TOXICITY OF DIISOPROPYL METHYLPHOSPHONATE AND DICYCLOPENTADIENE ON THE MALLARD (ANAS PLATYRHYNCHOS)

By

Ross Edward Jones Jr.

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Poultry Science

1977

ABSTRACT

TOXICITY OF DIISOPROPYL METHYLPHOSPHONATE AND DICYCLOPENTADIENE ON THE MALLARD (ANAS PLATYRHYNCHOS)

By

Ross Edward Jones Jr.

A toxicological study was performed on Mallards using diisopropyl methylphosphonate (DIMP) and dicyclopentadiene (DCPD). The LD_{50} for adult Mallards dosed with DIMP was 1490 $\pm$ 75.8 mg/kg and for ducks dosed with DCPD was greater than 40000 mg/kg, the highest level tested.

An LC_{50} could not be determined for either DIMP, with zero mortality of ducklings on diets up to 16000 ppm, or DCPD, with 30 percent mortality in ducklings on diets containing 60000 ppm DCPD. Predicted zero feed intake levels for the ducklings were calculated to be 23222 ppm of DIMP in the diet and 77290 ppm of DCPD in the diet.

On a chronic study (0, 1000, 3200, 10000 ppm DIMP, and 0, 32, 100, 320 ppm DCPD) during the Mallards' first reproduction period no significant difference in mortality of adults or hatched ducklings from the adults was found in any group. The hens on diets containing 10000 ppm DIMP had a significant decrease in the number of eggs laid. The ducks on diets containing DCPD were not affected in any parameter measured such as feed consumption, incubation parameters, eggshell thickness, and blood parameters.

to my wife and child

ACKNOWLEDGMENTS

I will to thank all those who have helped in this endeavor for the past two years, especially Dr. R. K. Ringer, Dr. R. J. Aulerich, Dr. T. H. Coleman, and Kathy Howell.

This research was supported by a grant from the U.S. Army Medical Research and Development Command, Washington, D.C.

FOREWARD

In conducting the research described in this thesis, the investigator 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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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 and near 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.

Background

In the past, a number of toxicological incidents allegedly related to the RMA and its disposal of waste material have occurred. These incidents have had environmental consequences as destruction of plants and animals, including wild birds, wild mammals, and domestic livestock, has been found near the RMA. The two compounds, 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 methyl isopropylphosphonofluoridate (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; they are readily available for toxicological testing and represent an aquatic form of avian wildlife.

LITERATURE REVIEW

Diisopropyl methylphosphonate (DIMP) (see Appendix A: Chemical Structures and Alternate Names for DIMP and DCPD), an organophosphorus compound which is a liquid at room temperature with a bulk density of 0.976 g/cc at 25°C (Dacre, 1975), is soluble in water at 1-2 g/l (Ringer, personal communication). DIMP odor detection limits have not been cited for humans or other species. It is, therefore, probable that ingestion of DIMP can occur fairly easily via one or more of the following routes: water, soil, plants, terrestrial invertebrates and vertebrates, and aquatic invertebrates and vertebrates.

Determined lethal doses of DIMP are listed in Table 1. No published information could be found on the effects of DIMP on humans.

McPhail and Adie (1960) stated that DIMP did not inhibit cholinesterase, an enzyme that is a measure of nerve activity, but no reference or experimental evidence was given. Jacobson (1953) reported moderate corneal damage to the eye of rabbits at a dose of 0.25 mm^3/kg. Using eye irritation studies on rabbits at an unspecified dosage, Hart (1976) produced variable results ranging from minimal redness to clouding of most or all of the cornea. Recovery was complete within seven days. In this same study, large

Table 1. Toxicity data on DIMP

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

[1] IV - Intravenous

[2] PC - Percutaneous

[3] SC - Subcutaneous

[4] IP - Intraperitoneal

amounts of dermally applied DIMP caused polycythemia vera in some of the rabbits with complete recovery within 10 days. Death occurred at some levels:

Level	Died	Abraded Skin
200 mg/kg	0	-
632 mg/kg	1/4	yes
2 gm/kg	3/4	2 of the 3

No information has been reported in the literature on detailed pathology caused by DIMP nor the effects on any avian species.

Dicyclopentadiene (DCPD) (see Appendix A: Chemical Structures and Alternate Names for DIMP and DCPD) is a waxy solid at room temperature with a melting point of 32°C and a bulk density at 20°C of 0.982 g/cc, but is considered insoluble in water as its calculated distribution coefficient, oil/water, is approximately 60,000. At 0.0004 mg/l, DCPD can be detected as a slight odor by humans. At 0.003 ppm the odor is increasingly unpleasant and can cause nausea and headaches (Shashkina, 1965). Thus, ingestion is probably very limited.

Determined lethal doses of DCPD are listed in Table 2. No published information could be found on the toxicological effects of DCPD on humans.

Shashkina (1965) found DCPD LC_{50} in rats to be 1.52 mg/l (1.37 - 1.69) by inhalation and in mice to be 0.74 mg/l (0.69 - 0.79). Reactions of rats to acute intoxication by

Table 2. Toxicity Data on DCPD

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

[1] mg/kg body wt.
[2] IP - Intraperitoneal
[3] Inh - Inhalation
[4] mg/l air

breathing DCPD at the moment of exposure included narcosis, tonic toxicity, excitability, and motor disturbances. Reactions following exposure included sharp increase in excitability with noise or physical contact, spasms, increased aggressiveness, and hemorrhage from the nose and eyes. Body temperature was lowered by 8.9°C or 24 percent and blood pressure by 4.4 mmHg or 6.2 percent.

Pathological changes in rats include pronounced plethora of organs, internal hemorrhage in the brain and lungs, emphysema in the lungs, albuminous dystrophic changes in the kidney, dystrophy of the liver, a depletion of lipoids in the cortex of the adrenals, and hyperfunction of the thyroid gland (Shashkina, 1965).

Changes in blood cells and hemoglobin of rats were not positive as both increases and decreases were noted over a six month period (Shashkina, 1965).

Rats administered DCPD subcutaneously showed pathological changes of general congestion, hyperemia and focal hemorrhages in the kidneys, intestine, stomach, bladder, and lungs (Kinkead et al., 1971). Most of these changes are typical of irritating hydrocarbons given in large doses (Frawley et al., 1952; Sherman et al., 1964; Keith and Mulla, 1966; Gage, 1970; Peckham, 1972).

Gage (1970) reported results with rats which inhaled DCPD at 100 ppm, 250 ppm, 1000 ppm, and 2500 ppm. At 100 ppm with fifteen 6-hour exposures no detrimental effects were seen. At 250 ppm with ten 6-hour exposures one died

after the second exposure and three survived. The survivors showed weight loss, nasal irritation, dyspnea, tremors hypersensitivity, and were lethargic. Blood parameters and organs were normal. At 1000 ppm with one 4-hour exposure, all the rats showed eye and nose irritation, dyspnea, muscular incoordination, tremors, and hypersensitivity before dying. Pathological changes included congestion of lungs and liver. At 2500 ppm with a 1-hour exposure all rats showed the same symptoms of the rats at 1000 ppm; one of the rats died with lung and liver congestion.

The toxicity appears to be higher in mammals when DCPD is given as a single dose by the oral and intraperitoneal route, than by the dermal route.

OBJECTIVES

1. To determine the single dose acute oral LD_{50} and dose-response curve for DIMP and DCPD to the adult Mallard.
2. To determine the eight day subacute dietary LC_{50} for DIMP and DCPD to the young Mallard.
3. To determine chronic toxicity, including long-range effects on reproduction parameters, blood parameters and general body changes, from feeding DIMP and DCPD to Mallards over their first reproductive cycle.

PROCEDURE

The research was divided into three experiments. Experiment 1 was concerned with the lethal dose for 50 percent of the animals (LD_{50}); experiment 2 dealt with the lethal chronic level (LC_{50}), and experiment 3 was a long term chronic study. All three experiments utilized Mallard ducks,[1] (Anas platyrhynchos). The Mallards were procurred from two locations:

1. Max McGraw Wildlife Foundation, Dundee, Illinois 60118
2. Frost Game Farm, Coloma, Wisconsin 54930

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

Experiment 1

This experiment was designed to determine the single oral dose LD_{50} of diisopropyl methylphosphonate (DIMP) and dicyclopentadiene (DCPD) 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

[1]Phenotypically indistinguishable from wild Mallards.

78.7 cm (w) X 35.6 cm (h) and there were ten ducks per battery for 960 cm^2/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 geometric scale 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 B: 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 chemicals. 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 for each chemical had a constant volume to body weight factor per animal. 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 LD_{50} 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.

Experiment 2

This subacute test was designed to determine the maximum repeated dosage tolerable to Mallard ducklings on DIMP and DCPD-treated diets. A random selection of healthy twelve-day-old ducklings were 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 unit[2] from one day of age through the end of the experiment.

A range finding pilot test was performed with both chemicals to determine their effect on feed consumption and body weight. A geometric scale 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 ration (Appendix B: 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 compounds did not have time to act. The test diets were a mixture of chemical (DIMP or DCPD) and corn oil, and duck starter (Appendix C: Ration Preparation). In the DIMP treated diets, the chemical-corn oil solution

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

was a constant two percent of the diet. The control diet consisted of two parts corn oil to 98 parts feed by weight. Because DCPD appeared to be relatively harmless (LD_{50} greater than 15000 mg/kg), the chemical-corn oil solution was greater than two percent. For each compound ten dietary treatments were used: for DIMP; 0, 2000, 4000, 6000, 8000, 10000, 12000, 14000, 16000, and 18000 ppm diets were used, and for DCPD; 0, 10000, 20000, 30000, 40000, 50000, 60000, 70000, 80000, and 90000 ppm diets were employed. Ten ducklings of undetermined sex were placed on each dietary treatment. Because all DCPD fed groups of ducklings in the initial test showed decreased feed consumption as compared to the control, the experiment was repeated using lower DCPD levels for a longer period of time. Young adult, male Mallards 23 weeks old $\pm$1 week were utilized. Diets used contained the following levels of DCPD: 0, 10, 100, 1000, 5000, and 10000 ppm (Appendix C: Ration Preparation). The birds were fed the treated diet for 32 days at the end of which necropsies were performed on all animals.

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

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.

Experiment 3

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

For each chemical, 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 by three. 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 C: Ration 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 22 and 24 weeks for DCPD and DIMP groups, respectively. 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 ration was fed for the first six weeks and breeder layer ration was fed for the remainder of the trail. Food consumption was measured at biweekly intervals during the entire experiment.

The room was kept at approximately 45°F (7°C) and six hours of light/day before egg production (December 28 to March 3) and raised to approximately 55°F (12.8°C) and 19 hours of light/day to induce egg production. Temperatures ranged from 47°F to 90°F (8.3°C to 32.3°C) for the rest of the study (March 4 to June 2 for DCPD and March 4 to June 13 for DIMP). The higher room temperatures generally occurred toward the end of the experiment.

Body weights 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. All survivors 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 55 to 60°F (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 99.5°F (37.5°C), with a range from 98.4°F (36.9°C) to 100.5°F (38.1°C), and at an average relative humidity of 56 percent (86 wet bulb), 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 99°F (37.2°C), with a range from 98.3°F (36.8°C) to 100.5°F (38.1°C) and a relative humidity of 65 to 70 percent (88 to 91 wet bulb). 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 regular feed. Mortality of all ducklings was recorded for the 14-day period and precent livability calculated.

At biweekly intervals all eggs from one day's collection were measured for eggshell thickness. Eggs to be

---

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

measured were cracked open at the girth, contents washed out, and 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 micrometer[4] calibrated to 0.01 mm units. Of all eggs laid 8.6 percent were utilized for these measurements.

Hematological Preparation

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

Hemoglobin concentration was determined by the cyanmethemoglobin method. Exactly 5 ml of Drabkin's Reagent (Appendix D: Preparation of Drabkin's Reagent) were placed into a clean cuvette. Twenty-five microliters of blood were added to the diluent and the pipette was rinsed several times with the diluent. After the blood was added, the tube was stoppered and inverted two or three times. The mixture of blood and reagent was allowed to stand ten minutes for maximum conversion of hemoglobin to cyanmethemoglobin. The cuvette was wiped clean and placed into a Spectronic 20 Calorimeter-Spectrophotometer[5] for reading. The percentage transmission at 540 nm was recorded and hemoglobin concentration was determined by comparing each

---

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

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

sample percent absorbance against the percent absorbance of standards with human hemoglobin concentration[6] (Appendix E: Determination of Hemoglobin Concentration).

Hematocrits were determined by collecting blood, from a venous puncture of the wing, into a heparinized capillary tube. After sealing one end of the capillary tube, it was centrifuged at 4500 rpm for 7.5 minutes in an International Microcapillary Centrifuge.[7] After centrifugation, the packed red cell volume in each tube was measured using a microcapillary reader.

Blood smears for differential counts were prepared using fresh flowing blood, containing no anticoagulants, and a clean glass slide. The blood was allowed to air dry before staining. The blood film was fixed by flooding with Wright's stain (Appendix F: Preparation of Wrights's Stain and Buffer) and left to stand for approximately five minutes. The buffer was then added to differentiate the cells. After five more minutes, distilled water was used to wash the slides which were drained and blotted dry.

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

---

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

[7]International Equipment Company, Boston, Massachusetts.

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, 1977).

RESULTS

Experiment 1

Rationale

The single oral median lethal dose (LD_{50}) was determined, because no information on the toxicity of either DIMP or DCPD to birds was known. Clinical signs from the LD_{50}, including time of mortality (instant or delayed), morbidity, and behavioral changes were needed to determine the toxicity of the compounds. Differences in clinical signs for each sex were noted. This information was used in the determination of dose levels in experiments 2 and 3.

LD_{50} values can be used as a guide for determining the dose levels to be used in extended toxicological studies, but care must be taken in that the LD_{50} figure often bears no relation to the maximum dose tolerated on prolonged administration. The LD_{50} is useful for comparison with other animals' reaction to the same chemical, though care must be taken because species variation is very large.

Preliminary testing on Mallards began at a level near the mammalian LD_{50} value. In range finding of DIMP, one animal was dosed at each level until a median dose of 1500 mg/kg was found after starting at 200 mg/kg and doubling the dose until an animal died at 1600 mg/kg. With DCPD, dosing

started at 800 mg/kg and was raised to 20000 mg/kg with no deaths. The "Federal Register" (1975) recommends a limit of 2 to 3 percent of the body weight as a maximum dose, but since no mortality occurred, a group of 20 ducks were dosed at four percent of their body weight (40000 mg/kg).

Results

Mortality for the ducks treated per os with DIMP is listed in Table 3. Determination of acute oral LD_{50} by the method of Litchfield and Wilcoxon (1949) for the compounds tested was:

Compound	Species	LD_{50} mg/kg
DIMP	Mallard duck	1490 $\pm$ 75.8
DCPD	Mallard duck	>40000

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.

Table 3. 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

[1]All 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.

