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THE EFFECT OF CHRONIC DIETARY ADMINISTRATION OF FLURIDONE ON SELECTED REPRODUCTIVE PARAMETERS OF BOBWHITES AND MALLARDS

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THE EFFECT OF CHRONIC DIETARY ADMINISTRATION OF FLURIDONE ON SELECTED REPRODUCTIVE PARAMETERS OF BOBWHITES AND MALLARDS

By

Christine Flaga

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ABSTRACT

THE EFFECT OF CHRONIC DIETARY ADMINISTRATION OF FLURIDONE ON SELECTED REPRODUCTIVE PARAMETERS OF BOBWHITES AND MALLARDS

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Christine Flaga

Four dietary concentrations of fluridone (1-methyl-3-phenyl-5-[3-(triflouromethyl-phenyl]-4(1H)-pyridinone) were fed to Bobwhites and Mallards to evaluate its effect on food consumption, body weight gain, and several reproductive parameters. Fluridone was fed at 0 ppm (control), 100 ppm (0.01%), 300 ppm (0.03%), and 1,000 ppm (0.10%) for approximately six months.

Chronic dietary administration of fluridone to Bobwhites and Mallards did not significantly affect mortality, food consumption, body weight gain, egg production, fertility, embryo survival, hatchability, offspring survivability, or eggshell thickness.

ACKNOWLEDGMENTS

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INTRODUCTION

The U.S. Environmental Protection Agency (EPA) has proposed, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), that avian reproduction studies be required to support the registration of a formulated pesticide if there exists any suggestion of bioaccumulation or chronic exposure to the avian species, especially during the breeding season (Federal Register, 1978). Mallards and Bobwhites, which are examples of a waterfowl and an upland game avian species, respectively, were indicated as test animals of choice.

This particular study was performed to determine the potential reproductive toxicity of chronic dietary exposure of fluridone to Bobwhites and Mallards. No such data had been available previously. Fluridone is an experimental herbicide produced by Eli Lilly and Company (Elanco).

LITERATURE REVIEW

1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1H)-pyridinone (fluridone) is an experimental herbicide developed by Eli Lilly and Company, Indianapolis, IN for use in aquatic plant management and in cotton fields. It is a white, odorless, crystalline solid with a molecular weight of 329.3. Fluridone melts at 151-154°C and is soluble in water at 12 ppm. See Appendix A for its chemical structure and a list of its solubilities in organic solvents. Fluridone has an n-octanol/water partition coefficient of 57.5 (log K_{ow} = 1.76) and a vapor pressure of 1 x 10^{-7} mm Hg at 25°C (Lilly, 1978).

Analytical Procedures

Methods for residue analysis from soil and plant tissue are discussed by West (1978).

West and Burger (1980) have described a gas liquid chromatographic method for determining the residues of fluridone and its major metabolite (1-methyl-3-(4-hydroxyphenyl)-5-[3-(trifluoromethyl)phenyl]-4-(1H)-pyridinone) in fish. Bioassay and gas chromatographic procedures were evaluated for their effectiveness in detecting fluridone from soil (Banks et al., 1979).

Herbicidal Properties

Waldrep and Taylor (1976) initially evaluated fluridone for its pre- and postemergence herbicidal efficacy on several weed species in cotton. They found it to be herbicidally active at low dosages for weeds, but cotton was relatively

resistant to its herbicidal action. Its herbicidal activity was greater when applied preemergence, controlling a wide variety of annual grasses and broadleaf weeds. Susceptible plants treated preemergence with fluridone emerged with chlorotic leaves which became necrotic, subsequently leading to plant death. The authors characterized fluridone as a slow-acting, translocated herbicide which appeared to inhibit chlorophyll synthesis. Specifically, fluridone inhibits carotenoid synthesis (Bartels and Watson, 1978) leading to chloroplast photodestruction and the observed loss of chlorophyll.

Plants susceptible to fluridone translocate the herbicide readily into the shoots (Berard et al., 1978). Cotton tolerates the herbicide primarily because of fluridone's limited translocation in this plant species. Any absorbed fluridone is retained in the roots and basal region of the stem. Root and shoot tissues from sago and Richardson pondweed concentrated fluridone with limited root to shoot translocation but negligible shoot to root transport (Marquis et al., 1981).

Sanders et al. (1981) reported a reduction in hydrilla biomass 84 days after treatment with $\geq$ 1.7 kg of fluridone/ha, while insufficient control occurred in plots treated at 0.84 kg/ha.

Persistence in Soils.

Fluridone strongly adsorbs to organic matter in soils. Soil microorganisms do not appear to be a major factor in its

dissipation. In cotton-producing areas, residues of fluridone may carry over to the next planting season causing slight injury to sorghum, soybeans, sugar beets, and tomatoes that follow in rotation (Weed Science Society of America, 1979).

Banks and Merkle (1979) reported that fluridone did not leach more than 1 cm in clay or sandy loam soils when up to 10 cm of water was passed through a soil column containing fluridone. Greater downward mobility (12 to 17 cm) occurred in a coarse sand when 5 or 10 cm of water was passed through a soil column.

Banks et al. (1979) determined the residue of fluridone in Miller clay and Lufkin fine sandy loam at various intervals after incorporated and nonincorporated field application. Incorporation increased fluridone's persistence in Lufkin fine sandy loam but not in Miller clay. Miller clay retained a low level of fluridone after 250 days, while up to 25% remained in Lufkin fine sandy loam after 285 days.

Persistence in Pond Water.

In 1979, fluridone's use as an aquatic herbicide was researched by W. R. Arnold of the Lilly Research Laboratories (Arnold, 1979). Fluridone was applied at various rates as a surface or bottom treatment to ponds. Little weed control was noticed until two to four weeks after treatment. Mature vegetation slowly decomposed and sank to the bottom gradually increasing the percentage of open water. Applied at 1.68 kg/ha, fluridone provided excellent control of hydrilla, common elodea, southern naiad, cattail, para grass, and

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several other species without adversely affecting phytoplankton, benthic organisms, or fish. Bluegills taken from the treated ponds were analyzed for fluridone residues which were detectable until 29 days after treatment (0.031 ppm with application of 1.68 kg/ha). At no time did the residue level in the fish exceed the concentration in the water. Observations, before and after treatment, were made of other aquatic organisms including crayfish, bass, catfish, turtles, frogs, watersnakes, and waterfowl. No adverse effects were observed in these species. It was also noted that a variety of waterfowl and shore birds continued to feed at the treated ponds. Determination of the fluridone content of water and hydrosoil indicated that most of the fluridone dissipated from the water into the hydrosoil. Minor additional dissipation was attributed to uptake by aquatic organisms and photodegradation.

McCowen et al. (1979) reported similar results when fluridone was applied to ponds by various methods at rates resulting in concentrations of 0.1 to 10 ppm. Fluridone provided control of many submersed and emersed aquatic plants. Residue levels were determined in water, hydrosoil, and fish. At an application rate resulting in a concentration of 0.1 ppm, fluridone residue levels were determined 54 days after application at < 0.0005 ppm and at 0.035 ppm in water and hydrosoil, respectively. One week after treatment, fluridone levels in water and hydrosoil were 0.021 and 0.220 ppm respectively. The half-life (t 1/2) of fluridone in ponds was determined to be 14 days or less. Fluridone was not

found to accumulate in fish. Mosquito fish (G. affinis) survived and reproduced at all rates of fluridone application. No adverse effects upon other aquatic life were observed.

Both of the above studies indicated that fluridone did not affect water quality parameters such as pH, BOD, color, dissolved solids, hardness, nitrate nitrogen, total phosphates, or turbidity.. Arnold (1979) did report an increase in the dissolved oxygen concentration but McCowen et al. (1979) reported no significant change.

West et al. (1979) applied fluridone to small ponds in the U.S. and Gatun Lake in the Panama Canal Zone. Fluridone dissipated rapidly from the water and the authors credited it to deposition in hydrosoil and uptake by aquatic plants, with photolysis as an additional contributing factor. The half-life of fluridone in pond water averaged 5 days. In fish, a maximum fluridone residue of 0.054 ppm was determined one day after treatment (DAT), decreasing steadily to a non-detectable level on 14 DAT. This data was determined from subsurface application of fluridone (formulated as a 4 lb./ gallon aqueous suspension) at a rate of 0.1 ppm relative to the total water column (equivalent to 1.12 kg/ha). The bioconcentration factor for fish ranged from 0 to 1.7 (determined as the concentration in fish divided by the concentration in the water). Bioconcentration was also very low in zooplankton and aquatic plants.

In another study (Muir et al., 1980), fluridone was applied to three small ponds at 70, 700, and 5,000 µg/l.

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Residue analysis was conducted on water, hydrosoil, duckweed, and minnows for 70 weeks after application. The half-life of fluridone in the water column (0.5 m depth) ranged from 4 (at 700 µg/l) to 7 days (70 µg/l). Results indicated that fluridone has a half-life of one year or more in the hydrosoil (at all treatment levels). Fluridone residue levels in minnow tissue ranged from < 0.02 to 0.14 µg/g (wet weight) throughout the sampling period, with bioconcentration factors ranging from 0 to 64. Fluridone levels in duckweed were proportional to the herbicide concentrations in the pond water with bioconcentration factors ranging from 19 to 85.

Other dissipation information was reported by West and Parka (1981). Fluridone was applied at 0.84 kg/ha of surface water to the surface and bottom of ponds. Half-lives in the water column were determined to be 21 and 26 days (surface and bottom application, respectively). No detectable residue remained in the hydrosoil 56 days after treatment by either method of application. Similar water and hydrosoil dissipation results were observed by Sanders et al. (1981). They stated that less than 15% of the applied compound remained after 56 days. Fluridone had been applied at levels of 0.84, 1.00, 1.70, 3.36, and 6.70 kg/ha to hydrilla test plots. No adverse effects were observed on dissolved oxygen or other water quality parameters nor were there any noticeable disturbances to the plankton and benthic communities.

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Toxicity of Fluridone.

Toxicity to wildlife and fish: Fluridone-contaminated diets were administered to 16-day-old Mallard ducklings and 10-day-old Bobwhite chicks for a period of five days. LC0 (maximum nonlethal conc.) values were calculated from the data produced during these studies. Acute LD0s for Bobwhites and Mallards were calculated by administering a range of single doses of fluridone. 96-hour static toxicity tests were conducted on bluegills and rainbow trout. Similar tests were conducted with Daphnia magna for 48 hours. Fathead minnows were used to determine a maximum acceptable toxicant concentration. Data from these studies are reported in Table 1.

Acute toxicity: Fluridone was administered to mice and rats as an oral or subcutaneous single dose. An aqueous suspension formulation containing 45% fluridone was also administered as a single oral dose to rats. Ocular and dermal toxicity tests were conducted with rabbits. The rat was utilized as the test animal in studies conducted to determine the toxicity of fluridone when inhaled for one hour. Results of these acute studies are listed in Table 2.

Subchronic toxicity: Fluridone toxicity was evaluated in rats, mice, and dogs for periods of three months. There were no treatment-related effects when fluridone was administered to mice at dietary doses of 330 ppm or to rats at dietary doses of 62 ppm (Lilly, 1981). However, rats and mice fed 2,000 ppm of fluridone showed slight histological changes in the liver and kidney (Weed Science Society of

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Table 1. General toxicity of fluridone to birds, fish and an invertebrate[1].

Species	Route	Toxicity
Mallard (Anas platyrhynchos)	Diet (5 days)	LC_0 > 5,000 ppm
Bobwhite (Colinus virginianus)	Diet (5 days)	LC_0 > 5,000 ppm
Bobwhite (Colinus virginianus)	Oral (acute)	LD_{50} > 2,000 mg/kg
Bluegill (Lepomis macrochirus)	Water (static)	LC50 >9<12.5 ppm
Rainbow trout (Salmo gairdneri)	Water (static)	LC50 11.7 ppm
Daphnia (Daphnia magna)	Water (static)	EC50 6.3 ppm
Fathead minnow (Pimephales promelas)	Water (flow through)	MATC[2] > 0.48 ppm < 0.96 ppm

[1] (Lilly, 1981)

[2] Maximum Acceptable Toxicant Concentration

Table 2. Acute mammalian toxicity data for fluridone[1].

Species	Material	Route	Toxicity
Rat (*Rattus norvegicus*)	Technical[2]	Oral	LD50 >10,000 mg/kg
	Technical	Subcutaneous	LD_0 >2,000 mg/kg
	Technical	Inhalation	LC_0 >2,130 mg/M^3 of air
	4 AS[3]	Oral	LD_0 >0.5 ml/kg
	4 AS	Inhalation	LC_0 >9.6 ml/M^3 of air
Mouse (*Mus musculus*)	Technical	Oral	LD50 >10,000 mg/kg
	Technical	Subcutaneous	LD50 >2,000 mg/kg
Cat (*Felis domesticus*)	Technical	Oral	LD_0 >250 mg/kg
Dog (*Canis familiaris*)	Technical	Oral	LD_0 >500 mg/kg
Rabbit (*Oryctolagus cuniculus*)	Technical	Dermal	LD_0 >500 mg/kg (no irritation)
	Technical	Ocular	Moderate irritant (44 mg/eye)
	4 AS	Dermal	LD_0 >2ml/kg (slight irritation)
	4 AS	Ocular	Very slight irritant (0.1 ml/eye)

[1] Lilly, 1981.

[2] Technical grade fluridone.

[3] 4 pound per gallon aqueous suspension (AS).

America , 1979). Further data relating to these changes are not currently available (Cochrane, 1981). No toxic effects were observed when fluridone was administered to dogs at doses of up to 200 mg/kg/day (Lilly, 1981).

Chronic toxicity: Fluridone-contaminated diets were administered to rats for either one or two years. No toxicological effects were observed at a dietary level of 200 ppm. There was also no evidence of a carcinogenic effect at this level. Similar long-term feeding studies were conducted in mice. No gross toxic effects were observed, although these studies have not been fully evaluated (Lilly, 1981).

Reproductive toxicity: Fluridone was administered to pregnant rats and rabbits during the organogenesis phase of gestation. At 200 mg/kg/day (rats) and 750 mg/kg/day (rabbits), no teratogenic effects were noted. The reproductive effects of fluridone were evaluated by maintaining three successive generations of rats on diets containing 2,000 ppm of fluridone. Fluridone did not produce impairment of fertility, live-born litter size, gestation length, progeny survival, or sex distribution. In addition, there was no indication of teratogenicity (Lilly, 1981).

Mutagenesis: In a modified Ames test, 1,000 µg/ml of fluridone produced no evidence of bacterial mutagenesis. Concentrations of up to 1,000 nanomoles of fluridone/ml did not induce cultured hepatocyte DNA repair synthesis. Administered as an oral dose of 2,000 mg/kg to male rats, fluridone did not produce dominant lethal mutations (Lilly, 1981).

Metabolism.

A detailed investigation of fluridone metabolism in the rat is currently in progress. Preliminary studies indicate that a single, oral dose is rapidly absorbed and extensively metabolized in the rat. The primary route of excretion is the feces.

The major biotransformation product of fluridone in fish is 1-methyl-3-(4-hydroxyphenyl)-5-[3-triflurorомethyl) phenyl]-4(1H)-pyridinone. This information was determined during carbon-14 fluridone metabolism studies utilyzing fat-head minnows and bluegills (Lilly, 1981).

