMP-dosed ducks is plotted in Figure 1. All deaths occurred within the first 24 hours after dosing with DIMP. There was no mortality nor clinical sign differences between the sexes among the treated groups. The first clinical signs occurred within 20 minutes after dosing. All the birds began to salivate and weave their heads. The salivation continued while the nutation increased. By the end of an hour, the animals were unable to lift their heads from the cage floor. Soon the birds became comatose with bradypnea and continued salivating. In many of those ducks that died, drowning on the copious amount of saliva was the attributing factor.

During the 14-day post-treatment period, no further signs of intoxication nor significant weight changes were noted except in the group dosed at 1800 mg/kg where a 14.8 percent loss in weight was observed (Table 2). 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.

Feed consumption, for the 14-day post-treatment period, is listed in Table 3 for the ducks dosed with DIMP. Feed consumption during the first week was depressed significantly from the control in the 1300, 1400, 1700, and 1800 mg/kg dosed groups by 22.6 percent, 37.8 percent, 23.4 percent, and 56.4 percent, respectively. During the second week, feed consumption was depressed significantly in the 1300 mg/kg group by 6.3 percent and by 39.8 percent in the 1800 mg/kg group; all others were equal to or above the control.

Table 1. Mortality of adult Mallard ducks during a 14-day period following a single per os dosing with DIMP

Treatment level (mg/kg)	Groups mean body weight (gms)	Mortality ¹		
		No. died/No. treated		Combined percent
		Male	Female	
0	1217	0/10	0/10	0
1300	1143	1/10	2/10	15
1400	1098	5/10	7/10	60
1500	1083	4/10	3/10	35
1600	1038	6/10	6/10	60
1700	1186	8/10	6/10	70
1800	1149	8/10	10/10	90

¹All deaths occurred within the first 24 hours after dosing.

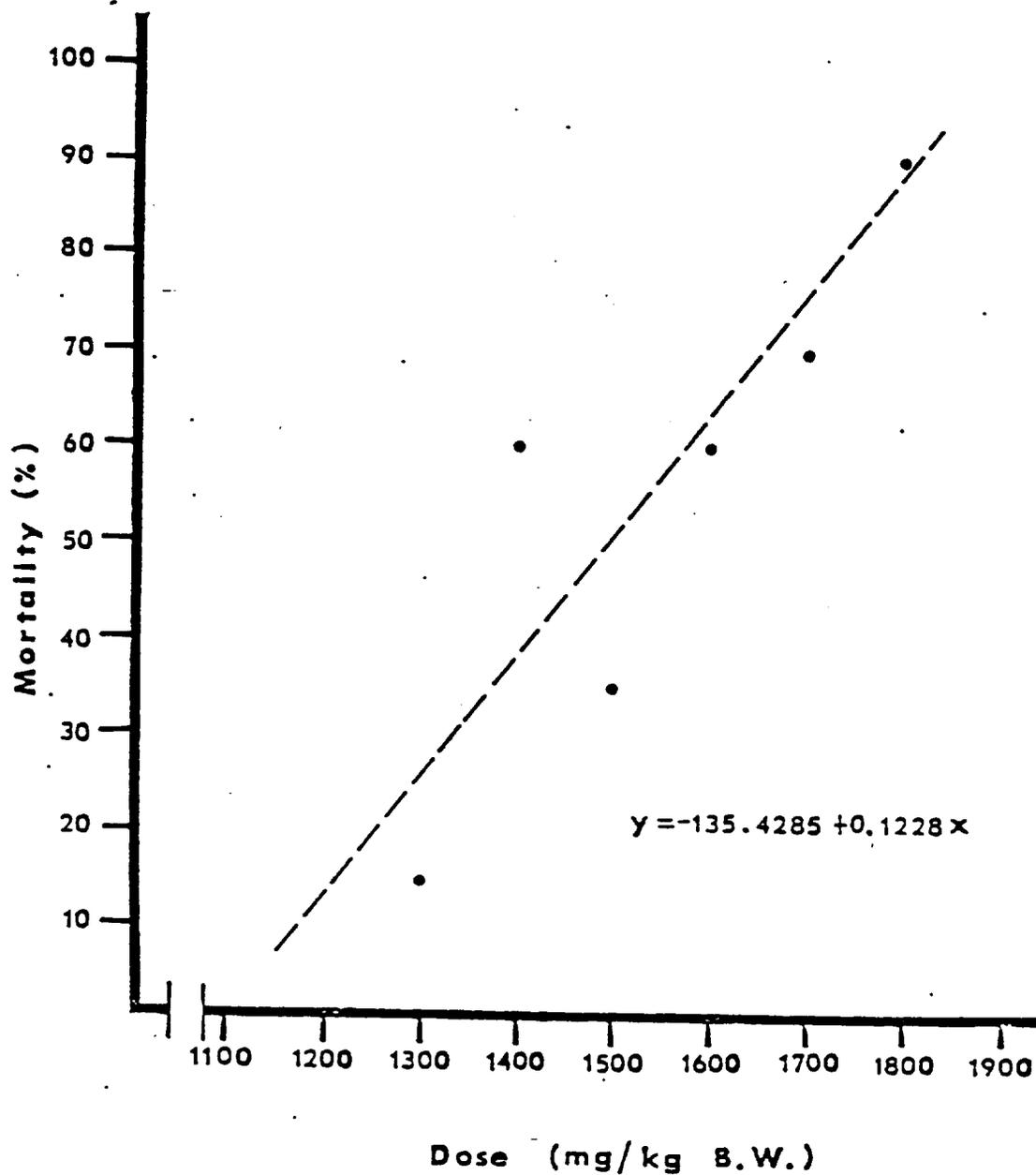


Figure 1. Percent mortality of adult Mallards, equal numbers of each sex, given a single oral dose of DIMP and observed for 14 days post-treatment. In the regression equation x = dose of DIMP in mg/kg of body weight and y = percent mortality.

Table 2. Body weight changes of Mallard ducks during 14 day post-treatment observation period following a single per os treatment with DIMP

Treatment	Treatment level (mg/kg)	n	Mean body weight		Mean change
			Day 0	Day 14	
DIMP	0	20	1217	1215	- 2 ¹ _a
DIMP	1300	17	1151	1190	39 _a
DIMP	1400	8	1111	1133	22 _a
DIMP	1500	13	1060	1126	66 _a
DIMP	1600	8	1052	1121	69 _a
DIMP	1700	6	1187	1232	45 _a
DIMP	1800	2	1263	1076	-187 _b

¹Means having the same subscript are not significantly different from their respective control ($P > 0.05$). Means having a different subscript are significantly different from control ($P = 0.01$).

Table 3. Feed consumption of Mallard ducks during 14-day post-treatment observation period following a single per os treatment with DIMP

Treatment	Treatment level (mg/kg)	n	Day 0-7 ¹ g/b/d	Day 8-14 ¹ g/b/d
DIMP	0	20	66.35 ± 1.745	61.55 ± 1.653
DIMP	1300	17	51.35 ₂ ± 1.892	57.70 ₂ ± 1.793
DIMP	1400	8	41.30 ₂ ± 2.758	69.84 ₄ ± 2.614
DIMP	1500	13	57.94 ₄ ± 2.164	67.32 ₂ ± 2.051
DIMP	1600	8	65.15 ₄ ± 2.758	73.40 ₂ ± 2.614
DIMP	1700	6	50.86 ₃ ± 3.185	64.93 ₄ ± 3.019
DIMP	1800	2	28.93 ₂ ± 5.517	37.07 ₂ ± 5.229

¹Data reported as treatment mean ± standard error.

² Significantly different from control (P < 0.0005)

³ Significantly different from control (P < 0.01)

⁴ Not significantly different from control (P > 0.05)

Discussion

The Mallard LD₅₀ for DIMP (1490 mg/kg) is, in general, higher than those reported for mammals. This value is in agreement with data presented in this report where the LD₅₀ value for Bobwhite was determined as 1000 (934.2 - 1070.5) mg/kg. The quail LD₅₀ is near that of the male mouse (1041 mg/kg) the male rat (1125 mg/kg) (Dacre and Hart, 1977), while the duck LD₅₀ range (1416 - 1568 mg/kg) is within the female mouse LD₅₀ range (1165 - 1594 mg/kg) (Dacre and Hart, 1977). The values for these animals place DIMP in the slightly toxic range, based on the following chart (Hodge and Sterner, 1949):

<u>Term</u>	<u>Range (mg/kg)</u>
Extremely toxic	1 or less
Highly toxic	1 - 50
Moderately toxic	50 - 500
Slightly toxic	500 - 5000
Practically nontoxic	5000 - 15000
Relatively harmless	> 15000

Since the slope of the dosage-mortality curve measures the change in mortality with a change in dose, then the "steeper" the slope of the curve the less variability expected. Consequently, a "flat" curve indicates extreme variability to that chemical. The dose-response slope (0.1228) for DIMP (Figure 1) is slightly "flat" and thus the variability of the data is to be expected.

No difference by sex was found in ducks dosed with DIMP. This lack of difference by sex in birds in response is consistent with Dahlen and Haugen (1954), Tucker and Crabtree (1970), Tucker and Haegele (1971), where no difference by sex was found in young non-breeding birds of 22 species treated with a maximum of 108 different pesticides. In mammals, such as rats and mice dosed with DIMP, a difference by sex was found (Dacre and Hart, 1977).

Of the two surviving ducks dosed at the highest level (1800 mg/kg), body weight and feed consumption were affected more than in any other group of surviving birds (Tables 2 and 3). All groups below 1800 mg/kg appeared, by the second week, to have recovered in their feed consumption, while the 1800 mg/kg dosed group had eaten only about 8 g/b/d more the second week than their first week consumption. This slight increase in the 1800 mg/kg second week consumption was 24.5 g/b/d lower than the control groups' second week consumption. Some internal damage may have occurred that caused a loss of appetite. An altered appetite may have resulted from the chemical altering the blood hormones, such as thyroxine or glucocorticoids, and/or circulating substates, such as glucose, glucagon, or amino acids which would affect the hypophysis (Leclercq-Meyer and Mailhe, 1970; Samsel et al., 1972; Karmann and Mialhe, 1973) and/or the hypothalamic satiety and hunger centers (Laurent and Mialhe, 1976). Another mechanism whereby appetite may be altered would be if the chemical had damaged the hunger center and the animal felt satisfied most of the time (Hawkes and George, 1975).

Also, if the chemical had damaged the gastrointestinal tract, then a decreased intake may have resulted while the intestinal wall was healing. - If damaged, the intestinal wall may not have been absorbing nutrients in the normal manner thus giving a decrease in body weight gains.

A list of compounds with LD₅₀'s from Tucker and Crabtree (1970) is presented in Table 4 along with LD₅₀'s of DIMP as a comparison of relative toxic levels. DIMP is 3.9 times less toxic for Mallards than dieldrin which is used as a standard for comparison in many studies. Toxicity index as calculated from Sun (1950) equals (LD₅₀ of standard/LD₅₀ of sample) x 100. For DIMP, the index is 25.57. As the route of administration is one of the most influential factors in modifying the LD₅₀, this index gives a more constant number for comparison between different routes of administration.

TEST 2 - SUBACUTE (LD₅₀)

Procedure

This subacute test was designed to determine the maximum repeated dosage tolerable to Mallard ducklings on DIMP-treated diets. A random selection of healthy twelve-day-old ducklings was employed for two reasons: (1) to avoid any possible interference of chemical intake by the yolk sac absorption and (2) to exclude any late hatching mortality. Sex of the bird was not taken into account, because determination of sex was not practical for birds of this age. The ducklings were held indoors in a Petersime Brood unit² from one day of age through the end of the test.

A range finding pilot test was performed to determine the effect of the chemical on feed consumption and body weight. A series of dosages was employed in the test to determine the point of zero feed consumption rather than 50 percent mortality, since no deaths occurred during range finding.

Testing

The ducklings were maintained on duck starter diet (Appendix A: Analysis of Feed). This feed was free of antibiotics and medication. Feed and water were provided ad libitum throughout the testing period. The test ran a total of eight days; the treated diets were fed for the first five days and untreated feed was provided for the last three days. The three days post-treatment period was used to avoid bias due to overestimating the dose by not taking into account mortality that would not have occurred because the compound did not have time to act. Treated feeds were prepared by adding a chemical: corn oil solution to the duck starter (Appendix B: Diet Preparation). In the DIMP-treated diets,

² Petersime Incubator Co., Gettysburg, OH 45328

Table 4. Comparative LD₅₀'s from the literature for the Mallard duck at various ages.

Compound	Primary use	Age (months)	Sex	LD ₅₀ mg/kg (95% conf. limits)
Thimet	I ¹	3-4	F	0.616 (0.367-1.03)
Parathion	I	2-3	F	1.90 (1.37-2.64)
Parathion	I	3-4	M	2.31 (1.54-2.96)
Diazinon	I	3-4	M	3.54 (2.37-5.27)
Methyl Parathion	I	3	M	10.0 (6.12-16.3)
Co-Ral.	I	3-4	M	29.8 (21.5-41.3)
Abate	I	--	M,F	80 - 100
Dieldrin	I	6-7	F	381 (141-1030)
Aldrin	I	3-4	F	520 (229-1210)
Chlordane	I	4-5	F	1200 (954-1510)
Malathion	I	3-4	F	1485 (1020-2150)
DIMP		12	M,F	1490 (1416-1568)
Lindane	I	3-4	M	>2000
Arochlors	Industrial	10	M	>>2000
DDT	I	3	F	>2240
Mires	I	3-4	M	>2400
Pyrethrum	I	3-4	F	>10000
DCPD	°	12	M,F	>40000

¹I = insecticide

the chemical-corn oil solution was a constant two percent of the diet. The control diet consisted of two parts corn oil to 98 parts feed by weight. For DIMP ten dietary treatments were used: 0, 2000, 4000, 6000, 8000, 10000, 12000, 14000, 16000, and 18000 ppm diets. Ten ducklings of undetermined sex were placed on each dietary treatment.

All signs of intoxication and abnormal behavior were noted throughout the eight days and all surviving animals were necropsied at the end of the test.

Estimates of average feed consumption with observation on excess spillage were made for determination of maximum repellency (estimated zero feed consumption).

Statistical Analysis

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

Results

Results of the five-day range finding trial were:

<u>Treatment</u>	<u>Level in diet (ppm)</u>	<u>Change in body wt. (g/b/d)</u>	<u>Feed consumed (g/b/d)</u>	<u>Percent mortality</u>
DIMP	6000	27.9	58.12	0
DIMP	9000	-1.6	6.26	0

Feed consumption of ducklings (Figure 2) on the 12000, 14000, and 16000 ppm diets was decreased as compared to that of those on the control diet by 57.4 percent, 43.0 percent, and 51.4 percent, respectively (mean decrease was 50.6 percent or 28.4 g/b/d), whereas intake of the 10000 ppm diet was decreased by only 20.8 percent (11.7 g/b/d). Thus, feed consumption of birds on the three highest levels (12000, 14000, and 16000 ppm) was decreased by more than two times that on any other diet. For the diets 0 through 8000 ppm the slope was only -0.465 while the diets of 8000 through 16000 ppm had a slope of -3.224 (Figure 3). Calculated zero feed consumption from the second slope equals 23222 ppm DIMP in the diet.

Body weight gain (Figure 4) showed changes similar to feed consumption. Birds on lower levels, 2000 to 8000 ppm, showed only a slight decrease of 21.2 percent (6.06 g/b/d) as compared to controls and a slope of -0.616 (Figure 5), while those on the higher levels, 10000 to 16000 ppm, showed a continuous decrease from 19.9 to 8.4 g/b/d (Figure 4) with a slope of -1.906 and a high correlation between feed consumed and level of DIMP in the diet of -0.996 as compared to the lower correlation for the lower DIMP treated levels of -0.630 (Figure 5). Predicted zero body weight gain was 20439 ppm DIMP in the diet. There was no mortality in

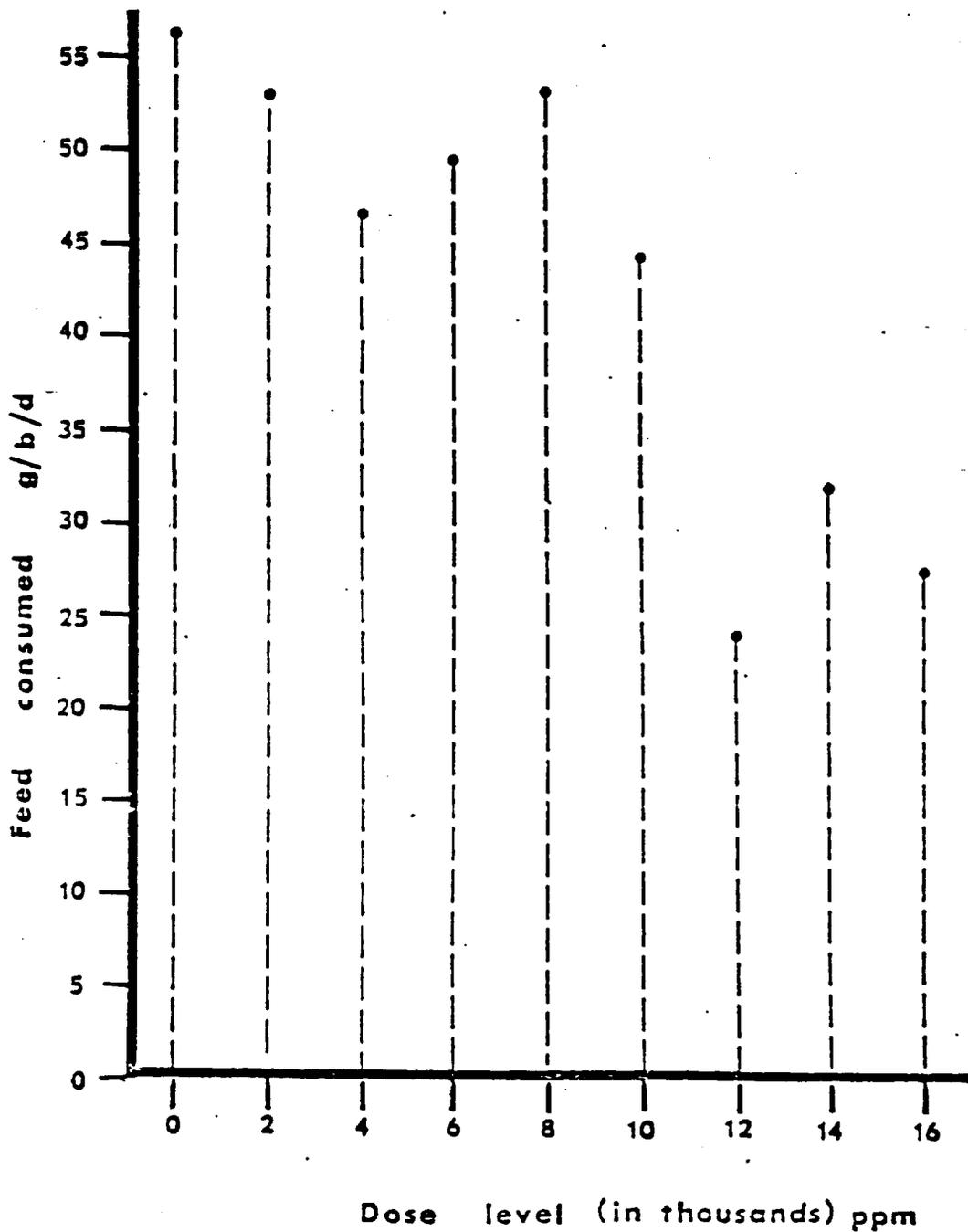


Figure 2. Effect of feeding DIMP at various levels in the feed for 5 days on feed consumption of 12-day-old Mallard ducklings.