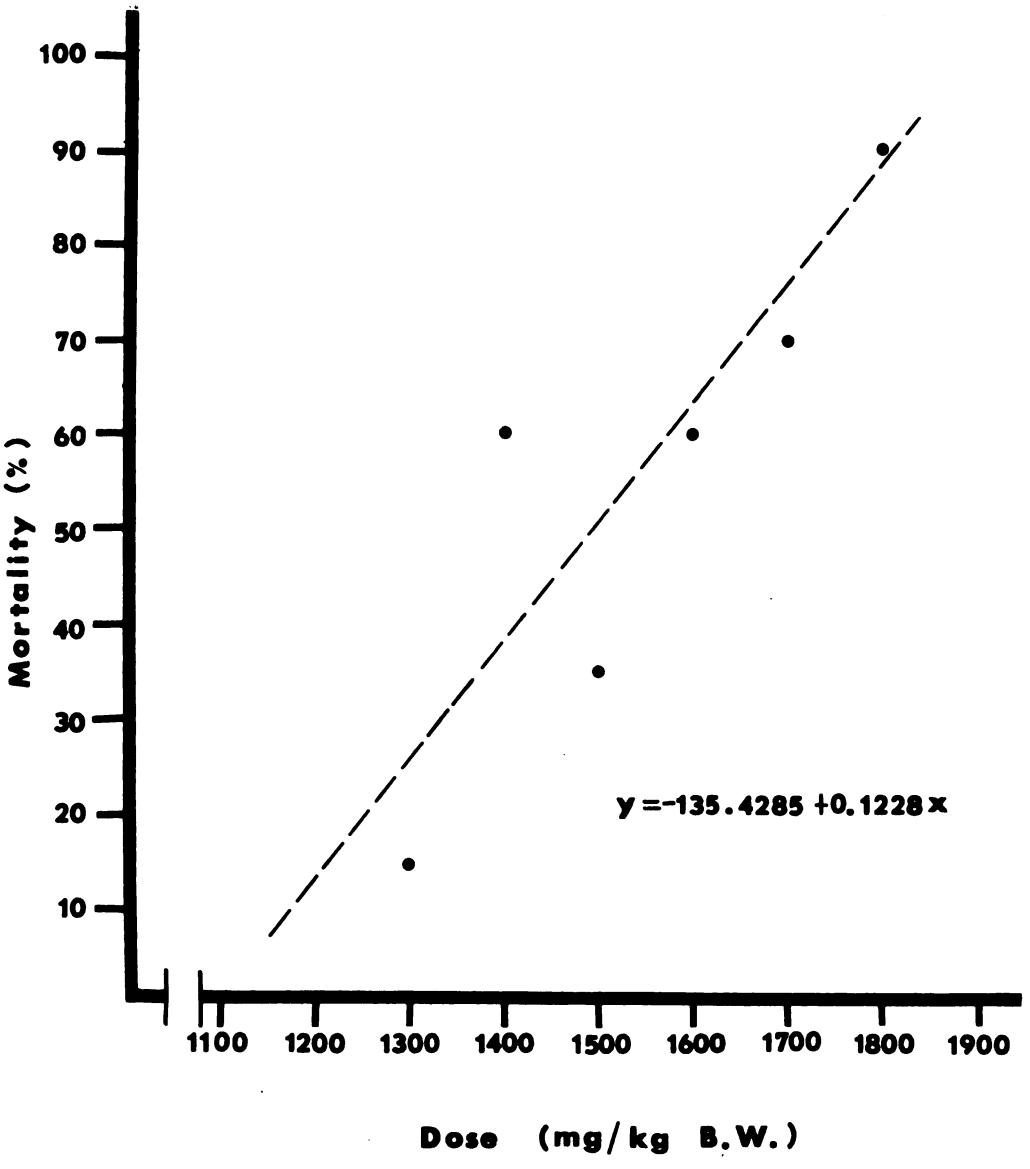
100
90
80
70
60
50
40
30
20
10
Mortality (%)
y =-135.4285 +0.1228 x
1100
1200
1300
1400
1500
1600
1700
1800
1900
Dose (mg/kg B.W.)

FIGURE 1

Though no deaths occurred with DCPD, responses were noticed. Responses to the 40000 mg/kg dose, which was given 5 cc (∿5000 mg) at a time over a maximum of two and one-half hours to prevent drowning, started to appear after approximately 20 to 30 cc had been given. Many birds showed no reaction to the chemical other than holding their mouths open during the first part of dosing. Of those that did show a response, only a slight intoxication was noticed and moderate tremors of the head and body in about ten percent of the birds. All the birds appeared to have recovered within two hours after dosing.

During the 14 day post-treatment period, no further signs of intoxication nor significant weight changes were noted for the ducks on either chemical except in the group dosed at 1800 mg/kg with DIMP where a 14.8 percent loss in weight was observed (Table 4). Necropsies of all birds, i.e., those that died and those that were sacrificed at the end of the post-treatment period, showed no gross pathological changes in ducks which had been administered either DCPD or DIMP.

Feed consumption, for the 14 day post-treatment period, is listed in Table 5 for the ducks dosed with DIMP or DCPD. 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

Table 4. Body weight changes of Mallard ducks during 14 day post-treatment observation period following a single per os treatment with DIMP or DCPD

Treatment	Treatment level (mg/kg)	n	Mean body weight		Mean change
			Day 0	Day 14	
DIMP	0	20	1217	1215	-2_a [1]
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
DCPD	0	20	1113	1151	38_c [1]
DCPD	40000	19	1169	1175	6_c

[1]Means 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 5. Feed consumption of Mallard ducks during 14-day post-treatment observation period following a single per os treatment with DIMP or DCPD

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
DCPD	0	20	57.28 ± 0.531	49.45 ± 0.539
DCPD	40000	19	44.28_2 ± 0.545	55.40_2 ± 0.553

[1]Data 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)

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. With DCPD, feed consumption was depressed significantly by 22.7 percent the first week but was above the control by 12.0 percent the second week.

Experiment 2

Rationale

In nature, animals are normally not exposed to just one dose (LD_{50}) of a contaminant, but to repeated doses usually via the feed. In toxicity testing a subacute test is conducted to establish the general toxicity of the compound being tested and the maximum dosage an animal will tolerate by repeated exposure over a period of several days. The five day feeding of treated diets in concentrations above and below the LD_{50} values allows for daily absorption, metabolism, and excretion and therefore does not provide the same information as the acute exposure test.

The levels of DIMP and DCPD used for the subacute experiment were partially derived from the lethal dose calculated, the variation in a groups' response to the same dose, the slope of the dosage-mortality curve, and the subacute range finding pilot experiment. Range finding is designed to provide a reasonable estimate of the maximum dosage an animal will tolerate on repeated intake of a compound. It is also a procedure to eliminate inappropriate dosages before starting the more extensive subacute and

chronic experiments.

Results of the five day dose-range finding trial were:

Treatment	Level in diet (ppm)	Change in body wt. (g/b/d)	Feed consumed (g/b/d)	Percent mortality
DIMP	6000	27.9	58.12	0
DIMP	9000	-1.6	6.26	0
DCPD	20000	21.1	39.32	0
DCPD	30000	17.4	31.66	0

Since DCPD did not cause any mortality during the acute test nor during range finding, seven of the ten levels were set above the maximum two percent levels recommended by the "Federal Register" (1975). This was done to establish a zero feed intake level if mortality did not reach 50 percent at any level. Since no birds died on the range finding trial with DIMP, but appeared to be more sensitive to lower levels than ducks treated with DCPD, the subacute levels were all set below two percent of the diet.

The subacute test was designed as a pilot experiment to guide the planning of the chronic test, from which it differs only in magnitude and duration.

Results

DCPD. The feed consumption of the ducklings on diets containing DCPD (Figure 2) was decreased in all treated groups as compared to the control group. This decrease ranged from 28.7 percent for birds receiving the 10000 ppm diet to 98.7 percent for those receiving the 80000 ppm diet.

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

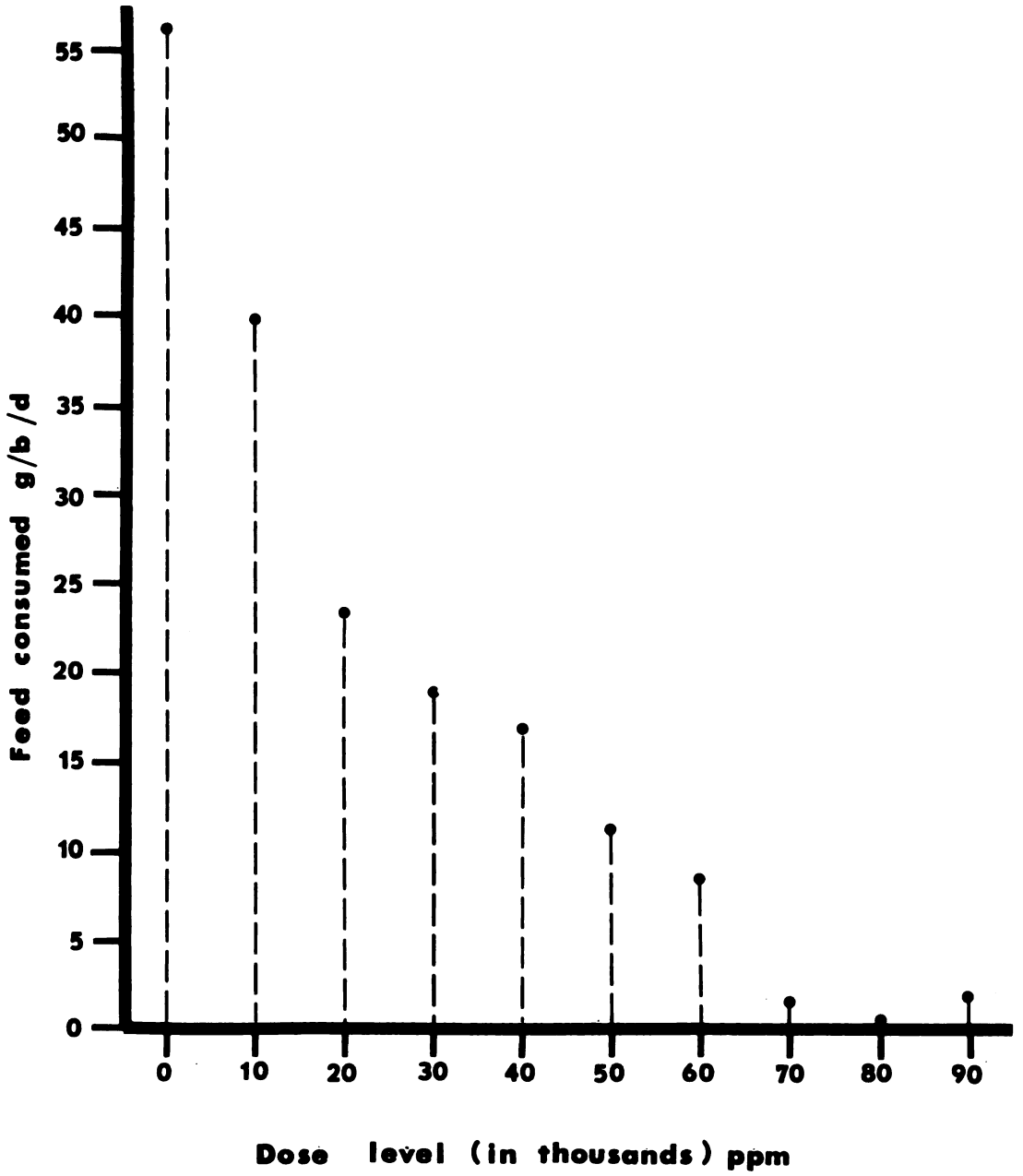
Feed consumed g/b /d
55
50
45
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
90
Dose level (in thousands) ppm

FIGURE 2

The feed consumption of those ducklings receiving the three highest levels of DCPD (70000, 80000, and 90000 ppm) was nearly zero (mean of 1.41 g/b/d for the three groups). The 10000 and 20000 ppm groups had the steepest rate of decline in feed consumption (Figure 3) with a slope of -0.0017 (or -1.657[1]) and a correlation between feed consumption and level of DCPD in the diet of -0.99987. The higher treatment groups, 30000 to 70000,[2] showed a smaller rate of decline with a slope of -0.4121; the predicted zero feed consumption was calculated off this line to be 77290 ppm DCPD in the diet. Body weight changes (Figure 4) showed that all treatment groups, with the exception of the birds on the 10000 ppm diet, lost from 1.64 to 19.08 g/b/d with an average loss of 6.74 g/b/d. Total intake of the chemical ranged from 340 to 3312 mg/kg/day (Table 6) with the least amount of intake in the three highest groups (70000, 80000, 90000 ppm) since they had refused to consume the feed. Mortality ranged from 0 to 30 percent (mean of 8 percent) and showed no trends (Table 6). The highest mortality occurred in the 60000 ppm group which consumed over 3000 mg/kg/day of chemical, but the 40000 ppm group which also consumed over 3000 mg/kg/day of DCPD had no mortatliy. Correlation between

---

[1]Equals -1.657 when calculated with dose divided by 1000. All subsequent slopes will be given in this manner.

[2]The 70000 to 90000 ppm groups were averaged and used as one point for regression analysis since none of the groups apparently ate any feed, but rather "tasted" it daily, thus giving a small calculated feed consumption.

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

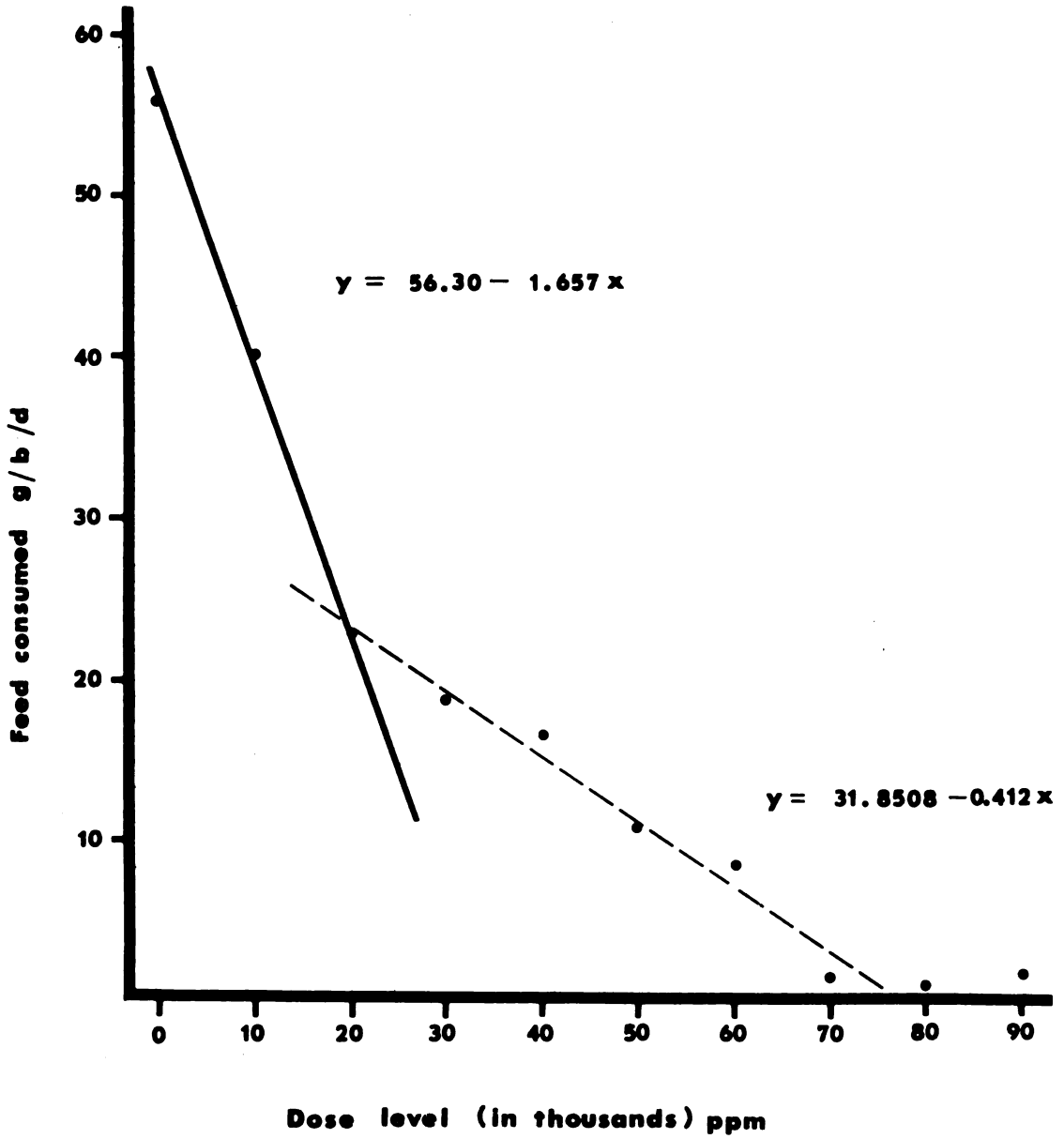
60
50
40
30
20
10
Feed consumed g/b/d
y = 56.30 − 1.657 x
y = 31.8508 −0.412 x
0
10
20
30
40
50
60
70
80
90
Dose level (in thousands) ppm