Current Status.

As of this date, fluridone is not fully registered by EPA as an aquatic herbicide or for use in cotton, and the additional existing toxicity and metabolism data are not available to the general public (Cochrane, 1981).

A temporary tolerance of 0.01 ppm was established for residues of fluridone in potable water (Federal Register, 1981) and in fish (Federal Register, 1980).

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OBJECTIVES

1. To determine the effect of chronic dietary administration of fluridone on food consumption and body weight gain of Bobwhites and Mallards.
2. To determine the effect of the chronic dietary administration of fluridone on egg production and fertility of Bobwhites and Mallards.
3. To determine the effect of chronic dietary administration of fluridone on embryo survival, hatchability and survivability (2 weeks) of the offspring.
4. To determine the effect of chronic dietary administration of fluridone on eggshell thickness.

MATERIALS AND METHODS

Mallards (Anas platyrhynchos) and Bobwhites (Colinus virginianus) were the two species used as test animals in this avian reproduction study. The Mallards were obtained from the Max McGraw Foundation, Elgin, IL while the Bobwhites were supplied by Barrett's Quail Farm of Houston, TX. When first received, the birds were placed in quarantine for one week. All birds were then individually banded, placed randomly into their respective testing cages and allowed to acclimate for two weeks. In addition, Mallards were wing-clipped to prevent their escape from the open-topped pens. The Mallards were 13-weeks-old and the Bobwhites were 16.5-weeks-old when testing began.

The Mallards and Bobwhites were housed in separate testing rooms at the Michigan State University (MSU) Poultry Research and Teaching Facility in East Lansing, MI. In the Mallard room, two males and five females were randomly assigned to each testing pen measuring 1.5 m x 1.6 m x 0.7 m (w x l x h). In the Bobwhite room, one male and one female were randomly assigned to each testing cage measuring 40 cm x 43 cm x 44.5 cm (w x l x h). Four pens of Mallards and fifteen cages of Bobwhites were utilized for each treatment level. Dietary concentrations of treated feed were randomly assigned to each pen or cage. Appendices B and C illustrate the testing room layouts.

Control and treated diets for the adult Mallards, adult

Bobwhites, Mallard ducklings, and Bobwhite chicks were prepared by Eli Lilly and Co. The mash-type feed and water were provided ad libitum to all birds. Diet compositions and analyses are listed in Appendices D through K. Each feed shipment, received every six weeks, was sampled and frozen for future fluridone analysis.

The dietary treatment levels used in this study were determined from a palatability test conducted at MSU during the summer of 1979. Some food refusal occurred at a level of 1.0% (10,000 ppm) fluridone for both Bobwhites ($P < 0.05$) and Mallards ($P < 0.01$). The highest concentration chosen for this reproduction study (1,000 ppm fluridone) was the highest level causing no change in food consumption during the palatability test. The four dietary concentrations used in this study were: 0 ppm (control), 100 ppm (0.01%), 300 ppm (0.03%), and 1,000 ppm (0.10%) fluridone.

Testing. Treated feed was administered to the birds on day one of the study's preproduction phase - September 18, 1979 for Mallards and September 19, 1979 for Bobwhites. This phase ended two months later when the photoperiod was increased from 7 hours light:17 hours dark to 16 hours light: 8 hours dark. The increased photoperiod stimulated egg laying thus beginning the study's production phase. Flow charts of the studies are given in Figures 1 and 2.

Eggs were collected daily when egg production reached fifty percent for all females within a testing room. Each egg collected was marked in pencil with the corresponding

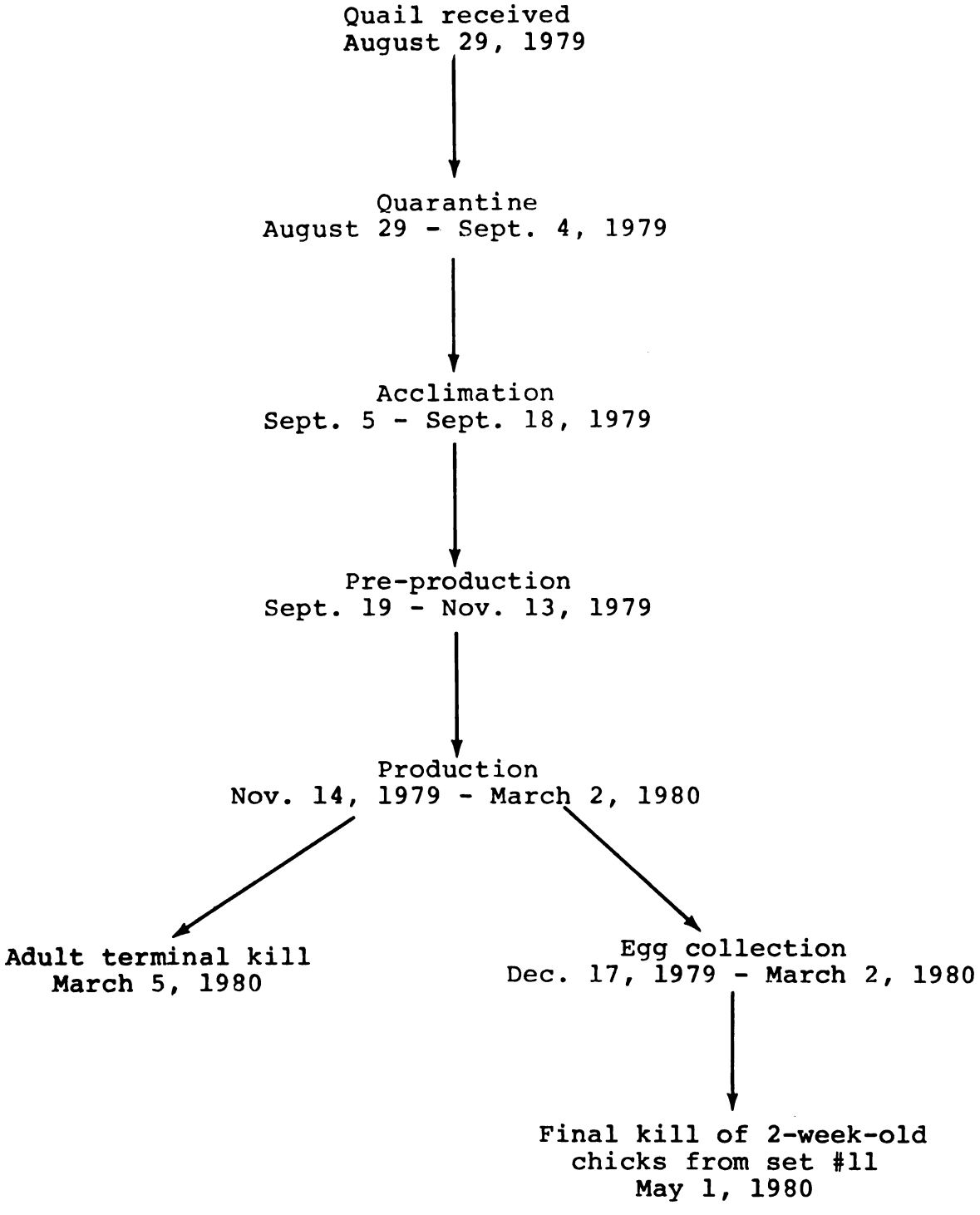

Figure 1. Chronology of study for Bobwhites fed fluridone.

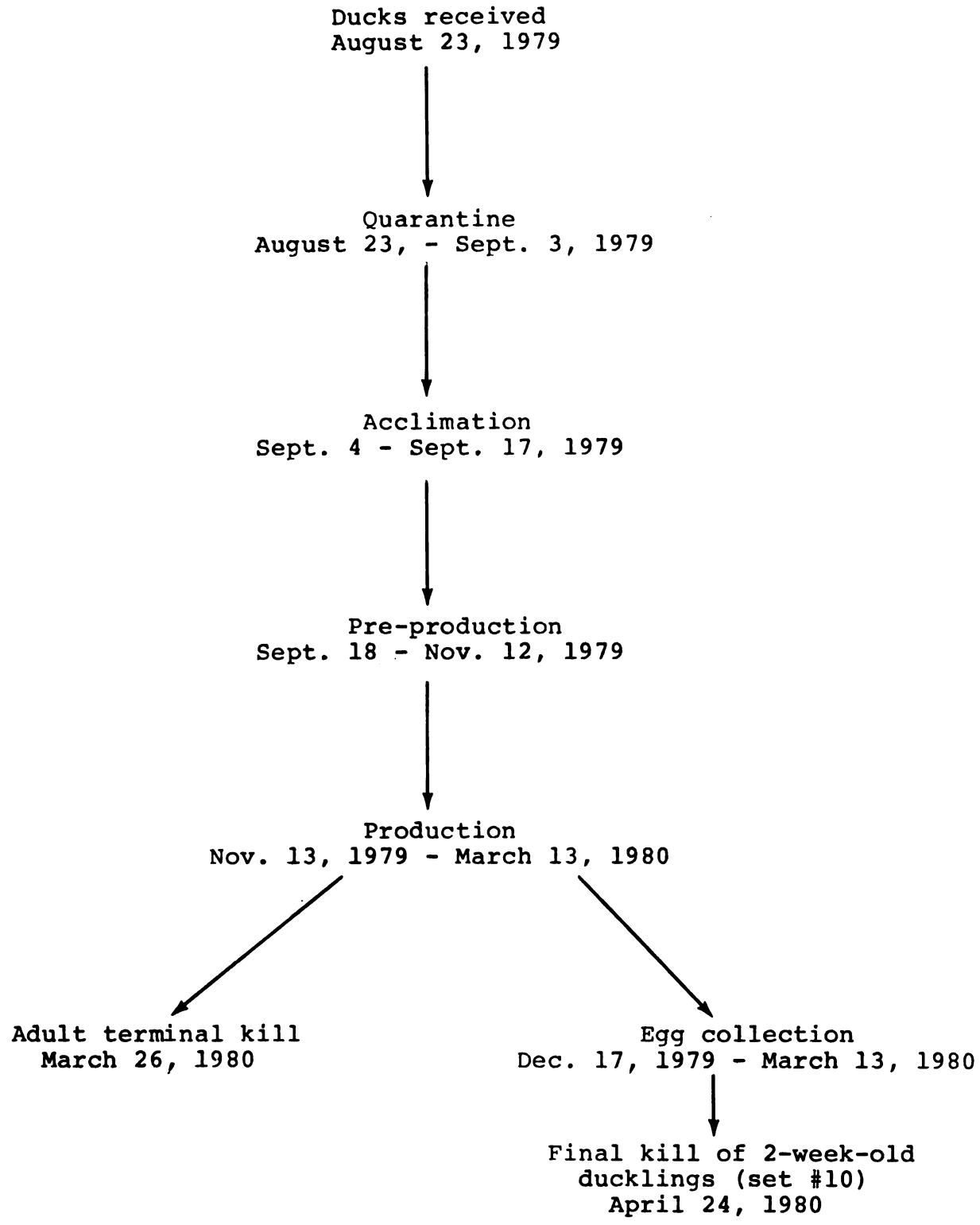

Figure 2. Chronology of study for Mallards fed fluridone.

date and cage number. Prior to this time, any eggs laid were discarded. Eggs were stored at 15.6°C (14.4-16.7°C) until one week's collection had accumulated. The Mallard eggs were collected for ten weeks. The Bobwhite collection period was extended an additional week because a set of eggs was not transferred from incubator to hatcher.

Eggs (one week's collection) were incubated weekly in a Jamesway, single stage, 252 incubator[1] maintained at dry and wet bulb temperatures of 37.5°C (37.2-37.8°C) and 30°C (29.4-30.6°C), respectively. Eggs were candled on day 11 for Bobwhites and on day 14 for Mallards to determine the number of fertile eggs, infertile eggs, and early dead embryos. Eggs were candled again on day 18 (Bobwhites) and day 21 (Mallards) of incubation for an interim death determination. On day 21 (Bobwhites) or day 23 (Mallards) of incubation, the eggs were transferred to a Jamesway incubator[1] with dry and wet bulb temperatures of 37.2°C (34.9-35.5°C) and 31.7°C (31.1-32.2°C), respectively. On day 24 for Bobwhites and day 27 for Mallards, all hatched, pipped, and unpipped offspring were removed from the hatcher and their numbers were recorded. Each hatched bird was individually wing-banded and recorded according to the parents' dietary fluridone concentration.

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

Hatchlings were transferred from Anthony Hall by automobile to Petersime 250-24 Battery brooders[1] located at the MSU Poultry Research and Teaching Facility. Temperatures in the brooders were maintained at 35°C. Starter mash and water were provided ad libitum. All morbidity and mortality was recorded daily. At the end of the two-week-period, surviving offspring were killed by exposure to chloroform.

Feed consumption of the adult birds was measured bi-weekly throughout the study. Separate feed containers for each pen or cage facilitated feed weighing. Mallard feed was weighed to the nearest 10 grams. Bobwhite feed was weighed to the nearest gram. Body weights were measured to the nearest gram at 0, 2, 4, 6, and 8 weeks and at termination. Body weights were not measured during the egg collection period to avoid any adverse effects caused by handling.

The adult birds were killed by CO_2 asphyxiation when the egg collection period was terminated. At this time, all birds were necropsied and tissues for histopathology were taken from at least five animals of each sex per treatment level. The following tissues were taken: kidney, liver, heart, lung, spleen, pancreas, proventriculus, gizzard, duodenum, jejunum, ileum, cecum, colon, testes, ovary, magnum, shell gland, pectoral muscle, leg muscle, thymus, sciatic nerve, and skin. Samples collected were placed in buffered formalin and transported to the Lilly Research

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[1]Petersime Incubator Company, Gettysburg, OH 45328.

Laboratories for histological processing and examination by Lilly personnel. Birds that died on test were necropsied and samples for histopathology were taken and preserved if the birds displayed any gross pathological abnormalities.

Eggs were collected once every other week for eggshell thickness measurements. The eggs were cracked in half, the contents were removed, and the shells were rinsed with tap water to remove the albumen. The shells were then left to air dry at room temperature for at least 48 hours. Eggshell (plus membranes) thickness was measured using an Ames Pocket Thickness Measure[1]. Four equidistant points around the shell circumference were approximated, and the thickness was measured to the nearest 0.01 mm. From these four data points, an average for that eggshell was calculated.

The amount of fluridone ingested (mg/kg/day) was calculated using food consumption and body weight data along with the dietary concentration. Since food consumption by sex was not known, body weights were calculated as a total average with no consideration given to sex.

Statistics.

Mallards: Data (except on test mortality) were analyzed by one-way analysis of variance and a Dunnet's t-test (Gill, 1978a and 1978b). Reproductive parameters were calculated as percentages of the previous parameter beginning with the number of eggs laid and continuing through the number of

[1]B. C. Ames Company, 111 Lexington St., Waltham, MA 02154.

the number of offspring surviving 14 days. This method accounts for all eggs laid by sequentially comparing reproductive parameters. These data, converted to a percentage of the number of eggs laid, can then be illustrated as a decreasing continuum. Figure 3 illustrates a hypothetical example of this continuum and the relationships and definitions of the reproductive parameters.