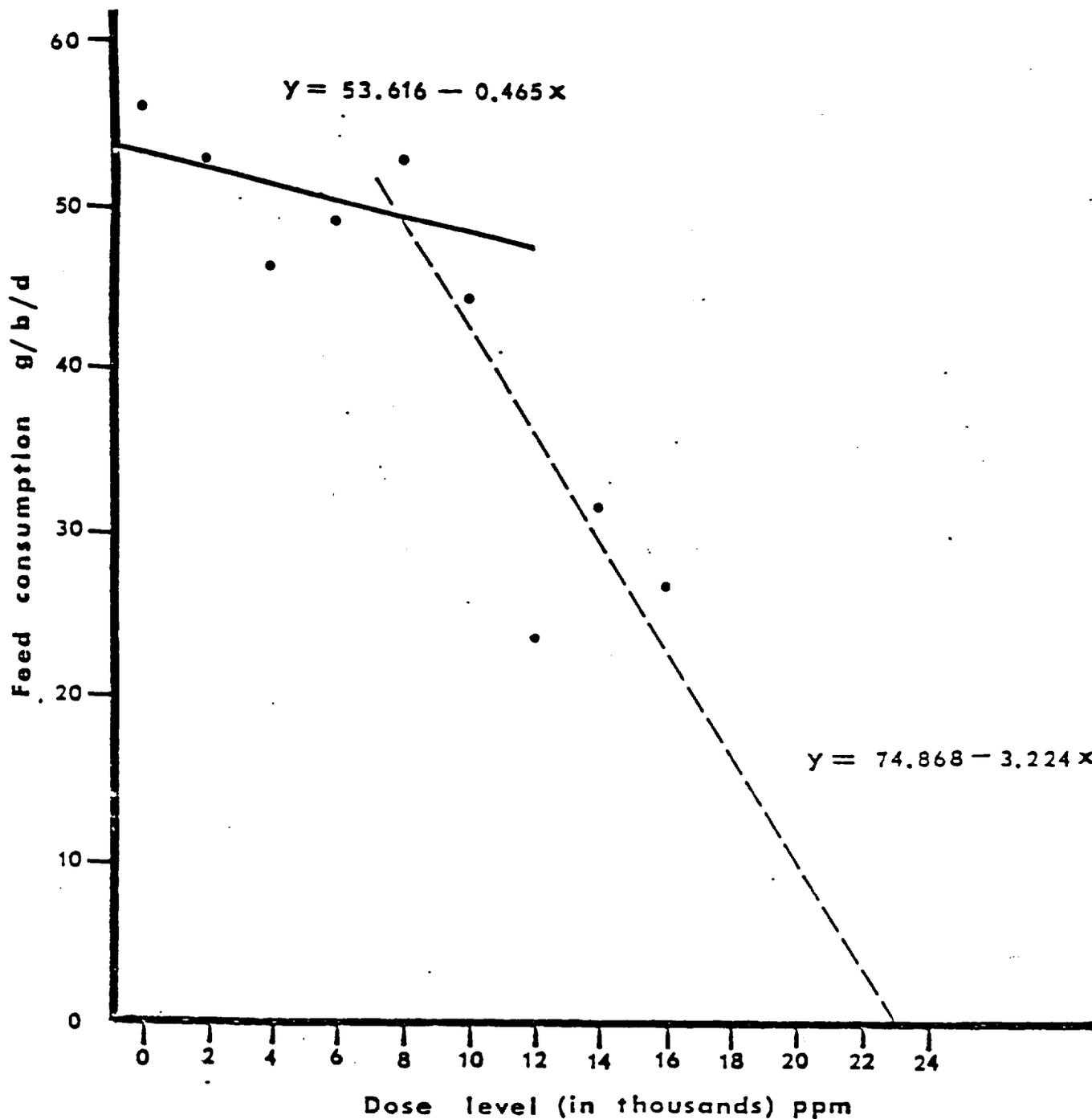


Figure 3. Regression equations of the data shown in Figure 2. In the regression equations x = ppm of DIMP in the feed and y = feed consumption in g/b/d.

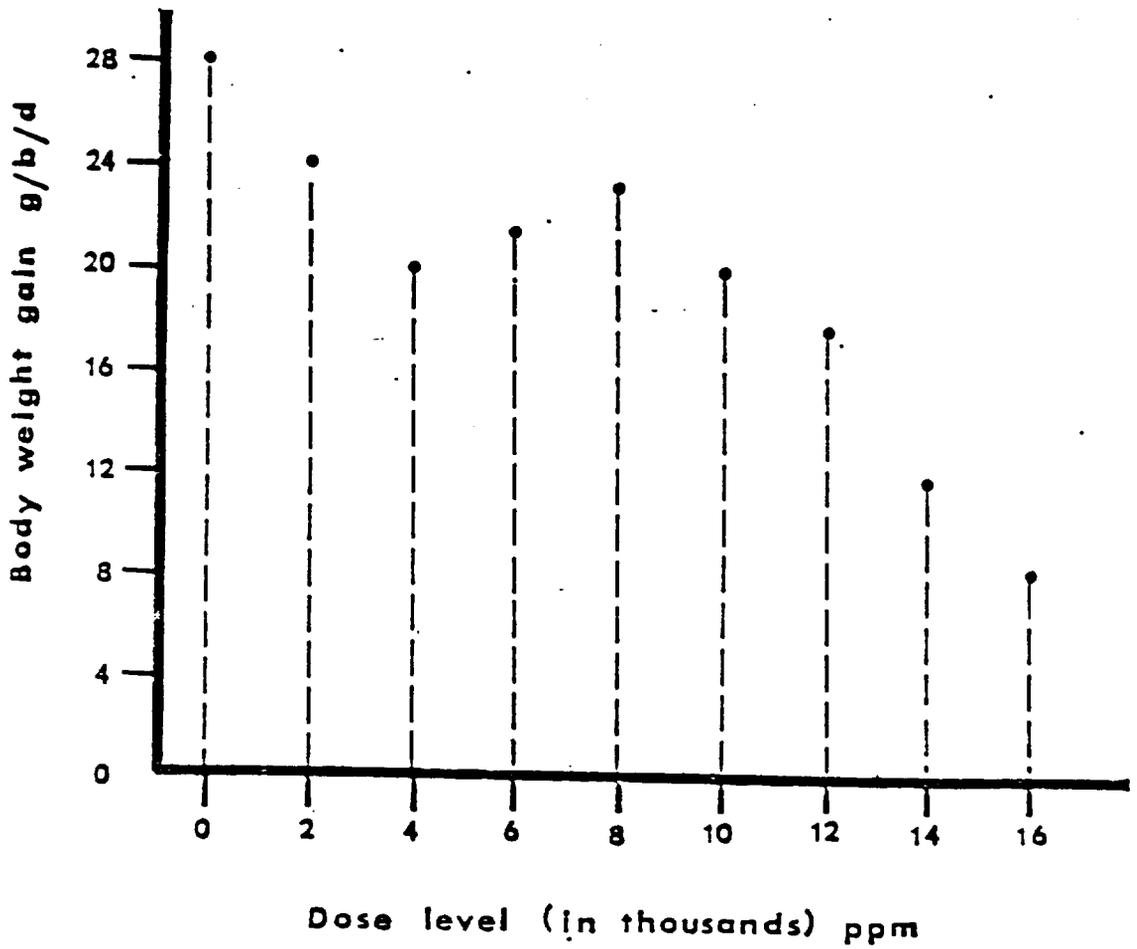


Figure 4. Effect of feeding DIMP at various levels in the feed for 5 days on body weight gain of 12-day-old Mallard ducklings.

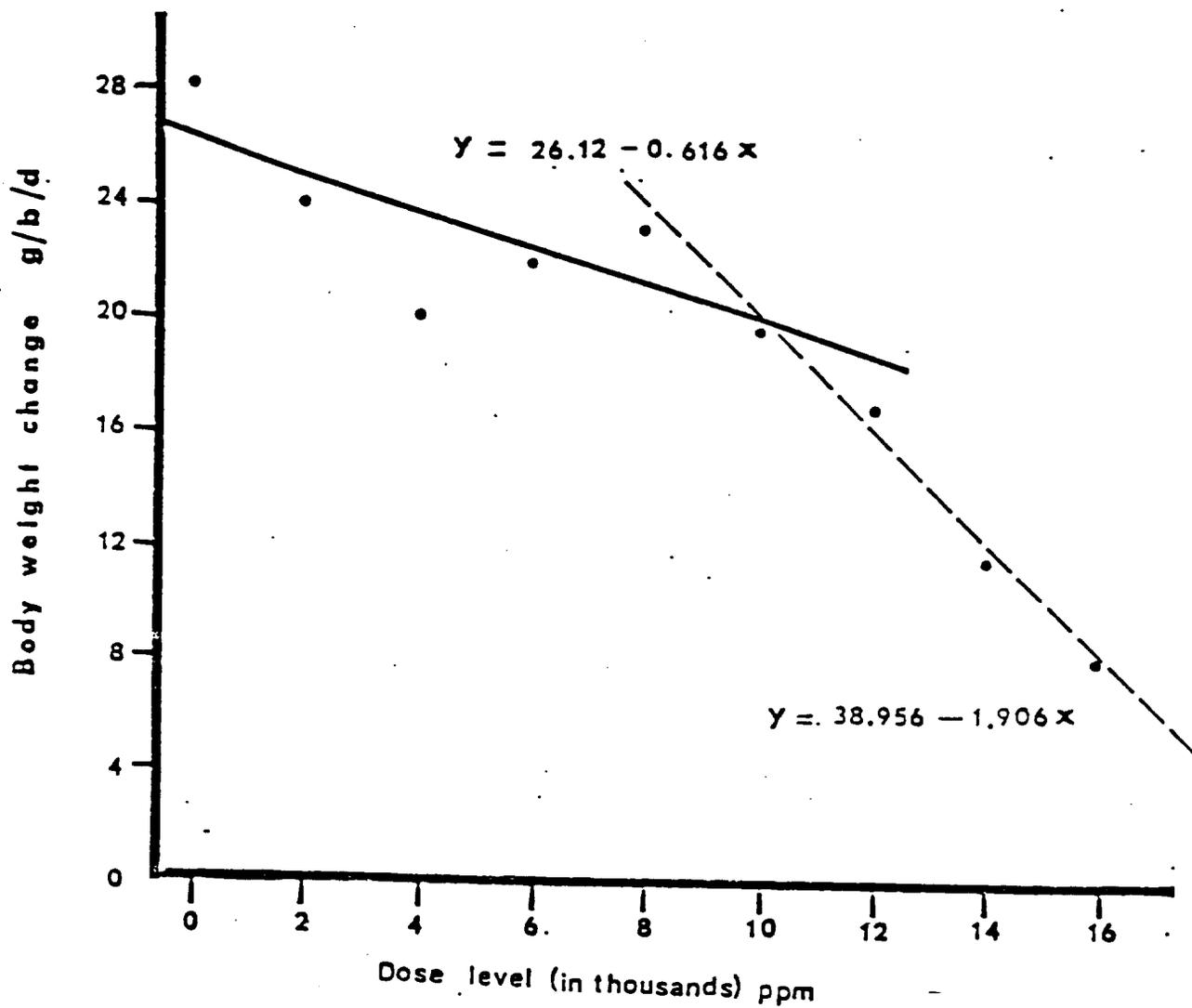


Figure 5. Regression of the data shown in Figure 4. In the regression equation x = ppm of DIMP in the feed and y = feed consumption in g/b/d.

any group even though the amount of DIMP ingested (Table 5) ranged from 403 to 2062 mg/kg/day which bracketed the LD₅₀ of 1490 mg/kg.

During the three-day post-treatment period, level of feed consumption (Figure 6) generally was higher in those groups of ducklings which had shown the greatest decrease in consumption during the five-day treatment period. The ducklings which had been receiving DIMP containing feed averaged 2.73 g/b/d greater than the control groups and showed a general increase toward the highest level, 16000 ppm, (slope +0.832, correlation between level of chemical in the diet and feed consumption was +0.885). The three lower levels, 2000, 4000, and 6000 ppm, during the 3 day post-treatment period showed a mean decrease of 6.85 g/b/d intake of feed as compared to the control group's consumption. Body weight changes during post-treatment (Table 6) show that all treatment groups, except the 6000 ppm group, gained more weight, from 0.6 to 14.3 g/b/d, than did the control. These seven treatment groups had a mean increase of 5.53 g/b/d as compared to the control.

Necropsies showed no gross pathological changes in treated groups as compared to controls.

Discussion

The lethality of a chemical mixed in the diet can differ markedly from that of the pure chemical administered as a single oral dose (Stickel *et al.*, 1965). This lethality difference appeared to be the case for DIMP, where no mortality occurred in the LD₅₀ test and the LD₅₀ was calculated at 1490 mg/kg.

A comparison of LC₅₀ values taken from Heath *et al.*,³ (1972) is listed in Table 7. There are a number of compounds with no LC₅₀ determinations, mostly in non-insecticides, as there was little or no mortality.

Of 12 compounds given in Tables 6 and 7, listed in order of relative toxicities (see Table 8), DIMP placed low on the list; thus, it is less toxic than most other compounds used in commerce.

For DIMP-treated ducks, a continual decrease in feed consumption did not occur until the level of DIMP ingested per day was higher than the determined LD₅₀ level (Table 5), i.e., 10000 ppm and greater. Fitshugh and Schouboe (1965) reported that it is unusual for animals to tolerate more than the LD₅₀ amount in mg/kg, per day. Levels of DIMP of less than 10000 ppm in the diet showed very little effect as intoxication from organophosphates tends to reverse more rapidly than intoxication from some other compounds such as DDT (Hill, 1971). The decrease in feed consumption at levels above 8000 ppm may have been from a loss of appetite, but

³ Except for DDT on 5-7 day old Mallard ducklings from Heath and Stickel (1965) and Mallards treated with DIMP or DCPD from this study.

Table 5. Calculated DIMP intake over 5 days and mortality over 8 days for 12-day-old Mallard ducklings on LC₅₀ trial

Dose (ppm)	Mg DIMP consumed/day	Mean body wt. (g)	Mg DIMP/kg/day	Percent mortality
0	0	277.3	0	0
2,000	106.1	263.1	403.3	0
4,000	187.4	285.3	656.9	0
6,000	297.0	286.2	1037.7	0
8,000	426.1	295.7	1441.0	0
10,000	444.8	249.1	1785.6	0
12,000	286.8	284.7	1007.4	0
14,000	448.0	249.4	1796.3	0
16,000	436.2	211.5	2062.4	0

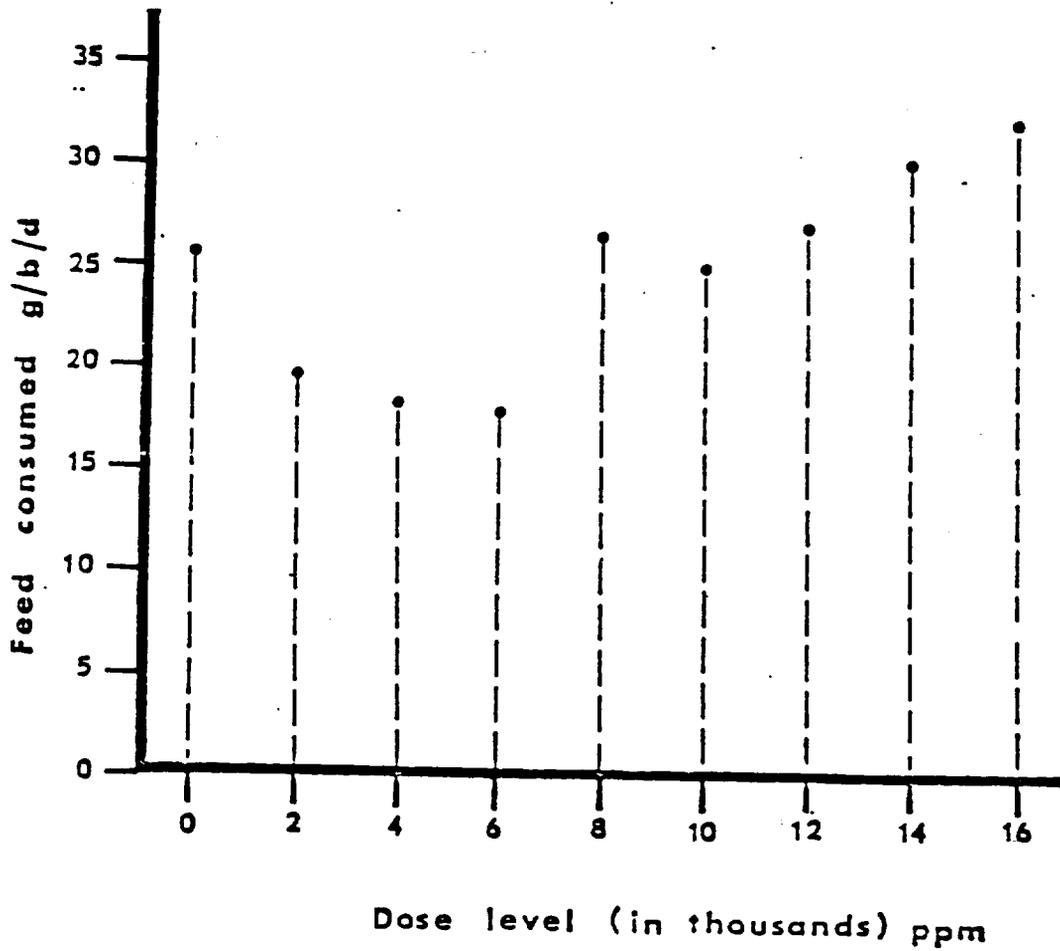


Figure 6. Feed consumption of 17-day-old Mallard ducklings fed non-treated diet during 3-day post-treatment after withdrawal of DIMP-treated diet.