FIGURE 3

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

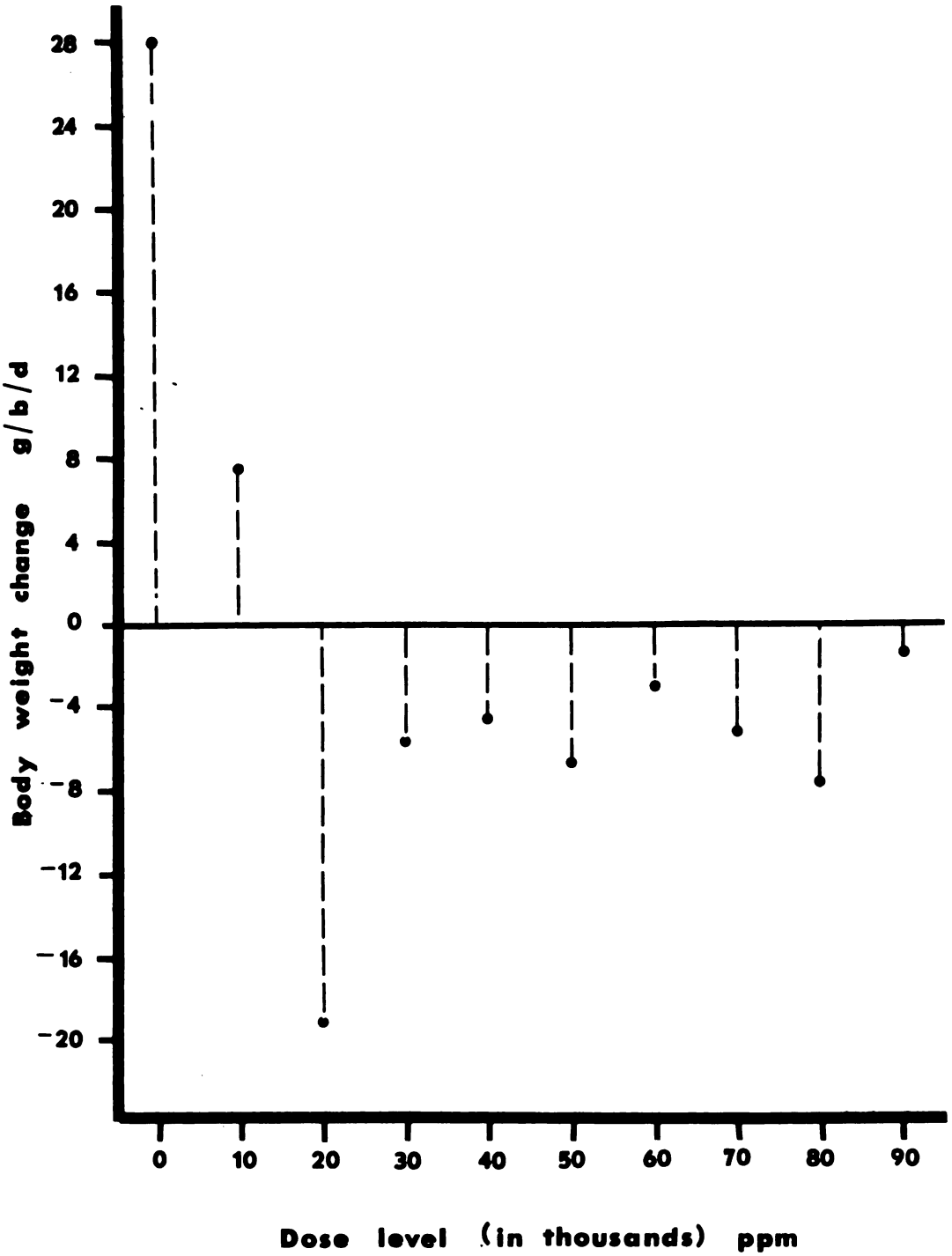
28
24
20
16
12
8
4
0
-4
-8
-12
-16
-20
Body weight change g/b/d
0
10
20
30
40
50
60
70
80
90
Dose level (in thousands) ppm

FIGURE 4

Table 6. Calculated DCPD intake over 5 days and mortality over 8 days for 12-day-old Mallard ducklings on LC_{50} trial

Dose (ppm)	mg DCPD consumed/day	Mean body wt. (g)	mg DCPD/ kg/day	Percent mortality
0	0	277.3	0	0
10,000	400.4	246.9	1621.7	0
20,000	460.0	240.7	1911.1	20
30,000	564.6	222.7	2535.2	10
40,000	674.4	203.2	3318.9	0
50,000	555.0	203.6	2725.9	10
60,000	519.0	170.7	3040.4	30
70,000	120.4	171.3	702.9	0
80,000	56.8	162.9	348.7	10
90,000	162.0	172.2	940.8	0

mortality and mg DCPD/kg/day ingested was only 0.441. During the three day post-treatment period groups previously on diets containing 30000 ppm, or more, DCPD had increased feed consumption over the control from 24.4 percent at 70000 ppm to 36.7 percent at 50000 ppm (Figure 5) with a mean increase of 32.2 percent (8.22 g/b/d). This increase is not present in the 10000 and 20000 ppm groups during the post-treatment as they were an average 15.2 percent (3.87 g/b/d) less than control birds. Body weight gains during post-treatment (Table 7) in the lower groups, 10000 to 40000 ppm, were 2.5 to 7.7 g/b/d with a mean gain of 5.81 g/b/d which was 2.45 g/b/d greater than the control; while the higher groups, 50000 to 90000 ppm, gained 21.4 to 29.7 g/b/d with a mean gain of 25.6 g/b/d which was 22.2 g/b/d more than the control birds.

In the DCPD treated repeat group of Mallards (Table 8) feed consumption was not affected by any level of the drug, but body weight was lost in increasing amounts by birds receiving the three highest levels; for 1000 ppm a decrease of 30.8 g/b from the control, the 5000 ppm was 83.9 g/b lower, and the 10000 ppm group was decreased by 183.6 g/b. Ingestion of DCPD ranged from 0.505 to 736.24 mg/kg/day and no mortality occurred during the 32 day period. There was a correlation of -0.992 between level of drug in the diet and body weight change.

DIMP. In the DIMP trial the data from the 18000 ppm treatment group were discarded because of experimental error.

Figure 5. Feed consumption of 17-day-old Mallard ducklings fed non-treated diet during 3 day post-treatment after withdrawal of DCPD treated diet

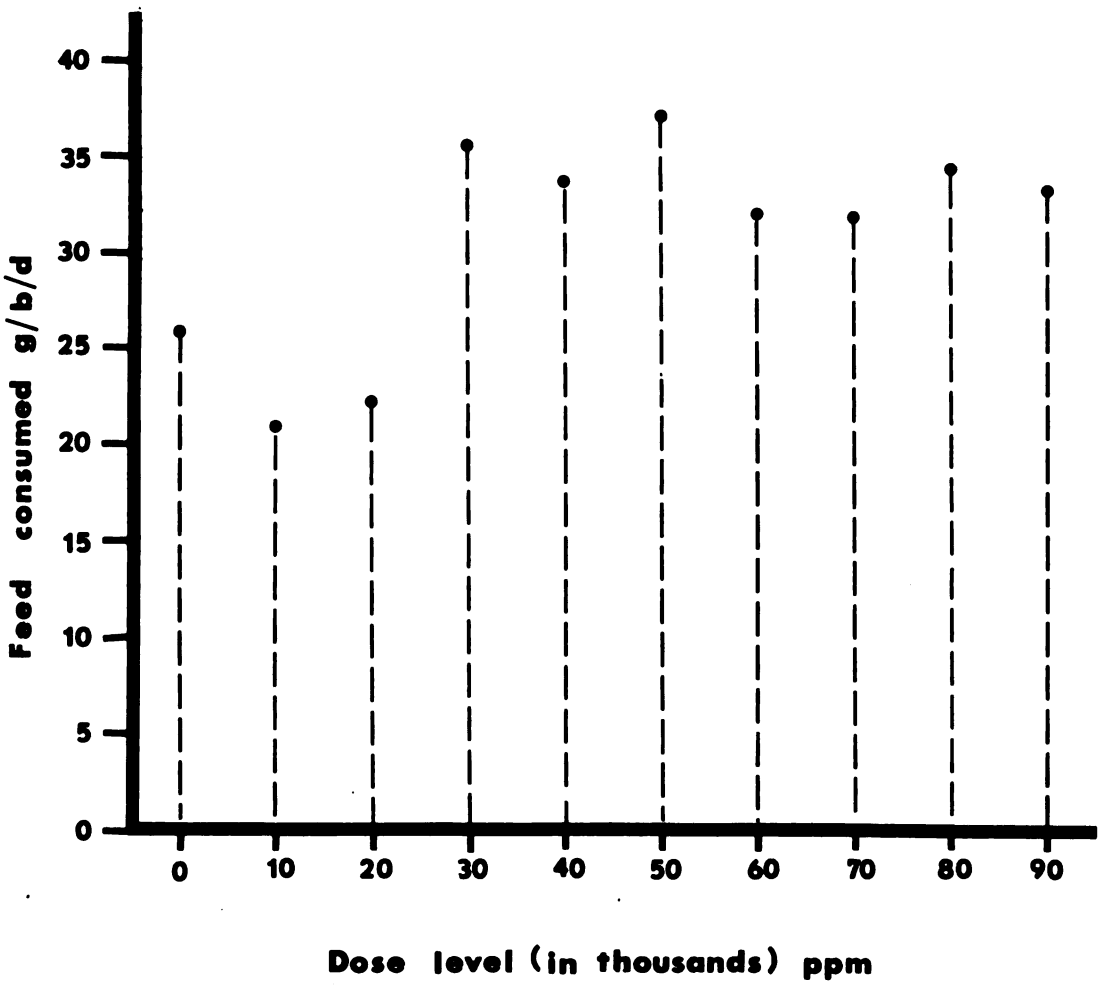
Feed consumed g/b/d
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
90
Dose level (in thousands) ppm

FIGURE 5

Table 7. Body weight gain of 17-day-old Mallard ducklings during 3-day post-treatment on non-treated feed after withdrawal of DCPD treated feed.

DCPD level in the diet (ppm)	Weight gain g/b/d	Feed consumed/ weight gain
0	3.36	7.59
10,000	2.46	8.51
20,000	6.50	3.43
30,000	6.55	5.44
40,000	7.73	4.29
50,000	23.66	1.55
60,000	29.20	1.09
70,000	21.40	1.48
80,000	29.70	1.15
90,000	24.00	1.38

Table 8. Feed consumption, body weight change, and amount of chemical ingested by 23 week old male Mallards, fed diets treated with DCPD, at various levels, for 32 days

DCPD level in diet (ppm)	Mean weight gain (loss)/bird (gms)	Feed consumed[1] g/b/d	Days	Mg DCPD consumed/day	Mg DCPD/ kg/day
0	17.4	$67.11 \pm 3.52_a$[2]	1-14	0.0	0.0
0			15-32	0.0	0.0
10	9.0	$66.31 \pm 3.52_a$	1-14	0.568	0.505
10			15-32	0.710	0.644
100	27.2	$66.22 \pm 3.52_a$	1-14	5.30	4.75
100			15-32	7.45	6.39
1000	(13.4)	$66.33 \pm 3.52_a$	1-14	56.9	46.00
1000			15-32	71.2	59.88
5000	(66.5)	$66.02 \pm 3.52_a$	1-14	284.3	249.39
5000			15-32	351.0	319.67
10000	(166.2)	$66.34 \pm 3.52_a$	1-14	569.0	543.46
10000			15-32	709.0	736.24

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

[2]Means with the same subscript are not significantly lower than the control group ($P > 0.05$).

Feed consumption of ducklings (Figure 6) on the 12000, 14000, and 16000 ppm diets was decreased as compared to 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 7). Calculated zero feed consumption from the second slope equals 23222 ppm DIMP in the diet.

Body weight gain (Figure 8) 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 slope of -0.616 (Figure 9), while those on the higher levels, 10000 to 16000 ppm, showed a continuous decrease from 19.9 to 8.4 g/b/d (Figure 8) 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 9). Predicted zero body weight gain was 20439 ppm DIMP in the diet. There was no mortality in any group even though the amount of DIMP ingested (Table 9) ranged from 403 to 2062 mg/kg/day which bracketed the LD_{50} of 1490 mg/kg.

Figure 6. Effect of feeding DIMP at various levels in the feed for 5 days on feed consumption of 12-day-old Mallard ducklings.

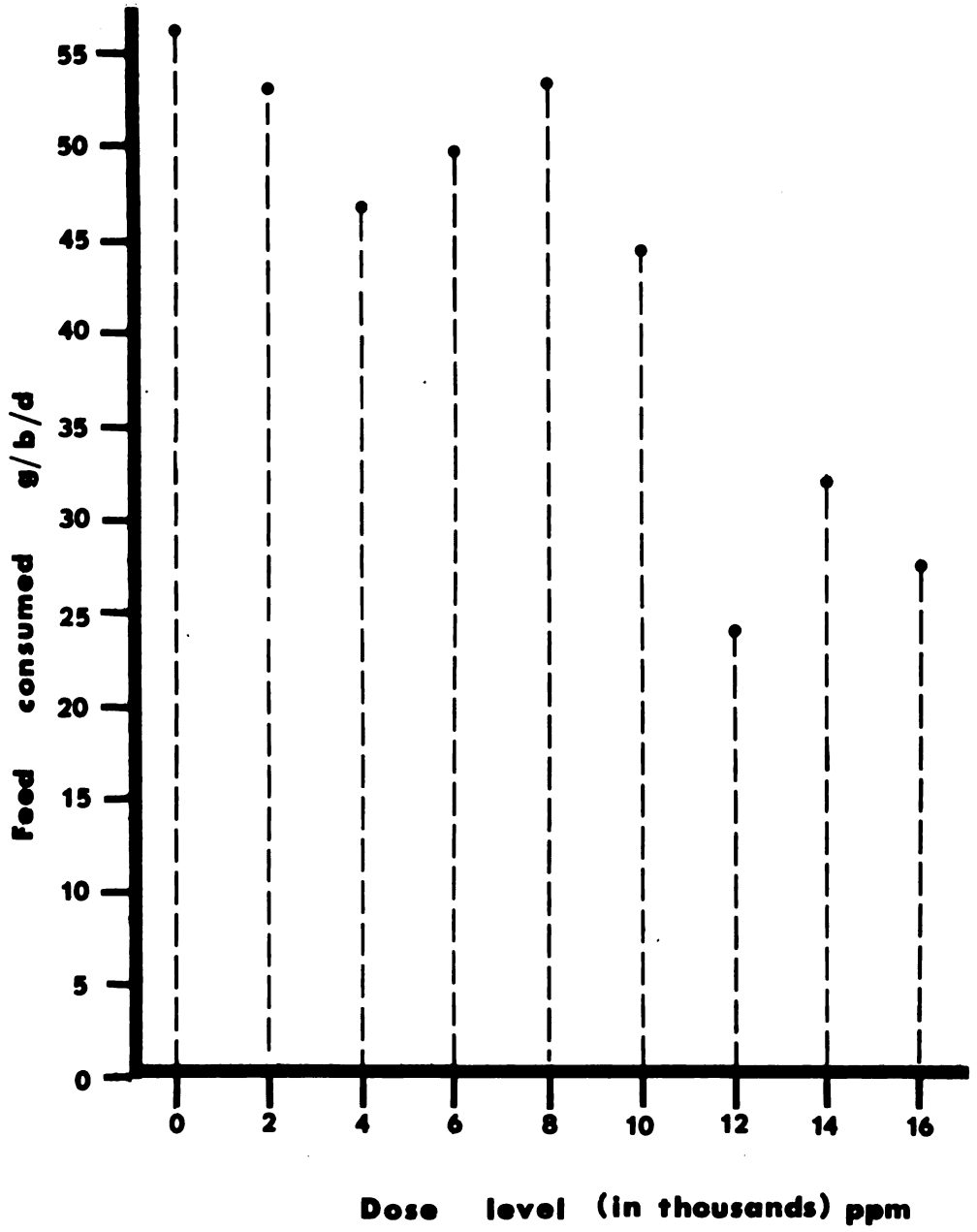
Food consumed g/b/d
55
50
45
40
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
16
Dose level (in thousands) ppm

FIGURE 6

Figure 7. Regression equations of the data shown in Figure 6. In the regression equations x = ppm of DIMP in the feed and y = feed consumption in g/b/d.