The on-test mortality data were analyzed by application of a Bonferroni Chi-Square test to a 2 x 2 contingency table comparing each treatment level's mortality to the control (Gill, 1978a and 1978b).

Adjustments were made in duckling survivability whenever dead offspring were found without wing bands. In such cases, the numbers of bandless, dead ducklings were apportioned among the stock cage numbers within their treatment level for that particular set number.

Bobwhites: The food consumption, body weight, and egg production data were analyzed by one-way analysis of variance (Gill, 1978a and 1978b). The remaining data were analyzed by application of a Bonferroni Chi-Square test to a 2 x 2 contingency table comparing each treatment group to the control group (Gill, 1978a and 1978b). Information pertaining to the reproductive parameters and offspring survivability as stated above under Mallards also applies to the Bobwhites.

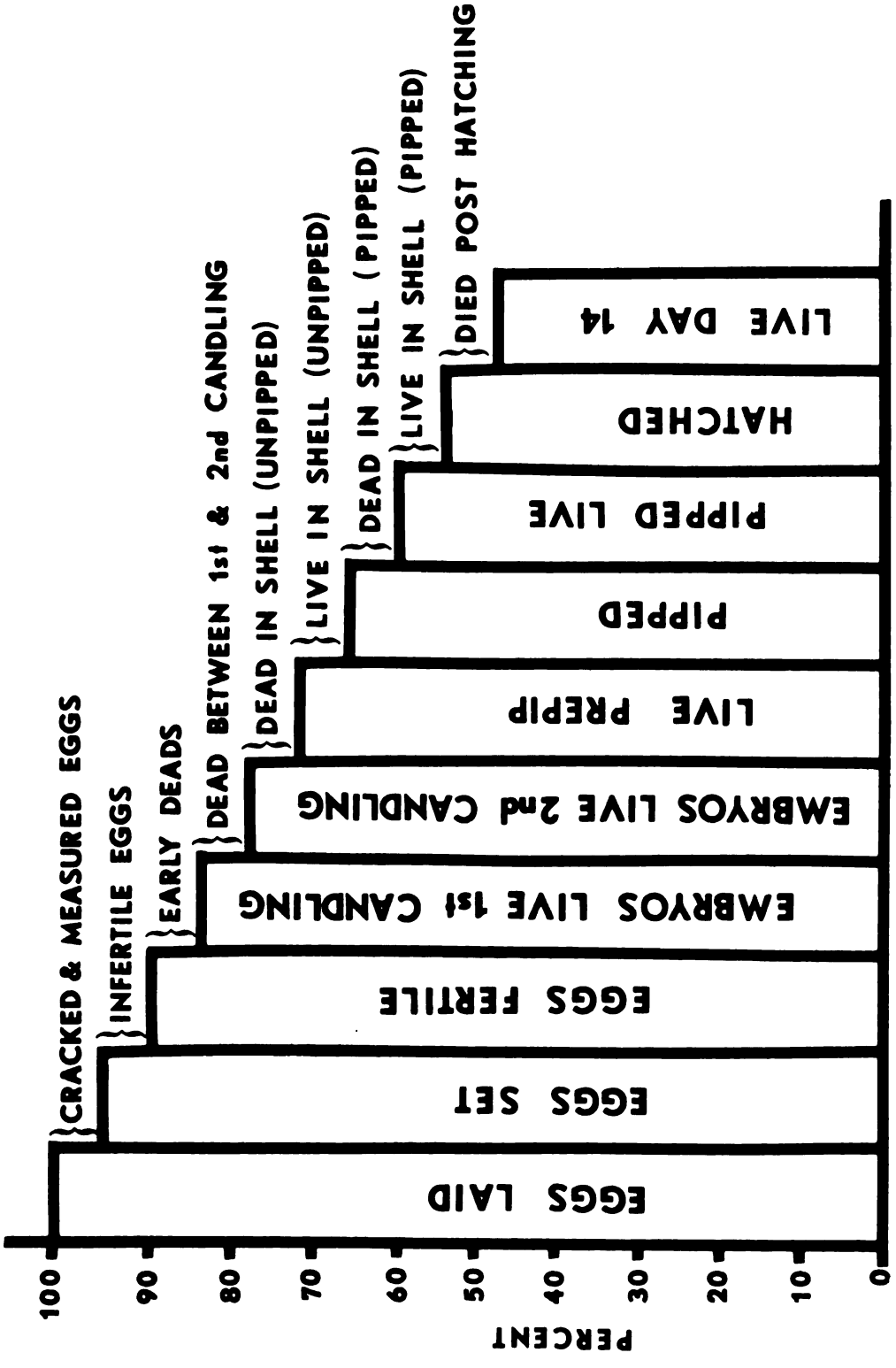

Figure 3. Example of reproductive parameters displayed in a continuum.

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RESULTS

Mallards. Mallard deaths occurring on test are listed chronologically in Table 3. Although no significant differences were calculated for either males or females, note that the first six dead birds are all males while the following ten birds are all female. Most interim deaths were caused by excessive aggressive trauma. The victims were treaded, cannibalized, and not allowed to feed. Prior to and at death, birds were devoid of many or most feathers with hematomas and abrasions located in the skin. Although body weights of the dead birds were not taken, they all appeared to have experienced a severe body weight loss. Most were noticeably moribund prior to death.

Feed consumption of ducks on the fluridone-treated diets was not significantly different from controls for most measurement periods (Table 4). A significant difference ($P < 0.05$) from the control value was calculated for the 100 ppm treatment group after 18 weeks on treated feed. However, the control value was lower than the treated value. A graphic representation of feed consumption over time is presented in Figure 4.

Analysis of variance revealed no significant differences for male or female body weight data (Tables 5 and 6, respectively) expressed as mean percent change of initial body weight. Graphs of the body weight data for male and females are illustrated in Figures 5 and 6, respectively.

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Table 3. Interim deaths for Mallards fed fluridone.

Pen #/ dietary level	Bird # (sex)	Date of death	Cause of death*
12/0.01%	5014 (M)	November 16	Excessive aggressive trauma
6/0.00%	5028 (M)	November 18	" " "
2/0.10%	5015 (M)	November 19	" " "
11/0.01%	5073 (M)	November 23	" " "
3/0.10%	5067 (M)	November 29	" " "
5/0.03%	5036 (M)	November 30	" " "
10/0.00%	5179 (F)	January 14	" " "
6/0.00%	5259 (F)	January 16	" " "
5/0.03%	5203 (F)	January 23	" " "
7/0.03%	5122 (F)	January 24	" " "
6/0.00%	5304 (F)	January 26	" " "
15/0.01%	5275 (F)	January 28	" " "
16/0.00%	5273 (F)	January 31	Systemic aspergillosis and excessive aggressive trauma.
9/0.03%	5155 (F)	February 3	Excessive aggressive trauma
15/0.01%	5193 (F)	February 18	Bacterial septicemia and excessive aggressive trauma.
8/0.10%	5408 (F)	February 25	Bacterial septicemia

*Cause of death diagnosed by a veterinarian from Eli Lilly and Co.

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Table 4. The effect of chronic dietary administration[1] of fluridone to Mallards upon feed consumption (grams/bird/day ± S.D.).

Weeks on Treatment	Dietary treatment (ppm)			
	0	100	300	1000
2	115.0[2] (± 24.39)	125.0 (± 30.10)$_a$[3]	117.0 (± 12.19)$_a$	124.8 (± 13.99)$_a$
4	128.0 (± 25.81)	137.0 (± 28.18)$_a$	117.5 (± 5.07)$_a$	133.3 (± 18.23)$_a$
6	127.0 (± 15.85)	143.5 (± 30.36)$_a$	163.5 (± 51.49)$_a$	136.5 (± 20.57)$_a$
8	123.3 (± 21.96)	140.5 (± 27.87)$_a$	123.8 (± 16.17)$_a$	137.3 (± 25.67)$_a$
10	79.5 (± 29.72)	91.5 (± 8.23)$_a$	89.5 (± 7.14)$_a$	86.3 (± 16.48)$_a$
12	122.3 (± 17.71)	133.3 (± 21.70)$_a$	118.0 (± 16.15)$_a$	122.0 (± 23.85)$_a$
14	140.0 (± 26.52)	157.8 (± 21.82)$_a$	141.0 (± 17.81)$_a$	138.0 (± 19.87)$_a$
16	164.0 (± 26.88)	194.3 (± 13.96)$_a$	179.3 (± 14.89)$_a$	169.3 (± 19.69)$_a$
18	187.8 (± 28.71)	233.5 (± 11.24)$_b$	207.0 (± 22.01)$_a$	186.0 (± 7.44)$_a$

cont'd

Table 4

Weeks on Treatme
20
22
24
26
28

[1]Food

[2]n =

[3]Mean
from
are

Table 4 cont'd.

Weeks on Treatment	Dietary treatment (ppm)			
	0	100	300	1000
20	201.5 (± 40.25)	231.3 (± 11.76)$_a$	202.8 (± 17.23)$_a$	199.5 (± 18.36)$_a$
22	198.3 (± 35.52)	211.3 (± 18.41)$_a$	196.8 (± 36.51)$_a$	192.0 (± 23.45)$_a$
24	187.8 (± 25.43)	200.0 (± 19.10)$_a$	197.3 (± 18.63)$_a$	191.3 (± 19.33)$_a$
26	163.3 (± 32.77)	173.8 (± 28.11)$_a$	164.5 (± 31.94)$_a$	163.3 (± 22.34)$_a$
28	178.3 (± 26.17)	188.0 (± 27.39)$_a$	176.0 (± 34.86)$_a$	169.8 (± 25.64)$_a$

[1]Food consumption values represent a biweekly average.

[2]n = 4 for all means.

[3]Means with subscript "a" are not significantly different from control values ($P > 0.05$); means with subscript "b" are significantly different from control values ($P < 0.05$).

Figure 4. The effect of chronic dietary administration of fluridone to Mallards upon mean food consumption.

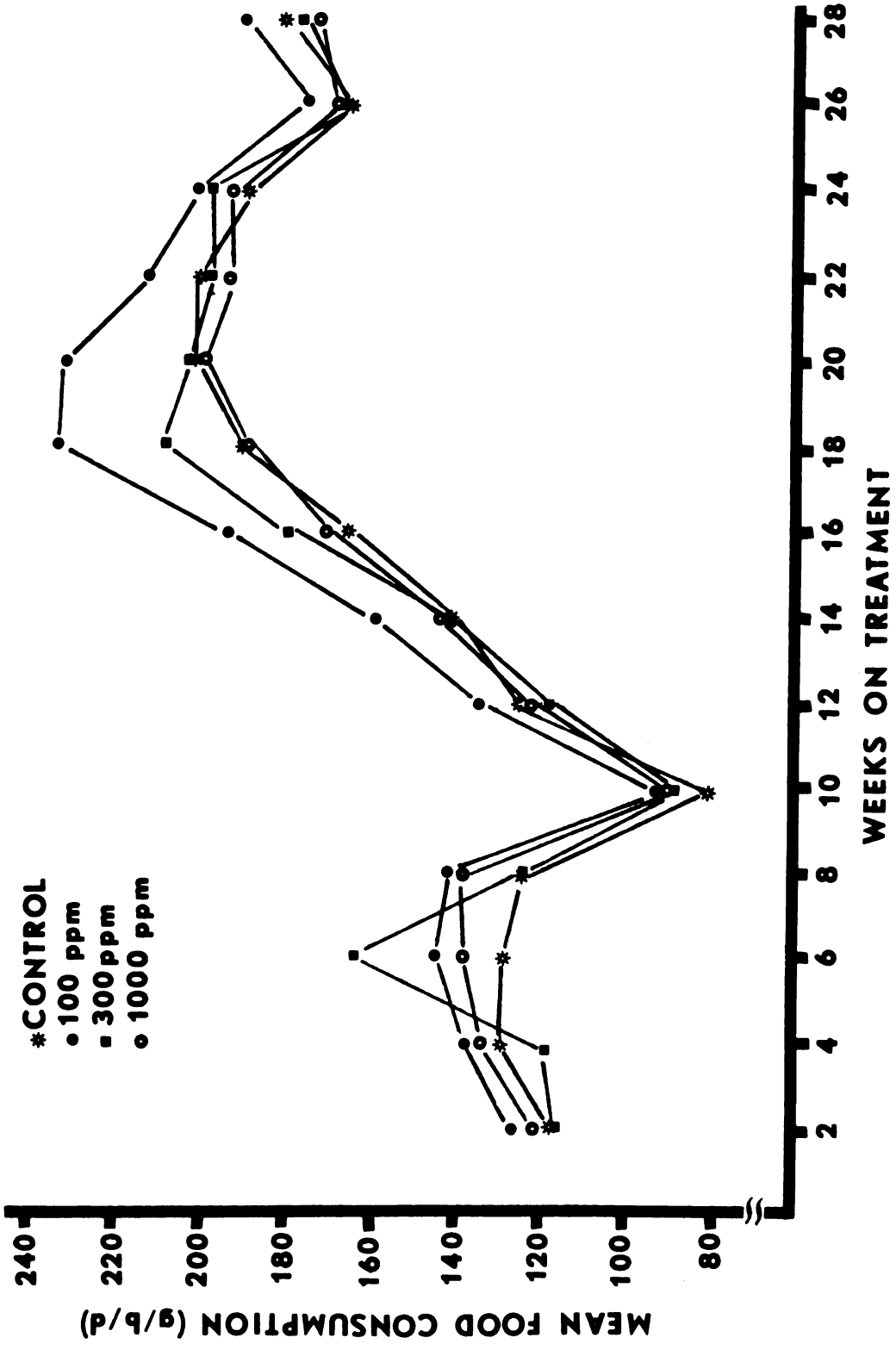

MEAN FOOD CONSUMPTION (g/b/d)
240
220
200
180
160
140
120
100
80
WEEKS ON TREATMENT
2
4
6
8
10
12
14
16
18
20
22
24
26
28
*CONTROL
• 100 ppm
▪ 300 ppm
○ 1000 ppm

Table 5. The effect of chronic dietary administration of fluridone to male Mallards upon mean[1] percent change of initial[2] body weight (± S.D.).

Table 5. The effect of chronic dietary administration of fluridone to male Mallards upon mean[1] percent change of initial[2] body weight (± S.D.).