Table 6. Body weight gain of 17-day-old Mallard ducklings during 3 day post-treatment on non-treated feed after withdrawal of DIMP-treated feed

DIMP level in the diet (ppm)	Weight gain g/b/d	Feed consumed/ weight gain
0	3.36	7.59
2,000	3.96	4.98
4,000	4.36	4.19
6,000	-10.80	-1.66
8,000	4.90	5.44
10,000	8.50	2.93
12,000	6.93	3.92
14,000	15.93	1.90
16,000	17.66	1.82

Table 7. Comparative LC₅₀'s from the literature, for Mallard ducklings two to three weeks old

Compound	Primary use	LC ₅₀ (ppm)	95% conf. limits
Endrin	I ¹	22	17-31
Aldrin	I	155	129-186
Dieldrin	I	185	152-217
Diazinon	I	191	138-253
Parathion	I	275	183-373
Methyl Parathion	I	682	541-892
Co-Ral	I	709	521-1032
DDT	I	875 ²	650-1140
Abate	I	894	575-1910
DDT	I	1869	1500-2372
DDD	I	4814	3451-7054
Lindane	I	40% mortality at 5000	
DDVP	I	30% mortality at 5000	
Amitrole	H ³	5000 ⁴	
Aramite	A ⁵	5000 ⁴	
Captan	F ⁶	5000 ⁴	
Mirex	I	5000 ⁴	
Nabam	F	5000 ⁴	
Picloram	H	5000 ⁴	
Tetradifon	I,A	5000 ⁴	
TFM	L ⁷	5000 ⁴	
DIMP		16000 ^{4,8}	
DICPD		30% mortality at 60000 ⁸	

¹I = insecticide

²5-7 days old

³H = herbicide

⁴No mortality

⁵A = acaricide

⁶F = fungicide

⁷L = lampricide

⁸11-13 days

Table 8. Overall toxicity of DCPD and DIMP compared with 10 commercial compounds

Chemical name	LD ₅₀ ¹ placing	LC ₅₀ placing	Overall placing
Parathion	1	4	1
Diazinon	2	3	1
Methyl Parathion	3	5	3
Co-Ral	4	6	6
Abate	5	7	7
Dieldrin	6	2	3
Aldrin	7	1	3
DIMP	8	11	10
Lindane	9	9	8
DDT	10	8	8
Mirex	11	10	11
DCPD	12	12	12

¹In decending order of toxicity. (1 = most toxic, 12 = least toxic).

was probably just a refusal to eat the diets containing higher concentrations of chemical (1.0 - 1.6 percent) in the diet. During the three-day post-treatment, increases in consumption were inversely related to the five-day treatment intake. The 16000 ppm group that had consumed the least during the first five days consumed the most during the post-treatment period (Figure 6); thus, showing no residual effects on appetite, if it had been affected.

Another parameter, related to feed consumption, is body weight change. Weight gains for ducks on DIMP-treated diets followed the same pattern as the food consumption data with the 16000 ppm group gaining the least (Figures 4 and 6). This observation conforms to the action of organophosphates. These compounds when given in the diet over a period of time are degraded by the body, as they are relatively unstable compounds. During the three-day post-treatment period, all groups gained more weight in relationship to feed intake than did the control, except for the 6000 ppm group which lost weight.

TEST 3 - CHRONIC

Procedure

This test was designed to determine the toxicological effects on adult Mallards and their progeny from continuous exposure to DIMP over a reproductive cycle.

Four test groups of randomly selected ducks were used. One group served as a control and three groups as treatment birds. Each group consisted of a pen of two males and five females and was replicated three times. All groups were randomly assigned to pens. The size of each pen was 1.47 m x 1.55 m x 0.7 m high with no top. Wing feathers were clipped to prevent the birds from escaping.

Testing

Diets were prepared by adding a chemical-corn oil solution to the pelleted feed (Appendix B: Diet Preparation). The control diet consisted of corn oil at two parts mixed to 98 parts of pelleted feed. Water and prepared diets were provided ad libitum throughout the entire 24 weeks. The animals were on the treated feed a minimum of ten weeks before commencement of egg production and a minimum of ten weeks after 50 percent production level was attained. Duck breeder-developer feed was fed for the first six weeks and breeder-layer feed was fed for the remainder of the trial. Food consumption was measured at biweekly intervals during the entire test.

The room was kept at approximately 7°C and six hours of light/day before egg production (December 28 to March 3) and raised to approximately 12.8°C and 19 hours of light/day to induce egg production. Temperatures ranged from 8.3°C to 32.3°C for the rest

of the study (March 4 to June 13). The higher room temperatures generally occurred toward the end of the test.

Body weight were taken at weeks 0, 2, 4, 6, 8, and at termination of treatment. During egg laying no weights were taken because of the adverse effects that handling may have had on egg production.

Mortality was recorded along with gross pathology of the animals. Morbidity and clinical signs were observed throughout the study. Any animals that died were necropsied, a gross examination performed, and the following organs weighed: liver, spleen, kidneys, pancreas, proventriculus, gizzard, gonad(s), heart, and brain.

Egg Collection, Storage, and Incubation

Percent egg production was based on hen-day production, where each day's collection is divided by the number of hens alive and multiplied by 100 to get a percentage. Eggs were collected and marked daily from each pen and stored at 12.8 to 15.6°C. Eggs were set once a week in a Jamesway, single stage, 252 incubator.³ The eggs were incubated for 23 days at an average temperature of 37.5°C, and at an average relative humidity of 56 percent, with a range from 52 to 65 percent. After the first 23 days of incubation, the eggs were transferred to a hatching unit at an average temperature of 37.2°C, with a range from 36.8°C to 38.1°C and a relative humidity of 65 to 70 percent. All eggs were candled on day 0 for shell cracks and on day 14 of incubation to measure fertility and early deaths of embryos. All eggs that did not hatch were checked for abnormalities and placed in one of the following categories: dead in shell, live in shell, pipped live, or pipped dead.

At hatching all ducklings were wing banded and housed in a Petersime battery brooder and observed for two weeks while on duck-starter feed. Mortality of all ducklings was recorded for the 14-day period and percent livability calculated.

At biweekly intervals all eggs from one day's collection were measured for eggshell thickness. Eggs to be measured were cracked open at the girth, contents washed out, and shell and membranes air dried for at least 48 hours before thickness was determined. Measurements were taken of the dried shell plus the shell membranes at four points around the girth using a micrometer⁴ calibrated to 0.01 mm units.

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

⁴ Federal Products Crop. (a subsidiary of Esterline Corp.), 1144 Eddy Street, Providence, RI 02901.

Histopathology

At the termination of the test all surviving animals were killed by cervical dislocation, a gross examination of the carcasses performed and the organs (liver, spleen, kidney, pancreas, proventriculus, gizzard, heart, and brain) excised and weighed. A sample of these organs plus lungs, adrenals, duodenum and sciatic nerve were then placed in ten percent neutral buffered formaldehyde (Luna, 1968) and prepared for histopathologic examination according to routine procedures, as described in Appendix C.

Hematological Preparation

Hemoglobin concentration, packed red cell volume (hematocrit value), and differential counts were determined for all birds at the termination of the experiment (see Appendix D, E, and F).

Statistical Analysis

Treatment groups were compared to their respective control by analysis of variance. Sample units were the individual pens within each experimental group except for body weights, organ weights, and hematology where sample units were the individual animals. Egg production and feed consumption were analyzed by split-plot design (Gill, 1978).

Results

The reproduction period was chosen as it offers a unique set of physiological and behavioral conditions in both parents and progeny. The endocrine changes in the parents, and embryo and prenatal developments in the young may accentuate any toxicological effects from the addition of a substance to the diet. Most notable effects are embryo mortality and teratogenicity, the induction of fetal malformations.

The purpose of the reproductive test was to establish an exposure level that may be absorbed over a long period without producing any toxicological effects characteristic for the same chemical when given in larger amounts; since a chemical may be innocuous in terms of acute mortality but still impair reproduction. Thus, if a compound significantly decreased spermatogenesis in the drake or had an adverse effect on the ovaries of the hen, then a decrease in fertility would result or possibly a decrease in numbers of eggs laid, such as reabsorption of developing follicles. Another objective was the determination of the long-term effects, if any, such as degenerative or carcinogenic changes, and/or unsuspected behavioral or physiological reaction not previously observed.

For the chronic study, including reproduction, animals were given the test substance in the feed for a period (minimum of 10 weeks) prior to onset of egg laying, and drug administration was continued throughout the reproductive cycle. Levels of chemical

employed in the chronic test were derived from the subacute test. Thus, DIMP, which did not affect body weights at levels below 10000 ppm but did decrease feed consumption and body weight gains at levels above 10000 ppm, was set at 10000 ppm and below for the chronic test.

Chemical intake is stated as ppm and not as mg/kg/day as in test 2. Expressing dose in mg/kg/day can be misleading when animals are exposed over a long time. Animals that die early, and have consumed less in terms of milligrams than surviving birds, point to the erroneous conclusion that lower dosages of a drug are more toxic than higher dosages. Furthermore, an accurate measurement of mg/kg/day is impossible during the egg laying period as birds would have to be weighed periodically. This handling might stress them sufficiently to cause cessation of egg laying or even cause mortality. Also, excretion of chemical through the urine and feces would need to be measured and chemical content determined to measure excretion of chemical per day, thus giving level of chemical in the body per day.

In the ducks treated with DIMP (Figure 7), those receiving the 3200 ppm diet had a significant increase in consumption during the reproductive period ($P = 0.161$), but feed consumption of those receiving the other two diets (1000 and 10000 ppm) was not significantly different than that of the control.

Mean body weight changes are reported in Table 9. All DIMP-treated groups lost less weight than did their control.

Body weight changes from before start of egg laying to end (or near end) of the egg production period are listed in Table 10. All treated groups gained weight with no significant difference between treated groups and the control.

For DIMP-treated ducks (Figure 8), only those receiving the 10000 ppm diet had a decrease in egg production of 14.42 percent overall (significant at $P < 0.096$). The other two groups, 1000 and 3200 ppm, were not significantly different.

Eggshell thickness for DIMP-treated Mallards is listed in Table 11. No significant difference was found between treated groups and the control. All eggs used for eggshell thickness measurements were not included in any calculated percentages other than production.

Incubation parameters for DIMP-treated ducks are listed in Table 12. The values for percent fertile eggs are based on the number of settable eggs. Percent hatchability, early dead, dead in shell, live in shell, pipped live, and pipped dead are based on the total number of fertile eggs. There were no significant differences between any treated group and the control, nor were there any trends. Livability of all ducklings for the 14-day period after hatching is listed in Table 13. There was no significant difference between any treated group of parents' ducklings and the control parents' ducklings.

Figure 7. Effect of feeding DIMP at various levels in the diet for 24 weeks on feed consumption of adult Mallards. Each point represents the mean of three cages of two males and five females each.

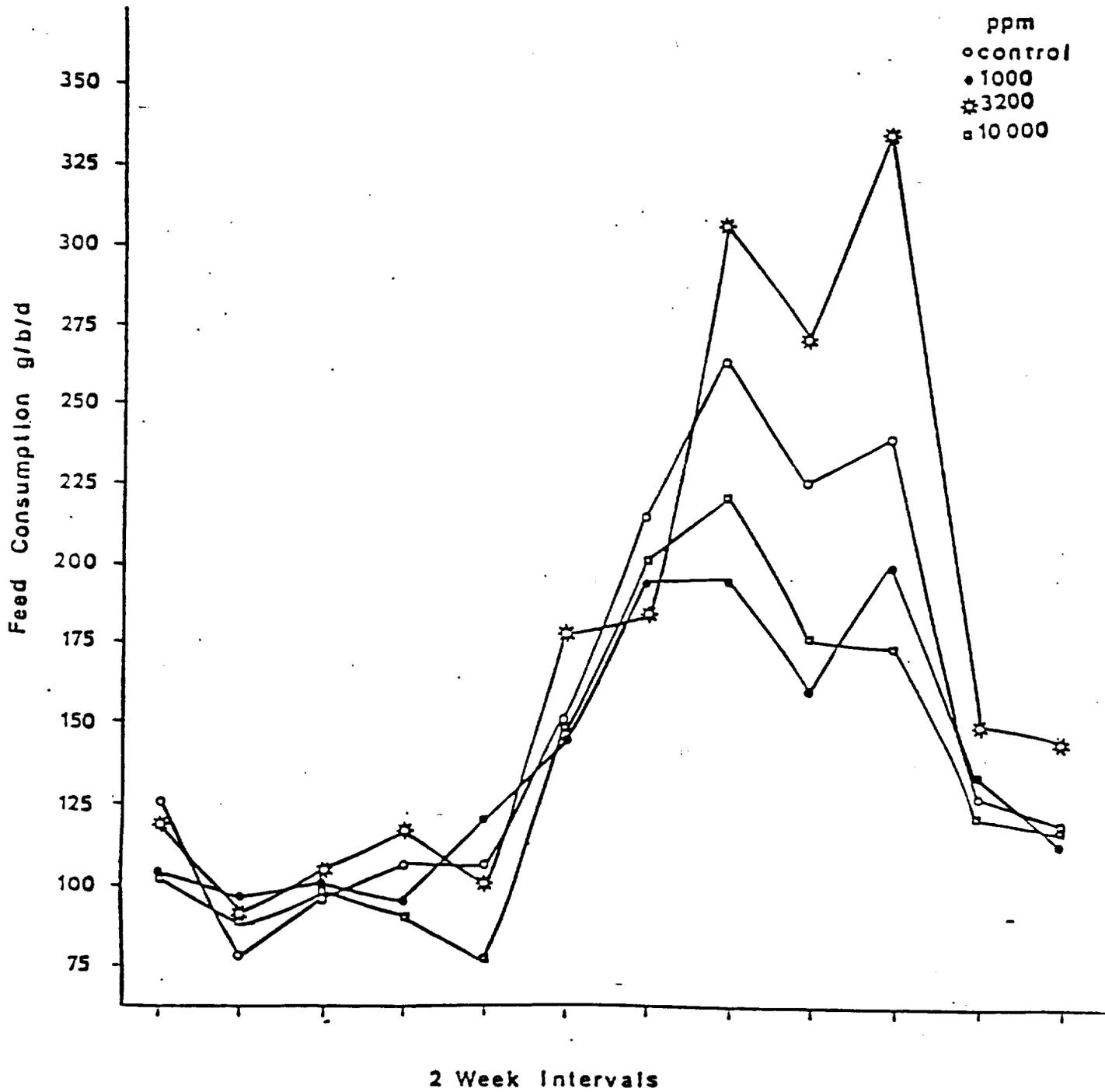


Table 9. Effect of feeding DIMP at various levels in the diet for eight weeks before commencement of egg production on body weight changes of adult Mallards.

Treatment	Level in the diet (ppm)	n	Mean body weight change					
			Weeks 1-4		Weeks 5-8		Combined	
			gms	As a % of body wt. ¹	gms	As a % of body wt. ¹	gms	As a % of body wt. ¹
DIMP	0	21	-22.90	-1.48	-4.40	-0.26	-27.30 _a ²	-1.74
DIMP	1000	21	-18.21	-1.48	-0.90	0.05	-19.11 _a	-1.43
DIMP	3200	21	- 6.12	-0.32	3.50	0.23	- 2.62 _a	-1.09
DIMP	10000	21	0.98	0.15	0.93	0.23	1.91 _a	0.38

¹Average percentage change by individual.

²Numbers with the same subscript are not significantly different from their respective control (P > 0.05).

Table 10. Effect of feeding DIMP at various levels in the diet before egg production starts and after egg production commences on body weight change of adult Mallards during their first reproductive cycle

Treatment	Level in the diet (ppm)	Mean body weight (gms)		Change	
		Before production	End of production	%	BW/gms
DIMP	0	1215.4	1300.0	6.96	84.6 ¹ _a
DIMP	1000	1200.0	1255.6	4.63	55.6 _a
DIMP	3200	1179.9	1241.1	5.19	61.2 _a
DIMP	10000	1208.2	1275.1	5.54	66.9 _a

¹Numbers with the same subscript are not significantly lower than their respective control group (P >0.05).

Figure 8. Effect of feeding DIMP at various levels in the diet for 24 weeks on egg production of adult Mallard hens in their first reproductive cycle. Each point represents the mean of three cages of five females each. Percents calculated from hen-day production.

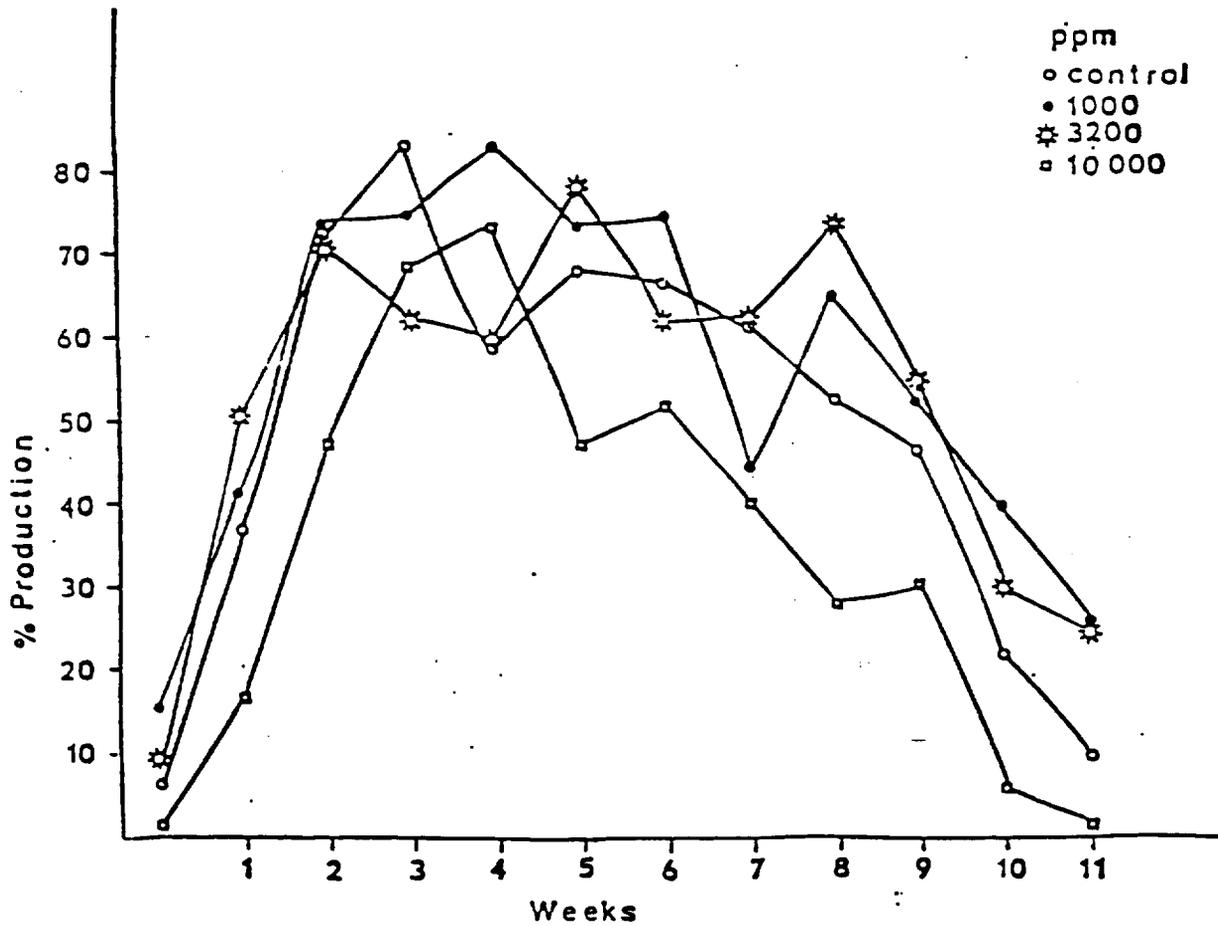


Table 11. Effect of feeding DIMP at various levels in the feed for 24 weeks on eggshell thickness values of adult Mallard eggs from females during their first reproductive cycle

Treatment	Level in the diet (ppm)	Cage	N	Mean thickness ¹ (mm x 10 ⁻²)	Combined	
					N	Mean
DIMP	0	5	18	40.3 + .513	53	40.30 + .381 _a ²
	0	22	13	41.9 + .822		
	0	23	22	39.3 + .623		
	1000	1	16	39.5 + .557	45	39.26 + .414 _a
	1000	12	11	39.1 + .959		
	1000	16	18	39.1 + .590		
	3200	8	22	38.9 + .557	69	38.84 + .334 _a
	3200	19	28	38.7 + .618		
	3200	21	19	38.9 + .565		
	10000	9	12	38.6 + .539	36	38.88 + .462 _a
	10000	13	10	38.6 + .973		
	10000	14	14	39.3 + .933		

¹Data given as group mean + standard error.