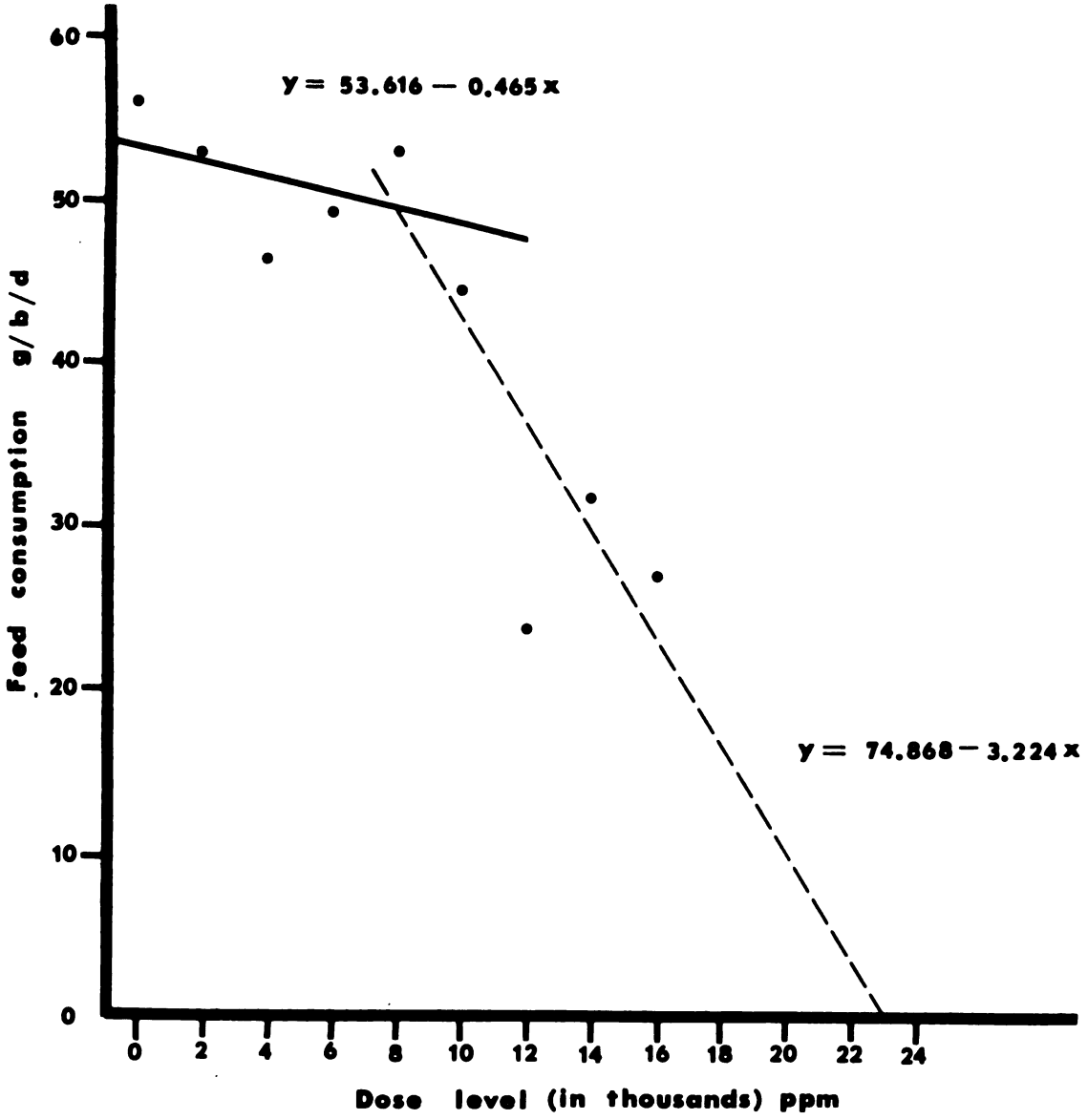
y = 53.616 — 0.465x
y = 74.868 — 3.224x
Feed consumption g/b/d
Dose level (in thousands) ppm
0
10
20
30
40
50
60
0
2
4
6
8
10
12
14
16
18
20
22
24

FIGURE 7

Figure 8. Effect of feeding DIMP at various levels in the feed for 5 days on body weight gain of 12-day-old Mallard ducklings

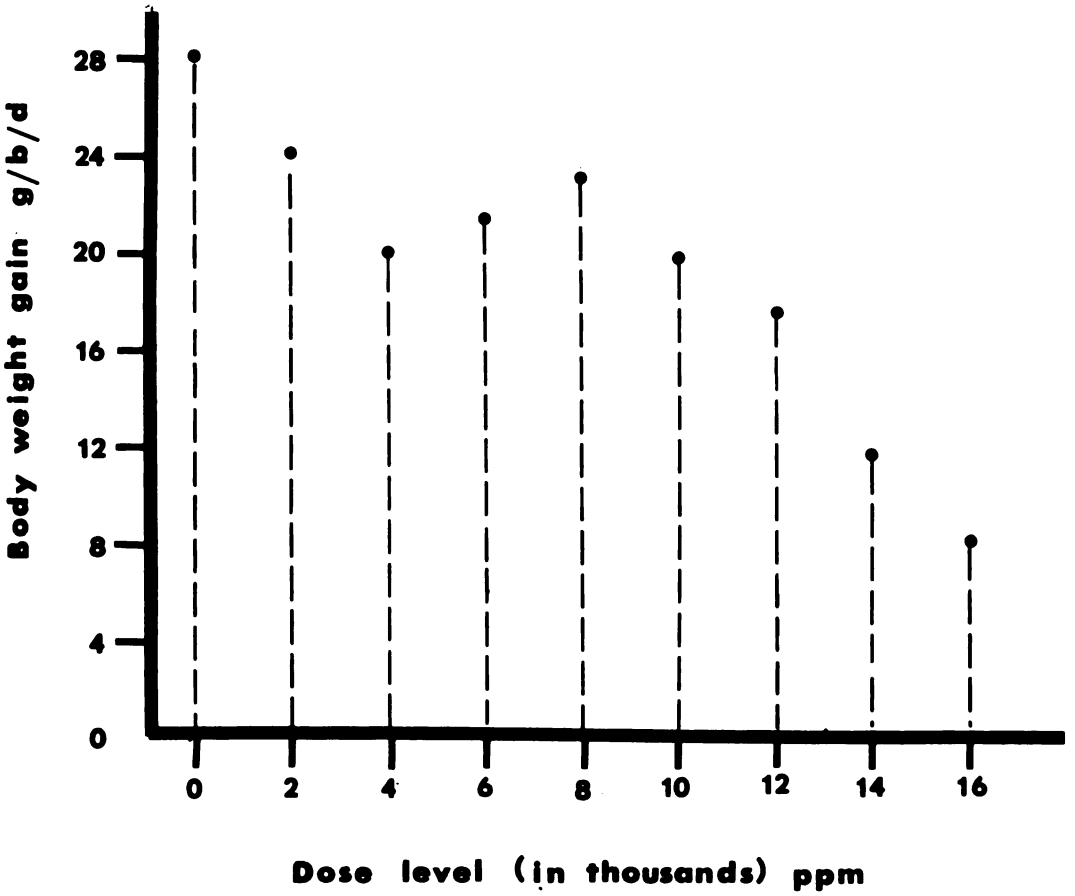
Body weight gain g/b/d
28
24
20
16
12
8
4
0
0
2
4
6
8
10
12
14
16
Dose level (in thousands) ppm

FIGURE 8

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

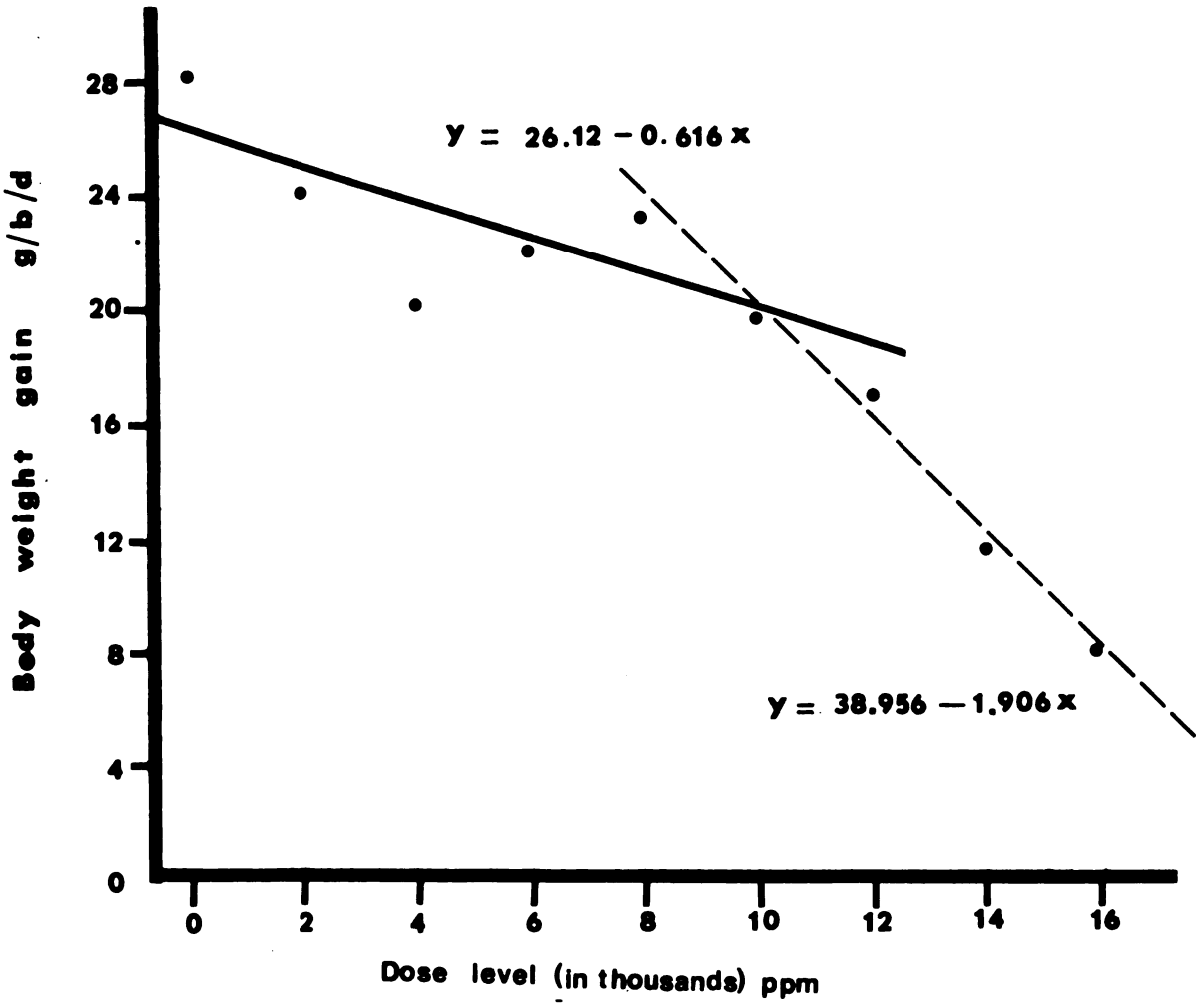
y = 26.12 − 0.616 x
y = 38.956 − 1.906 x
Body weight gain g/b/d
Dose level (in thousands) ppm
0
4
8
12
16
20
24
28
0
2
4
6
8
10
12
14
16

FIGURE 9

Table 9. Calculated DIMP intake over 5 days and mortality over 8 days for 12-day-old Mallard ducklings on LC_{50} 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

During the three day post-treatment period, level of feed consumption (Figure 10) 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 groups consumption. Body weight changes during post-treatment (Table 10) show that all treatment groups, except the 6000 ppm group, gained more weight, from 0.6 to 14.3 g/b/d, than 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 DCPD nor DIMP treated groups from controls.

Experiment 3

Rationale

Migratory species such as the duck were exposed to repeated intake of DIMP and DCPD only twice a year as they migrated through the contaminated area of RMA near Denver, Colorado. The chronic test performed was thus shortened to a single generation reproduction test and not a continuous

Figure 10. Feed consumption of 17-day-old Mallard ducklings fed non-treated diet during 3 day post-treatment after withdrawal of DIMP treated diet.

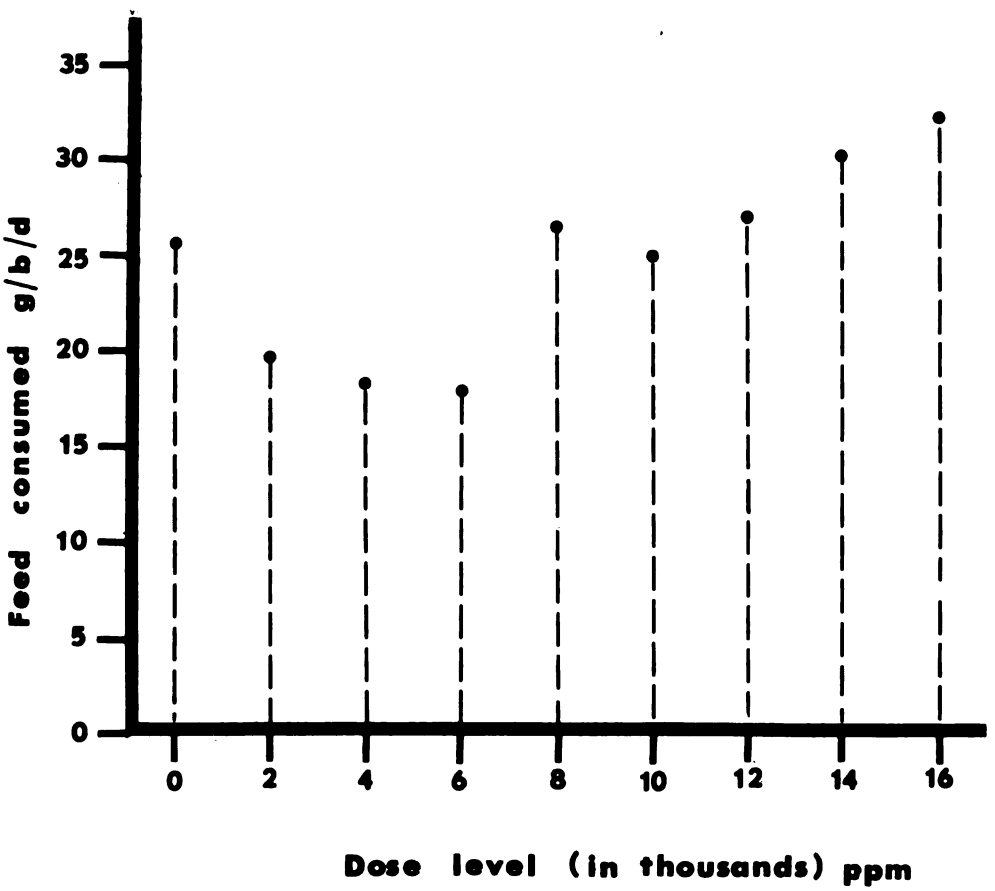
Feed consumed g/b/d
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
16
Dose level (in thousands) ppm

FIGURE 10

Table 10. 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

year long test.

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.[3] Thus, DCPD, which adversely affected body weight gains at levels of 1000 ppm and above, was set at 320 ppm and below. 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 experiment 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.

---

[3]Data from the repeat group of DCPD treated Mallards in experiment 2 were used more in determining the levels of DCPD to be used in the chronic test than the first trial group.

Results

Feed consumption is plotted in Figure 11 for the ducks treated with DCPD. Each point plotted is the mean of three cages of seven ducks per cage. There was no significant difference in any DCPD treated group as compared to their control. In the ducks treated with DIMP (Figure 12), 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 for all birds receiving either of the two compounds are reported in Table 11. All DIMP treated groups lost less weight than did their control. There was no significant difference in body weight change of any DCPD treated group as compared with their control group.

Body weight changes from before start of egg laying to end (or near end) of the egg production period for all birds receiving either compound are listed in Table 12. All treated groups (DCPD and DIMP) gained weight with no significant difference between treated groups and their respective control.

Egg production for DCPD treated ducks is plotted in Figure 13. Each point plotted is the mean of three cages of five hens per cage. Percent production was based on hen-day production. There was no significant difference

Figure 11. Effect of feeding DCPD at various levels in the diet for 22 weeks on feed consumption of adult Mallards. Each point represents the mean of three cages of two males and five females each.