Dietary concentration (ppm)	Weeks on treatment					
	0	2	4	6	8	28
0	104.0[3] (± 3.04)	104.1 (± 6.90)	104.9 (± 7.70)	106.2 (± 8.09)	109.1 (± 7.81)	109.0 (± 8.66)
100	101.0[4] (± 4.17)$_a$	101.7 (± 4.0)$_a$	101.3 (± 5.73)$_a$	105.8 (± 5.67)$_a$	108.7 (± 6.08)$_a$	109.6 (± 5.32)$_a$
300	104.0 (± 3.23)$_a$	105.4 (± 5.64)$_a$	106.5 (± 5.44)$_a$	110.2 (± 4.97)$_a$	112.6 (± 6.71)$_a$	111.7 (± 5.61)$_a$
1000	104.5 (± 1.69)$_a$	103.4 (± 4.56)$_a$	107.1 (± 4.75)$_a$	110.5 (± 4.86)$_a$	114.3 (± 5.64)$_a$	114.9 (± 7.22)$_a$

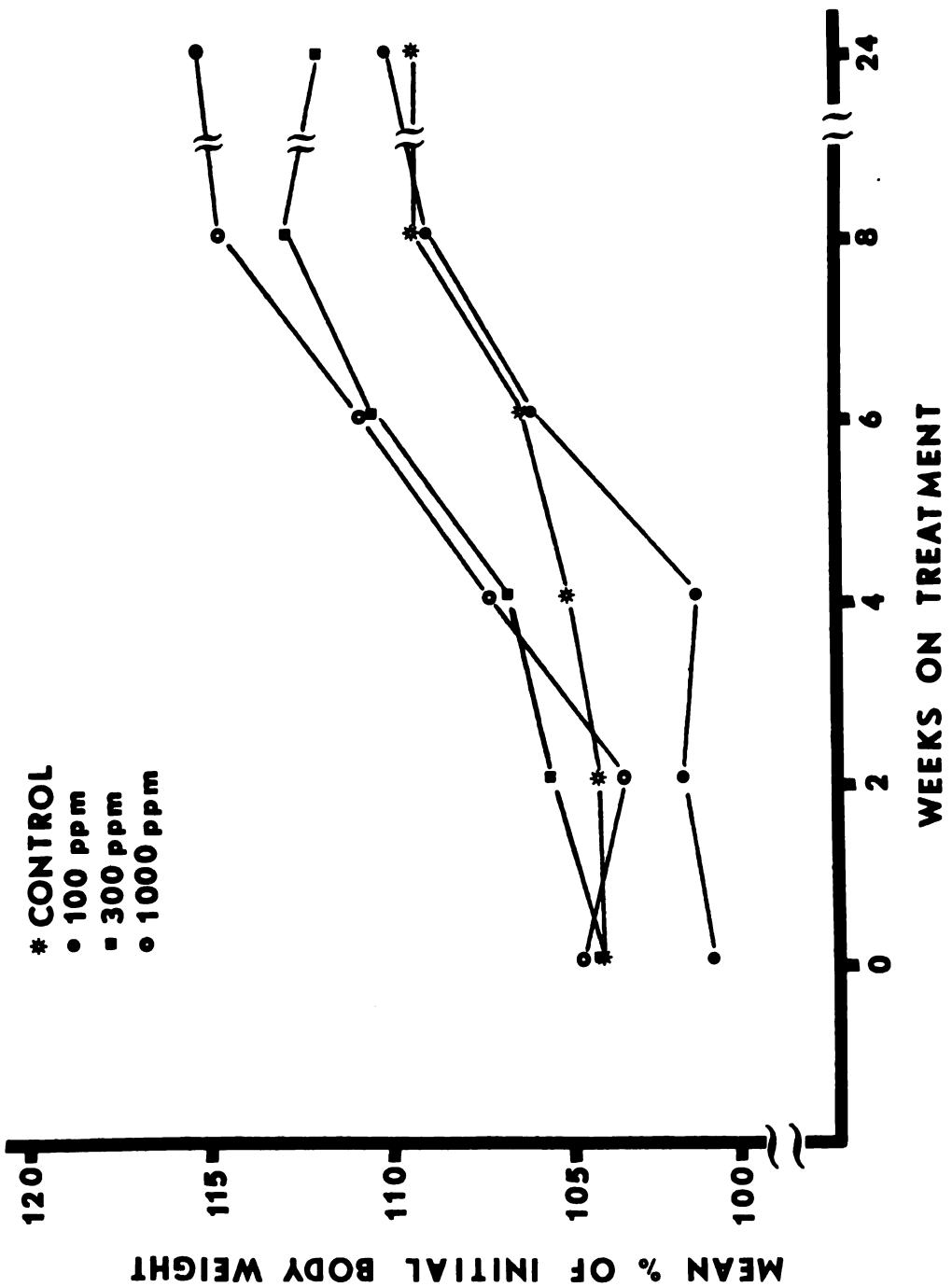
[1] Initial body weight taken on 9-4-79 (2 weeks before week 0).

[2] n = 4 for all means.

[3] Values represent a "%" change.

[4] Means with subscript "a" show no significant difference from control values ($P > 0.05$).

Figure 5. The effect of chronic dietary administration of fluridone to male Mallards upon mean percent change of initial body weight.


* CONTROL
• 100 ppm
▪ 300 ppm
• 1000 ppm
MEAN % OF INITIAL BODY WEIGHT
120
115
110
105
100
0
2
4
6
8
24
WEEKS ON TREATMENT

administration of fluridone to female Mallards

Table 6. The effect of chronic dietary administration of fluridone to female Mallards upon mean[1] percent change of initial[2] body weight (± S.D.).

Dietary treatment (ppm)	Number of weeks on treatment					
	0	2	4	6	8	24
0	102.2[3] (± 4.43)	103.5 (± 3.99)	105.9 (± 4.44)	112.2 (± 5.39)	116.2 (± 6.34)	115.0 (±10.89)
100	102.4[4] (± 3.80)$_a$	102.8 (± 5.17)$_a$	108.0 (± 4.87)$_a$	115.3 (± 6.20)$_a$	118.3 (± 6.47)$_a$	116.8 (±10.83)$_a$
300	103.9 (± 3.79)$_a$	105.3 (± 4.54)$_a$	109.8 (± 6.84)$_a$	114.9 (± 7.94)$_a$	119.4 (± 8.57)$_a$	115.4 (± 9.76)$_a$
1000	102.3 (± 3.45)$_a$	104.8 (± 4.48)$_a$	104.6 (±23.55)$_a$	114.9 (± 7.34)$_a$	119.3 (± 6.69)$_a$	113.9 (± 8.01)$_a$

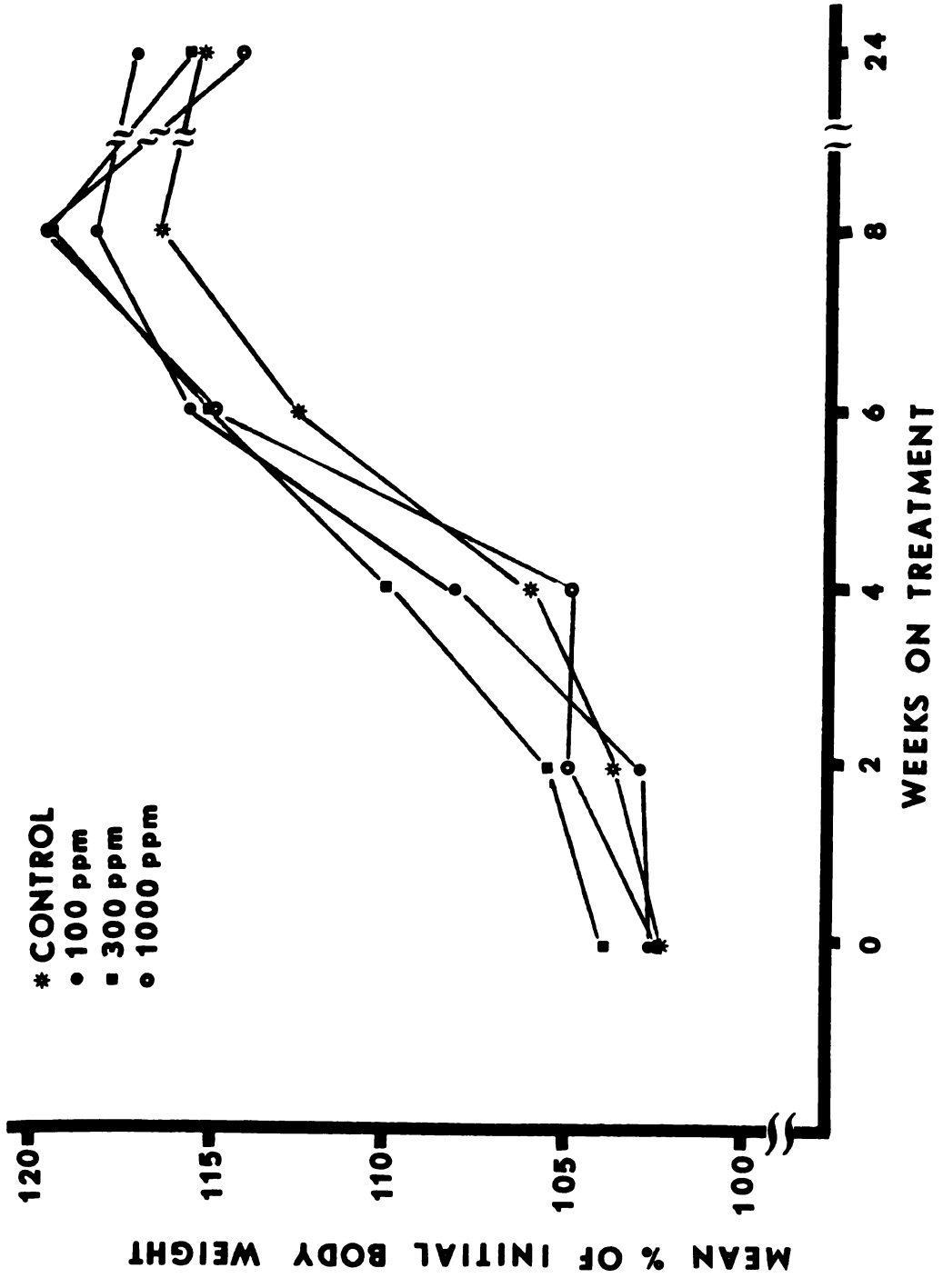
[1] Initial body weight taken on 9-4-79.

[2] n = 4 for all means

[3] Values represent a "%" change.

[4] Means with subscript "a" show no significant difference from control values ($P > 0.05$).

Figure 6. The effect of chronic dietary administration of fluridone to female Mallards upon mean percent change of initial body weight.


MEAN % OF INITIAL BODY WEIGHT
120
115
110
105
100
0
2
4
6
8
24
WEEKS ON TREATMENT
* CONTROL
• 100 ppm
▪ 300 ppm
○ 1000 ppm

Egg production data (egg/hen/day) for treatment groups display no significant differences from their respective control values. These data are shown in Table 7. Mean egg production plotted against time (weeks) is presented in Figure 7.

Data from all sets per treatment group were combined for analysis of the reproductive parameters. No significant differences were noted for percent set, percent fertile, percent live day 14, percent live day 21, percent live pre-pip, percent pipped, percent hatched, or percent survived. In the percent pipped live category, 100 and 300 ppm treatment groups were shown to be significantly lower than the control values (Table 8). Mallard reproductive parameters were converted to a percent of the number of eggs laid and are displayed as a histogram in Figure 8 (Figures in Table 9).

Analysis of the eggshell measurements failed to show a significant difference between treatment groups and the control (Table 8).

The pathology findings provided by Eli Lilly and Co. are presented in Table 10. Gross and microscopic examinations did not reveal any fluridone-related pathologic alterations.

Bobwhites: Chronic ingestion of dietary fluridone did not result in greater mortality for either female or male Bobwhites. Most deaths were caused by indeterminable factors (Table 11).

Feed consumption of the birds on treated diets was not significantly different from the controls as shown in Table 12.

Table 7. The effect of chronic dietary administration of fluridone to Mallards upon egg production (eggs/hen/day ± S.D.)

Set number	Dietary treatment (ppm)			
	0	100	300	1000
1	0.493[1] (±0.0943)	0.600[2] (±0.1598)$_a$	0.643 (±0.0824)$_a$	0.429 (±0.1418)$_a$
2	0.618 (±0.0888)	0.643 (±0.1409)$_a$	0.586 (±0.0855)$_a$	0.500 (±0.0677)$_a$
3	0.666 (±0.1079)	0.564 (±0.3000)$_a$	0.575 (±0.1395)$_a$	0.693 (±0.1638)$_a$
4	0.549 (±0.1365)	0.513 (±0.1483)$_a$	0.475 (±0.0883)$_a$	0.500 (±0.1268)$_a$
5	0.447 (±0.1876)	0.323 (±0.0980)$_a$	0.294 (±0.0935)$_a$	0.443 (±0.1201)$_a$
6	0.278 (±0.0553)	0.395 (±0.0518)$_a$	0.234 (±0.1003)$_a$	0.393 (±0.1366)$_a$
7	0.319 (±0.1719)	0.340 (±0.0984)$_a$	0.217 (±0.2103)$_a$	0.307 (±0.1655)$_a$
8	0.207 (±0.0706)	0.207 (±0.1885)$_a$	0.179 (±0.2040)$_a$	0.109 (±0.0870)$_a$
9	0.244 (±0.0930)	0.260 (±0.1077)$_a$	0.241 (±0.1897)$_a$	0.054 (±0.1070)$_a$
10	0.101 (±0.1554)	0.050 (± 0.0588)$_a$	0.116 (±0.1683)$_a$	0.009 (±0.1800)$_a$

[1] n = 4 for all means.

[2] Means with subscript "a" are not significantly different from control values ($P > 0.05$).

Figure 7. The effect of chronic dietary administration of fluridone to Mallards upon mean egg production.

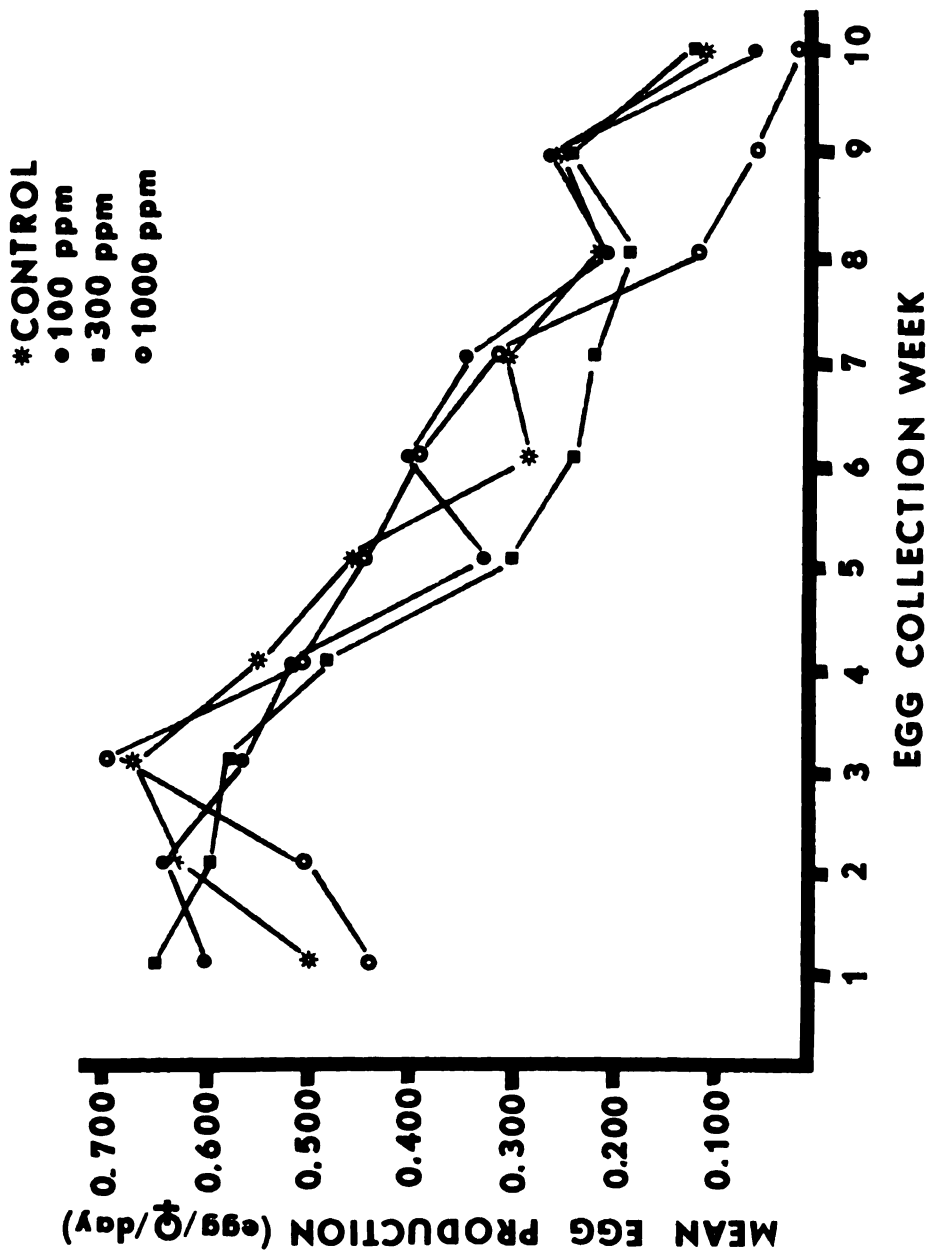

*CONTROL
•100 ppm
▪300 ppm
∘1000 ppm
MEAN EGG PRODUCTION (egg/♀/day)
0.700
0.600
0.500
0.400
0.300
0.200
0.100
1
2
3
4
5
6
7
8
9
10
EGG COLLECTION WEEK

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Table 8. The effect of chronic dietary administration of fluridone to Mallards on mean reproductive parameters (± S.D.) for all sets combined.