²Numbers with the same subscript are not significantly different from their respective control (P > 0.05).

Table 12. Effect of feeding DIMP at various levels in the diet for 24 weeks on incubation parameters of Mallard duck eggs laid in March, April, and May, 1977

Parameter	Level in diet (ppm)	March	April	May	Combined
Cracked	0	2.78	6.79	4.31	5.01 ¹
	1000	4.62	4.50	5.71	4.72 ^a
	3200	2.50	3.42	1.74	2.65 ^a
	10000	5.95	4.15	1.35	3.09 ^a
Fertile	0	80.71	90.00	65.77	82.49 ¹
	1000	92.12	92.04	89.39	91.47 ^b
	3200	77.56	79.88	53.85	71.77 ^b
	10000	86.08	84.23	86.30	84.77 ^b
Hatched	0	80.53	57.14	52.05	62.33 ¹
	1000	77.63	69.92	59.32	69.78 ^c
	3200	78.51	66.67	51.65	66.82 ^c
	10000	83.82	67.98	61.91	70.06 ^c
Early dead	0	2.66	6.35	8.22	5.71 ¹
	1000	1.32	9.40	7.63	6.72 ^d
	3200	2.48	4.76	7.69	4.74 ^d
	10000	4.41	14.78	1.59	10.18 ^d
Dead in shell	0	7.97	29.76	32.88	24.66 ¹
	1000	17.11	15.04	27.97	18.47 ^e
	3200	12.40	20.00	31.87	20.38 ^e
	10000	10.29	13.79	30.16	16.17 ^e
Live in shell	0	1.77	0.00	0.00	0.46 ¹
	1000	0.66	0.38	0.00	0.37 ^f
	3200	0.83	0.95	1.10	0.95 ^f
	10000	0.00	0.00	0.00	0.00 ^f
Pipped live	0	5.31	5.95	4.11	5.48 ¹
	1000	3.29	4.14	2.54	3.54 ^g
	3200	4.96	5.24	2.20	4.50 ^g
	10000	0.00	2.96	0.00	1.80 ^g
Pipped dead	0	1.77	0.79	2.74	1.37 ¹
	1000	0.00	1.13	2.54	1.12 ^h
	3200	0.83	2.38	5.50	2.61 ^h
	10000	1.47	0.49	6.35	1.80 ^h

¹Means with the same subscript are not significantly different from their respective control (P > 0.05).

Table 13. Effect of feeding DIMP at various levels in the diet over the first reproductive cycle on the mean 14-day livability of progeny over 16 hatch periods, one hatch/week.

Treatment	Level in parents' diet (ppm)	Percent of hatched ducklings alive at end of 14 days	No. died/ no. hatched
DIMP	0	99.63 ¹ _a	1/273
	1000	99.20 _a	3/374
	3200	99.65 _a	1/282
	10000	96.58 _a	8/234
Total		98.88	13/1163

¹Means with the same subscript are not significantly different from their respective control ($P > 0.05$).

Histopathologic examination of the tissues taken from the treated groups of Mallards revealed no differences from the controls.

Hemoglobin (Hb) values for DIMP-treated Mallards are listed in Table 14. There was no significant difference by sex nor by level of chemical in the diet as compared to the control. Hematocrit (Hct) values for DIMP-treated Mallards are listed in Table 15. There was no significant difference by sex, nor by level of chemical in the diet as compared to the control. Mean corpuscular hemoglobin concentration (MCHC) was determined by the formula: $MCHC = (Hb \times 100) / Hct$, where Hb equals hemoglobin gm/dl and Hct equals packed cell volume. MCHC is listed in Table 16 for DIMP-treated ducks. Ranges for DIMP-treated Mallards were 26.80 to 35.29 percent for 0 ppm, 26.67 to 32.00 percent for 1000 ppm, 27.24 to 37.50 percent for 3200 ppm, and 27.22 to 30.95 percent for 10000 ppm. There was no significant difference in MCHC between sexes, nor between treatment levels as compared to the control. Leukocyte counts of the Mallards treated with DIMP are listed in Table 17. There was no significant difference between any treated group and its control for any type of leukocyte.

Organ weights for DIMP-treated Mallards are listed in Tables 18 and 19. The liver and gonads showed differences by sex. Thus, they were divided into male, females with developing follicles, and females without developing follicles. There were very few males in the reproductive state at the time of termination and, thus, they were not divided into reproductive state groups. There was no significant difference in any organ weight on any treatment level as compared to the organ weight of the controls.

Mortality and birds removed from cages are listed in Table 20. Ducks were removed either for reasons of cannibalism from other ducks or, in the case of some females, excessive forced mating. The ducks had been harassed to such an extent that they would have died if left in the cage. Most of the deaths were from cannibalism by the more aggressive males. There was no significant difference in mortality between dietary treatment groups.

Discussion

In contrast to the subacute test, the chronic study determines whether a small amount of the compound given for a long time differs from the effects of a larger amount of the chemical given for a short time.

Food consumption followed the typical pattern during the egg production period (Figures 7 and 8), that is, feed intake increased during the reproductive period to accommodate for the increase in metabolism and decrease in intake as production terminated (Scott et al., 1969). The ducks receiving 3200 and 10000 ppm levels of DIMP consumed more feed than did the control. The 3200 ppm group was significantly greater and the 10000 ppm group was above the control's feed consumption, though not significantly. This increase

Table 14. Effect of feeding DIMP at various levels in the diet for 24 weeks on hemoglobin values of adult Mallard ducks at the end of their first reproductive cycle.

Treatment	Level (ppm) in the diet	N	Male Hb gm/dl	N	Female Hb gm/dl	N	Combined ¹ Hb gm/dl
DIMP	0	6	13.05	13	13.08	19	13.07 ± .228 ² _a
DIMP	1000	6	12.72	14	12.85	20	12.18 ± .222 _a
DIMP	3200	3	12.87	15	13.01	18	12.98 ± .234 _a
DIMP	10000	3	13.13	15	12.84	18	12.89 ± .234 _a
Total		18	12.92	57	12.94	75	12.94 ± .113

¹Data reported as treatment mean ± standard error.

²Means with the same subscript are not significantly different from their respective control (P > 0.05).

Table 15. Effect of feeding DIMP at various levels in the diet for 24 weeks on hematocrit values of adult Mallard ducks at the end of their first reproductive cycle.

Treatment	Level (ppm) in the diet	N	Male Hct %	N	Female Hct %	N	Combined ¹ Hct %
DIMP	0	6	43.96	13	45.90	19	45.30 ± .726 _a ²
DIMP	1000	6	43.50	14	44.36	20	44.10 ± .707 _a
DIMP	3200	3	44.83	15	44.08	18	44.21 ± .746 _a
DIMP	10000	3	43.50	15	44.00	18	43.92 ± .746 _a
Total		18	43.87	57	44.54	75	44.38 ± .363

¹Data reported as treatment mean ± standard error.

²Means with the same subscript are not significantly different from their respective control (P > 0.05).

Table 16. Effect of feeding DIMP at various levels in the diet for 24 weeks, on mean corpuscular hemoglobin concentration of adult Mallard ducks (calculated from the data in Table 14 and 15).

Treatment	Level (ppm) in the diet	N	Male MCHC %	N	Female MCHC %	N	Combined ¹ MCHC %
DIMP	0	6	29.74	13	28.52	19	28.91 ± .417 ² _a
DIMP	1000	6	29.24	14	28.97	20	29.05 ± .406 _a
DIMP	3200	3	28.68	15	29.70	18	29.53 ± .428 _a
DIMP	10000	3	30.17	15	29.18	18	29.35 ± .428 _a
Total		18	29.47	57	29.12	75	29.20 ± .206

¹Data reported as treatment mean ± standard error.

²Means with the same subscript are not significantly different from their respective control (P > 0.05).

Table 17. Effect of feeding DIMP in the diet at various levels for 24 weeks on leukocyte counts of adult Mallard ducks at the end of their first reproductive cycle.

Cell	Level DIMP in diet (ppm)	N	Mean ¹	Range
Basophil	0	19	2.05 + .309 ²	0-6
	1000	20	1.50 + .301 ^a	0-4
	3200	18	1.50 + .317 ^a	0-4
	10000	18	1.00 + .317 ^a	0-3
Total		75	1.52 + .155	0-6
Eosinophil	0	19	1.58 + .443 ²	0-6
	1000	20	2.65 + .432 ^b	0-7
	3200	18	1.72 + .455 ^b	0-9
	10000	18	2.33 + .455 ^b	0-8
Total		75	2.08 + .223	0-9
Heterophil	0	19	19.84 + 2.63 ²	6-52
	1000	20	22.85 + 2.56 ^c	10-55
	3200	18	24.39 + 2.70 ^c	3-50
	10000	18	17.06 + 2.70 ^c	7-46
Total		75	21.07 + 1.32	3-55
Lymphocyte	0	19	73.00 + 2.71 ²	40-89
	1000 ³	20	69.25 + 2.64 ^d	39-83
	3200	18	67.78 + 2.79 ^d	40-89
	10000	18	76.11 + 2.79 ^d	46-87
Total		75	71.49 + 1.37	39-89
Monocyte	0	19	3.53 + .491 ²	1-7
	1000	20	3.57 + .479 ^e	0-7
	3200	18	4.61 + .540 ^e	0-10
	10000	18	3.50 + .504 ^e	0-9
Total		75	3.84 + .247	0-10

¹Data given as group mean + standard error.

²Means with the same subscript are not significantly different from their respective control (P > 0.05).

³Some lymphocytes showing magenta granules in 5 of the 20 ducks.

Table 18. Effect of feeding DIMP at various levels in the diet for 24 weeks on liver and gonad(s) weights in adult Mallard ducks at the end of their first reproductive cycle

Organ	Level of DIMP in diet (ppm)	Mean organ weight (gms)						Organ weight as percent of					
		N		F ¹		F ²		Body weight			Brain weight		
		N	M	N	F ¹	N	F ²	M	F ¹	F ²	M	F ¹	F ²
Liver	0	6	33.0	13	26.9 ³	-	--	2.26	2.19	--	581.3	560.0	--
	1000	6	23.8 ^a	13	27.5 ^b	1	48.4 ³	1.77	2.25	3.45	440.4	560.0	1001.0
	3200	3	29.7 ^a	12	29.7 ^b	3	53.8 ^c	2.11	2.49	4.11	561.8	609.2	1147.7
	10000	3	35.3 ^a	15	32.5 ^b	-	-- ^c	2.26	2.68	--	658.8	654.5	--
Combined		18	29.7	53	29.3	4	52.4	2.07	2.41	3.95	544.0	598.5	1111.0
Gonad (s)	0	6 ⁴	2.89 ^d	13	0.71 ^e	-	--	0.21	0.057	--	53.3	14.4	--
	1000	6 ⁴	19.46 ^d	13	0.77 ^e	1	54.0 ^f	1.46	0.063	3.85	366.0	15.7	1117.2
	3200	3	2.20 ^d	12	0.66 ^e	3	53.0 ^f	0.17	0.054	4.05	52.6	13.5	1129.8
	10000	3	3.00 ^d	15	0.62 ^e	-	-- ^f	0.20	0.051	--	57.1	12.6	--
Combined		18	8.32	53	0.69	4	53.3	0.62	0.056	4.00	156.4	14.0	1126.7

¹Females without developing follicles.

²Females with developing follicles.

³Means with the same subscript are not significantly different from their respective control (P > 0.05).

⁴Five of the six males were still in a reproductive state; no other males were.

Table 19. Effect of feeding DIMP at various levels in the diet for 24 weeks on organ weights in adult Mallard ducks at the end of their first reproductive cycle

Organ	Level in diet (ppm)	N	Mean organ weight (gms)	Organ weight as percent of:	
				Body weight	Brain weight
Spleen	0	19	0.683 ^l	0.053	13.43
	1000	20	0.688 ^a	0.055	13.62
	3200	18	0.567 ^a	0.046	11.51
	10000	18	0.619 ^a	0.049	12.25
Kidney	0	19	8.76b ^l	0.677	173.08
	1000	20	8.67 ^b	0.689	172.37
	3200	18	8.57 ^b	0.694	175.46
	10000	18	8.45 ^b	0.668	168.32
Pancreas	0	19	3.99 ^l	0.307	78.67
	1000	20	3.86 ^c	0.306	76.37
	3200	18	3.97 ^c	0.319	80.71
	10000	18	3.90 ^c	0.307	77.71
Proven-triculus	0	19	3.72 ^d	0.287	73.45
	1000	20	3.72 ^d	0.293	73.41
	3200	18	4.22 ^d	0.339	85.94
	10000	18	4.04 ^d	0.320	80.31
Gizzard	0	19	36.47 ^l	2.80	718.21
	1000	20	34.06 ^e	2.70	671.73
	3200	18	33.77 ^e	2.73	684.78
	10000	18	36.43 ^e	2.85	721.50
Heart	0	19	8.57 ^f	0.678	173.32
	1000	20	8.88 ^f	0.706	175.75
	3200	18	8.06 ^f	0.653	164.26
	10000	18	8.34 ^f	0.656	165.74
Brain	0	19	5.069 ^l	--	--
	1000	20	5.054 ^g	--	--
	3200	18	4.925 ^g	--	--
	10000	18	5.040 ^g	--	--

^l Means with the same subscript are not significantly different from their respective control (P > 0.05).

Table 20. Dates of mortality and removals¹ of adult Mallards during the DIMP chronic test, 12/27/76 to 6/14/77

Compound	Level	Sex	Date of:		Cage
			Mortality	Removal	
DIMP	0	F	4/25		22
	0	F	5/1		22
	1000	F	4/6		16
	3200	M		3/15	19
	3200	M	3/24		21
	3200	M	4/7		8
	10000	M		1/30	13
	10000	M	3/15		9
	10000	M		3/15	14

¹Birds were removed from a group because of either cannibalism from other birds or, in the case of some females, excessive rape (Lebret, 1961; McKinney, 1975; Barash, 1977) by males.

in consumption shows a trend to eat more of a feed that contains less nutrients and less energy. High levels of any non-nutrient ingredient added to a diet would give less energy per gram of feed. Since birds normally eat to satisfy their energy requirement, they would tend to consume more feed to meet their requirement (Scott et al., 1976).

The pre-egg production feed intake (77.9 to 126 g/b/d) for ducks that weighed about 1200 grams was similar to that reported by Gasaway and Buss (1972) of 36.0 to 73.7 g/b/d for Mallards weighing about 900 grams. Irby et al. (1967) reported feed consumption of 45 to 68 g/b/d for Mallards weighing about 900 to 1100 grams.

Changes in body weight for DIMP-treated ducks ranged from -2.16 to +0.38 percent of their weight, at the beginning of the experiment. This change was less than that reported by Gasaway and Buss (1972) for control Mallards of 96 to 104 percent of the animals' weight at the start of their study. These larger changes may have been because of the lighter weight (900 grams) or the fact they only had three birds of each sex. Grandy et al. (1968), using 18-month-old Mallard drakes as controls, reported body weight changes of 8 percent over a 30-day period. Irby et al. (1967) recorded changes in the controls of 14 percent in a 60-day period with 24 ducks of 18 months of age. Changes in body weight while going through a reproductive phase was consistent with normal cycles for birds in that they gained weight for the reproductive period and lost weight at the end, or near the end of their reproductive cycle (Scott et al., 1976).

Total number of eggs laid for all hens on all treatments of DIMP was 2349 in 77 days with an average of 41.2 eggs per hen per season. Normal values range from 28 to 38 eggs per hen per season (Heath et al., 1969; Davison and Sell, 1974; "Federal Register," 1975). Only DIMP at 10000 ppm decreased eggs laid to 29.2 eggs per hen per season which was a 34 percent decrease from all other groups. A decrease in egg laying may be from the fact that any non-nutrient additive at 10000 ppm would give a decrease in the number of eggs laid as there are less nutrients and energy available in the diet. The 10000 ppm group did not increase their feed intake enough to offset the decrease in eggs laid as the 3200 ppm group appeared to have done. Other mechanisms that would have decreased the number of eggs laid might have been an increase in oviposition time or if the chemical had interfered with calcium metabolism. The overall increase in egg numbers as compared to previous reports, may be due to the fact that all eggs were collected, as the ducks were in cages rather than uncaged and/or the strain of duck used was partially domesticated. Egg production curves followed the normal shape; a sharp rise after initiation of egg production followed by a maintained level of 55 to 75 percent for a few weeks, thereafter declining though not as rapidly as the increase in the beginning (Hafez, 1974).

Eggshell thickness conformed to reports by Heath et al. (1969), Longcore et al. (1971), Heath and Spann (1973), Heinz (1974), Davison and Sell (1974), though their means were slightly lower,

ranging from 35 to 39 mm x 10⁻². This difference may have been due to a difference in procedure or strain of Mallard used. Exterior shell quality was not affected as no significant numbers of abnormally shaped eggs nor increased numbers of soft shell eggs were noted.

Normal comfort movements noted were the body-shake (körperschütteln), wing-shake (Flugelschütteln), head-shake (köpfschütteln), and wing-flap (Sich-Flugeln) and were in agreement with observations by McKinney (1965; 1975). The body-shake starts with a tail-wag followed by the erection of many body feathers. The shake moves forward on the body to the wings and then head. The wing-shake proceeds as above except there is no head movement and the tail-wag may not occur. The head-shake consists of shaking the bill laterally from side to side. The wing-flap occurs when the bird rises up to its toes slightly and fully opens the wings then flaps them a few times, as in flight.

Sexual behavior also appeared normal, as it was consistent with the findings of Lebret (1961) and Deforges and Wood-Gush (1975a; 1975b; 1976). Pumping of the head in a prelude to mating, social display ("Gesellschaftsspiel") with the head drawn firmly between the shoulders and head feathers erected were noted. Rape (Lebret, 1961; McKinney, 1975; Barach, 1977) was observed by repulsive actions from the harassed female, and is a normal occurrence during the reproduction period in Mallards.