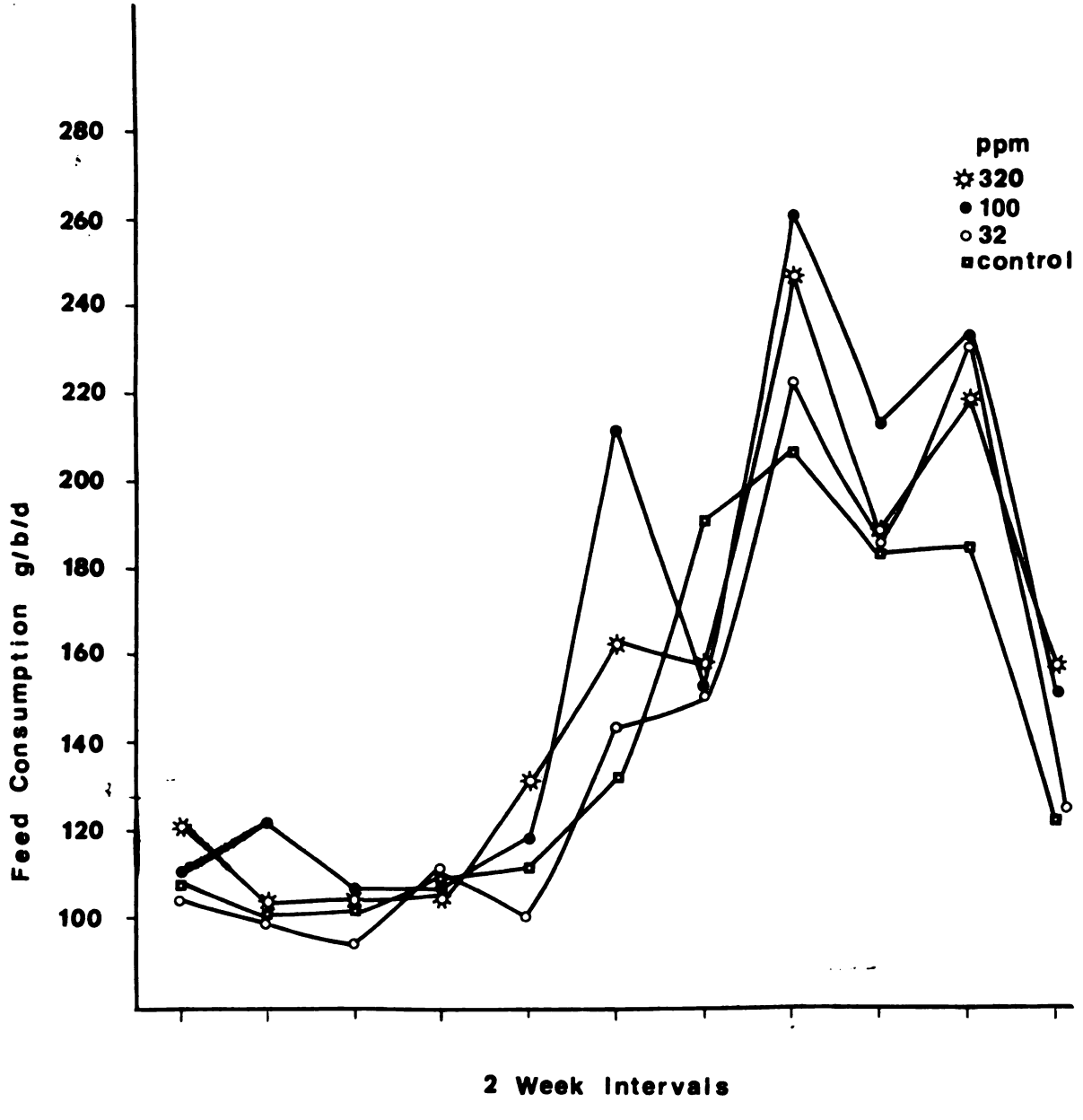
280
260
240
220
200
180
160
140
120
100
Feed Consumption g/b/d
ppm
320
100
32
control
2 Week Intervals

FIGURE 11

Figure 12. 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.

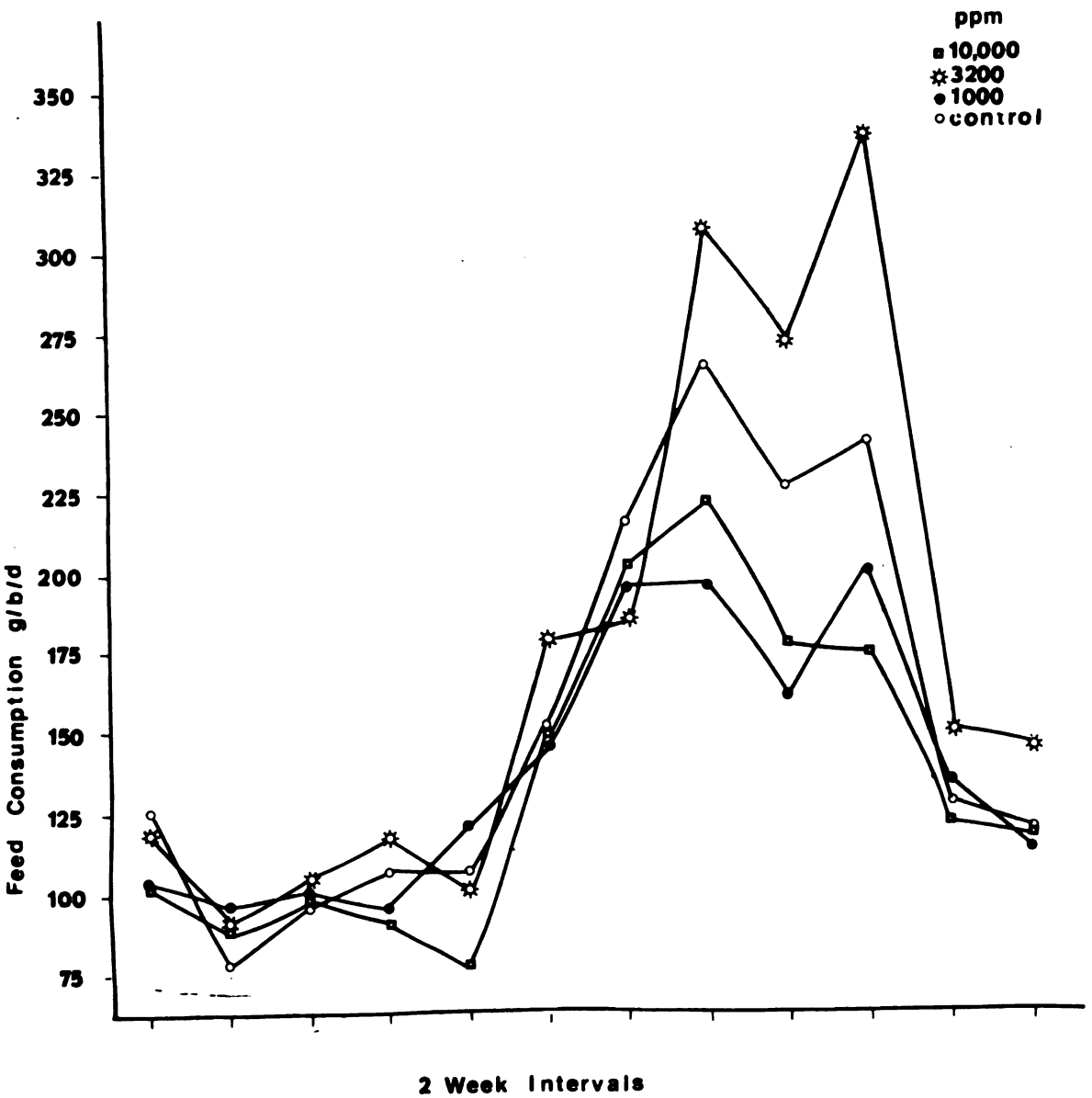
ppm
10,000
3200
1000
control
350
325
300
275
250
225
200
175
150
125
100
75
Feed Consumption g/b/d
2 Week Intervals

FIGURE 12

Table 11. Effect of feeding DIMP and DCPD 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.[2]	gms	As a % of body wt.[2]	gms	As a % of body wt.[2]
DIMP	0	21	-22.90	-1.48	-4.40	-0.26	-27.30_a[1]	-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	$- 2.62_a$	-0.09
DIMP	10000	21	0.98	0.15	0.93	0.23	1.91_a	0.38
DCPD	0	21	- 8.69	-0.70	-5.02	-0.41	-13.71_b	-1.11
DCPD	32	21	-19.86	-1.67	-5.55	-0.49	-25.41_b	-2.16
DCPD	100	21	- 8.48	-0.44	-1.62	0.09	-10.10_b	-0.35
DCPD	320	21	-15.62	-1.31	2.16	0.24	-13.46_b	-1.07

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

[2]Average percentage change by individual.

Table 12. Effect of feeding DCPD or 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_a[1]
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
DCPD	0	1175.7	1295.7	10.21	120.0_b[1]
DCPD	32	1185.3	1252.2	5.66	67.1_b
DCPD	100	1241.6	1319.4	6.27	77.8_b
DCPD	320	1200.3	1306.9	8.88	106.6_b

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

Figure 13. Effect of feeding DCPD at various levels in the diet for 22 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.

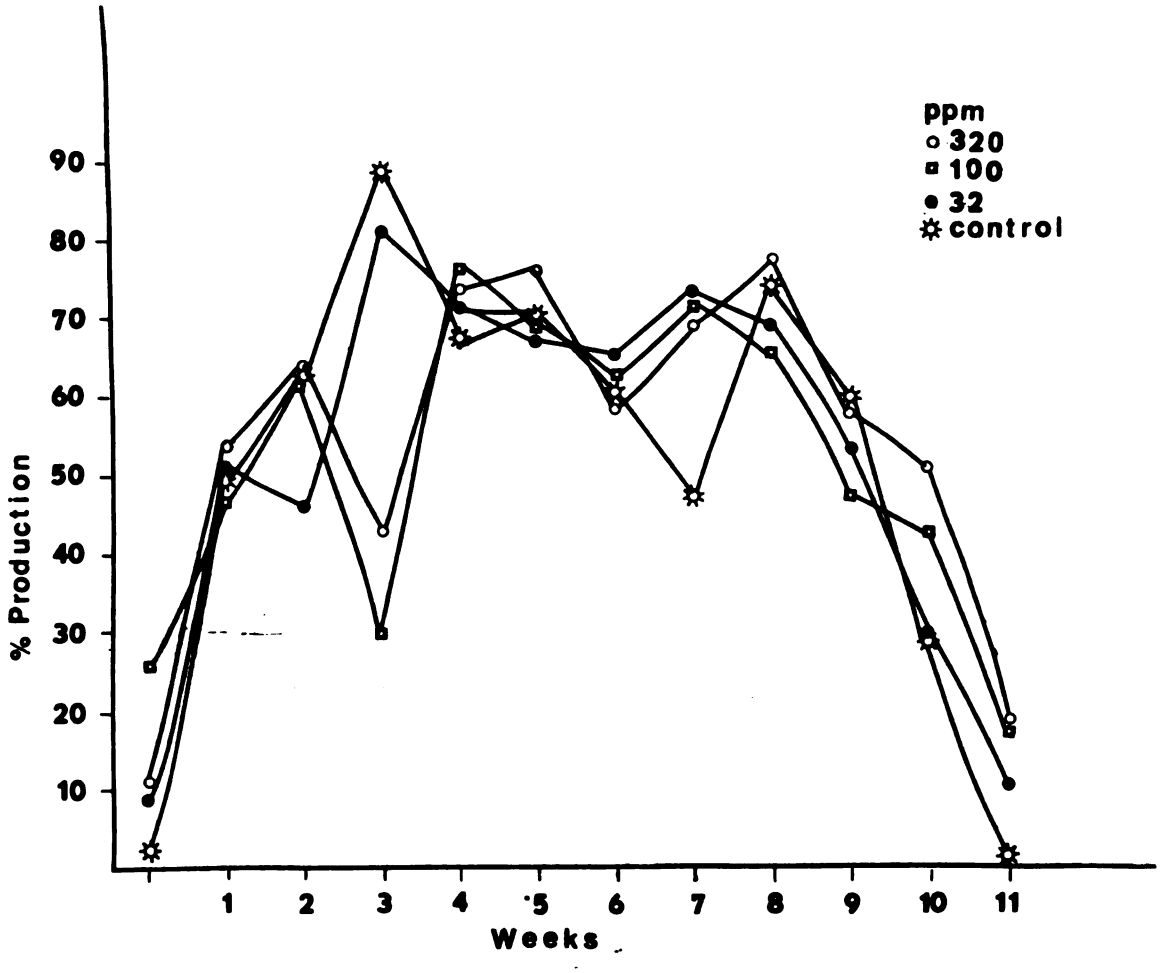
ppm
320
100
32
control
% Production
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
Weeks

FIGURE 13

between the treated groups' overall egg production as compared to the control.

For DIMP treated ducks (Figure 14), 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 both DCPD and DIMP treated Mallards is listed in Table 13. No significant difference was found between treated groups and their respective control for eggshell thickness from birds fed either compound. All eggs used for eggshell thickness measurements were not included in any calculated percentages other than production.

Incubation parameters for the ducks on DCPD treated feed are listed in Table 14. There was no significant difference between any treated group and the control in any parameter. The values for percent fertile eggs are based on the number of setable 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. Incubation parameters for DIMP treated ducks are listed in Table 15. 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 16. There was no significant difference between any treated group of parents' ducklings

Figure 14. 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.

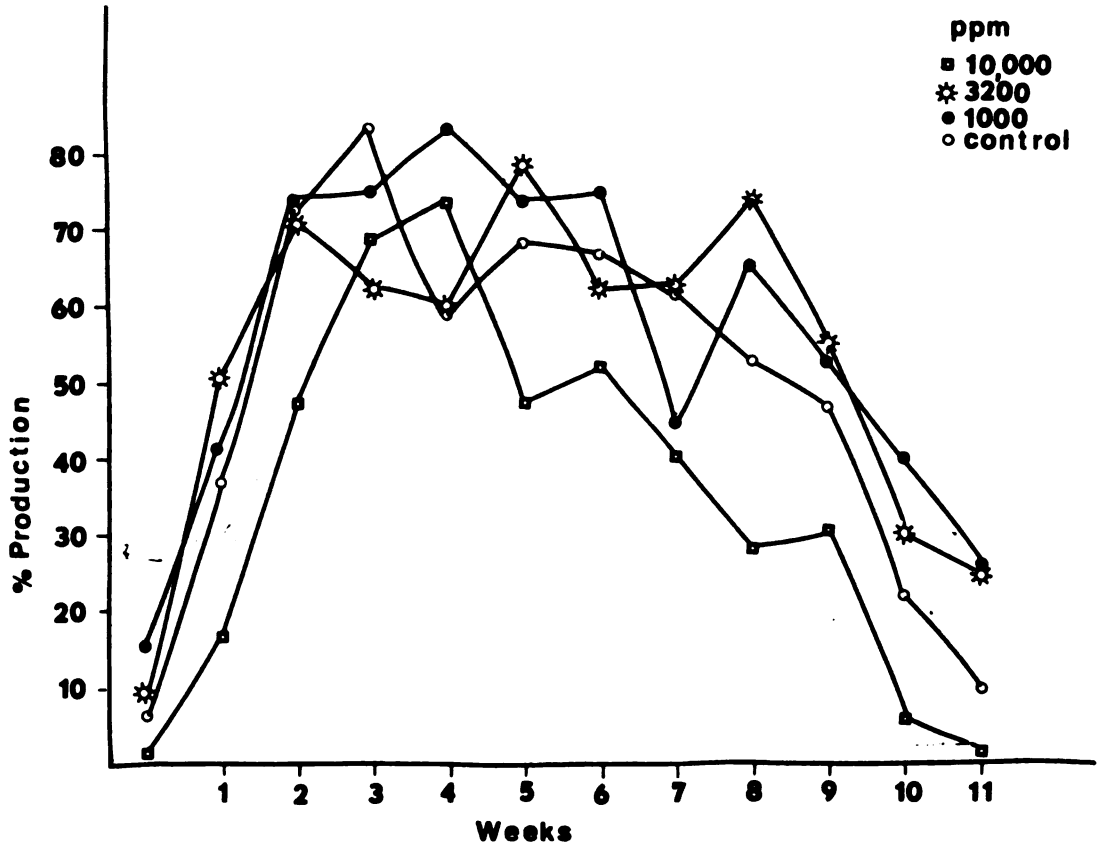
ppm
10,000
3200
1000
control
% Production
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
Weeks

FIGURE 14

Table 13. Effect of feeding DIMP or DCPD at various levels in the feed for 24 and 22 weeks respectively 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[2] ($mm \times 10^{-2}$)	Combined N	Combined Mean
DCPD	0	6	18	40.7 ± .775		
	0	10	19	41.8 ± .552	53	40.90 ± .374$_a$[1]
	0	18	16	40.0 ± .734		
	32	4	16	40.8 ± .655		
	32	11	23	40.0 ± .719	55	39.99 ± .367$_a$
	32	20	16	29.2 ± .815		
	100	2	15	39.7 ± .595		
	100	15	19	39.7 ± .525	57	39.29 ± .364$_a$
	100	24	23	38.6 ± .426		
	320	3	22	41.1 ± .600		
	320	7	18	40.6 ± .563	56	41.10 ± .361$_a$
	320	17	16	41.6 ± .536		
DIMP	0	5	18	40.3 ± .513		
	0	22	13	41.9 ± .822	53	40.30 ± .381$_b$[1]
	0	23	22	39.3 ± .623		
	1000	1	16	39.5 ± .557		
	1000	12	11	39.1 ± .959	45	39.26 ± .414$_b$
	1000	16	18	39.1 ± .590		
	3200	8	22	38.9 ± .557		
	3200	19	28	38.7 ± .618	69	38.84 ± .334$_b$
	3200	21	19	38.9 ± .565		
	10000	9	12	38.6 ± .539		
	10000	13	10	38.6 ± .973	36	38.88 ± .462$_b$
	10000	14	14	39.3 ± .933		

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

[2]Data given as group mean ± standard error.