Reproductive parameter[1]	Dietary concentration (ppm)			
	0	100	300	1000
% set	80.2[1] ±4.73	84.0 ±2.80[2]$_a$	85.5 ±2.50$_a$	84.9 ±3.97$_a$
% fertile	93.2 ±3.69	79.2 ±11.38$_a$	86.2 ±10.95$_a$	84.9 ±17.19$_a$
% live day 14	98.3 ±0.61	96.9 ±0.613$_a$	96.3 ±2.17$_a$	98.1 ±2.78$_a$
% live day 21	99.1 ±0.600	98.6 ±1.81$_a$	96.8 ±2.23$_a$	98.0 ±1.86$_a$
% live prepip	80.7 ±9.78	80.4 ±10.81$_a$	88.7 ±3.59$_a$	85.5 ±1.80$_a$
% pipped	99.7 ±0.600	99.3 ±1.35$_a$	98.8 ±0.826$_a$	100.0 ±0$_a$
% pipped live	97.5 ±1.20	94.6 ±0.789$_b$	95.9 ±1.59$_a$	94.2 ±1.86$_b$
% hatched	96.0 ±1.67	92.4 ±2.01$_a$	94.5 ±3.42$_a$	97.7 ±1.68$_a$
% survived	94.7 ±2.21	98.8 ±1.62$_a$	98.6 ±1.94$_a$	95.5 ±4.37$_a$
Eggshell thickness (mm)	0.429 ±0.0121	0.430 ±0.0208$_a$	0.424 ±0.0248$_a$	0.420 ±0.0171$_a$

[1] Reproductive parameters (except eggshell thickness) expressed as a percentage of the previous parameter.

[2] Means with the subscript "a" are not significantly different from their respective controls ($P > 0.05$); means with the subscript "b" are significantly different from their respective controls ($P < 0.05$).

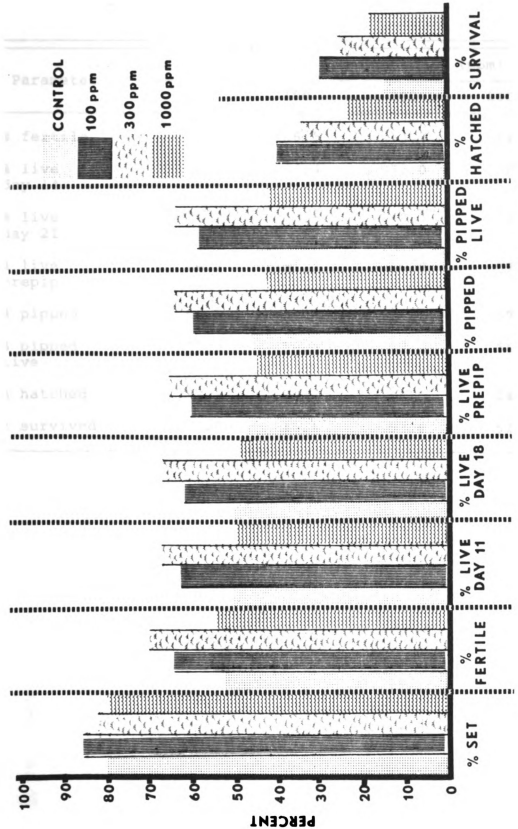

Figure 8. Mallard reproduction parameters expressed in a cointuum as a percent of the number of eggs laid.

Table 9 . Mallard reproductive parameters expressed as a percentage of eggs laid.

Parameter	Dietary concentration (ppm)			
	0	100	300	1000
% fertile	74.7	66.5	73.7	72.1
% live day 14	73.4	64.4	71.0	70.7
% live day 21	72.7	63.5	68.7	69.3
% live prepip	58.7	51.1	60.9	59.5
% pipped	58.5	50.7	60.2	59.5
% pipped live	57.0	50.0	57.2	56.0
% hatched	54.7	46.2	54.5	54.7
% survived	51.8	45.6	53.6	52.2

Table 10. Pathology report for the Mallards.†

Diagnosis	0 ppm		100 ppm		300 ppm		1,000 ppm	
	M‡	F‡	M	F	M	F	M	F
Died on test	1*	4	2	2	1	3	2	1
Terminal kill, no substantitive micro tissue alteration	5	5	5	5	5	5	5	6
Terminal kill, no substantive gross tissue alteration	3	9	1	13	2	12	1	13
General								
gross trauma	1	3	2	1	1	3	2	0
systemic aspergillosis		1						
bacterial septicemia				1				1
Magnum/shell gland								
obstructed gross		1						

† Lilly, 1980.

‡ Male and female.

* Values represent the numbers of birds in that particular category; 8 males and 20 females per treatment group.

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Table 11. Interim deaths for Bobwhites fed fluridone.

Dietary level	Cage #	Bird #	Sex	Date of death	Cause of death
0.00%	16	8553	F	October 12	Unknown
0.01%	29	8671	M	January 5	Unknown
0.10%	3	8580	M	January 18	Starvation
0.03%	57	8574	F	January 18	Myocardial degeneration & myositis of skeletal muscle
0.10%	56	8505	M	January 24	Unknown

Table 12. The effect of chronic dietary administration of fluridone to Bobwhites upon food consumption[1] (grams/bird/day ± S.D.).

Weeks on treatment	Dietary treatment (ppm)			
	0	100	300	1000
2	16.8[2] (± 1.14)	16.8 (± 1.41)[3]$_a$	17.0 (± 1.59)$_a$	17.7 (± 1.83)$_a$
4	17.7 (± 1.89)	16.5 (± 1.85)$_a$	17.8 (± 1.70)$_a$	18.6 (± 2.14)$_a$
6	18.7 (± 1.55)	18.3 (± 1.53)$_a$	18.9 (± 1.56)$_a$	19.0 (± 1.48)$_a$
8	19.0 (± 1.35)	18.6 (± 1.00)$_a$	18.9 (± 1.28)$_a$	19.4 (± 1.74)$_a$
10	19.9 (± 1.78)	18.6 (± 1.96)$_a$	20.5 (± 1.74)$_a$	20.2 (± 2.21)$_a$
12	21.8 (± 2.40)	21.4 (± 1.81)$_a$	23.6 (± 2.82)$_a$	22.2 (± 2.63)$_a$
14	23.7 (± 2.78)	23.3 (± 1.91)$_a$	24.5 (± 2.21)$_a$	23.8 (± 2.55)$_a$
16	24.2 (± 2.65)	25.1 (± 2.66)$_a$	24.8 (± 3.60)$_a$	25.6 (± 3.81)$_a$
18	26.0 (± 3.17)	26.0 (± 2.44)$_a$	27.3 (± 3.81)$_a$	27.3 (± 4.16)$_a$
20	27.6 (± 4.67)	27.3 (± 3.52)$_a$	26.5 (± 3.94)$_a$	26.7 (± 5.00)$_a$
22	30.5 (± 5.75)	28.8 (± 3.74)$_a$	29.1 (± 5.36)$_a$	29.8 (± 5.64)$_a$
24	28.8 (± 8.27)	30.6 (±10.03)$_a$	27.6 (± 5.17)$_a$	28.1 (± 5.52)$_a$

[1]Food consumption values represent a biweekly average.

[2]n = 15 per treatment for all weeks.

[3]Means with subscript "a" are not significantly different from their respective controls ($P > 0.05$).

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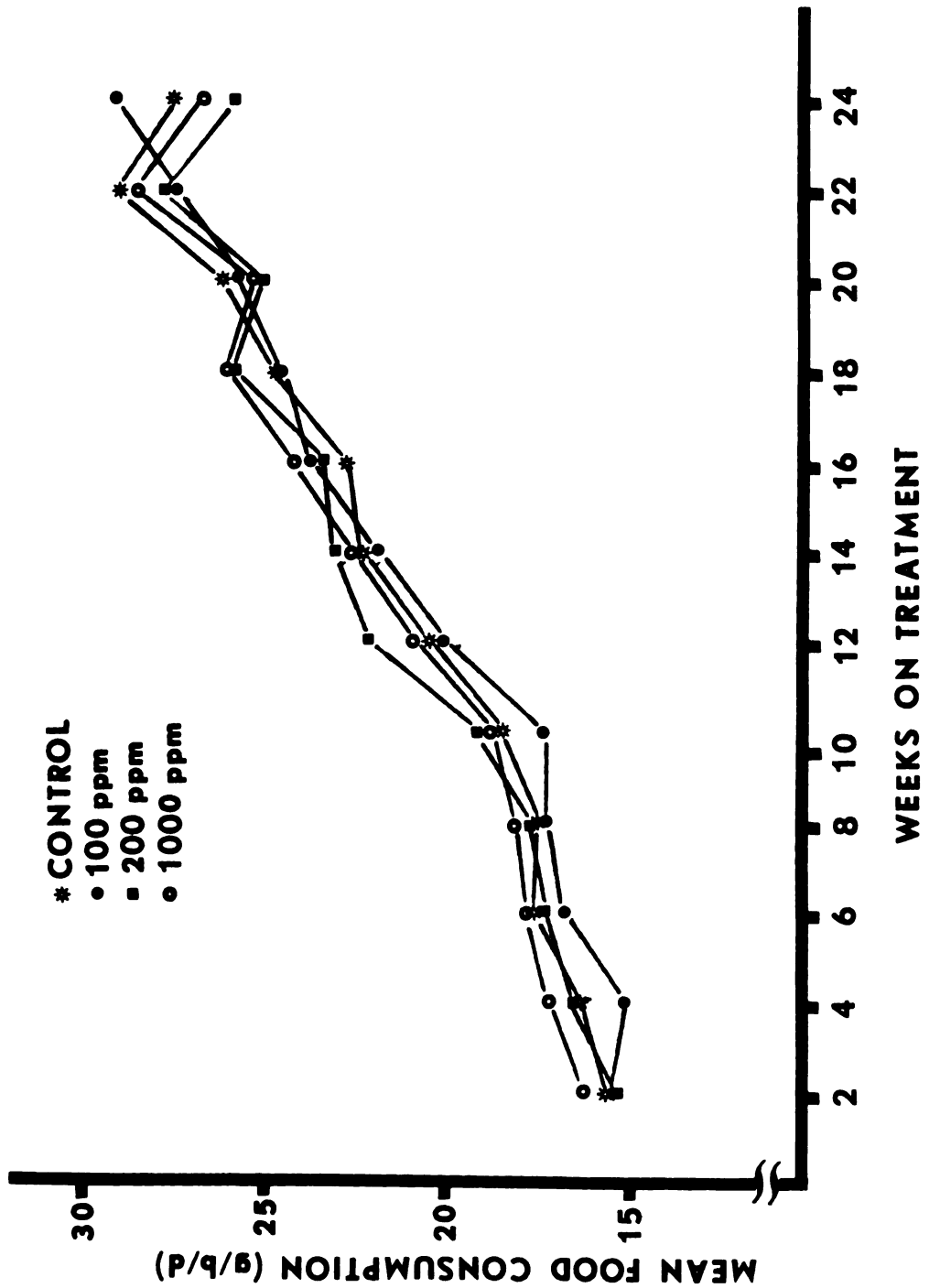
A graphic representation of these data is presented in Figure 9.

Neighter male nor female Bobwhites fed the treated diets exhibited significant body weight differences (expressed as a percent change of initial body weight) from the controls (Tables 13 and 14). Figures 10 and 11 display these changes over time for the males and females, respectively.

Analysis of the egg production data revealed no significant differences between the control birds and the birds on treated diets except for the 100 ppm treatment group during the fourth set. Egg production for the 100 ppm group was significantly higher than the control group (Table 15). These data are presented graphically in Figure 12.

Significant differences were present in two of the reproductive parameters (Table 16). Percent fertility and percent hatchability of the 100 ppm treatment group was significantly greater than the control group. The 300 ppm treatment group was also significantly greater than the control for percent fertility. Note the discrepancies in Table 16 where notated with astericks. These value differences are due to the elimination of sets one and five when the data were no longer available. Loss of data from set five was due to the accidental lack of egg transfer from incubator to hatcher. Loss of data from set one was due to the accidental absence of parent stock information. The reproductive parameters were converted to a percent of the

Figure 9. The effect of chronic dietary administration of fluridone to Bobwhites upon mean food consumption.


MEAN FOOD CONSUMPTION (g/b/d)
30
25
20
15
2
4
6
8
10
12
14
16
18
20
22
24
WEEKS ON TREATMENT
*CONTROL
•100 ppm
▪200 ppm
•1000 ppm

Table

Weeks treatme	
0	
2	
4	
8	
10	
24	

Body w
before

n = 15
1000 p

Means
from t

Table 13. The effect of chronic dietary administration of fluridone to male Bobwhites[1] upon mean percent change of initial body weight (± S.D.)

Weeks on treatment	Dietary Concentration (ppm)			
	0	100	300	1000
0	103.7[2] (± 4.84)	105.4[3] (± 6.51)$_a$	104.4 (± 4.32)$_a$	107.0 (± 6.27)$_a$
2	106.5 (± 5.14)	108.9 (± 5.82)$_a$	106.6 (± 4.57)$_a$	109.4 (± 4.22)$_a$
4	111.0 (± 4.07)	113.1 (± 5.86)$_a$	111.0 (± 5.43)$_a$	113.9 (± 5.23)$_a$
8	113.9 (± 5.35)	116.8 (± 6.34)$_a$	114.8 (± 6.08)$_a$	117.6 (± 6.51)$_a$
10	112.7 (± 5.08)	117.0 (± 5.62)$_a$	113.9 (± 5.92)$_a$	117.4 (± 6.10)$_a$
24	115.8 (± 9.70)	117.0 (±11.15)$_a$	113.0 (± 7.50)$_a$	117.8 (± 7.66)$_a$

[1] Body weight on 9-5-79 taken as initial body weight (2 weeks before week 0).