Incubation parameters for the eggs laid by Mallards treated with DIMP are comparable to values given by Prince *et al.* (1968; 1969b; 1970), Heath *et al.* (1969), Heath and Spann (1973), Davison and Sell (1974):

Parameter	Ranges	
	Reported	DIMP
	%	%
Cracked	2.6-3.0 5.0 7.0-11.9	3.2-6.2
Fertile	50-100 75-89 81-89	65-89
Hatched	52-74 61-73 63-68	55-73

Greatest mortality during incubation occurred from approximately the 19th day until hatching as was noted by percent dead in shell (Table 12). This high mortality is consistent with the 38 to 66 percent of total mortality for the same period reported by Prince *et al.* (1969a).

Livability of the hatched ducklings raised for two weeks ranged from 96.6 to 99.6 percent (Table 13) and was within the range of normal values of 94 to 99 percent stated in the "Federal Register" (1975).

Hemoglobin gives an indication of the blood's oxygen carrying capacity since one gram of hemoglobin can combine with 1.34 ml of O₂ (Sturkie, 1976). Mean hemoglobin values of drakes treated with DIMP ranged from 12.7 to 13.1 gm/dl. Mean hemoglobin values for hens treated with DIMP ranged from 12.8 to 13.1 gm/dl (Table 14). These values are consistent with other reported values:

Species	Sex	Reported Value (gm/dl)	Reference
Mallard adult	-	9-21	Altman and Dittmer, 1964
3 mo.-1 yr.	-	7.5-16.5	Hemm and Carlton, 1967
7-15 weeks	-	18.8	Gasaway and Buss, 1972
Wild duck	-	14.0	Hemm and Carlton, 1967
Domestic duck	M	13.8	Hemm and Carlton, 1967
Domestic duck	F	12.2	Hemm and Carlton, 1967
Pekin	M	14.2	Sturkie, 1976
Pekin.	F	12.7	Sturkie, 1976
Indian	M	13.3	Sturkie, 1976
Indian	F	12.7	Sturkie, 1976
Diving duck	M	15.2	Sturkie, 1976
Diving duck	F	13.3	Sturkie, 1976

The reported values for the adult Mallard, 3 mo.-1 yr.-old Mallard, domestic female duck, and female Pekin and Indian ducks were in the same range as the DIMP-treatment group of ducks of 9.7 to 15.0 gm/dl. Hemoglobin values of diving ducks are higher as compared to dabbling ducks, since diving ducks need additional oxygen carrying capacity during dives.

Hematocrit values give an indication of red blood cell numbers, but the size of the RBC's also influence the packed cell volume. Thus, an increase in RBC numbers with a decrease in size of the cells may make no significant change in the hematocrit value. It was observed that ducks have two sizes of red blood cells which could also give varying results. Mean hematocrit values for the drakes treated with DIMP ranged from 43.5 to 44.83 percent. For the hens treated with DIMP, values ranged from 44.0 to 45.9 percent. These values are comparable to reported values:

Species	Sex	Reported Value (%)	Reference
Mallard	M	47-50	Gasaway and Buss, 1972
Mallard	F	45-50	Gasaway and Buss, 1972
Mallard	-	43.0	Hemm and Carlton, 1967
Pekin	-	41-49	Hemm and Carlton, 1967
Indian	M	40.7	Sturkie, 1976
Indian	F	38.1	Sturkie, 1976
Pekin	M	46.7	Sturkie, 1976
Pekin	F	44.2	Sturkie, 1976
Mallard	-	43.0	Sturkie, 1976

The hematocrit means of DIMP-treated Mallards are comparable to the Mallard values reported by Sturkie (1976) and Hemm and Carlton (1967), while the hematocrit range of ducks treated with DIMP of 32.0 to 51.0 percent was within the range of all reported values.

Though the mean corpuscular hemoglobin concentration (MCHC) is important in the diagnosis of anemic conditions, values for the Mallard have not been reported in the literature. MCHC reflects the overall morphology of the red blood cells (normocytic, macrocytic, or microcytic) being produced by the bone marrow in the animal. This size determination reflects the condition of the bone marrow, metabolic capacity of the red blood cell, and hemoglobin content (Coles, 1974; Sturkie, 1976). One value of MCHC for Mallards of 33.6 percent was reported by Hemm and Carlton (1967), though numbers of animals used were not mentioned. This reported MCHC value is higher than the means for Mallards treated with DIMP of 29.2 percent, but is within the range of 26.8 to 37.5 percent. There could be a problem with the interpretation of mean corpuscular values in ducks, because they have two types of red blood cells. One cell type is elongated and narrow with denser chromatin in the nucleus (leptochromatic type) while the other cell type is shorter and rounder with less dense chromatin in the nucleus (pachychromatic type) (Lucas and Jamroz, 1961).

Leukocyte numbers can change with certain chemicals given to an animal. Though a slight change may be a result of a compound, it may be the influence of stress, starvation, or other factors. Comparative differential counts in the literature vary greatly depending on numbers counted, age, physical condition, wild or domestic, and species of duck. Values reported are:

Species	Cell				
	B	E	H	L	M
Duck ¹	1.5	2.1	24.3	61.7	10.8
Duck ² 1 ½-4 yr.	2.1	2.6	44.1	47.4	1.3
Duck ² 3-12 mo.	1.0	1.6	46.1	45.8	4.4
Duck ²	2.4	7.1	44.4	40.4	5.3
Pekin male ²	3.1	9.9	52.0	31.0	3.7
Pekin female ²	3.3	10.2	32.0	47.0	6.9
DIMP (treated Mallards)	1.5	2.1	21.1	71.5	3.8
DCPD (treated Mallards)	1.7	2.4	23.6	68.0	4.3

B = Basophil; E = eosinophil; H = heterophil; L = lymphocyte;
M = monocyte

¹ Sturkie, 1976

² Hemm and Carlton, 1967

The duck values cited in Sturkie (1976) had the closest leukocyte count in comparison to the Mallards treated with DIMP while the other authors cited indicated a higher heterophil count. There were more lymphocytes than heterophils in the DIMP-treated ducks, which is generally true for most avian species (Sturkie, 1976). DIMP-treated ducks' differential counts showed extreme ranges which was consistent with all investigators:

Species	Cell				
	B	E	H	L	M
Duck ¹	0-4	0-9	8-40.5	45.5-83	4-20
3-12 mo. ¹	0-4.5	0-5	19.5-82	13-73.5	.5-11.5
1 ½-4 yr. ¹	0-6	0-8.5	17.5-76.5	18.5-70	0-5
Duck ¹	0-5	0-18.5	12.5-82	11-75	0.5-13.5
Wild duck ²	2-11	3-11	31-57	24-49	3-15
Combined	0-11	0-18.5	8-82	11-83	0-20
DIMP (treated ducks)	0-6	0-9	3-55	39-89	0-10
DCPD (treated ducks)	0-5	0-9	4-67	25-92	0-11

B = basophil; E = eosinophil; H = heterophil; L = lymphocyte;
M = monocyte

¹ Hemm and Carlton, 1967

² Lucas and Jamroz, 1961

Magenta bodies, which are granules that appear to be produced during a disease state, were found in lymphocytes of Mallards treated with DIMP at 1000 ppm. This may have shown an acute reaction to the low level, whereas, the ducks on the higher levels, 3200, and 10000 ppm, may have passed through the acute phase early in the test. Magenta granules have been found in lymphocytes of wild male Mallards (Lucas and Jamroz, 1961) though the birds could have had some type of infection that may have produced the granules.

There is generally some difficulty in differentiating eosinophils from heterophils in the duck (Hemm and Carlton, 1967). The features used to distinguish between them for the differential counts on DIMP-treated ducks were: (1) heterophil's nucleus stains fainter or with more variability than the eosinophil's, (2) heterophil's cytoplasm is clear while the eosinophil has a light blue cytoplasm and (3) the heterophil's granules are characteristically round. The whole area of duck hematology, especially differential counts and mean corpuscular values, needs much additional work so that correct interpretations can be made.

Individual organ weights can give an indication of pathologic changes occurring in that organ; especially hypertrophy, hyperplasia, and atrophy. All organs from the treated ducks appeared normal at the time of sacrifice, except that some of the spleens showed discoloration in a number of the controls and those on treatment. No trends in appearance or weight difference were noted for any other organ. All organs were normal in weight as is noted when compared to the controls and other reported values:

Organ weights as a percent of body weight

Organ	15-week-old Mallards ¹	Pekin ²	DIMP		DCPD	
			Control	Trts ³	Control	Trts ³
Liver	1.97	4.20	2.23	2.26	2.21	2.32
Gonads-M	0.46	--	0.21	0.61	0.24	0.61
Gonads-F	0.10	--	0.06	0.06	0.19	0.12
Pancreas	0.22	0.60	0.31	0.31	0.32	0.29
Spleen	--	0.10	0.05	0.05	0.05	0.06
Kidney	0.27	--	0.68	0.68	0.67	0.67

¹ Gasaway and Buss, 1972

² Carlton, 1966

³ Trts = Mean of treatments

The Pekin's organ weights, expressed as a percent of body weight, were consistently twice the Mallards, while the 15-week-old Mallards were similar to the DIMP-treated ducks except for the kidney. The controls were consistent with the treatment groups except for the male gonads, because there were some males still in a reproductive state in the treatment groups and not in the control group.

CONCLUSIONS

- Oral LD₅₀: DIMP is slightly toxic to Mallards considering mortality, body weight changes, and feed consumption. The LD₅₀ is 1490 mg/kg with a 95% confidence interval of 1416.1 - 1567.7 mg/kg.
- Oral LC₅₀: An LC₅₀ could not be obtained at levels as high as 16000 ppm, a level that yielded daily consumption of the chemical in excess of the LD₅₀. Mallards on DIMP showed decreasing body weight but no mortality occurred. Thus, they were not able to ingest enough of the compound to cause mortality.
- Oral Chronic: Mallards fed DIMP-treated feed were adversely affected as feed consumption decreased at 3200 ppm and egg production decreased at 10000 ppm. No effects were seen in body weight, cracked eggs, incubation parameters, normal ducklings, 14-day-old survivors, eggshell thickness, teratogenicity, behavior, gross pathology, histopathology, blood parameters or mortality of adults.

Toxicity of DIMP to Bobwhite Quail

TEST 1 - ACUTE (LD₅₀)

The research consisted of the determination of: the lethal dose for 50% of the test subjects (LD₅₀), the lethal dietary concentration for 50% of the test subjects (LC₅₀) and the chronic toxicity of DIMP to Bobwhite quail (Colinus virginianus). The tests 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.

Procedure

This test was designed to determine the single, 14-day, oral dose LD₅₀ of DIMP to Bobwhite.

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 379cm².

Body weights of all birds were recorded following 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. A series 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 a quail breeder feed (Appendix G: Composition 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 utilized twenty birds, ten of each sex, per dose level. Weights were recorded immediately preceding 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 substance. 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 at the 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 LD₅₀ 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.

Results

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 21.

Mortality for the quail treated per os with DIMP is listed in Table 22. Determination of acute oral LD₅₀ by the method of Litchfield and Wilcoxon (1949) for the compound tested was 1000 mg/kg with a 95% confidence interval of 934.2-1070.5 mg/kg.

The mortality curve of DIMP for the Bobwhite is plotted in Figure 9. Most deaths occurred within the first 24 hours after dosing with DIMP. 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.

During the 14-day post-treatment period, no further signs of intoxication nor significant weight changes of birds in treated groups from that of the control were noted (Table 23). Necropsies of all birds that died and those that were sacrificed at the end of the post-treatment period showed no gross pathological changes.

Feed consumption, for the 14-day post-treatment period, is listed in Table 24. Feed consumption in the 900 and 1200 mg/kg dosed groups appeared to have been depressed during the first week.

Table 21. Results of DIMP LD₅₀ range finding trials

Chemical	DIMP level (mg/kg body wt.)	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

Table 22. Mortality of adult Bobwhite quail during a 14-day period following a single per os dosing with DIMP.

Treatment level (mg/kg)	Mortality		Combined (%)
	No. died/No. treated male	No. died/No. treated female	
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

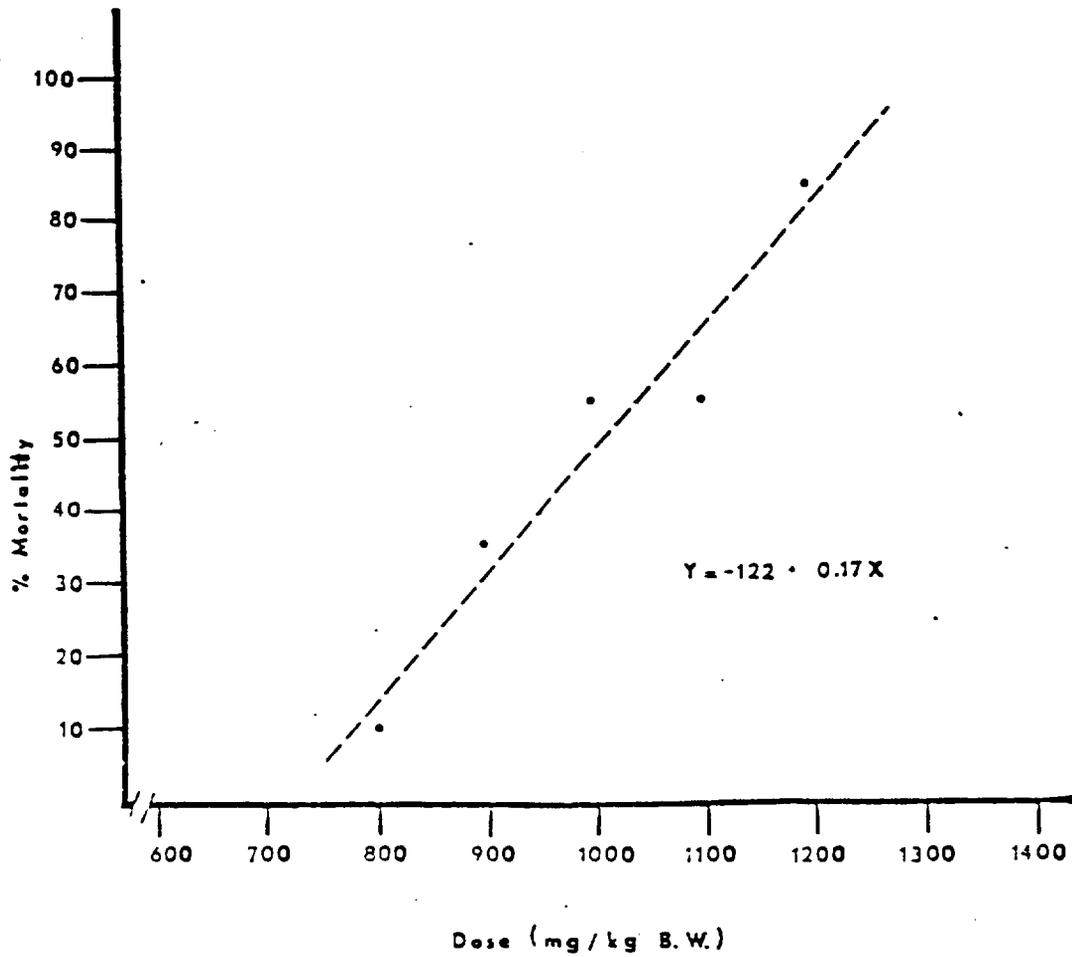


Figure 9. Percent mortality of adult Bobwhites (equal numbers of each sex) given a single per os dose of DIMP and observed for 14 days post-treatment. In the regression equation x = dose of DIMP in mg/kg of body weight and y = percent mortality.

Table 23. Quail body weight changes during post treatment for LD₅₀ (mean values).

DIMP level mg/kg	n	Mean body weight (g)		Mean change (g/b/d)
		Day 0	Day 14	
0	20	202.95	207.05	+0.293 _a ¹
800	18	199.33	195.28	-0.290 _a
900	13	190.23	195.23	+0.357 _a
1000	9	203.22	197.33	-0.421 _a
1100	9	193.67	195.22	+0.111 _a
1200	3	199.67	182.00	-1.262 _a

Table 24. Quail feed consumption (g/b/d) during post-treatment for LD₅₀.

DIMP level mg/kg	n	Days	
		0-7	8-14
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

¹ Means having the same subscript are not significantly different from their respective control (P>0.05).

At the 1000 mg/kg level, feed consumption may have been affected for the entire two-week period.

Discussion

The LD₅₀ of DIMP for Bobwhites is similar to the LD₅₀ of DIMP for rats and mice reported by Dacre and Hart (1977), but approximately 30 percent less than the LD₅₀ of DIMP for Mallard ducks.

Based on the chart on Page 29 DIMP is slightly toxic to the Bobwhite. Table 25 lists the LD₅₀'s of several compounds for the Bobwhite. The DIMP LD₅₀ for the Bobwhite is included for comparison purposes.

The slope of the dose-response curve of DIMP for Bobwhites can be considered flat. 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. Dacre and Hart (1977) found no difference in response to treatment with DIMP by sex for mice. Their results were similar to data in this report for Mallard ducks. 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. Similar inconsistent data in this report have been shown 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 the findings that no significant difference in body weight change of Mallard ducks resulted from dosing 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. In the Mallard duck, deaths occurred within 24 hours after dosing with DIMP. Dacre and

Table 25. The LD₅₀ values of twelve compounds for the Bobwhite quail at various ages.

Compound	Primary use	Sex	Age	LD ₅₀ mg/kg (95% Conf. limits)
Azodrin	I ¹	male	1-2yr.	0.944 (0.749-1.19)
Furadan	I	female	3 mo.	5.040 (3.64-6.99)
Aldrin	I	female	3-4mo.	6.59
Dieldrin	I	both	2-3mo.	12-14
Accothion	I	male	2-3mo.	27.4 (19.0-37.1)
DDT oil sol.	I	both	---	60-85
Toxaphene	I	male	3mo.	85.4 (59.2-123)
Lindane	I	male	2-3mo.	120-130
DDT crystalline	I	both	---	300
SD 15418	H ²	female	3-5mo.	400-500
DIMP	—	both	adult	1000 (934.2-1070.5)
DCPD	—	both	adult	1010 (933.2-1093.1)
Ceresan L	F ³	male	2-3mo.	1060 (841-1330)

¹ I = insecticide

² H = herbicide

³ F = fungicide

Hart (1977) reported most deaths occurred during the first 24 hours and no deaths occurred more than 48 hours after administration of DIMP to rats and mice.

TEST 2 - SUBACUTE (LC₅₀)

Procedure

This test was conducted to determine the minimum repeated oral dosage (mg/kg/day) of DIMP that was lethal to Bobwhite chicks.

Range finding pilot studies were conducted with DIMP to determine the effect on mortality, feed consumption, and body weight.

Levels of DIMP employed for the subacute test were partially determined by the LD₅₀ 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 eight-day range finding pilot test utilized six birds for each dietary treatment. Dietary treatments consisted of 4000, 8000, and 16000 ppm DIMP. Treated feed was fed for the initial five days of the test and untreated feed for the remaining three days. The three-day (untreated feed) period was included to avoid overestimation of the lethal dosage by calculating mortality before the 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 untreated feed for the birds and weighing the remainder on days five and eight of the test, respectively.