Table 14. Effect of feeding DCPD at various levels in the diet for 22 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	3.90	5.78	3.29	4.51[a][1]
	32	5.39	4.69	2.06	4.29[a]
	100	3.49	7.92	7.24	6.23[a]
	320	2.14	4.21	2.69	3.18[a]
Fertile	0	80.40	92.06	63.27	81.64[b][1]
	32	93.50	82.53	67.83	83.69[b]
	100	63.86	66.42	65.25	65.38[b]
	320	89.78	91.19	95.51	89.29[b]
Hatched	0	61.35	62.35	59.14	61.4[c][1]
	32	76.52	67.84	63.92	69.16[c]
	100	87.74	68.18	65.22	72.99[c]
	320	62.60	51.68	52.56	54.54[c]
Early dead	0	3.36	7.45	5.38	6.00[d][1]
	32	3.48	5.49	4.12	4.7[d]
	100	6.60	3.41	5.44	4.81[d]
	320	5.69	7.56	4.49	6.19[d]
Dead in shell	0	15.13	25.10	34.41	24.41[e][1]
	32	9.57	21.57	26.80	19.70[e]
	100	4.72	20.46	28.26	17.91[e]
	320	14.63	33.19	33.33	28.82[e]
Live in shell	0	3.36	0.39	0.00	1.07[f][1]
	32	0.00	0.00	0.00	0.00[f]
	100	0.94	0.00	0.00	0.27[f]
	320	0.81	0.84	0.00	0.58[f]
Pipped live	0	11.76	3.14	0.00	4.71[g][1]
	32	9.57	4.71	3.09	5.57[g]
	100	0.00	7.39	0.00	3.48[g]
	320	11.38	5.88	7.69	7.74[g]
Pipped dead	0	5.04	1.57	1.08	2.36[h][1]
	32	0.87	0.39	2.06	0.86[h]
	100	0.00	0.57	1.09	0.53[h]
	320	2.44	0.84	1.92	1.55[h]

[1]Means 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 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]

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

Table 16. Effect of feeding DIMP or DCPD 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_a [1]	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
DCPD	0	98.61_b [1]	4/287
	32	98.76_b	4/323
	100	99.27_b	2/273
	320	99.29_b	2/282
Total		98.97	12/1165

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

and the control parents' ducklings; nor was there any significant difference between ducklings from parents treated with either DIMP or DCPD.

Hemoglobin values for DIMP and DCPD treated groups of Mallards are listed in Table 17. There was no significant difference by sex nor by level of chemical in the diet as compared to their respective control group for ducks treated with either compound. Hematocrit values for both DIMP and DCPD treated groups of Mallards are listed in Table 18. There was no significant difference by sex, nor by level of chemical in the diet as compared to their respective control group for ducks treated with either compound. Mean corpuscular hemoglobin concentration (MCHC) was determined by the formula: MCHC = (Hb x 100)/ Hct, where Hb equals hemoblobin gm/dl and Hct equals packed cell volume. MCHC is listed in Table 19 for DIMP and DCPD 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. Ranges for DCPD treated Mallards were 26.82 to 30.92 percent for 0 ppm, 25.81 to 31.90 percent for 32 ppm, 26.03 to 30.70 percent for 100 ppm, and 25.00 to 30.23 percent for 320 ppm. There was no significant difference in MCHC between sexes, nor between treatment levels as compared to their respective control group for either DIMP or DCPD treated Mallards. Leukocyte counts of the Mallards treated with DCPD are listed in Table 20 and for Mallards treated

Table 17. Effect of feeding DIMP or DCPD at various levels in the diet for 24 and 22 weeks, respectively, 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$_a$[2]
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
DCPD	0	6	11.93	14	12.09	20	12.05 ± .266$_b$[2]
DCPD	32	6	12.87	14	12.44	20	12.57 ± .266$_b$
DCPD	100	5	12.20	14	11.90	19	11.99 ± .273$_b$
DCPD	320	6	12.72	15	11.62	21	11.94 ± .259$_b$
Total		23	12.44	57	12.01	80	12.135± .133

[1]Data reported as treatment mean ± standard error.

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

Table 18. Effect of feeding DIMP or DCPD at various levels in the diet for 24 and 22 weeks, respectively, 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 T
DIMP	0	6	43.96	13	45.90	19	$45.30 \pm .726_a$[2]
DIMP	1000	6	43.50	14	44.36	20	$44.10 \pm .707_a$
DIMP	3200	3	44.83	15	44.08	18	$44.21 \pm .746_a$
DIMP	10000	3	43.50	15	44.00	18	$43.92 \pm .746_a$
Total		18	43.87	57	44.54	75	$44.38 \pm .363$
DCPD	0	6	41.50	15	42.65	21	$42.32 \pm .791_b$[1]
DCPD	32	6	43.33	14	43.86	20	$43.70 \pm .811_b$
DCPD	100	5	42.55	14	43.27	19	$43.08 \pm .832_b$
DCPD	320	6	44.67	15	42.17	21	$42.88 \pm .791_b$
Total		23	43.03	58	42.97	81	$42.98 \pm .399$

[1]Data reported as treatment mean ± standard error.

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

Table 19. Effect of feeding DIMP or DCPD at various levels in the diet for 24 and 22 weeks, respectively, on mean corpuscular hemoglobin concentration of adult Mallard ducks (calculated from the data in Table 17 and 18)

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	$28.91 \pm .417_a$[2]
DIMP	1000	6	29.24	14	28.97	20	$29.05 \pm .406_a$
DIMP	3200	3	28.68	15	29.70	18	$29.53 \pm .428_a$
DIMP	10000	3	30.17	15	29.18	18	$29.35 \pm .428_a$
Total		18	29.47	57	29.12	75	$29.20 \pm .206$
DCPD	0	6	28.82	14	28.10	20	$28.32 \pm .331_b$[2]
DCPD	32	6	29.69	14	28.31	20	$28.72 \pm .331_b$
DCPD	100	5	28.71	14	27.61	19	$27.89 \pm .339_b$
DCPD	320	6	28.53	15	27.53	21	$27.82 \pm .323_b$
Total		23	28.95	57	27.88	80	$28.19 \pm .162$

[1]Data reported as treatment mean ± standard error.

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

Table 20. Effect of feeding DCPD in the diet at various levels for 22 weeks on leukocyte counts of adult Mallard ducks at the end of their first reproductive cycle

Cell	Level DCPD in diet (ppm)	N	Mean[2]	Range
Basophil	0	21	1.48 ± .324 [1]	0-4
	32	20	1.70 ± .332$_a$	0-5
	100	19	1.79 ± .340$_a$	0-5
	320	21	1.95 ± .324$_a$	0-5
Total		81	1.73 ± .165	0-5
Eosinophil	0	21	1.76 ± .440 [1]	0-6
	32	20	2.25 ± .451$_b$	0-5
	100	19	2.58 ± .462$_b$	0-7
	320	21	2.86 ± .440$_b$	0-9
Total		81	2.36 ± .224	0-9
Heterophil	0	21	23.90 ± 2.86 [1]	4-57
	32	20	20.30 ± 2.93$_c$	5-67
	100	19	22.42 ± 3.01$_c$	8-40
	320	21	27.43 ± 2.86$_c$	10-61
Total		81	23.58 ± 2.46	4-67
Lymphocyte	0	21	69.00 ± 2.92 [1]	37-92
	32	20	70.40 ± 2.99$_d$	25-92
	100	19	69.63 ± 3.07$_d$	54-82
	320	21	63.47 ± 2.92$_d$	34-83
Total		81	68.06 ± 1.48	25-92
Monocyte	0	21	3.86 ± .456 [1]	0-7
	32	20	5.35 ± .467$_e$	1-11
	100	19	3.58 ± .479$_e$	0-5
	320	21	4.29 ± .456$_e$	1-10
Total		81	4.27 ± .232	0-11

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

[2]Data given as group mean ± standard error.

with DIMP in Table 21. There was no significant difference between any treated group of either chemical and its respective control for any type of leukocyte.

Organ weights for DIMP treated Mallards are listed in Tables 22 and 23. 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 a 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. Organ weights for DCPD treated animals are listed in Tables 24 and 25, and were divided as stated above. There was no significant difference in any organ weight on any treatment level as compared to the organ weight of the control, nor were the organ weights of ducks fed DCPD different from DIMP treated Mallards.

Mortality and birds removed from cages are listed in Table 26. 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 for either chemical.

Table 21. 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[3]	Range
Basophil	0	19	2.05 ± .309 [a][1]	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 [b][1]	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 [c][1]	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 [d][1]	40-89
	1000[2]	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 [e][1]	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

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

[2]Some toxic lymphocytes showing magenta granules in 5 of the 20.

[3]Data given as group mean ± standard error.

Table 22. 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					
								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_b[3]	-	--[3]	2.26	2.19	--	581.3	560.0	--
	1000	6	23.8_a	13	27.5_b	1	48.4_c[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[3]	13	0.71_e[3]	-	--[3]	0.21	0.057	--	53.3	14.4	--
	1000	6[4]	19.46_d	13	0.77_e	1	54.0_f[3]	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

[1]Females without developing follicles.

[2]Females with developing follicles.

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

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

Table 23. 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	Organ weight as percent of: Brain weight
Spleen	0	19	0.683_a [1]	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	8.76_b [1]	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_c [1]	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 [1]	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_e [1]	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 [1]	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_g [1]	--	--
	1000	20	5.054_g	--	--
	3200	18	4.925_g	--	--
	10000	18	5.040_g	--	--

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

Table 24. Effect of feeding DCPD at various levels in the diet for 22 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:					
								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	26.7[3] $_a$	13	31.9[3] $_b$	2	36.8[3] $_c$	1.87	2.55	2.93	496.4	658.6	739.6
	32	5	25.9 $_a$	13	29.1 $_b$	2	43.6 $_c$	1.96	2.37	3.59	510.6	611.8	887.9
	100	5	33.7 $_a$	10	30.7 $_b$	4	44.3 $_c$	2.49	2.39	3.33	639.0	613.4	936.0
	320	6	25.5 $_a$	11	37.2 $_b$	4	43.5 $_c$	1.88	2.83	3.61	480.2	760.0	917.6
Combined		22	27.8	47	32.1	12	42.7	2.03	2.53	3.40	527.6	659.8	889.1
Gonad(s)	0	6[4]	3.55[3] $_d$	13	2.48[3] $_e$	2	31.0[3] $_f$	0.24	0.19	2.44	66.2	52.0	621.0
	32	5[4]	10.34 $_d$	13	1.23 $_e$	2	37.8 $_f$	0.78	0.10	3.13	207.0	26.0	773.2
	100	5[5]	3.77 $_d$	10	1.68 $_e$	4	51.2 $_f$	0.29	0.13	3.91	72.0	33.2	1094.5
	320	6[5]	9.86 $_d$	11	1.75 $_e$	4	30.8 $_f$	0.75	0.14	2.55	191.0	35.8	651.8
Combined		22	6.86	45	1.79	12	38.8	0.51	0.14	3.08	133.6	37.0	814.5

[1]Females without developing follicles.

[2]Females with developing follicles.

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

[4]Three out of the five males were in a reproductive state.

[5]One out of the six males was in a reproductive state.

Table 25. Effect of feeding DCPD at various levels in the diet for 22 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
Speen	0	21	0.669_{a} [1]	0.053	13.38
	32	20 [2]	0.697_{a}	0.055	14.24
	100	18 [2]	0.753_{a}	0.057	14.84
	320	21	0.692_{a}	0.054	13.92
Kidney	0	21	8.57_{b} [1]	0.666	171.52
	32	20	8.68_{b}	0.691	179.63
	100	19	8.68_{b}	0.662	173.51
	320	21	8.63_{b}	0.666	174.29
Pancreas	0	21	4.06_{c} [1]	0.316	80.93
	32	20	3.56_{c}	0.286	74.08
	100	19	3.82_{c}	0.291	76.27
	320	21	3.71_{c}	0.288	74.90
Proven-triculus	0	21	3.97_{d} [1]	0.308	79.01
	32	20	3.79_{d}	0.304	78.70
	100	19	3.94_{d}	0.300	78.61
	320	21	4.07_{d}	0.313	81.91
Gizzard	0	21	36.53_{e} [1]	2.82	725.07
	32	20	32.55_{e}	2.62	675.16
	100	19	32.61_{e}	2.48	646.09
	320	21	32.52_{e}	2.48	652.09
Heart	0	21	8.73_{f} [1]	0.681	174.47
	32	20	8.27_{f}	0.667	172.17
	100	19	8.58_{f}	0.650	171.07
	320	21	8.89_{f}	0.686	179.20
Brain	0	21	5.03_{g} [1]	--	--
	32	20	4.83_{g}	--	--
	100	19	5.03_{g}	--	--
	320	21	4.99_{g}	--	--

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

[2] One spleen was lost during the necropsy.

Table 26. Dates of mortality and removals[1] of adult Mallards during the chronic test, 12/27/76 to 6/2/77 for DCPD and to 6/14/77 for DIMP

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
DCPD	32	F	5/8		11
	100	M	4/2		15
	100	F	5/10		2

[1]Birds 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.

DISCUSSION

Experiment 1

The most common expression for the acute toxicity of a compound is the LD_{50}. This standard of comparison is utilized because the dosage required to obtain 50 percent mortality in the animals tested is more reproducible than any other dosage; also it is more accurate, statistically, than any other percentage.

The Mallard LD_{50} for DIMP (1490 mg/kg) is, in general, higher than those reported for mammals. This value is in agreement with Howell (personal communication) where the LD_{50} value for Bobwhite was determined to 1000 $\pm$ 68 mg/kg. The quail LD_{50} is near the male mouse (1041 mg/kg) and male rat (1125 mg/kg) (Hart, 1976), while the duck LD_{50} range (1414 - 1566 mg/kg) is within the female mouse LD_{50} range (1165 - 1594 mg/kg) (Hart, 1976). The values for these animals places DIMP in the slightly toxic range, based on the following chart (Hodge and Sterner, 1949):

Term	Range (mg/kg)
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 the rats and mice dosed with DIMP, a difference by sex was found (Hart, 1976).

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 4 and 5). 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 substrates, such as glucose, glucagon, or amino acids which would affect the hypophysis (Leglercq-Meyer and Mialhe, 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 some of 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 after dosing, 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.

Ducks dosed with DCPD up to 40000 mg/kg[1] showed no terminal effects nor any body weight or feed consumption differences over the 14-day post-treatment period (Tables 4 and 5). This classifies DCPD in the relatively harmless range (see page 89). The LD_{50} (>40000 mg/kg) is more than 114 times the average mammalian LD_{50} of 350 mg/kg (Table 2) and more than 40 times the Bobwhite LD_{50} of 1010 mg/kg (Howell, personal communication). Therefore, the Mallard lies outside the general rule of response within a 10-fold

---

[1]For toxicity purposes administration of doses beyond 5000 mg/kg in the acute oral test is not of practical value. "Federal Register" (1975).

range. An exception in the mammals was found in mink by Aulerich (personal communication). Mink dosed orally were not affected other than regurgitation and/or suffocation on the liquid DCPD with levels up to 960 mg/kg. Mink were affected by intraperitoneal injections which caused 100 percent mortality at 960 mg/kg. It may be that DCPD is not absorbed from the gastrointestinal tract in any significant amounts in ducks and mink. Further tests with these compounds (DIMP and DCPD), tagged with radioactive tracers, are being conducted at this time to determine their metabolism and excretion by Bobwhite and Mallards.

These LD_{50} values for DIMP and DCPD of birds and mammals are in agreement with Grollean and Giban (1966); Tucker and Haegele (1971); and Machin et al., (1975), who reported that sensitivity in one species, as compared to others tested, did not differ from any other and that each species varied widely in its sensitivity to any one compound. Thus, Tucker and Haegele (1971) recommended ". . . that extrapolation of toxicity data from one species to another be avoided." In this same study it was shown that LD_{50}'s for 16 pesticides for the Mallard ranged from 2.13 mg/kg for Parathion[2] to 1130 mg/kg for Mobam.

The toxicity of five organophosphorus mosquito larvicides to Mallards was determined by Keith and Mulla

---

[2]Chemical names of all compounds listed in discussion are found in the reference cited.