[2] n = 15 for all means on all dates, except 100 ppm (n = 14) and 1000 ppm (n = 13) for week 24.

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

Figure 10. The effect of chronic dietary administration of fluridone to Bobwhite males upon mean percent change of initial body weight.

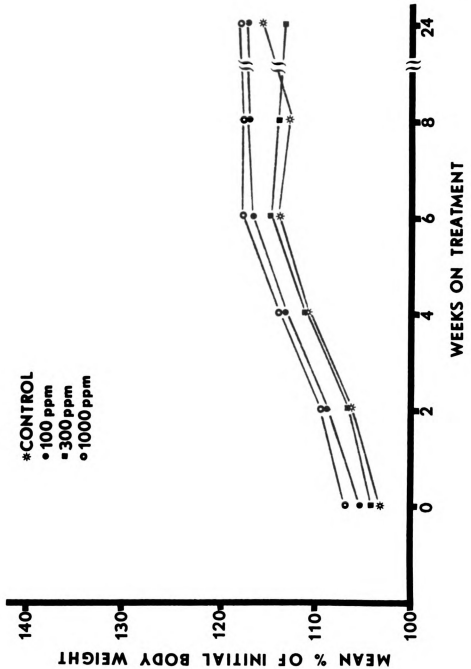

MEAN % OF INITIAL BODY WEIGHT
140
130
120
110
100
0
2
4
6
8
24
WEEKS ON TREATMENT
*CONTROL
•100 ppm
▪300 ppm
○1000 ppm

Table 14. The effect of chronic dietary administration of fluridone to female Bobwhites upon mean percent change of initial[1] body weight (±S.D.)

Weeks on treatment	Dietary concentration (ppm)			
	0	100	300	1000
0	103.9[2] (± 5.30)	106.6 (± 4.39)[3]$_a$	107.5 (± 4.74)$_a$	106.1 (± 3.94)$_a$
2	109.9 (± 5.43)	107.6 (± 7.07)$_a$	107.9 (± 4.58)$_a$	108.0 (± 3.57)$_a$
4	112.6 (± 6.79)	111.6 (± 5.86)$_a$	114.4 (± 5.93)$_a$	112.1 (± 3.92)$_a$
6	117.1 (± 5.97)	116.4 (± 4.87)$_a$	119.0 (± 7.02)$_a$	118.0 (± 4.36)$_a$
8	119.9 (± 9.23)	118.9 (± 7.57)$_a$	119.1 (± 9.66)$_a$	121.5 (± 9.66)$_a$
24	128.9 (±12.87)	132.2 (± 6.07)$_a$	128.9 (±13.75)$_a$	129.8 (± 8.11)$_a$

[1] Body weight on 9-5-79 taken as initial body weight.

[2] n = 15 except controls (n = 14) after 10-3-79 and 300 ppm (n = 14) on 3-5-80.

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

Figure 11. The effect of chronic dietary administration of fluridone to Bobwhite females upon mean percent change of initial body weight.

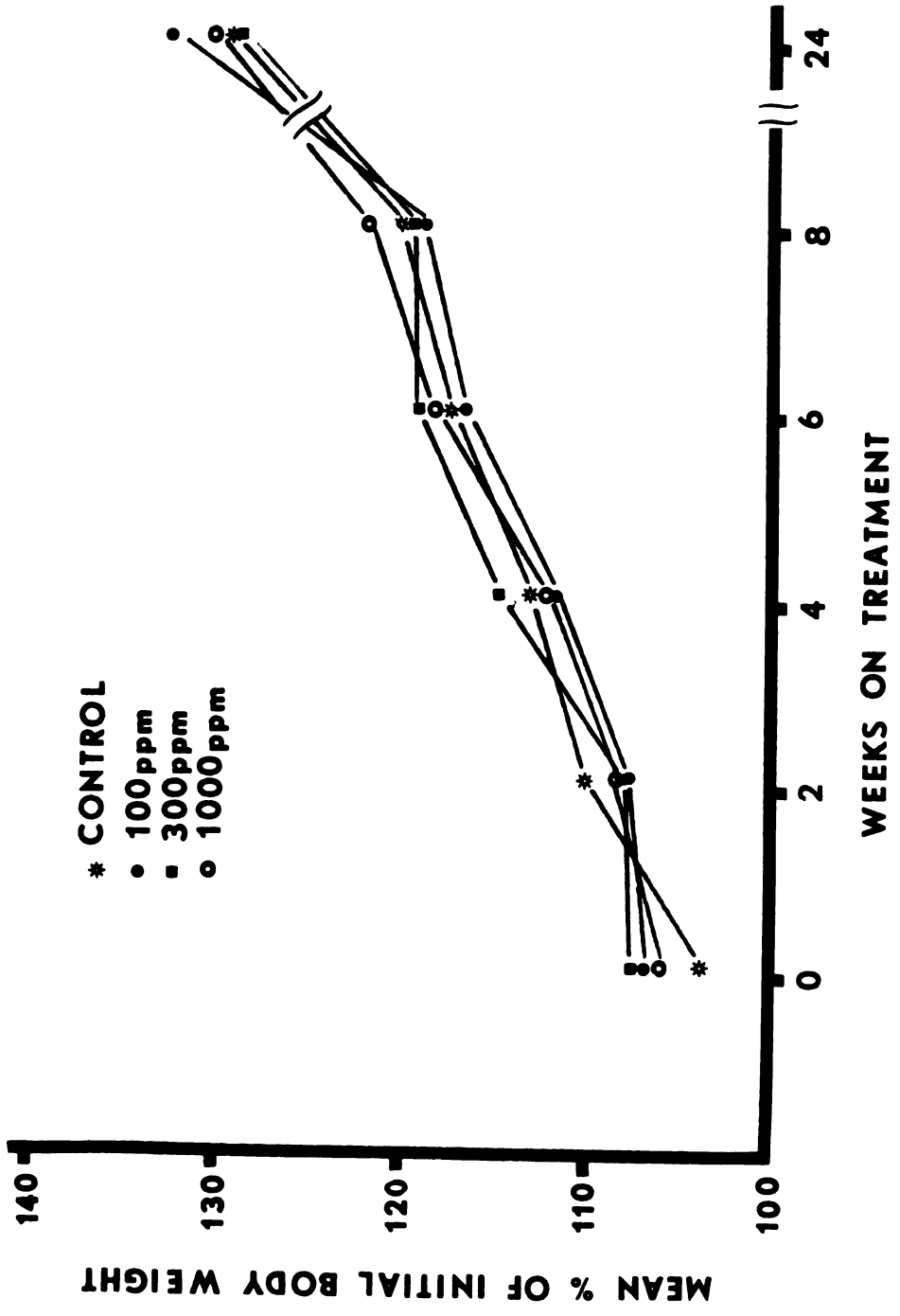

MEAN % OF INITIAL BODY WEIGHT
140
130
120
110
100
0
2
4
6
8
24
WEEKS ON TREATMENT
* CONTROL
• 100ppm
▪ 300ppm
o 1000ppm

Table 15. The effect of chronic dietary administration of fluridone to Bobwhites upon egg production (eggs/female/day ± S.D.)

Set number	Dietary concentration (ppm)			
	0	100	300	1000
1	0.408[1] (± 0.3995)	0.705 (± 0.3028)[2]$_a$	0.600 (± 0.2815)$_a$	0.543 (± 0.3464)$_a$
2	0.408 (± 0.4224)	0.724 (± 0.2442)$_a$	0.600 (± 0.3110)$_a$	0.571 (± 0.3327)$_a$
3	0.418 (± 0.4247)	0.743 (± 0.2034)$_a$	0.629 (± 0.3138)$_a$	0.562 (± 0.3214)$_a$
4	0.418 (± 0.4018)	0.771 (± 0.2006)$_b$	0.524 (± 0.3084)$_a$	0.552 (± 0.3884)$_a$
5	0.408 (± 0.4334)	0.666 (± 0.1762)$_a$	0.592 (± 0.3060)$_a$	0.571 (± 0.2906)$_a$
6	0.439 (± 0.4320)	0.695 (± 0.1779)$_a$	0.520 (± 0.3995)$_a$	0.657 (± 0.3650)$_a$
7	0.337 (± 0.3783)	0.581 (± 0.2441)$_a$	0.306 (± 0.3307)$_a$	0.467 (± 0.3718)$_a$
8	0.337 (± 0.3945)	0.457 (± 0.2655)$_a$	0.276 (± 0.314)$_a$	0.333 (± 0.3399)$_a$
9	0.357 (± 0.3531)	0.514 (± 0.2741)$_a$	0.306 (± 0.3536)$_a$	0.362 (± 0.3450)$_a$
10	0.388 (± 0.3605)	0.533 (± 0.2723)$_a$	0.316 (± 0.3322)$_a$	0.381 (± 0.3222)$_a$
11	0.398 (± 0.3848)	0.543 (± 0.2966)$_a$	0.388 (± 0.3038)$_a$	0.362 (± 0.2797)$_a$

[1] n = 15 except controls (n=14 for all sets) and 300 ppm (n=14 from set 5 through set 11).

[2] Means with the subscript "a" are not significantly different from their respective control values ($P > 0.05$); means with the subscript "b" are significantly different from their respective control values ($P < 0.05$).

Figure 12. The effect of chronic dietary administration of fluridone to Bobwhites upon mean egg production.

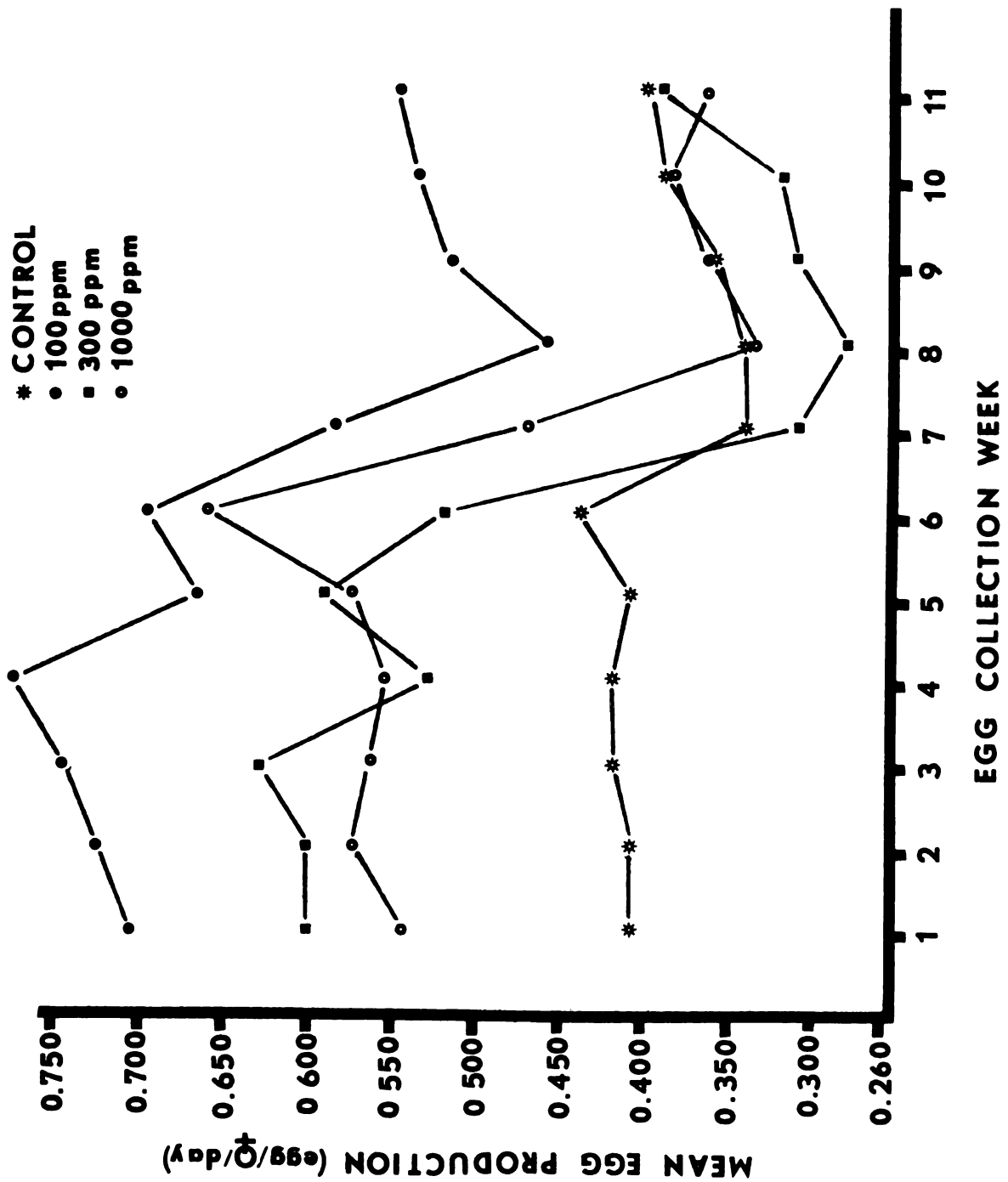

* CONTROL
• 100 ppm
▪ 300 ppm
○ 1000 ppm
MEAN EGG PRODUCTION (egg/♀/day)
0.750
0.700
0.650
0.600
0.550
0.500
0.450
0.400
0.350
0.300
0.260
1
2
3
4
5
6
7
8
9
10
11
EGG COLLECTION WEEK

Table 16. The effect of chronic dietary administration of fluridone to Bobwhites on mean reproductive parameters for all sets combined.

Dietary conc. (ppm)	# set	# laid	% set	# fertile	# set	% fertile
0	343	424	80.9	225	343	65.6
100	627	724	86.6^{1}_{a}	470	627	75.0_{a}
300	410	512	80.1_{a}	348	410	84.9_{b}
1000	467	563	82.9_{a}	318	467	68.1_{a}

ppm	# live day 11	# fertile	% live day 11	# live day 18	# live day 11	% live day 18
0	215	225	95.6	214	215	99.5
100	461	470	98.1_{a}	453	461	98.3_{a}
300	334	348	96.0_{a}	324	334	97.0_{a}
1000	291	318	92.1_{a}	287	293	98.0_{a}

ppm	# live prepip	# live day 18	% prepip[2]	# pipped	# live prepip	% pipped
0	177	187	94.7	173	177	97.7
100	394	406	97.0_{a}	387	394	98.2_{a}
300	286	293	97.6_{a}	280	286	97.6_{a}
1000	240	261	92.0_{a}	234	240	97.5_{a}

cont'd

Table 16 (con't).

ppm	# pipped live	# pipped	% pipped live	hatched	# pipped live	% hatched
0	171	173	98.8	75	171	43.9
100	382	387	98.7_a	261	382	68.3_b
300	279	280	99.6_a	150	279	53.8_a
1000	227	234	97.0_a	126	227	55.5_a

ppm	# survived	# hatched	% survived [3]	ppm	Eggshell thickness (mm)
0	48	64	75.0	0	0.249 _ 0.0096
100	174	233	74.7_a	100	0.243 ± 0.0183
300	95	129	73.6_a	300	0.249 ± 0.0171
1000	85	111	76.6_a	1000	0.248 ± 0.0144

[1] Percentages with the subscript "a" are not significantly different ($P > 0.05$) from their respective control values; percentages with the subscript "b" are significantly different ($P < 0.05$) from their respective control values.