Results of the range-finding test were:

DIMP level in diet (ppm)	Mean change in body wt. (g/b/d)	Mean feed consumption (g/b/d)	Mort- ality (%)
4000	+3.150	7.055	0
8000	+0.185	5.650	33.3
16000	+0.340	4.235	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 any mortality that did occur appeared unrelated to the dietary levels of DIMP, a series 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 Unit² and maintained on a standard quail starter diet (Appendix G: Composition 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 ten dietary treatments. At the initiation of the experiment, one bird from the control group and one from 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 untreated feed was fed for the remaining three days. Feed and water were provided ad libitum throughout the test period.

The test diets were prepared by dissolving the chemical in corn oil, and hand mixing this with quail starter to make a premix. The premix was then added to a standard quail ration to yield the appropriate dietary level (Appendix H: Diet Preparation). The DIMP-treated diets' chemical-corn oil solution constant was greater than two percent of the diet. The control diet consisted of two parts corn oil to 98 parts feed by weight. The ten dietary treatments used for testing were as follows: 0, 4000, 8000, 12000, 16000, 20000, 24000, 28000, 32000, and 36000 ppm DIMP.

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 (untreated feed) to provide estimates of average feed consumption. Observations on feed 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

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

Results

Feed consumption (Figure 10) of chicks on the three lowest levels (4000, 8000, and 12000 ppm) was increase 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), as compared to that of the control.

² Petersime Incubator Company, Gettysburg, OH 45328

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 was -0.00008 , with a correlation between feed consumption and level of DIMP in the diet of -0.8514 (Figure 11). The predicted zero feed consumption calculated from this line was 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 12). 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, 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 was $-.00007$ and the correlation between the level of DIMP in the diet and body weight was -0.8540 (Figure 13). Predicted zero body weight gain was 44,547 ppm DIMP in the diet.

Mortality was limited and showed no trends (Table 26). 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 LD₅₀ value of 1000 mg/kg. Quail in all groups showing no mortality, except the 4000 ppm group, had values of DIMP ingested above the LD₅₀ value. The correlation coefficient between mortality and mg DIMP ingested was $+0.0905$.

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 consumption during the five-day treatment period (Figure 14). Increases ranged from 105 percent (3.39 g/b/d) at 36000 ppm level to 22 percent (1.33 g/b/d) at 20000 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 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 was -0.5222 (Table 27). All treated 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.

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

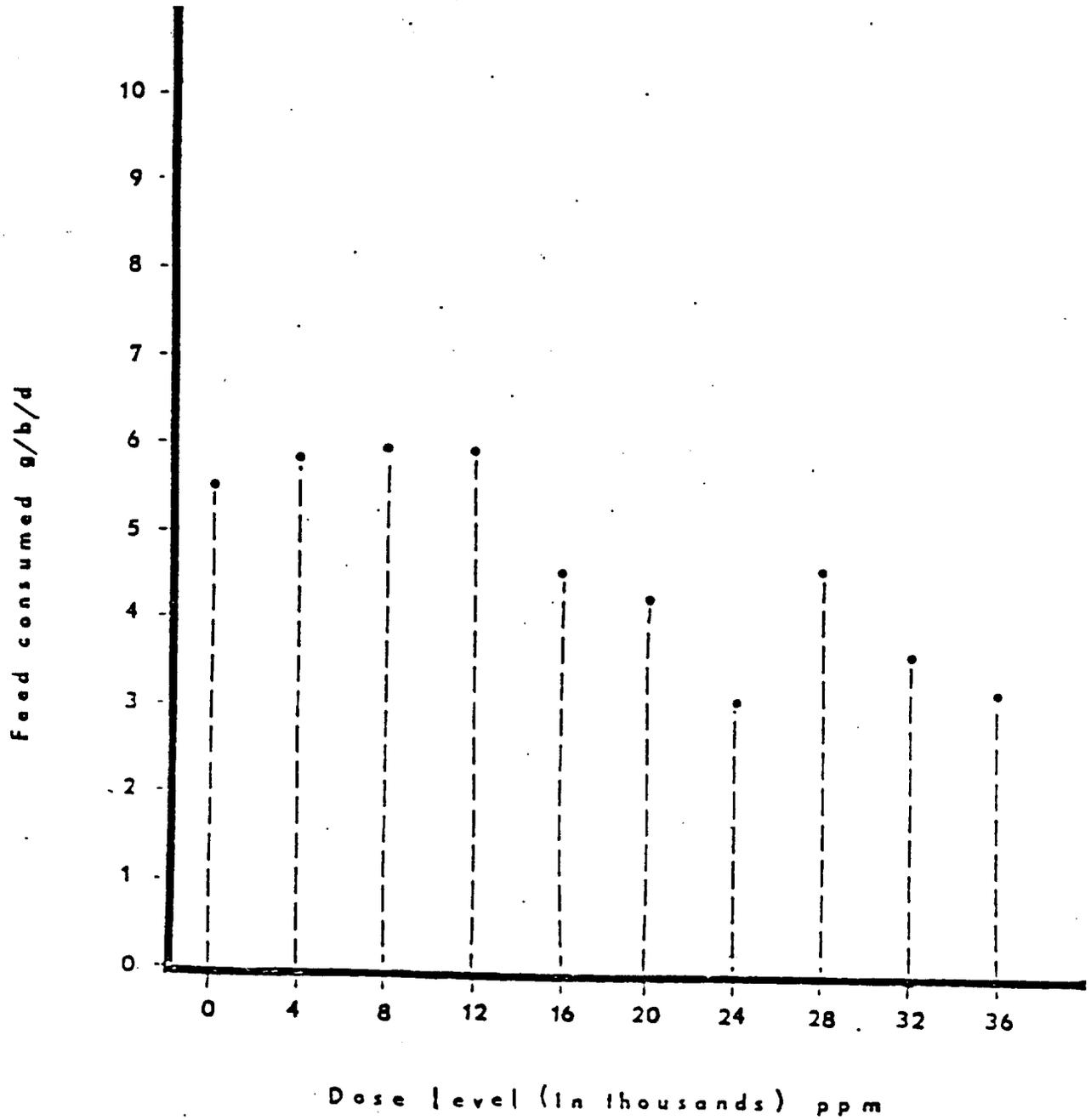


Figure 10. Effect of feeding various levels of DIMP in the diet for five days on feed consumption of 14-day-old Bobwhite chicks.

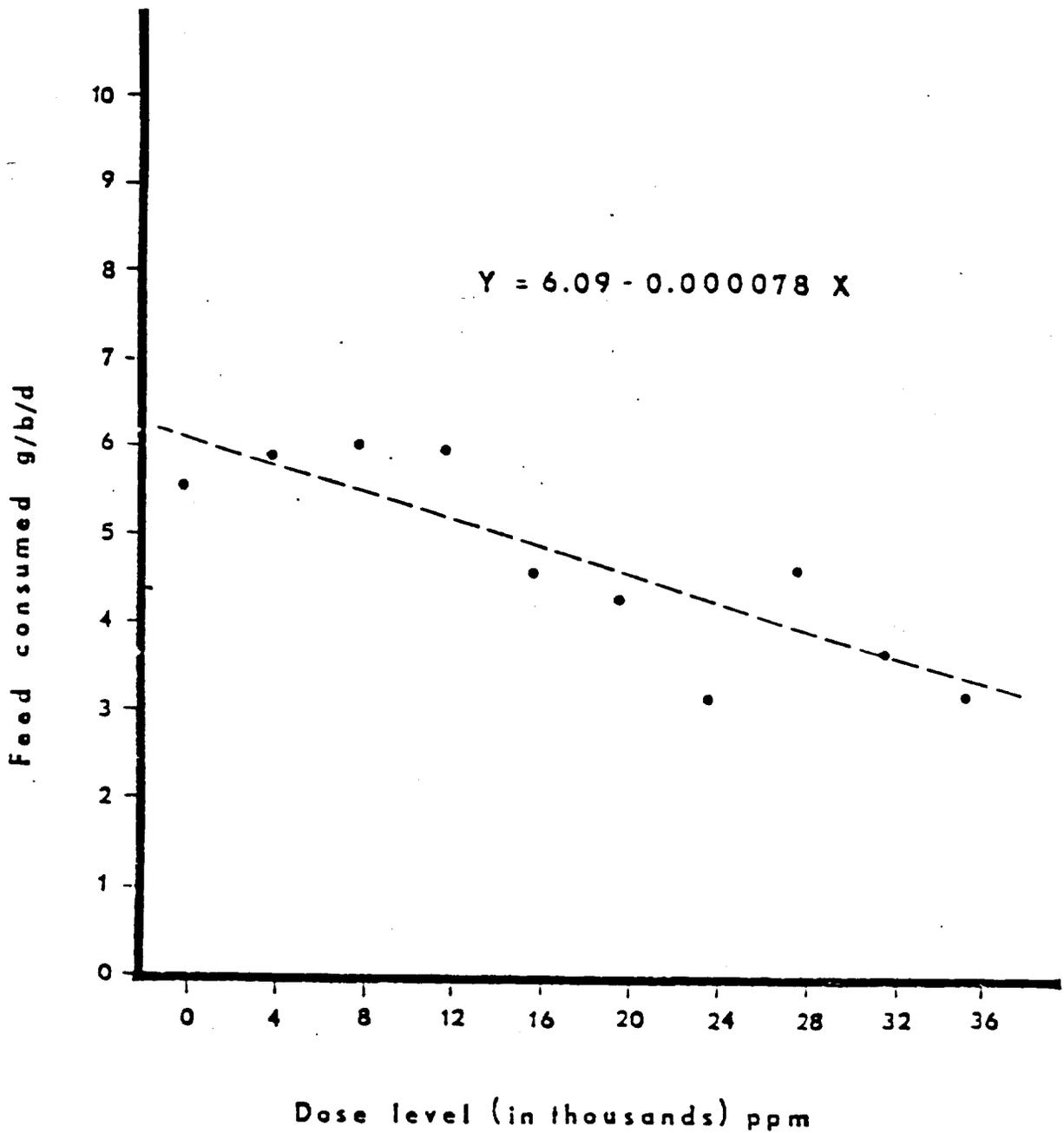


Figure 11. Regression equation of the data shown in Figure 10. In the regression equation x = ppm of DIMP and y = feed consumption in g/b/d.

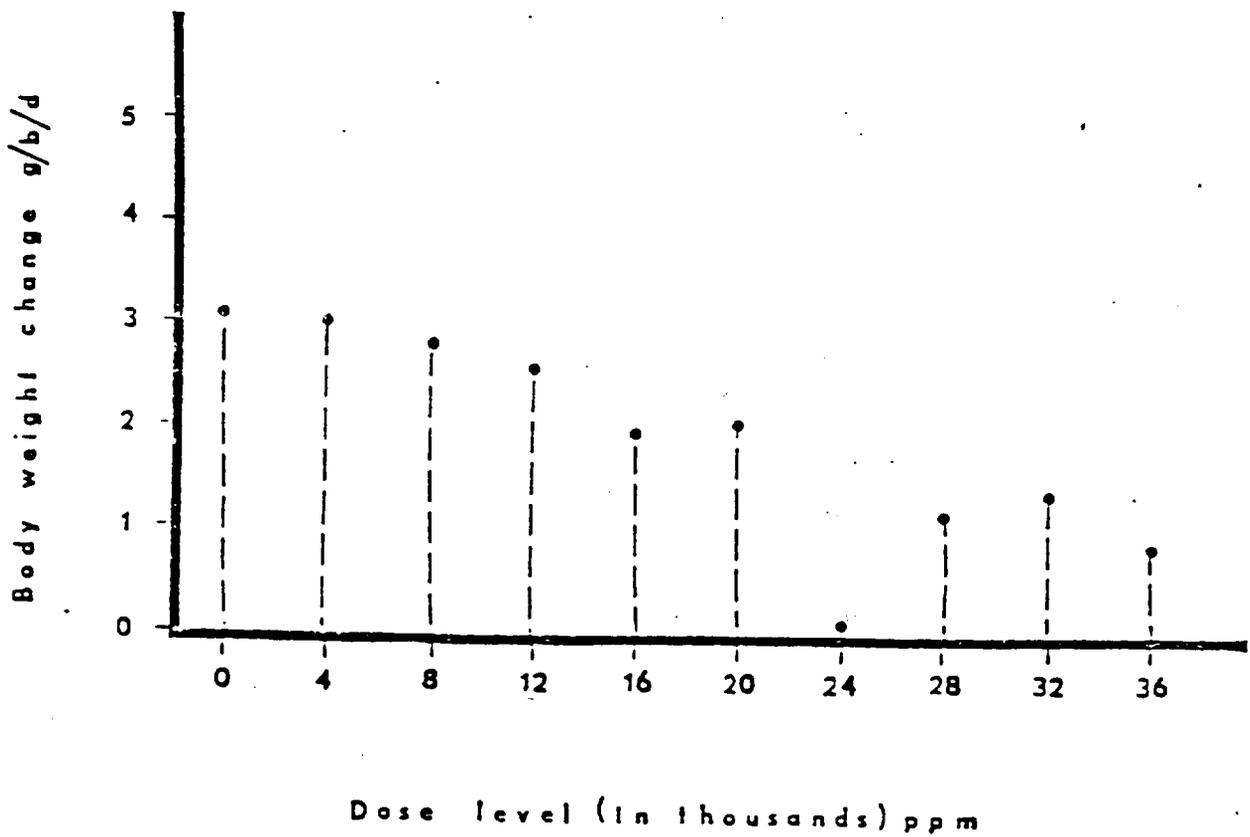


Figure 12. Effect of feeding various levels of DIMP in the diet for five days on body weight change of 14-day-old Bobwhite chicks.

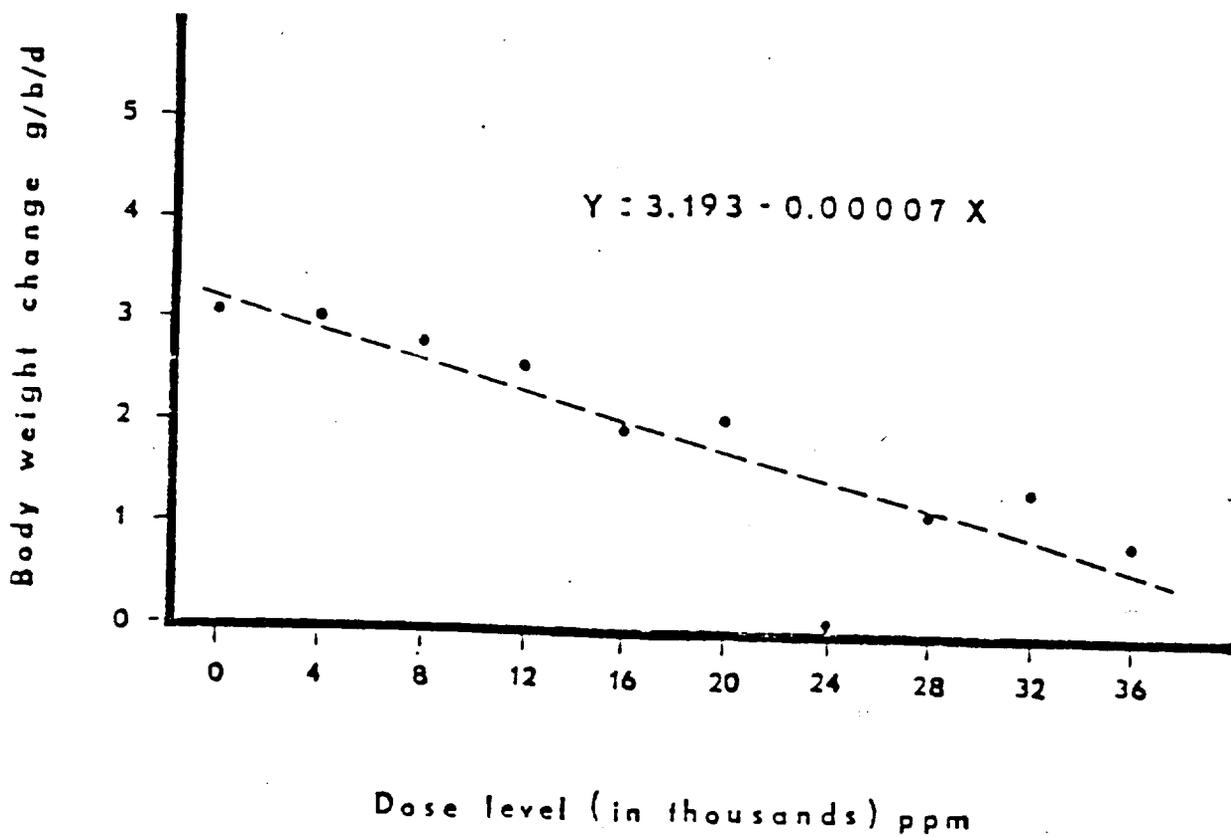


Figure 13. Regression equation of the data shown in Figure 12. In the regression equation x = ppm of DIMP and y = body weight change in g/b/d.

Table 26. Calculated DIMP intake over 5 days and mortality over 8 days for 14-day-old Bobwhite chicks on LC₅₀ trial.

DIMP level in diet (ppm)	DIMP consumed/day (mg)	Mean ¹ body wt. (g)	DIMP consumed (mg/kg/day)	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.