(1966). LD_{50}'s ranged from 1-2 mg/kg for Baytex and Parathion to 400 mg/kg for SD-7438.

A list of compounds with LD_{50}'s from Tucker and Crabtree (1970) is presented in Table 27 along with LD_{50}'s of DIMP and DCPD 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 (LD_{50} of Standard/ LD_{50} of sample) x 100. For DIMP, the index is 25.57 and, for DCPD, the index is less than 0.95. As the route of administration is one of the most influential factors in modifying the LD_{50}, this index gives a more constant number for comparison between different routes of administration. Though DCPD did not kill ducks when administered in a single dose, this can be misleading. Coburn and Treichler (1946) could not kill ducks or starlings with a single dose of DDT, nor were robins killed by DDT in an LD_{50} study by Hickey and Hunt (1960). Yet, DDT has very toxic cumulative properties. Also, Dougherty (1962) could not kill Mallard ducklings with Korlan if they were allowed to regurgitate.

Experiment 2

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 LC_{50} test and the LD_{50} was

Table 27. Comparative LD_{50}'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	--[2]	12	M,F	1490 (1414-1566)
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	--[2]	12	M,F	>40000

[1] I = insecticide

[2] See Literature Review.

calculated at 1490 mg/kg. There was no lethality difference for DCPD.

Though an LC_{50} could not be determined in Mallards for either chemical, as DCPD treated ducks had only 30 percent maximum mortality in any one group and DIMP treated ducks had zero mortality, a point of zero feed consumption was reached for Mallards eating DCPD treated diet at about 77300 ppm and a zero intake for Mallards eating DIMP treated diet calculated at 23222 ppm. Both of these compounds are far above the normal maximum of 5000 ppm used in most LC_{50} testing. These undeterminable LC_{50}'s are in agreement with the findings of Howell (personal communication) where an LC_{50} could not be determined in Bobwhite on diets containing DIMP or DCPD, but neither was a zero feed consumption level determined with levels in the diet up to 36000 ppm for DIMP and up to 18000 ppm for DCPD because of near zero slope lines (no difference between the control and the group with the highest level of chemical in the diet) on feed consumption and body weight gains.

A comparison of LC_{50} values taken from Heath et al.,[3] (1972) is listed in Table 28. There are a number of compounds with no LC_{50} determinations, mostly in non-insecticides, as there was little or no mortatliy. In this same study, there was no mention of feed consumption for any

---

[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 28. Comparative LC_{50}'s from the literature, for Mallard ducklings two to three weeks old

Compound	Primary use	LD_{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	---[8]	16000[4,9]	
DCPD	---[8]	30% mortality at 60000[9]	

[1] I = insecticide

[2] 5-7 days old

[3] H = herbicide

[4] No mortality

[5] A = acaricide

[6] F = fungicide

[7] L = lampricide

[8] See Lieterature Review

[9] 11-13 days old

compound nor any predicted zero consumption values. Mallards in comparison to Bobwhite, Japanese quail, and pheasants were less sensitive to 14 organochlorines and 15 organophosphates (Heath et al., 1972). The organophosphates are generally less toxic in the diet than aldrin or dieldrin; of 23 organophosphate compounds tested on Mallards, only two were more toxic (Heath et al., 1972). Of 12 compounds listed in both Tables 27 and 28, placed 1 to 12 in order of relative toxicities (see Tables 29), DIMP and DCPD overall placed low on the list; thus, they are less toxic in comparison to most other compounds used commercially.

For ducklings treated with DCPD, decreased food consumption at levels above 20000 ppm was probably due to a refusal to eat the very high concentrations of chemical (odor was very strong) and not due to an altered appetite. When placed on clean feed, they consumed more than the control group (Figures 2 and 5). If appetites were affected by DCPD then its effect must have had a very short duration, as there was no intake effect during post-treatment. All ducklings that consumed feed with more than 20000 ppm DCPD when put on regular feed increased their intake above the control. This increase in consumption was apparently an attempt to compensate for their lack of intake during the preceding five days of subjection to a treated diet (Figure 5).

Table 29. Overall toxicity of DCPD and DIMP compared with 10 commercial compounds

Chemical name	LD_{50} placing[1]	LD_{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

[1]Descending order ot toxicity in comparison to each other; 1 = most toxic, 12 = less toxic.

The DCPD treated repeat group of Mallards showed no effect until the level of DCPD in the diet reached 1000 ppm (Table 8) at which level body weight was lost; though feed consumption was not affected by any level. This finding may have been because these ducks were older and were not affected by the chemical via repulsion or decreased appetite, but by some unknown mechanism causing decreased uptake of nutrients. A decreased uptake could be at either the intestine, by slowing absorption of nutrients, or the liver, where enzyme activity may be decreased; thus not allowing for enough endogenous constituents to be available for both conjugation and normal growth (Dinman, 1974).

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 LD_{50} level (Table 9), i.e., 10000 ppm and greater. Fitzhugh and Schouboe (1965) reported that it is unusual for animals to tolerate more than the LD_{50} 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 was probably just a refusal to eat the diets containing higher concentrations of chemical (1.0 - 1.6 percent) in the diet. This decrease in feed consumption is similar to that observed under DCPD treated ducks where percentages

in the diet were 1.0 to 9.0 percent and a decrease in consumption was noted at all levels. 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 10); 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 6 and 8). 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.

Body weights for ducklings treated with DCPD were not affected in the same manner as was feed consumption. All groups fed over 10000 ppm lost weight with the 20000 ppm group losing the most even though they ate more than any higher concentration group (Figure 4). If the chemical had affected uptake of nutrients, then the 30000 and 60000 ppm groups should have lost as much, if not more weight, than the 20000 ppm group as they took in more mg/kg/day of

the chemical (Table 6). During the post-treatment period, all groups previously on DCPD, except 10000 ppm, were more efficient in their feed utilization (Table 7) than the control as the feed consumption/body weight gain ratio was less than the control. All groups above 40000 ppm had feed efficiencies of less than 1.60 or at least 4.75 times better than the control.

In the repeat group of DCPD treated Mallards, the loss of weight in the three highest levels, 1000, 5000, and 10000 ppm, was proportional to the ppm in the diet. The 5000 ppm group, which is 5 times the 1000 ppm level, lost 4.96 times as much weight as the 1000 ppm group and the 10000 ppm group which is 10 times the 1000 ppm level, lost 12.4 times as much weight as the 1000 ppm group. There was also a constant proportion of the mg/kg/day chemical ingested and weight loss of 3.95, 4.28, and 3.85 times for 1000, 5000, and 10000 ppm, respectively.

Experiment 3

In contrast to the subactue 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 11 and 12); increased intake during the reproductive period to accomodate for the increase in metabolism and decreased intake as

production terminated (Scott et al., 1969). The ducks receiving 3200 and 10000 ppm levels of DIMP consumed more feed than 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 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 an energy requirement, they would tend to consume more feed to meet their requirement (Scott et al., 1969).

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 and DCPD 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., 1969).

Total number of eggs laid for all hens on all treatments of DIMP and DCPD was 4958 in 77 days with an average of 42.2 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 is 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. All seven other groups, three DIMP and four DCPD, were consistent with each other during the entire period (Figure 13 and 14). The high level treatment groups, 100 and 320

ppm, of DCPD showed a decrease in egg production during week 3, for unknown reasons, then recovered completely by the next week. The overall increase in egg numbers as compared to previous reports may be that every egg was collected as they were in cages rather than outside 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 (körperschutteln), wing-shake (Flugelschutteln), head-shake (köpfshutteln), 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; Barash, 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 either compound 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	DCPD
	---------- % ----------		
Cracked	2.6-3.0 5.0 7.0-11.9	3.2-6.2	2.7-5.0
Fertile	50-100 75-89 81-89	65-89	72-91
Hatched	52-74 61-73 63-68	55-73	62-70

Live three-week embryos were not measured for two reasons: (1) to reduce time out of the incubator and (2) the difficulty involved in accurately determining which embryos had died. Greatest mortality during incubation occurred from approximately the 19th day until hatching as was noted by percent dead in shell (Tables 14 and 15). 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 16) and was within the range of normal values of 94 to 99 percent stated in the "Federal Register" (1975). However, lower values have been reported for ducks in other experiments, but they will not be mentioned because of the high values obtained in this experiment.

Hemoglobin gives an indication of the blood's oxygen carrying capacity since one gram of hemoglobin can combine with 1.34 ml of O_2 (Sturkie, 1976). Mean hemoglobin values of drakes treated with DIMP ranged from 12.7 to 13.1 gm/dl and from 11.9 to 12.9 gm/dl for DCPD treated males. Mean hemoglobin values for hens treated with DIMP ranged from 12.8 to 13.1 gm/dl and from 11.6 to 12.4 gm/dl for DCPD treated females (Table 17). 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 of 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 and the DCPD treated group of ducks of 9.0 to 15.0 gm/dl. Hemoglobin values of diving ducks are higher, as is expected for those species, 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 and from 41.5 to 44.67 percent for drakes treated with DCPD. For the

hens treated with DIMP values ranged from 44.0 to 45.9 percent and from 42.17 to 43.86 percent for hens treated with DCPD. 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 and DCPD treated Mallards are comparable to the Mallard values reported by Sturkie (1976) and Hemm and Carlton (1967), while the hematocrit ranges of ducks treated with DIMP of 32.0 to 51.0 percent and of ducks treated with DCPD of 35.25 to 51.5 percent were 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 MCHC value is higher than the means for Mallards treated with DIMP of 29.2 percent and of Mallards treated with DCPD of 28.2 percent but is within the range of the ducks treated with DIMP 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 1/2-4 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 cited in Sturkie (1976) had the closest leukocyte count in comparison to the Mallards treated with DCPD or DIMP while the other authors cited indicated a higher heterophil count. There were more lymphocytes than heterophils in the DIMP and DCPD treated ducks, which is generally true for most avian species (Sturkie, 1976). DIMP and DCPD 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 1/2-4 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 actue phase early in the experiment. 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 and DCPD 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 rod shaped while eosinophil'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 treatments of both DIMP and DCPD. 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	Organ weights as a percent of body weight					
	15-week-old Mallards	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	-	0.057	0.056	0.19	0.12
Pancreas	0.22	0.60	0.31	0.31	0.32	0.29
Spleen	-	0.10	0.053	0.05	0.053	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, as a percent of body weight, were consistently twice the Mallards, while the 15-week-old Mallards were similar to the DIMP and DCPD 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 groups.

Negative finding (non-significant differences) are encouraging but they do not show, in themselves, non-toxicity of a chemical. Since DIMP and DCPD are both fat and organic soluble, they may tend to accumulate in fatty tissue of the animal and be held in storage until the fat is used for metabolic purposes or the chemical metabolized into a water soluble derivative. An evaluation of the accumulated effects of the chemicals over several generations should be performed. Also, since plants are affected by DIMP and DCPD separately and together synergically,

(Ringer, personal communication), then a test of the effect of plants grown on soil treated with the compounds and fed to the ducks might be performed.

Saturation dosage (SD) in the air as calculated by the formula from Kenaga (1968) is:

$$\frac{\text{(vapor pressure at "y"°C) x (Molecular wt.) x 1000}}{\text{(air pressure in mm) x (factor 0.082) x (273 + "y"°C) x 16}} = \text{SD}$$

Saturation dosage is given in pounds per 1000 cubic feet at "y"°C. For DIMP; vapor pressure at 10°C equals 0.0768 mmHg, molecular weight equals 180, thus, the saturation dosage equals 1.241 lb/1000 cu. ft. at 10°C. For DCPD; vapor pressure at 10°C equals 47.6 mmHg, molecular weight equals 132, thus, SD equals 564.1 lb/1000 cu. ft. at 10°C. Because of these high values, especially for DCPD, inhalation test should be performed.

CONCLUSIONS

LD_{50}: DIMP is slightly toxic to ducks considering mortality, body weight changes, and feed consumption. DCPD is relatively harmless as it did not effect the animals except for feed consumption the first week after dosing.

LC_{50}: Ducks on DCPD reached zero (essentially) feed consumption at about 70000 ppm but mortality only reached 30 percent at 60000 ppm. Thus, they probably cannot ingest enough chemical to reach an LC_{50}. Ducks on DIMP showed decreasing body weight but no mortality occurred. Thus, they may not be able to ingest enough of the compound to cause mortality.

Chronic: Only DIMP affected the ducks and only in feed consumption at 3200 ppm and egg production 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, blood parameters or mortality of adults. Even though no effects were found, some positive findings might be obtained by using a different criteria of toxicity, prolongation of the test or a different species.

APPENDICES

APPENDIX A

CHEMICAL STRUCTURES AND ALTERNATE NAMES FOR DIMP AND DCPD

APPENDIX A

CHEMICAL STRUCTURES AND ALTERNATE NAMES FOR DIMP AND DCPD

DIMP

Structural formula

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

Alternative names

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

DCPD

Structural formula

$$C_{10}H_{12}$$

Alternative names

Dicyclopentadiene; Bicyclopentadiene; Biscyclopentadiene; 3a,4,7,7a-Tetrahydro-4,7-Methanoindene.

APPENDIX B

ANALYSIS OF FEED

APPENDIX B

ANALYSIS OF FEED

Table A1.

Feed[1]	Crude Protein Not < Percent	Crude Fat Not < Percent	Crude Fiber Not> Percent
Duck Starter	19	3	6
Breeder Developer	14	2.5	10
Breeder Layer	17	2.5	7.5

All feed obtained from Ralston Purina Co., 5620 Millett Road, Lansing, Michigan 48917.

[1]Complete analysis not available, these feeds are in a closed book formula (privileged information).

APPENDIX C

RATION PREPARATION

APPENDIX C

RATION PREPARATION

Experiment 2

Ducklings. A pre-mix of DCPD or DIMP was prepared by adding the 97 percent pure chemical to corn oil and mixing this by hand to duck starter ration. The final individual diets were prepared in 0.4 to 4 kg quantities (depending on predicted amount, from range finding test, to be consumed) by combining a quantity of pre-mix with the duck starter ration (Table C1). All final ration mixing was done on a Paul G. Abbe feed mixer[2] by tumbling the mixture for 15 minutes in a seven kilogram capacity feed can. The total amount of chemical-corn oil solution was not more than 2 percent of the diet containing DIMP.

Young male ducks. For the DCPD repeat group of ducks the diets were made by adding the chemical-corn oil solution to the duck breeder developer ration and mixing in seven kilogram capacity feed cans on a Paul G. Abbe, Inc., feed mixer. Total chemical-corn oil mixture was approximately two percent of the four kilogram diets made (Table C2).

---

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

Table C2.

DCPD (gms)	Corn oil (gms)	Feed (gms)	Total (Kg)	ppm
0.00	80	3920	4	0
0.04	80	3920	4	10
0.4	79.6	3920	4	100
4.0	76	3920	4	1000
20.00	60	3920	4	5000
40.00	40	3920	4	10000

Table C1.

Premix

Chemical	Amount (gms)	Feed (gms)	Total (gms)	ppm
DCPD	400	3600	4000	100000
DIMP	100	4900	5000	20000

Diets

Chemical	Premix (gms)	Feed (gms)	Total (gms)	ppm
DCPD	400	3600	4000	10000
	600	2400	3000	20000
	600	1400	2000	30000
	400	600	1000	40000
	250	250	500	50000
	300	200	500	60000
	350	150	500	70000
	400	100	500	80000
	450	50	500	90000
DIMP	400	3600	4000	2000
	800	3200	4000	4000
	900	2100	3000	6000
	1200	1800	3000	8000
	200	200	400	10000
	240	160	400	12000
	280	120	400	14000
	320	80	400	16000
	360	40	400	18000

Experiment 3

Diets were made by adding a chemical-corn oil solution to the duck breeder developer or breeder layer ration and mixing in a Mix-mill[3] for 25 minutes. Only 80 kg of diet were prepared at a time so that the diets would be fresh at all times, (Table C3). A pre-mix was not made since the chemical-corn oil solution was found to be well distributed on the pelleted feed in the Mix-mill.

Table C3.

Chemical	Amount Added (gms)	Oil Added (gms)	Feed (kg)	Total (kg)	ppm
DCPD	0.0	1600	78.4	80	0
	2.56	1597	78.4	80	32
	8.0	1592	78.4	80	100
	25.6	1574	78.4	80	320
DIMP	0.0	1600	78.4	80	0
	80.0	1520	78.4	80	1000
	256.0	1344	78.4	80	3200
	800.0	800	78.4	80	10000

[3]Mix-mill, Incorporated, Bluffton, Indiana 46714

APPENDIX D

PREPARATION OF DRABKIN'S REAGENT

APPENDIX D

PREPARATION OF DRABKIN'S REAGENT

1000	mg	Sodium Bicarbonate $NaHCO_3$
50	mg	Potassium Cyanide KCN
200	mg	Potassium Ferricyanide $K_3Fe(CN)_6$
1250	mg	

Mix to dissolve and dilute to 1 liter.