[2] From this point on, one set (set #5) was eliminated; number live day 18 values will not coincide with values in the previous block.

[3] From this point on, an additional set (set #1) was eliminated; number hatched values will not coincide with values in the previous block.

due to the accidental absence of parent stock information. The reproductive parameters were converted to a percent of the number of eggs laid and are displayed as a histogram in Figure 13 and are listed in Table 17.

The pathology findings provided by Eli Lilly and Co. are listed in Table 18. Gross and microscopic examination presented no compound-related pathological alterations.

Table 17. Bobwhite reproductive parameters expressed as a percentage of eggs laid.

Parameter	Dietary concentration (ppm)			
	0	100	300	1000
% set	80.9	86.1	82.9	80.1
% fertile	53.3	64.6	70.4	54.4
% live day 11	50.7	62.8	67.8	49.8
% live day 18	50.4	62.2	67.4	48.8
% live prepip	47.7	60.3	65.8	44.9
% pipped	46.1	59.2	64.2	43.8
% pipped live	45.5	58.6	63.8	42.5
% hatched	20.0	39.9	34.2	23.6
% survived	15.0	29.8	25.2	18.1

Table 18. Pathology report for the Bobwhites.†

Diagnosis	0 ppm		100 ppm		300 ppm		1,000 ppm	
	M‡	F‡	M	F	M	F	M	F
Died on test		1*	1			1	2	
no substantive tissue alteration		1	1				1	
Killed - no substantive microscopic tissue alteration	5	5	4	4	5	4	5	4
Killed - no substantive gross tissue alteration	10	8	9	9	10	7	7	9
Skeletal muscle myositis						1		
Heart								
inflammatory foci								1
myocardial degeneration						1		
Duodenum - mild enteritis			1					
Colon - mild colitis						1		
Liver								
amyloidosis		1		1		2		1
inflammatory foci							1	

Continued

Table 18 cont'd.

Diagnosis	0 ppm		100 ppm		300 ppm		1,000 ppm	
	M	F	M	F	M	F	M	F
Uterine obstruction				1				
General - starvation			1					

†Lilly, 1980.

‡Male and Female.

*Values represent the numbers of birds in that particular category; 15 female and 15 males per treatment group.

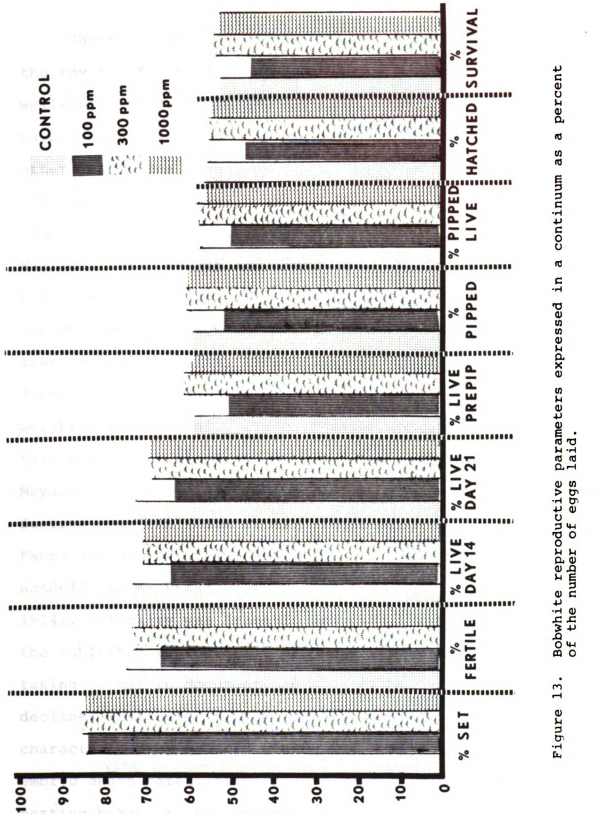


Figure 13. Bobwhite reproductive parameters expressed in a continuum as a percent of the number of eggs laid.

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DISCUSSION

Shortly after the introduction of DDT in the 1940's the toxic effects of pesticides on avian wildlife became well-documented. Although the organochlorine compounds have caused most of the pesticide-related toxicity problems, other pesticides have been incriminated as well (McEwen and Stephenson, 1979). Some of these adverse effects include increased mortality (Fergin and Schafer, 1977; Wallace, 1959; Pearce and Peakall, 1977; Turtle et al., 1963; Hunt, 1960; Ratcliffe, 1969), decreased food consumption (Heinz, 1979; Davison and Sell, 1974; Ernst and Ringer, 1968; Keith and Mulla, 1966; Linduska and Springer, 1957), decreased body weight (Nusz et al., 1976), increased organ weights (Strik et al., 1980), changes in the levels of certain enzymes or blood parameters (Iturri et al., 1978; Meydani and Post, 1979; White, 1976), and sublethal reproductive problems (Blus et al., 1979; Faber et al., 1972; Faber and Hickey, 1973; Frank et al., 1975; Odsjo and Sondell, 1976; Peakall, 1970; Jefferies, 1967; and Heinz, 1974). Lethality was dramatic and easily observed while the sublethal reproductive problems were more subtle thereby taking longer to document and understand. Population declines attributed to reproduction problems have been characterized by reduced clutch size, egg breakage, high embryo death rates, high chick mortality rates, and unusual nesting behavior (McEwen and Stephenson, 1979). The most

documented and researched effect is that of eggshell thinning (Ratcliffe, 2967; Hickey and Anderson, 1968; Cooke, 1973; Davison and Sell, 1974).

In an effort to predict and control these pesticide-related effects on avian species, the U.S. Environmental Protection Agency (EPA) has proposed the requirement of avian toxicity studies for partial fulfillment of pesticide registration (Federal Register, 1978). These studies, along with other data provided by the manufacturer, enable EPA to evaluate the hazards posed to birds as the result of a particular pesticide's use (Federal Register, 1978). The proposed rules require both an avian single-dose oral LD50 and an avian dietary LC50 to support the registration of all manufacturing-use products and all formulated products intended for outdoor application. A subsequent avian reproduction study would be required if any of the following conditions exist: 1) the pesticide is persistent in the environment to the extent that toxic amounts on avian feed could be expected under normal use; 2) the pesticide is stored or accumulated in plant or animal tissues; 3) the pesticide is intended for use under conditions where birds may be subjected to repeated or continued exposure to the pesticide especially during the breeding season; or 4) any exisiting test information indicates that reproduction may be adversely affected by the use of the pesticide (Federal Register, 1978).

Acute oral toxicity tests measure the inherent toxicity

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of a chemical (Tucker and Crabtree, 1970). Data that such tests provide are used mainly for comparisons of species' sensitivity or a pesticide's relative toxicity. This method involves the administration (by peroral intubation) of the test chemical as a single dose to groups of five or more birds. The birds are observed for fourteen days during which time mortality is recorded. Sufficient dosage levels are used to statistically calculate an LD50. The LD50 is defined as the dosage of test chemical that is lethal to fifty percent of the experimental population (Federal Register, 1978).

The avian dietary LC50 is a subacute test which measures a species' response to a diet contaminated with the test chemical (Heinz, 1979). Subacute studies are different from acute studies in the sense that they allow for metabolic changes that occur more readily in repeated dietary exposure. Data provided by subacute studies are often more useful in wildlife toxicology assessments than acute data since the test chemical is consumed in a manner more similar to actual field situations. The speed of incapacitation and reversibility of compound-related effects can also be determined via subacute studies. The avian dietary LC50 is run for a total of eight days - five days on treated feed followed by three days on uncontaminated feed. Toxicity is expressed as the median lethal concentration (LC50) of a chemical in dry diet. The concentration is most commonly presented as parts per million (ppm) in feed (Federal Register, 1978).

The present study is an example of the third type of proposed avian study and adheres to the guidelines proposed by EPA (Federal Register, 1978). Avian reproduction studies determine a pesticide's effect on all aspects of reproduction. More detailed information can be obtained from the Materials and Methods section of this thesis.

Other conditional avian studies have been proposed and would be requested on a case-by-case basis. These include a short-term (small pen) field test, a long-term (large) pen field test, and a full scale field test which would evaluate hazard to wildlife, including birds. These studies provide exposure through pesticide application as would occur in the field situation.

Toxicity of Fluridone.

Since chemicals vary in their ability to produce lethality, dose categories have been devised which rate a compound in terms of its toxicity. Classification systems exist for acute oral LD50's, dermal LD50's, aquatic 96-hour LC50's, and subacute dietary LC50's. These rating systems are presented in Tables 19 and 20.

With the information provided in the above mentioned tables and fluridone toxicity data from Tables 1 and 2, it is obvious that fluridone was not very toxic to the mammalian and avian species tested. However, it was moderately to highly toxic to fish.

Herbicides are generally less toxic to animals than insecticides. This makes sense when consideration is given

Table 19. Classification systems used to rate the toxicity of chemicals.[1]

Rating	Oral LD50	Dermal LD50	Aquatic 96 hour LC50
Extremely toxic	<5 mg/kg[2]	<5mg/kg	<1 mg/l[3]
Highly toxic	5-50 mg/kg	5-200 mg/kg	1-10 mg/l
Moderately toxic	>50-500 mg/kg	>200-500 mg/kg	>10-100 mg/l
Slightly toxic	>0.5-5 g/kg	>0.5-5 g/kg	>100-1,000 mg/l
Relatively nontoxic	>5 g/kg	>5 g/kg	>1,000 mg/l

[1] Michigan Department of Natural Resources, 1980.

[2] mg of chemical per kg of body weight.

[3] mg of chemical per liter of water.

Table 20. Classification system used to rate or compare the subacute dietary LC50s of chemicals[1].

Rating	Subacute dietary LC50 (ppm)
Highly toxic	<40
Very toxic	41-200
Moderately toxic	201-1,000
Slightly toxic	1,001-5,000
Probably not toxic	>5,000

[1] Heinz, 1979.

to the fact that plants differ from animals in major aspects of their morphology and physiology (Doull et al., 1980). The lower toxicity of herbicides over insecticides is demonstrated in Table 21 which lists the LC50s of several herbicides and insecticides administered to Bobwhites. The LC50 of fluridone to Bobwhites is > 5,000 ppm and therefore, is relatively non-toxic (Table 1). The LD0 (highest non-lethal dose) of fluridone to Mallards is 21,000 mg/kg (Table 1). A list of the acute oral LD50s of several other pesticides administered to Mallards is presented in Table 22. Fluridone is less toxic than most of the other pesticides listed.

LC50s and LD50s provide minimal information when evaluating a compound's potential hazard to avian life. Chronic studies can provide valuable information for use in calculating the maximum daily dietary intake of a compound tolerated without adverse effects (Kenaga, 1973). Comparison of the level of pesticide tolerated by birds in toxicity studies with the level expected in field situations provides a basis for assessing a pesticide's hazard to birds.

The approximate daily intake of fluridone for Mallards and Bobwhites during the course of the present study is reported in Tables 23 and 24, respectively. Prior to calculation, the fluridone intake was expected to be greater for Bobwhites than for Mallards. This assumption was based on the fact that smaller sized animals consume more food on a percent body weight basis due to the increased surface area

Table 21. Subacute dietary LC50s of certain insecticides and herbicides administered to Bobwhites[1].

Insecticide	LC50 (ppm)	Herbicide	LC50 (ppm)
Aldrin	37	Atrazine	>5,000
Carbaryl	>2,000	2,4-D	>5,000
Chlordane	331	Diquat	2,932
DDT	611	Diuron	1,730
Diazinon	245	Monuron	>5,000
Dichlorvos	298	Picloram	>5,000
Dieldrin	37	Propachlor	>5,000
Endrin	14	Silvex	12,979
Fenitrothion	157	Simazine	>5,000
Lindane	882	2,4,5-T	2,776
Malathion	3,497	Triclopyr	2,935
Methoxychlor	>5,000		
Mirex	>2,511		
Parathion	194		
Phosphamidon	24		
Trichlorfon	720		
Toxaphene	828		

[1]Tucker and Crabtree, 1970.

Table 22. Acute LD50s (Mg/kg) of certain herbicides and insecticides administered orally to Mallards.[1]

Insecticide	LD50 (mg/kg)	Age & sex of Mallards	Herbicide	LD50 (mg/kg)	Age & sex of Mallards
Aldrin	520	3-4 mo. females	Aminotriazole	>2,000	3-4 mo. males
Baygon	11.9	4-6 mo. females	Atrazine	>2,000	6 mo. females
Chlordane	1,200	4-5 mo. females	Balan	>2,000	3-4 mo. females
DDT	>2,240	3 mo. females	Casoron	>2,000	3 mo. females
Diazinon	3.54	3-4 mo. females	Chloroxuron	>2,000	3-4 mo. females
Dieldrin	381	6-7 mo. females	Dichlone	>2,000	
Endrin	5.64	10-13 mo. females	Dichlobenil	>2,000	
Guthion	136	3-4 mo. males	Dinoseb	9-13	
Heptachlor	>2,000	3 mo. males	Diquat	564	3-4 mo. males
Lindane	>2,000	3-4 mo. males	Diuron	>2,000	3-4 mo. males
Malathion	1,485	3-4 mo. females	Elgetol	22.7	5-7 mo. males
Methoxychlor	>2,000	3-4 mo. males	Picloram	>2,000	3-4 mo. males
Mirex	>2,400	3-4 mo. males	Propachlor	512	
Parathion	2.13	3-4 mo. males	Silvex	>2,000	3-4 mo. males
Phosphamidon	3.05	3 mo. females	Trifluralin	>2,000	
Rotenone	>2,000	3-4 mo. females	2,4-D	>2,025	3-5 mo. males & females
Sevin (Carbaryl)	>2,179	3 mo. females			
Strychnine	2.9	6 mo. females & males	2,4,5-T	>2,000	
Toxaphene	70.7	3-5 mo. females			

[1]Tucker and Crabtree, 1970.

Table 23. Amount of fluridone (mg/kg/day) ingested by Mallards.

Weeks on Feed	Dietary Concentration (ppm)		
	100	300	1,000
2	12.2	34	118.5
4	12.6	39.9	121.3
6	12.8	42.6	119.1
8	11.8	31.2	115.4
18	19.8	53.4	158.4
28	15.9	32.7	146.9

Table 24. Amount of fluridone (mg/kg/day) ingested by Bobwhites.

Weeks on Feed	Dietary Concentration (ppm)		
	100	300	1,000
2	8.8	26.2	90.3
4	8.2	26.1	91.3
6	8.8	27.0	89.4
8	9.2	27.1	89.3
16	11.8	35.4	116.2
24	13.2	38.1	126.5

to body weight ratio (Wilson, 1972; Prosser, 1973). One possible explanation for the results is the Mallard feed wastage. Pen-reared birds such as ducks and pheasants waste a considerable amount of feed (Kenaga, 1973). Empirical observations indicated that feed wastage was considerable for the Mallards in this study. Since no mathematical compensations were made during food consumption calculations, the Mallard food consumption data may be erroneously high. Feed wastage by the Bobwhites was minimal.