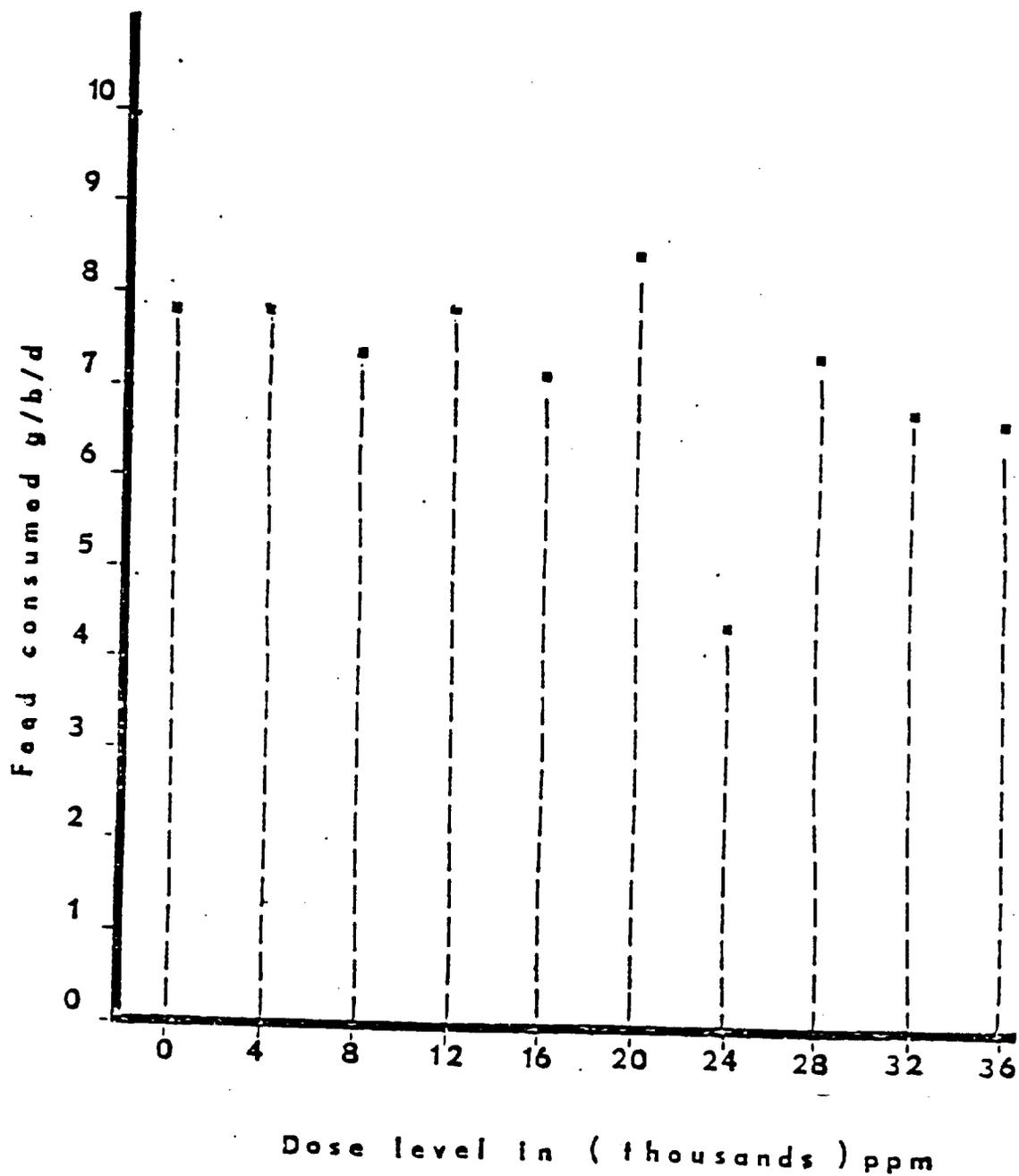


Figure 14. Feed consumption of Bobwhite chicks fed untreated feed during three-day post-treatment period after withdrawal of DIMP-treated diets.

Table 27 . Body weight change of Bobwhite chicks during 3-day period after withdrawal of DIMP-treated diets.

DIMP level in 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

Discussion

LC₅₀ values of DIMP could not be determined for the Bobwhite due to insufficient mortality, even though the average mg of compound consumed per bird per day was greater than the respective LD₅₀ value. Most of 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 was above 70000 ppm. These results are in agreement with the reported undeterminable LC₅₀ values for the Mallard duck fed DIMP-treated diets.

Values taken from LC₅₀ determinations (Heath et al., 1972) of 89 pesticidal chemicals are listed in Table 28. The LC₅₀ value of DDT in Table 28 was taken from results by Heath and Stickel (1965).

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. These observations on feed consumption were made during the first five days of the eight-day test period. This reduction of feed intake was undoubtedly due to a repellent effect of the compounds rather than a toxic effect since the birds increased their feed consumption when fed untreated feed during the three day post-treatment period. Voluntary feed restriction of treated diets is not uncommon; 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 Bobwhites.

Body weight gains were generally reduced in Bobwhites fed DIMP diets; the least weight gains occurred in birds fed the highest dietary levels. The reduced body weight gain of the birds fed DIMP diets was fairly consistent with the decrease in feed consumption.

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

All birds on dietary levels of DIMP, other than the lowest level, consumed a greater amount of chemical (mg/kg/day) than the LD₅₀ value. Fitshugh and Schouboe (1965) reported that animals tolerating an amount of chemical in their diet greater than the LD₅₀ 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. Heath et al. (1972) reported that exposure of a compound via the diet is often gradual, allowing sufficient time for the degradation of unstable compounds, but not necessarily the stable compounds.

Table 28. Median lethal concentrations (LC₅₀'s) of various pesticidal chemicals for Bobwhite quail chicks two to three weeks of age.

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 - 796
DDT	I	611	514 - 724
Diazinon	I	245	178 - 334
Dieldrin	I	39	37 - 41
Diquat	H ⁵	2932	1811 - 5256
Endrin	I	14	11 - 24
Fenitrothion	I	157	135 - 193
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

TEST 3 - CHRONIC

Procedure

This test was designed to determine the effects of continuous long term exposure of DIMP to the adult Bobwhite throughout a single reproductive cycle.

The 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 fifteen times. The birds were allowed a two week acclimatization period before the initiation of the test.

Dietary levels of the test substance were determined via the results of the LC₅₀ experiment and consultations with the Project Officer (United States Army Medical Command). Decreased feed consumption (as compared to the control) at levels about 12000 ppm DIMP coupled with an increase in body weight loss (as compared to the control) at levels about 16000 ppm DIMP and the 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. Subsequent mortality at the 3800 and 12000 ppm DIMP levels prompted a reduction in these levels to 380 and 0 ppm DIMP, respectively. This decision was reached by general consensus of the principal investigator and the project officer (U.S. Army Medical Command).

Testing

Test diets were prepared by the addition of a premix to a standard quail breeder ration to attain the appropriate dietary levels. (Appendix H: Diet Preparation). The control diet 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 test.

Feed consumption was measured biweekly for the 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) the testing rooms were maintained at approximately 18°C with six hours of light provided per day. To induce egg production, the lighting schedule was increased to 16 hours per day. This schedule was maintained throughout the production period (Jan. 8 to May 28). Temperature of the test room during the production period ranged from 15° to 28°C.

Egg production, mortality, morbidity, and any observable clinical signs of intoxication were recorded daily. All birds that died

during the study were subjected to gross necropsy. Hemoglobin concentrations, packed red cell volume (hematocrit value), and differential counts were determined for all surviving birds at the termination of the test.

Egg Collection, Storage, and Incubation

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

Eggs were set at weekly intervals in a Jamesway 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. Fertile, developing eggs were put into 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 were 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. Untreated 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 recorded in one of the following categories; pipped live, pipped dead, live in shell, or dead in shell.

Eggs from one day's production were collected at biweekly intervals to be measured for eggshell 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 micrometer⁴ calibrated to 0.01 mm units.

Histopathology

At the termination of the test all surviving animals were killed by cervical dislocation, a gross examination of the carcasses performed, and the organs (liver, kidney, pancreas, proventriculus,

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

⁴ Federal Products Corporation (a subsidiary of Esterline Corp.) 1144 Eddy Street, Providence, RI 02901.

gizzard, heart, and brain) excised and weighed. A sample of these organs plus lungs, adrenals, duodenum, and sciatic nerve were then placed in ten percent neutral buffered formaldehyde (Luna, 1968) and prepared for histopathologic examination according to routine procedures, as described in Appendix C.

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 (see Appendix D, E, and F).

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 hematological parameters where the sample units were the individual birds.

Dunnett's t-test (with modification for unequal replication where applicable) was used to compare all treatment groups to the control for each variable, except percent livability of progeny. The latter was analyzed by the split-plot design (Gill, 1978) with arcsin transformation.

Results

Feed consumption data for adult Bobwhites fed the DIMP-treated and control diets are presented in Figure 15. 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, these 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 29. 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 30. The data were analyzed separately for males

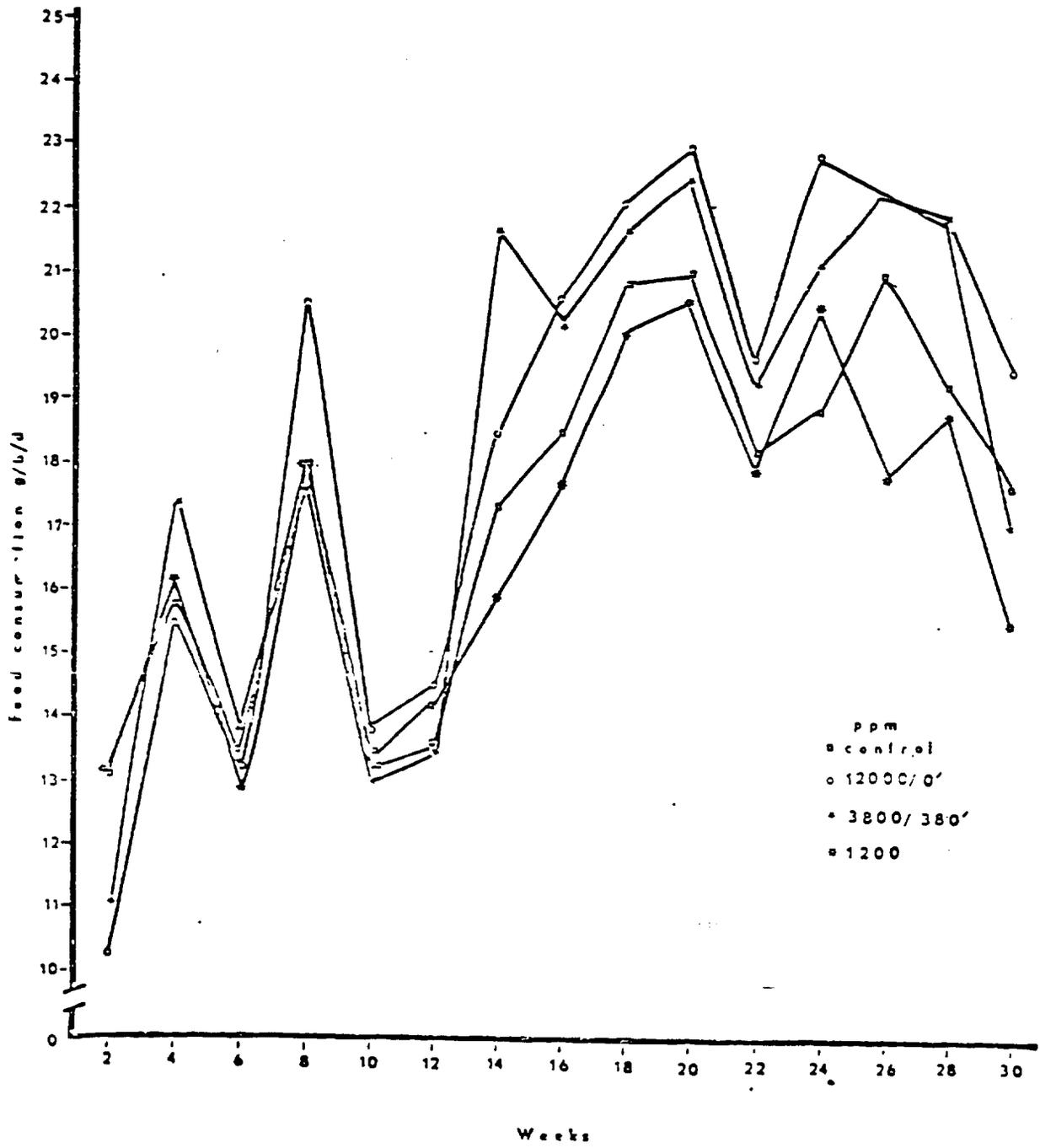


Figure 15. Effect of feeding various levels of DIMP in the diet for 29 weeks on feed consumption of adult Bobwhites. Each point represents the mean feed consumption of fifteen cages, each containing one male and one female bird.

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 31). 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-treated Bobwhites are plotted in Figure 16. Each point is the mean of approximately 15 cages of one hen per cage. 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 the control diet 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 an unmated female + 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 32 contains the incubation parameter data.

Eggshell thickness data for the Bobwhites fed DIMP are given in Table 33. 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 included only in the percent egg production calculations.

Fourteen-day survival of progeny of DIMP-treated Bobwhites is plotted in Figure 17. Each point is the 14-day 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 34 and 35. Due to weight differences attributed to sex differences, the liver and gonad(s) weights were categorized according to sex (Table 34). Liver and gonad weights of females were further separated into "producing" and "non-producing" categories. Males were not differentiated by reproductive capacities. No significant differences between organ weights could be attributed to any treatment.

Histopathologic examination of tissues taken from all birds revealed no changes attributable to DIMP treatment.

Hemoglobin values for DIMP-treated or control Bobwhites are given in Table 36. There was no significant difference found between the hemoglobin values of DIMP-fed Bobwhites and control Bobwhites of either sex.

Table 29. Effect of feeding DIMP at various levels on body weight change of Bobwhites for the 10 weeks prior to the onset of egg production.

DIMP level (ppm)	n	Biweekly body weight change (%) ¹				
		2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
0	30	- 8.09 (+3.94)	+ 7.04 (+13.63)	- 0.16 (+4.77)	+ 5.00 (+4.74)	- 2.24 (+2.92)
1200	30	- 6.17 (+3.64)	+ 7.15 (+3.76)	+ 0.52 (+3.34)	+ 5.58 (+3.41)	- 3.90 (+2.46)
3800/380 ²	26	- 8.64 (+6.69)	+10.72 (+7.16)	+ 0.77 (+3.81)	+ 4.14 (+3.91)	- 3.87 (+2.80)
12000/0 ³	26	-11.43 (+8.89)	+12.64 (+13.85)	- 2.51 (+6.92)	+11.64 (+9.08)	- 3.17 (+2.40)

¹ Data reported as mean \pm standard deviation. All values are nonsignificantly different from the control ($P > 0.05$).

² 3800 ppm reduced to 380 ppm after 26 days.

³ 12000 ppm reduced to 0 ppm after 18 days.

Table 30. Effect of feeding DIMP at various levels for 29 week on body weight change of Bobwhites during the 10 week reproductive period.

Sex	DIMP level (ppm)	n	Mean body weight (gms)		Body weight change (%) ¹
			Pre-production	Termination	
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>0.05).

Table 31. Effects of feeding DIMP on mortality of Bobwhites during the 29-week chronic study.

DIMP level (ppm)	Mortality wks. 1-4 ¹	Total mortality	Mortality (%)
0	0	4/30	13.33
1200	0	2/30	6.67
3800/380 ²	4	5/30	16.67
12000/0 ³	4	4/30	13.33

¹ Eight of the total 15 deaths occurred within the first four weeks of the test and prompted changes in DIMP levels (see text for details).

² 3800 ppm reduced to 380 ppm after 26 days.

³ 12000 ppm reduced to 0 ppm after 18 days.

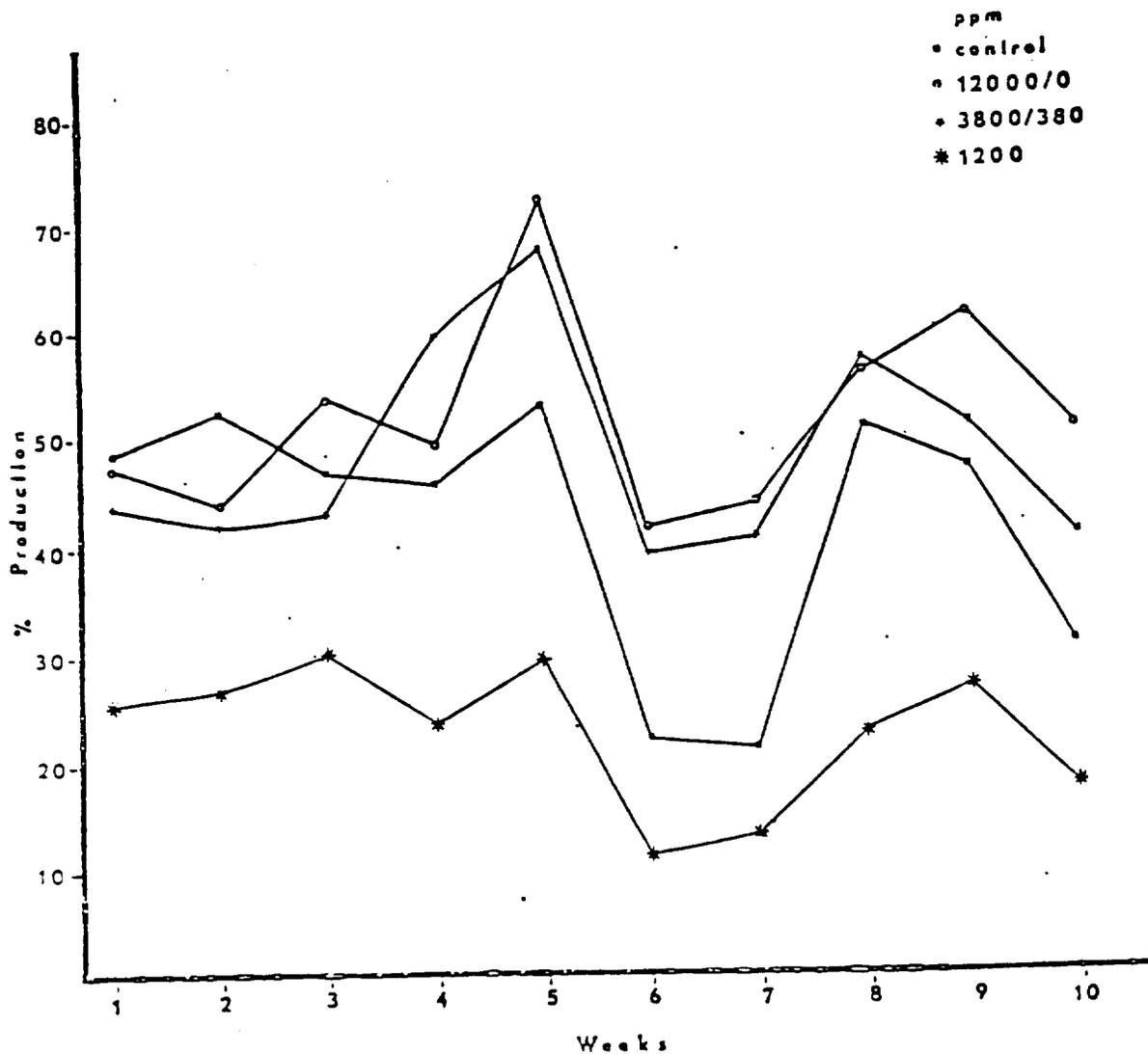


Figure 16. Effect of feeding various levels of DIMP in the diet for 29 weeks on egg production of adult Bobwhites in their first reproductive cycle. Each point represents the mean egg production of fifteen females. Percents calculated from hen-day production.

Hematocrit values for Bobwhites on the DIMP study are presented in Table 36. 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.

Listed in Table 37 are mean corpuscular hemoglobin concentration data. 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.

Leukocyte counts of Bobwhites on the DIMP study are presented in Table 38. No significant difference in leukocyte counts was found between the treated birds and their respective control.

Discussion

Feed consumption was unaffected in Bobwhites fed 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 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 accommodate for the increased energy expenditure of egg production then decreases as egg production declines.

Pre-production body weight change of Bobwhites fed DIMP-treated diets coincided with the feed consumption results and showed no treatment effects. These results are consistent with results reported for Mallards.

During the reproductive period, body weight change of Bobwhites fed DIMP-treated diets showed no treatment effects. Female birds showed a greater weight gain than male birds in all groups. This 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).

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

Table 32. 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 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 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 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 ²
	1200	9.09	13.73	3.45	8.76 + 5.15 ^d
	3800/380	5.33	5.51	3.61	5.00 + 1.22 ^d
	12000/0	2.63	5.51	8.33	5.49 + 2.35 ^d
Dead in shell	0	3.85	2.44	2.97	3.09 + 0.71 ²
	1200	3.03	7.84	13.97	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 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 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 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>0.05).