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

APPENDIX E

DETERMINATION OF HEMOGLOBIN CONCENTRATION

Figure 15. Sample hemoglobin concentration calculation. The line is constructed by plotting the percent absorbance of each standard against its known hemoglobin concentration.

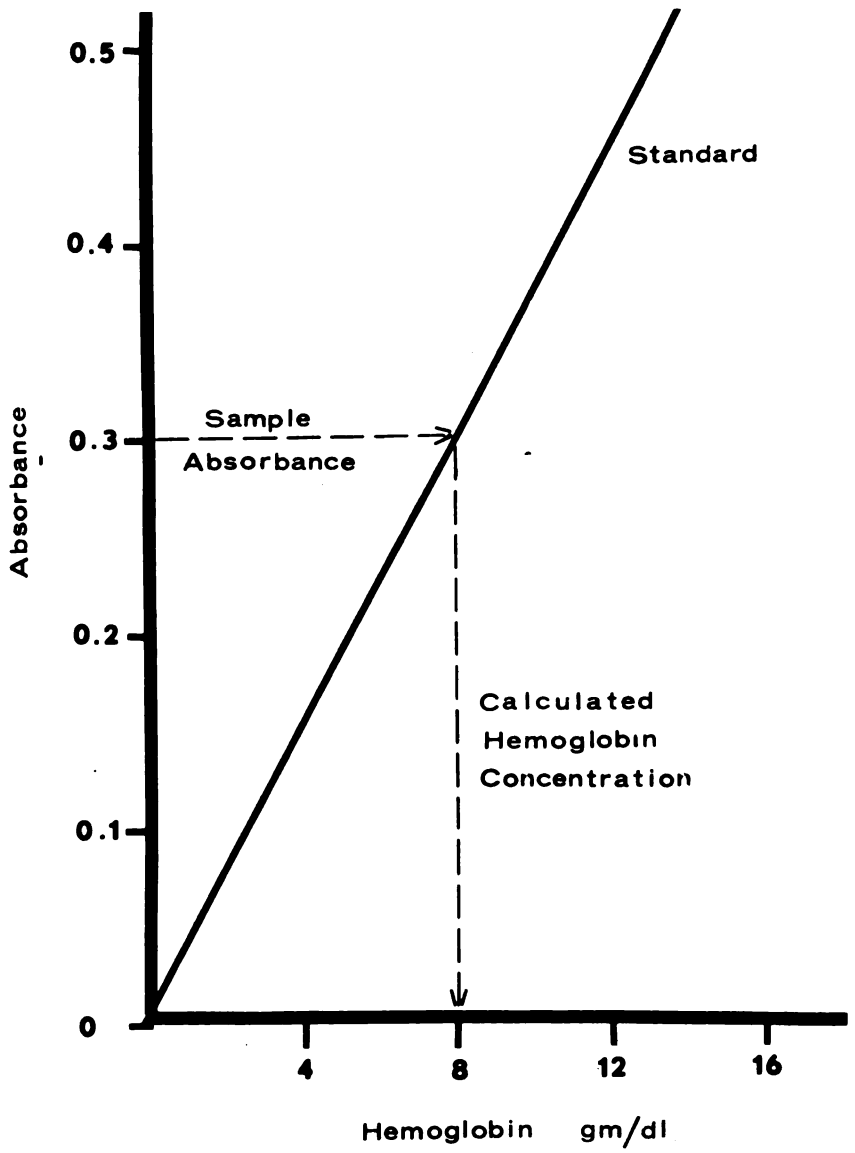
0.5
0.4
0.3
0.2
0.1
0
Absorbance
Standard
Sample
Absorbance
Calculated
Hemoglobin
Concentration
4
8
12
16
Hemoglobin gm/dl

FIGURE 15

APPENDIX F

PREPARATION OF WRIGHT'S STAIN AND BUFFER

APPENDIX F

PREPARATION OF WRIGHT'S STAIN AND BUFFER

Wright's Stain

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

Buffer

3.80 gm Na_2HPO_4

5.47 gm KH_2PO_4

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

BIBLIOGRAPHY

BIBLIOGRAPHY

Altman, P. L. and D. S. Dittmer, 1964. Biology Data Book. Federation of American Societies for Experimental Biology. Washington D.C.

Aulerich R., Personal communication.

Barash, D. P., 1977. Sociobiology of rape in Mallards (Anas platyrhynchos): responses of the mated male. Science, 197 (4305):788-89.

Carlton, W. W., 1966. Experimental coal tar poisoning in the white Pekin duck. Avian Diseases, 10:484-502.

Christensen, H. E., T. T. Lugenbyth, and B. S. Carroll, 1974. The Toxic Substance List. U.S. Department of Health, Education, and Welfare.

Coburn, D. R. and R. Treichler, 1946. Experiments on toxicity of DDT to wildlife. J. Wildl. Man., 10 (3):208-16.

Coles, E. H., 1974. Veterinary Clinical Pathology. 2nd. ed. W. B. Saunders Co., Philadelphia, Pa.

Dacre, J., 1975. Fact Sheet-DIMP Toxicity. U. S. Army Chemical Center, Maryland.

Dahlen, J. H. and A. O. Haugen, 1954. Acute toxicity of certain insecticides to the Bobwhite quail and Mourning Dove. J. Wildl. Man., 18(4):477-81.

Davison, K. L. and J. L. Sell, 1974. DDT thins shells of eggs from Mallard ducks maintained on ad libitum or controlled-feeding regimes. Arch. Environ. Contam. Toxicol., 2(3):222-32.

Desforges, M. F. and D. G. M. Wood-Gush, 1975a. A behavioural comparison of domestic and Mallard ducks: Habituation and flight reactions. Anim. Behav., 23:692-97.

Desforges, M. F. and D. G. M. Wood-Gush, 1975b. A behavioural comparison of domestic and Mallard ducks: Spatial relationships in small flocks. Anim. Behav., 23:698-705.

Desforges, M. F. and D. G. M. Wood-Gush, 1976. Behavioural comparison of Aylesbury and Mallard ducks: Sexual behaviour. Anim. Behav., 24:391-97.

Dinman, B. D., 1974. Chemical agents and disease. In Pathologic Physiology. 5th ed. W. A. Sodeman, Jr. and W. A. Sodeman, ed. W. B. Saunders, Philadelphia, Pa.

Dougherty, E., III, 1962. Fly sprays can poison ducks. Farm Res., 28(1):14.

Federal Register, Wednesday, June 25, 1975. Environmental Protection Agency; Pesticide program, guidelines for registering pesticides in United States. Vol. 40, No. 123, Part II.

Fitshugh, O. G. and P. J. Schouboe, 1965. Subacute toxicity. In Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Association of Food and Drug Officials of the United States, P.O. Box 1494, Topeka, Kansas.

Ford-Moore, A. H. and B. J. Perry, 1948. The chemistry of the alkane fluorophosphonates: Part VI The dialkanepyrophosphonates. Summary of Porton Technical Paper No. 68. U. S. Army Chemical Center, Maryland.

Frawley, J. P., E. C. Hagan, and O. G. Fitzhugh, 1952. A comparative pharmacological and toxicological study of organic phosphate-anticholinesterase compounds. J. Pharmacol. Exp. Ther., 105:156-65.

Gage, J. C., 1970. The subacute inhalation toxicity of 109 industrial chemicals. Brit. J. Industr. Med., 27:1-18.

Gasaway, W. C. and S. O. Buss, 1972. Zinc toxicity in the Mallard duck. J. Wildl. Man., 36(4):1107-17.

Gill, J. L., 1977. Unpublished work.

Grandy, J. W., L. N. Locke, and G. E. Bagley, 1968. Relative toxicity of lead and five proposed substitute shot types to pen-reared Mallards. J. Wildl. Man., 32(3):483-88.

Grollean, G. and J. Giban, 1966. Toxicity of seed dressings to game birds and theoretical risks of poisoning. J. Appl. Ecol., 3(supplement):199-212.

Hafez, E. S. E., 1974. Reproduction in Farm Animals. Lea and Febiger, Philadelphia, Pa.

Hart, E. R., 1976. Unpublished data.

Hawkes, M. P. G. and J. C. George, 1975. Effects of hypothalamic lesions on levels of plasma free fatty acids in the Mallard duck. Arch. Int. Physiol. Biochem., 83:763-70.

Heath, R. G. and J. W. Spann, 1973. Reproduction and related residues in birds fed Mirex. In Pesticides and the Environment: A continuing controversy.

Heath, R. G., J. W. Spann, E. F. Hill, and J. F. Kreitzer, 1972. Comparative Dietary Toxicities of Pesticides to Birds. U. S. Fish and Wildlife Service Special Scientific Report-Wildlife. No. 152, 57pp.

Heath, R. G., J. W. Spann, and J. F. Kreitzer, 1969. Marked DDE impairment of Mallard reproduction in controlled studies. Nature, 224(5214):47-48.

Heath R. G., and L. F. Stickel, 1965. Protocol for testing the acute and relative toxicity of pesticides to penned birds. In The Effects of Pesticides on Fish and Wildlife. U. S. Fish and Wildlife Circular 226.

Heinz, G., 1974. Effects of low dietary levels of methyl mercury on Mallard reproduction. Bull. Env. Cont. Toxicol., 11:386-92.

Hemm, R. and W. W. Carlton, 1967. Review of duck hematology. Poultry Sci., 46(4):956-62.

Hickey, J. J. and L. B. Hunt, 1960. Initial songbird mortality following a Dutch elm disease control program. J. Wildl. Man., 24(3):259-65.

Hill, E. F., 1971. Toxicity of selected mosquito larvicides to some common avian species. J. Wildl. Man., 35(4): 757-62.

Hodge, H. C. and J. H. Sterner, 1949. Tabulation of toxicity classes. Am. Ind. Hyg. Assoc. Quart., 10:93.

Horton, R. G., 1948. Screening Candidate Chemical Agents. U. S. Army Chemical Center, Maryland.

Howell, K. Personal communication.

Irby, H. D., L. N. Locke, and G. E. Bagley, 1967. Relative toxicity of lead and selected substitute shot types to game farm Mallards. J. Wildl. Man., 31(2): 253-57.

Jacobson, K. H., 1953. Report No. 17. The Acute Toxicity of Some Intermediates in GB Manufacture, U. S. Army Chemical Center, Maryland.

Karmann, H. and P. Mialhe, 1973. Glucose-glucagon feedback mechanism in normal diabetic geese and ducks. Diabetologia, 9:74.

Keith, J. O. and M. S. Mulla, 1966. Relative toxicity of five organophosphorus mosquito larvicides to Mallard ducks. J. Wildl. Man., 30(4):553-63.

Kenaga, E. E., 1968. Guidelines for evaluating the properties of pesticides for safe use in the wildlife environment. Down to Earth, 23(4):11-18.

Kinkead, E. R., U. C. Pozzani, D. L. Geary, and C. P. Carpenter, 1971. The Mammalian toxicity of dicyclopentadiene. Tox. and Appl. Pharmaco., 20:552-61.

Laurent, F. and P. Mialhe, 1976. Insulin and the glucose-glucagon feedback mechanism in the duck. Diabetologia, 12:23-33.

Lebret, T., 1961. The pair formation in the annual cycle of the Mallard, Anas platyrhynchos L., Ardea, 49: 97-158.

Leclercq-Meyer, V. and P. Mialhe, 1970. Hypophysectomy and glucagon secretion in the duck. Diabetologia, 6:636.

Litchfield, L. J. and F. Wilcoxon, 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Theo., 96(2):99-113.

Longcore, J. R., F. B. Sampson, and T. W. Whittendale Jr., 1971. DDE thins eggshells and lowers reproductive success of captive black ducks. Bull. Env. Cont. Toxicol., 6:485-90.

Lucas, A. M. and C. Jamroz, 1961. Atlas of Avian Hematology. Agric. Monogr. 25, U. S. Dept. Agric., Washington, D.C.

Machin, A. F., H. Rogers, A. J. Cross, M. P. Quick, L. C. Howells, and N. F. Jones, 1975. Metabolic aspects of the toxicology of Diazinon I. Hepatic metabolism in the sheep, cow, pig, guinea-pig, rat, turkey, chicken, and duck. Pestic. Sci., 6:461-73.

McKinney, F., 1965. Comfort movements in the Anatidae. Behavior, 25:120-220.

McKinney, F., 1975. The evolution of duck displays. In Function and Evolution in Behavior. Oxford University Press, London.

McPhail, M. K. and P. A. Adie, 1960. The distribution of radioactive phosphorus in the blood and tissues of rabbits treated with tagged isopropyl methylphosphonofluoridate (sarin). Can. J. Biochem. and Phy., 38:945-51.

Peckham, M. C., 1972. Poisons and Toxins. In Diseases of Poultry. The Iowa State University Press, Ames, Iowa.

Prince, H. H., P. B. Siegel, and G. W. Cornwell, 1968. Embryonic growth of Mallard and Pekin ducks. Growth, 32:225-33.

Prince, H. H., P. B. Siegel, and G. W. Cornwell, 1969a. Hatchability, clutch position, and hatching sequence in Mallards. The Auk, 86(4):762-63.

Prince, H. H., P. B. Siegel, and G. W. Cornwell, 1969b. Incubation environment and the development of Mallard embryos. J. Wildl. Man., 33(3):589-95.

Prince, H. H., P. B. Siegel, and G. W. Cornwell, 1970. Inheritance of egg production and juvenile growth in Mallards. The Auk, 87(2):342-52.

Ringer, R. K. Personal communication.

Samsel, J., P. Mialhe, and H. Karmann, 1972. Effect of arginine on secretion of pancreatic glucagon, intestinal glucagon and insulin in the duck. Diabetolgia, 8:65.

Scott, M. L., M. C. Nesheim, and R. J. Young, 1969. Nutrition of the Chicken. Scott and Associates, Publishers, Ithaca, New York.

Shashkina, L. F., 1965. Materials for substantiation of maximum permissible concentration of cyclopentadiene and of its dimer-dicyclopentadiene-in the atmosphere of industrial premises. Gigiena Truda i Prof. Zabolevaniya, 9:13-19.

Sherman, M., E. Ross, and M. T. Y. Chang, 1964. Acute and subacute toxicity of several organophosphorus insecticides to chicks. Toxicol. Appl. Pharmacol., 6:147-53.

Smyth, H. F., C. P. Carpenter, C. S. Weil, U. C. Pozzani, and J. A. Striegel, 1962. Range-finding toxicity data: List VI. Amer. Indus.

Stickel, W. H., W. E. Dodge, W. G. Sheldon, J. B. DeWitt, and L. F. Stickel, 1965. Body condition and response to pesticides in Woodcocks. J. Wildl. Man., 29(1): 147-55.

Sturkie, P. D., 1976. Avian Physiology. 3rd ed. Springer-Verlag, New York, N. Y.

Sun, Yun-Pei, 1950. Toxicity index-an improved method of comparing the relative toxicity of insecticides. J. of Econ. Entomol., 43(1):45-53.

Tucker, R. K. and D. G. Crabtree, 1970. Handbook of toxicity of pesticides to wildlife. U. S. Bureau of Sport Fisheries and Wildlife, Resource Publ. 84.

Tucker, R. K. and M. A. Haegele, 1971. Comparative acute oral toxicity of pesticides to six species of birds. Toxicol. Appl. Pharmacol., 20:57-65.

GENERAL REFERENCES

Ariens, E. J., A. M. Simonis, and J. Offermeier, 1976. Introduction to General Toxicology. Academic Press, New York, N. Y.

Boyland, E. and R. Goulding, 1968. Modern trends in Toxicology I. Appleton-Century-Crofts, New York, N. Y.

DuBois, K. P. and E. M. K. Geiling. 1959. Textbook of Toxicology. Oxford University Press, Inc., New York, N. Y.

Goodman, L. S. and A. Gilman, 1975. The Pharmacological Basis of Therapeutics. MacMillan Publishing Co., Inc., New York, N. Y.

Jones, L. M., N. H. Booth, and L. E. McDonald, 1977. Veterinary Pharmacology and Therapeutics. The Iowa State University Press, Ames, Iowa.

Loomis, T. A., 1968. Essentials of Toxicology. Lea and Febiger, Philadelphia, Pa.