Table 33. Effect of feeding DIMP at various levels for 29 weeks on shell thickness values of adult Bobwhite eggs.

Dietary level (ppm)	n	Shell thickness ¹ (mm x 10 ⁻²)
0	38	21.17 ± 2.08 _a ²
1200	18	21.83 ± 2.19 _a
3800/380 ³	35	22.13 ± 2.11 _a
12000/0 ⁴	26	22.17 ± 1.38 _a

¹ Data reported as mean ± standard deviation.

² Numbers with the same subscript are not significantly different from their respective control (P>0.05).

³ 3800 ppm reduced to 380 ppm after 26 days.

⁴ 12000 ppm reduced to 0 ppm after 18 days.

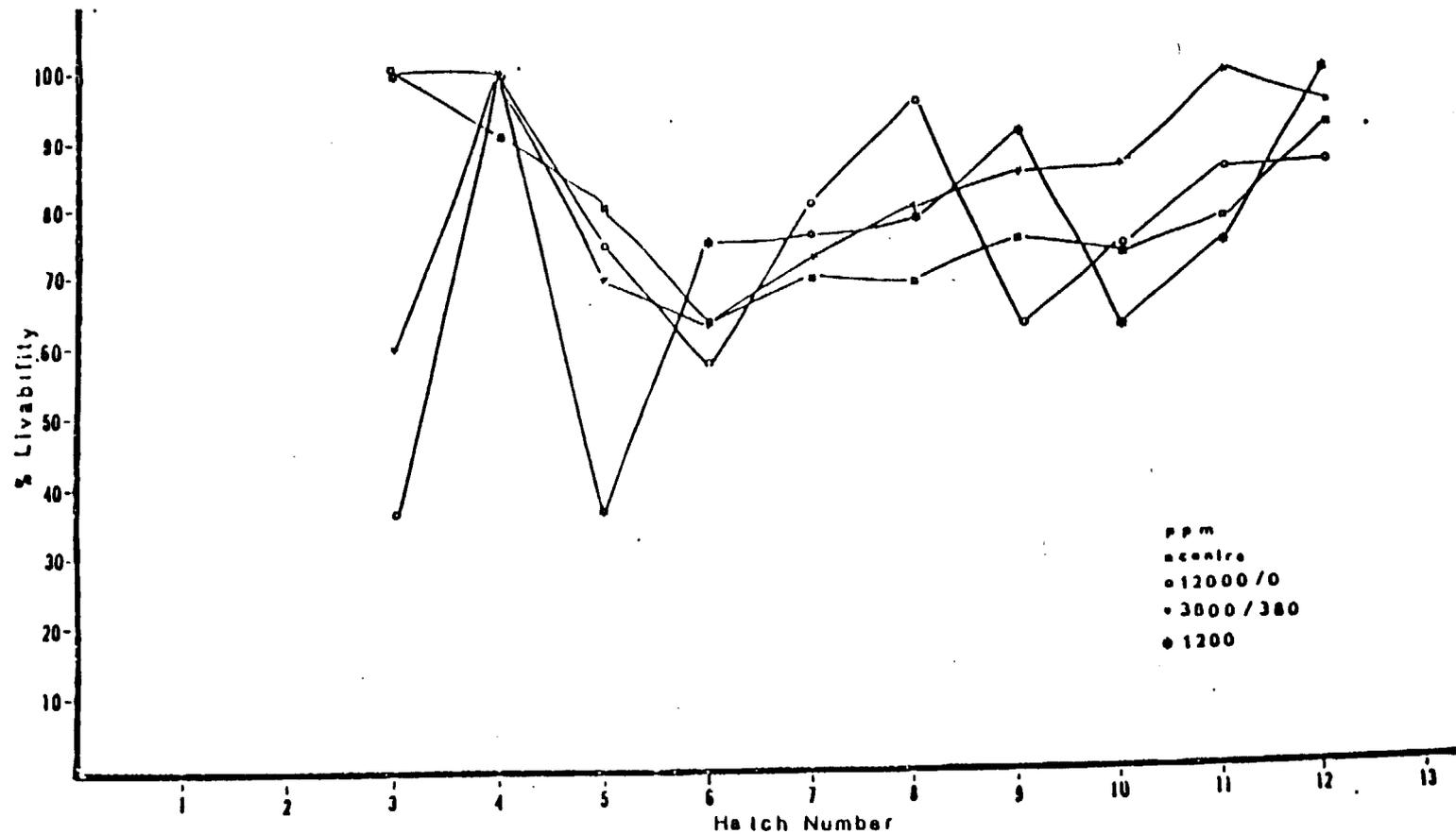


Figure 17. Percent livability of offspring of adult Bobwhites fed various levels of DIMP in their diet for 29 weeks.

Table 34. Effects of feeding DIMP at various levels in the diet for 29 weeks on gonad and liver weights of adult Bobwhites.

Sex	Organ	DIMP level (ppm)	n	Mean organ wt. (gms)	Organ wt. as % of		
					Body wt.	Brain wt. ¹	
Female ²	Ovary	Control	7	1.51	0.74	125.98 ±	11.00 ⁴
		1200	3	2.33	1.07	202.99 ±	13.43 ^a
		3800/380	7	2.93	1.33	269.68 ±	24.51 ^a
		12000/0	9	2.59	1.21	229.80 ±	14.54 ^a
	Liver	Control	7	7.44	3.58	628.96 ±	21.10 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	Control	5	0.46	0.229	41.24 ±	2.54 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	Control	5	5.11	2.58	464.24 ±	10.30 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	Control	14	1.05	0.51	87.01 ±	15.89 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	Control	14	3.81	2.58	320.01 ±	21.67 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>0.05).

Table 35. Effect of feeding DIMP at various levels for 29 weeks on organ weight of adult Bobwhites.

Organ	DIMP level (ppm)	n	Mean organ wt. (gms)	Organ wt. as % of	
				Body wt.	Brain wt. ¹
Kidneys	0	26	1.47	0.75	125.74 ± 2.26 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
	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
Proventri- culus	0	26	0.92	0.46	78.98 ± 1.45 ^c
	1200	28	1.11	0.57	98.39 ± 3.64 ^c
	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
	1200	28	4.93	2.49	448.70 ± 15.79 ^f
	3800/380	25	4.48	2.23	410.75 ± 10.49 ^f
	12000/0	26	4.48	2.20	389.15 ± 6.09 ^e
Heart	0	26	0.99	0.51	81.74 ± 2.27 ^g
	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	—	—
	1200	28	1.15 ± 0.03	—	—
	3800/380	25	1.11 ± 0.02	—	—
	12000/0	26	1.16 ± 0.01	—	—

¹ Data reported as mean ± standard deviation.

² Means having the same subscript are not significantly different from their respective controls (P>0.05).

Table 36. Effect of feeding DIMP at various levels for 29 weeks on hemoglobin and hematocrit values of adult Bobwhites.

Sex	DIMP level (ppm)	n	Mean hemoglobin (gm/dl) ¹	n	Mean hematocrit (%) ¹
Female	0	10	10.01 ± 1.37	12	37.4 ± 4.40 ₂
	1200	14	9.98 ± 1.45	14	36.4 ± 4.76 _b
	3800/380	11	10.60 ± 0.57	12	37.2 ± 2.77 _b
	12000/0	10	10.20 ± 0.71	13	37.2 ± 3.52 _b
	Overall	45	10.19 ± 1.12	51	37.00 ± 3.87
Male	0	13	11.3 ± 0.94	14	41.5 ± 3.35 ₂
	1200	14	10.9 ± 1.00	14	40.7 ± 2.79 _d
	3800/380	12	11.7 ± 1.02	13	42.3 ± 4.46 _d
	12000/0	12	11.2 ± 1.13	13	40.9 ± 3.91 _d
	Overall	51	11.27 ± 1.05	54	41.36 ± 3.61

¹ Data reported as mean ± standard deviation.

² Means having the same subscript are not significantly different from their respective controls (P > 0.05).

Table 37. Effects of feeding DIMP at various levels for 29 weeks on mean corpuscular hemoglobin concentration (MCHC)¹ of adult Bobwhites.

DIMP level (ppm)	n females	Mean MCHC (%) females	n males	Mean MCHC (%) males	n combined	Mean MCHC (%) ² combined ³
0	10	26.91	13	27.40	23	27.18 ± 1.38 _a ³
1200	14	27.36	14	26.50	28	27.39 ± 2.20 _a
3800/380	11	28.69	12	27.84	23	28.25 ± 1.83 _a
12000/0	10	27.48	12	27.24	22	27.35 ± 1.95 _a

¹ Calculated from data in Table 36.

² Data reported as mean ± standard deviation.

³ Means having the same subscript are not significantly different from their respective controls (P>0.05).

Table 38. Effect of feeding DIMP at various levels for 29 weeks on leukocyte counts of adult Bobwhites.

Cell	DIMP level (ppm)	n	Mean ¹	Range
Basophil	0	26	2.57 ± 1.28 ²	0-6
	1200	28	2.63 ± 1.78 ^a	0-8
	3800/380	25	2.76 ± 2.09 ^{aa}	1-10
	12000/0	26	3.65 ± 2.47 ^{aa}	0-9
Eosinophil	0	26	4.27 ± 2.63 ²	0-9
	1200	28	2.85 ± 2.91 ^b	0-10
	3800/380	25	3.44 ± 2.26 ^{bb}	0-11
	12000/0	26	2.73 ± 2.34 ^{bb}	0-9
Heterophil	0	26	19.23 ± 10.47 ²	4-46
	1200	28	23.04 ± 12.95 ^c	2-56
	3800/380	25	21.04 ± 14.36 ^{cc}	1-63
	12000/0	26	22.19 ± 11.26 ^{cc}	2-43
Lymphocyte	0	26	65.77 ± 13.01 ²	39-93
	1200	28	62.56 ± 14.85 ^d	40-84
	3800/380	25	63.24 ± 18.55 ^{dd}	14-89
	12000/0	26	61.88 ± 15.45 ^{dd}	32-88
Monocyte	0	26	7.69 ± 4.24 ²	1-21
	1200	28	9.00 ± 4.08 ^{ee}	1-20
	3800/380	25	9.40 ± 5.63 ^{eee}	4-24
	12000/0	26	9.50 ± 5.62 ^{eee}	2-21

¹ Data reported as mean ± standard deviation.

² Means having the same subscript are not significantly different from their respective controls (P > 0.05).

of the control birds, the 12000/0 ppm birds and the 3800/380 ppm birds was less than the standard reported by Coleman (1930) 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 the DIMP tests. The percentages of cracked eggs for each of the dietary groups and the control group were above the normal range reported in the Federal Register (1975) but no percentages differed significantly from the 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).

Normal values for egg production, fertility, hatchability, and number of cracked eggs of Mallard ducks fed DIMP-treated diets were in agreement with the results of this quail study.

Two-week livability of Bobwhite hatchlings from parents treated with 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).

Eggshell thickness data from 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).

In general, the feeding of 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. The proventriculi and gizzards of Bobwhites fed 1200 ppm DIMP weighted more than did the same organs of the control birds. Organ weights of Mallard ducks fed DIMP-treated diets were unaffected.

Two blood parameters, hemoglobin concentration and hematocrit (packed cell volume), plus the calculated mean corpuscular hemoglobin concentration, were measured in the DIMP-treated Bobwhites and found to be unaffected by treatment. 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.

The mean hematocrit values for male or female were within the normal ranges reported by Spiers (1978) and very near the values reported by Bond and Gilbert (1958) and Ernst et al. (1971).

Leukocyte differentials of Bobwhites fed DIMP were unaffected. The normal leukocyte differentials of Bobwhites are in agreement with DIMP-fed Mallards.

The Bobwhites suffered high mortality during the first four weeks of the test 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.

Mortality, other than the case mentioned, was sporadic and not treatment related.

CONCLUSIONS

LD₅₀: Observations on mortality, feed consumption, and body weight change of Bobwhite quail show DIMP to be slightly toxic. The LD₅₀ for DIMP is 1000 mg/kg with 95% confidence intervals of 934.2 - 1070.5 mg/kg.

LC₅₀: Bobwhites fed DIMP-treated diets showed decreasing feed consumption coincident with decreasing body weight gain as the dietary level of DIMP increased. These results coupled with no trend in mortality suggest that the Bobwhite voluntarily restricted feed intake and thus did not consume a sufficient amount of chemical to cause sufficient deaths to calculate a LC₅₀ value.

Chronic: Few parameters of the DIMP-treated quail were significantly different from the control group. The parameters that did show significant changes showed no consistent treatment-effect results. Thus, the results suggest that DIMP has 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.

Toxicity of DIMP to Mink

TEST 1 - ACUTE (LD₅₀)

Procedure

Testing

To ascertain the effect of an acute oral exposure of DIMP to mink, 29 adult female mink were singly dosed intragastrically with the compound. The following progression of doses (and number of mink per dose) were used:

0.0 mg/kg (2); 75 mg/kg (2); 150 mg/kg (4); 300 mg/kg (4); 450 mg/kg (4); 500 mg/kg (6); 550 mg/kg (5); and 600 mg/kg (4).

The larger doses (300 mg/kg and greater) were administered by gavage. This was accomplished by inserting a plexiglass rectangle (approximately 20 x 50 x 3 mm) with a 9 mm hole in the center, between the jaws of a restrained animal, and introducing the tube into the esophagus through the hole in the plexiglass. This consisted of a length of polyethylene tubing (premeasured for average esophageal length) attached to a 3 ml syringe with an 18 gauge needle.

Smaller doses were introduced into the stomach by gelatin capsule. The capsules were pushed down the esophagus by means of a length of polyethylene tubing to the level of the stomach.

Mortality and signs of intoxication were recorded during a 2 hour observation period after dosing and daily thereafter for 14 days. The mink were then killed by cervical dislocation, and examined for gross pathomorphological changes.

Statistical Analysis

The determination of the acute oral - LD₅₀ was made by the method of Litchfield and Wilcoxon (1949).

Results

The dose related mortality of mink to a single acute oral exposure of DIMP is presented in Table 39. The acute oral LD₅₀ as determined by the method of Litchfield and Wilcoxon (1949) was 503 mg/kg with a 95% confidence interval of 379-668 mg/kg. A least-squares regression line of the probit analysis data shown in Table 39 is presented in Figure 18.

The clinical signs of acute intoxication with DIMP included salivation, lethargy, myasthenia, immobilization, vomiting, and death. The mink exposed to 300-550 mg/kg that did not die, were immobilized to varying degrees, but eventually recovered. Recovery was complete within several hours of dosing.

Table 39. Acute oral toxicity of DIMP to adult female mink.

Dose (mg/kg) ¹	No died/no. tested	Mortality (%) ²	Probits ³
0	0/3	0	--
75	0/2	0	--
150	0/4	0	--
300	1/4	25	3.55 ⁴
450	1/4	25	4.33
500	4/6	66.7	5.45
550	2/5	40	4.75
600	4/4	100	--

¹ Administered by gavage

² Taken at 24 hours post-dosing

³ Determined by method of Litchfield and Wilcoxon (1949)

⁴ Represents adjusted value (Litchfield and Wilcoxon, 1949)

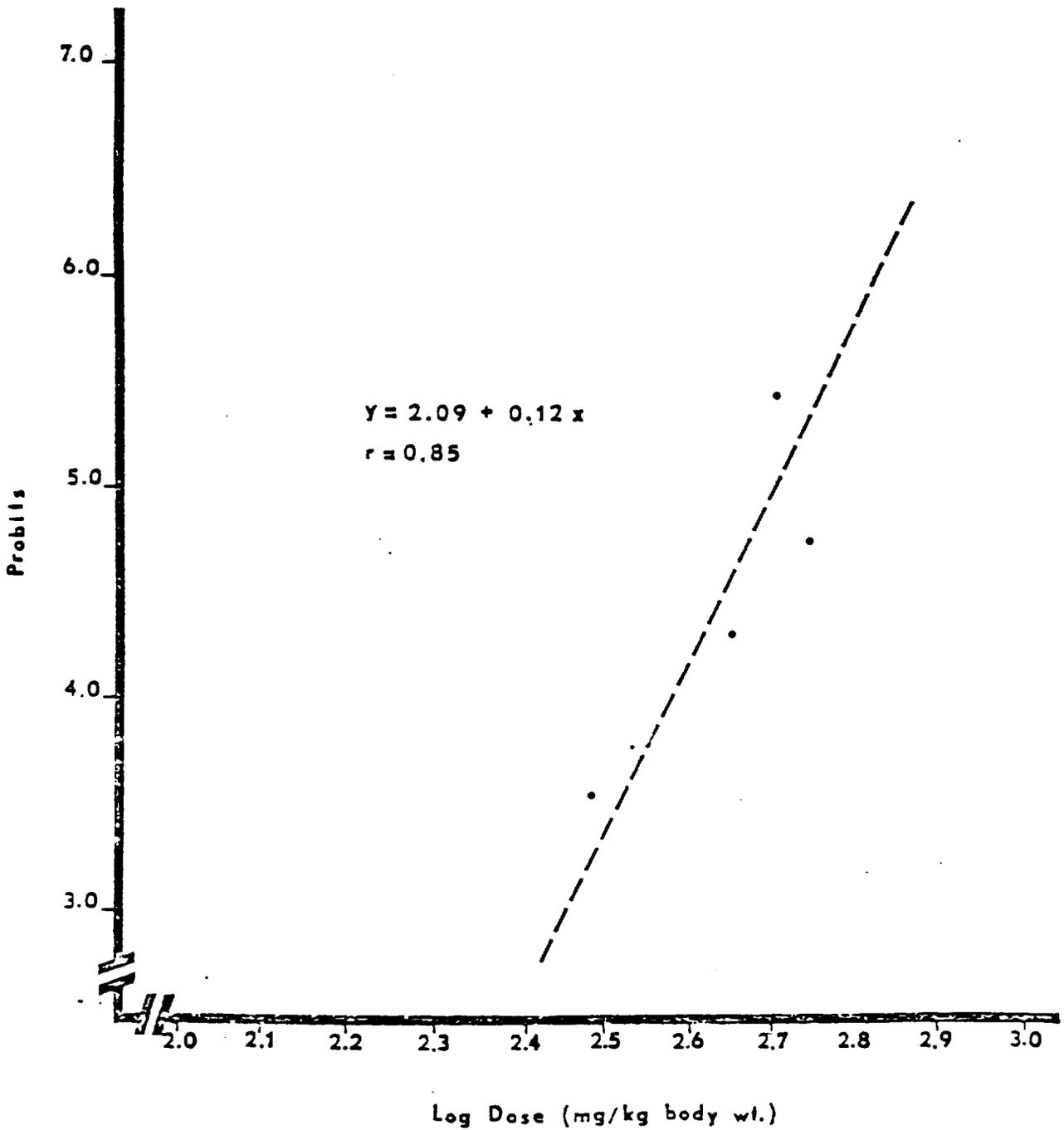


Figure 18. Regression equation of the data shown in Table 39 in the regression equation $x = \log$ dose DIMP in mg/kg body weight, $y =$ probits.