The highest daily fluridone intake by Mallards was 158.4 mg/kg while for Bobwhites the highest value was 126.5 mg/kg (Tables 23 and 24). Both values were calculated from the 1,000 ppm treatment groups whose values were not significantly lower than the control groups in any of the analyzed parameters. This information can now be compared to the quantity of residues expected in the environment, providing important data necessary to evaluate this pesticide's hazard to birds. As an example, the Mallard's daily intake of fluridone can be used to make this comparison especially since the reported residue data pertains to aquatic use.

Eli Lilly and Co. has established recommended fluridone application rates for use in research trials (Lilly, 1981). This information, plus data from a study by West et al. (1979) was used to approximate and evaluate a daily intake level for Mallards presuming they consumed a quantity of plant material equal to the quantity of diet consumed during the reproduction study. This is only a rough approximation

since many variables determine food consumption and actual residue intake. The approximation assumes similar caloric content of both foods (plant material and prepared feed).

The application rate on one of the ponds utilized in the study by West et al. (1979) was 2.7 kg/ha. This rate is higher than the 0.55-1.1 kg/ha rate suggested by Lilly for a pond of equivalent size (Lilly, 1981). Residue analysis calculated 3.98 ppm of fluridone residue on aquatic plants three days after treatment. This was the highest value reported in the study. If a Mallard were to consume 155 grams of plant material (average food consumption during the study) when residue levels were 3.98 ppm, it's daily intake would be 54.9 mg/kg/day. This figure is based on an average body weight of 1123.2 grams (calculated from body weight data obtained from this study). Even though this hypothetical situation was maximized in terms of application rate and dietary habits, a daily intake lower than values estimated during this study was calculated. This estimation supports the argument that fluridone was administered in this study at doses high enough to be of value in extrapolating to a real field situation. The estimation also suggests that fluridone, when used as recommended, should not present a hazard to Mallards.

Examination of the Study's Results.

Mallard mortality which occurred during the study was high but was not caused by fluridone administration. The Mallard drakes that died on test started to die shortly

after induction of the study's production phase. All six drakes were dead before the first female death was reported. A similar trend was shown by Breslin (1981) and Jones (1977). All of the male deaths and most of the female deaths were caused by aggressive behavior which developed in response to the increased photoperiod (Table 3). Increasing day-length stimulates hormone production which leads to sexual maturation and competitive behavior (Welty, 1979; Sturkie, 1976).

Bobwhite mortality was less frequent with no deaths attributed to aggressive behavior. Similar mortality data were reported by Howell (1981) and Breslin (1981) whose studies also included one pair per cage.

Body weight changes for both Mallards and Bobwhites (Tables 3, 4, 11, and 12 and Figures 5, 6, 10, and 11) and feed consumption data for Bobwhites (Table 10 and Figure 9) were comparable to figures and trends reported in the literature (Howell, 1981; Jones, 1977; Breslin, 1981; Scott et al., 1976). Feed consumption for Mallards (Table 2 and Figure 4) was comparable to one study (Breslin, 1981), slightly higher than another (Jones, 1977), and considerably higher than a third (Davison and Sell, 1974).

Mallard egg production data and percentages for other reproductive parameters were quite high (Tables 5 and 6 and Figures 7 and 8) and in agreement with other results reported in the literature (Breslin, 1981; Jones, 1977; Nestler et al., 1944; Federal Register, 1978; Heath et al., 1969;

Davison and Sell, 1974).

Although significant in only one case ($P < 0.05$), the Bobwhite egg production data were lowest for the control group (Table 13 and Figure 12). However, egg production values reported for all treatment groups were within the ranges provided by other studies (Breslin, 1981; Howell, 1981; Fergin and Schafer, 1977). Fertility and hatchability of the control group was also lower than other treatment groups (significantly lower in two instances). These results were unexpected and are difficult to explain since birds and dietary concentrations were randomly assigned to the pens and all pens were treated similarly. Although the control group was generally less productive than the other groups and meaningful comparisons were more difficult to make, the lack of a dose response trend by treatment groups reaffirms that there were no fluridone-related effects.

Some of the Bobwhite reproductive data (egg production and embryo survival) is comparable to data from other studies (Wilson and Holland, 1974; Howell, 1981; Federal Register, 1978; and Breslin, 1981). Other parameters, primarily fertility, hatchability, and survivability range lower or are in the lower limits of the ranges provided by other studies (Howell, 1981; Federal Register, 1978; Breslin, 1981; Wilson and Holland, 1974). Figure 18 illustrates where the most dramatic losses have occurred in the continuum of reproductive parameters.

Bobwhite and Mallard eggshell thickness measurements

were both greater than those reported in the literature (Jones, 1977; Howell, 1981; Federal Register, 1978; Heath et al., 1969).

CONCLUSIONS

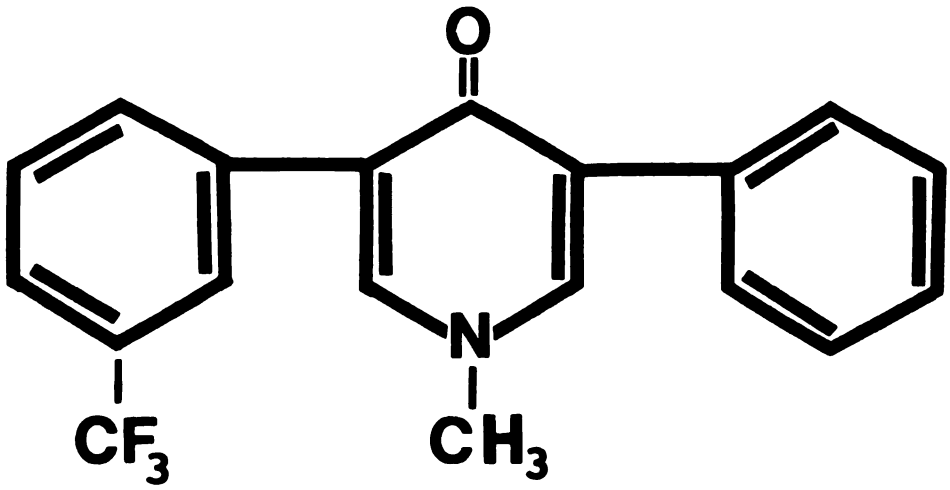
1. Chronic dietary administration of fluridone did not affect the food consumption or body weight gain of Bobwhites or Mallards.
2. Chronic dietary administration of fluridone did not affect egg production or fertility of Bobwhites or Mallards.
3. Chronic dietary administration of fluridone did not affect embryo survival, hatchability, or survivability (2 weeks) of the offspring (Bobwhites and Mallards).
4. Chronic dietary administration of fluridone did not affect eggshell thickness (Bobwhites and Mallards).

APPENDICES

APPENDIX A

STRUCTURE AND SOLUBILITIES OF FLURIDONE

CHEMICAL STRUCTURE OF FLURIDONE:



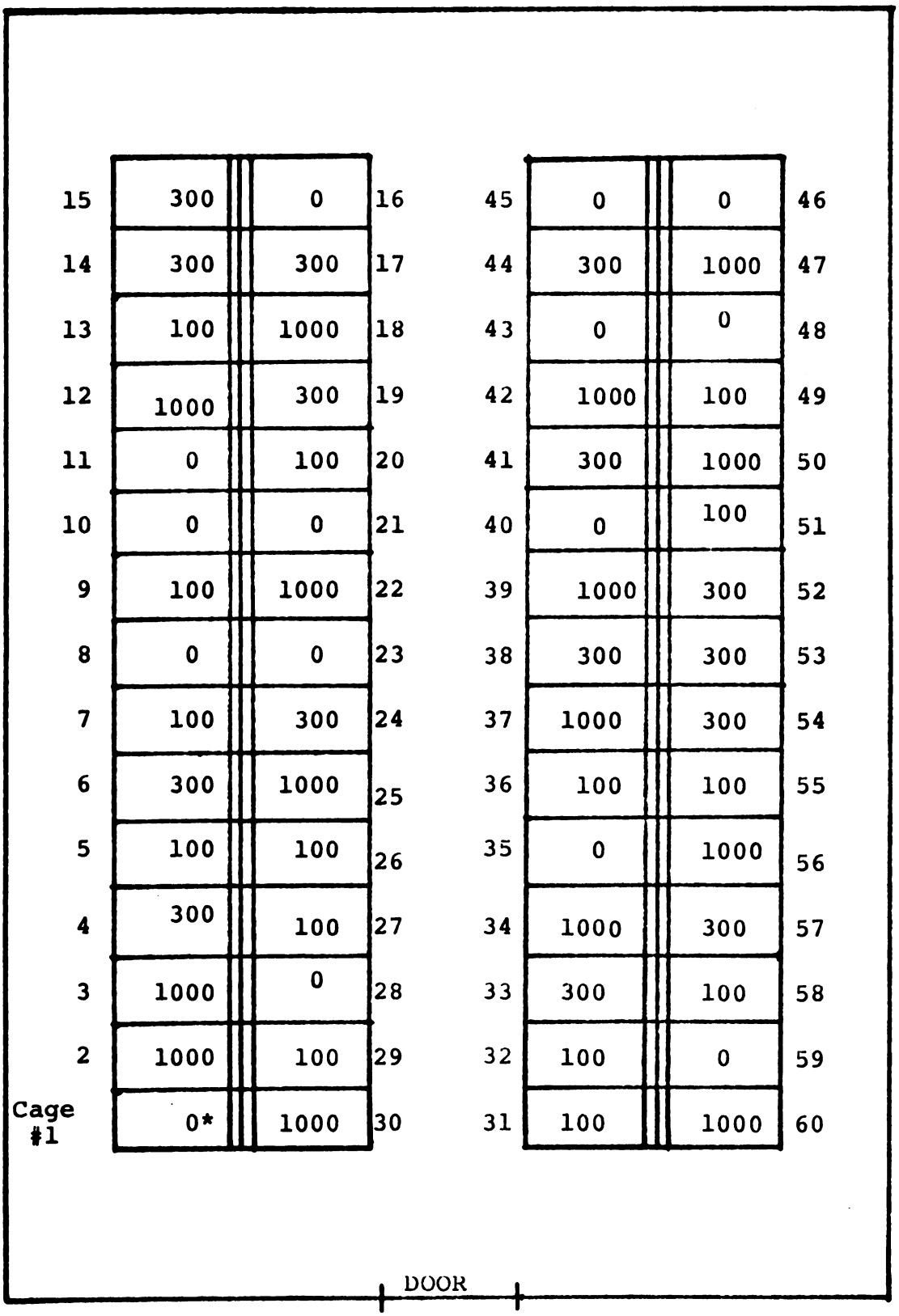
MOLECULAR FORMULA: $C_{19}H_{14}F_3NO$

SOLUBILITY:

Solvent	Solubility (mg/ml)
methanol	> 10.0
diethylether	> 1.0
ethylacetate	> 5.0
chloroform	> 10.0
hexane	< 0.5
dimethyl sulfoxide	240-250
tetraethyleneglycol	20-25
ethanol	30-50
dimethylformamide	400-450

APPENDIX B

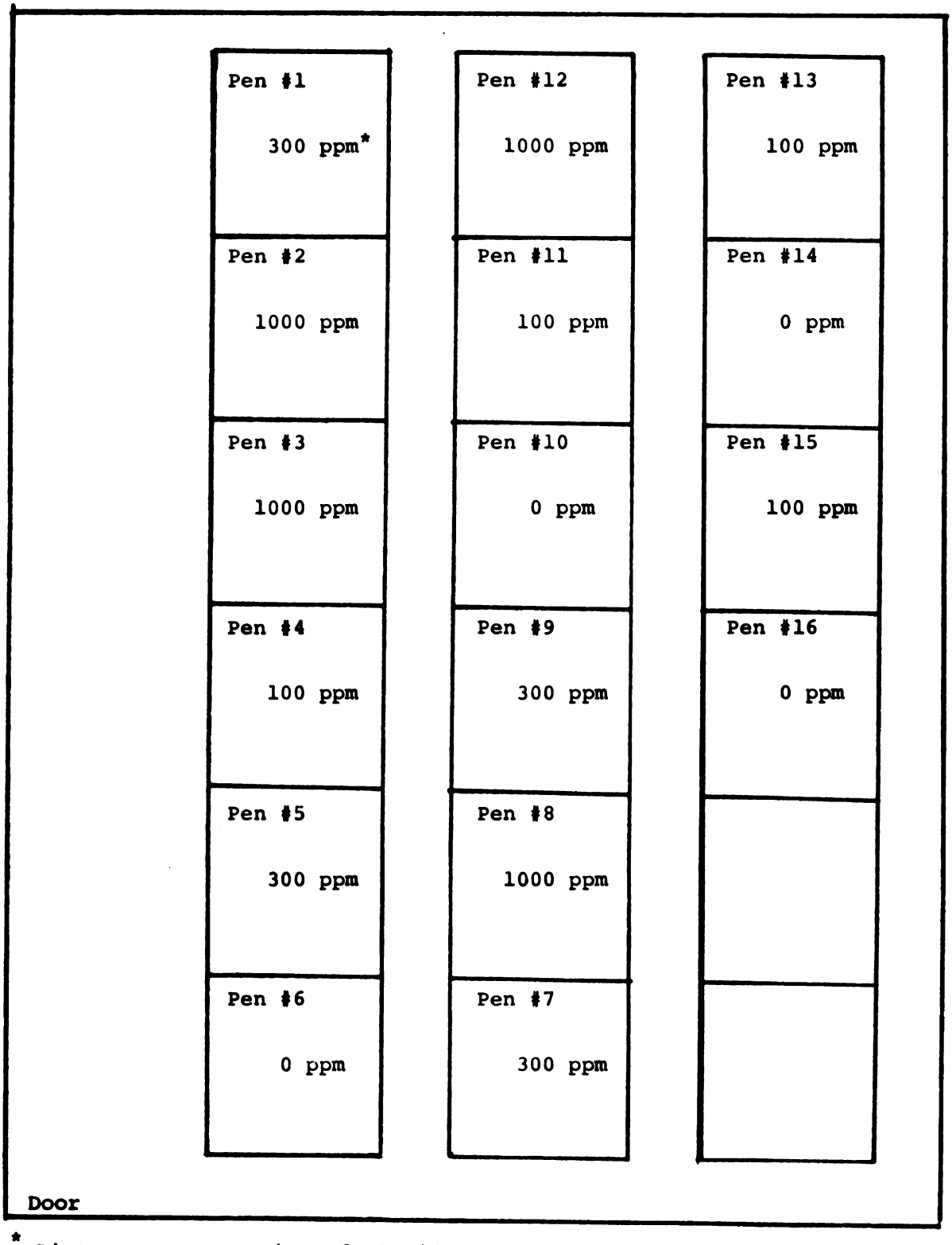
LAYOUT OF BOBWHITE TESTING ROOM



*Dietary Concentration (ppm)

APPENDIX C

LAYOUT OF MALLARD TESTING ROOM



* Dietary concentration of Fluridone.

APPENDIX D

COMPOSITION OF ADULT DUCK MASH [1]

INGREDIENTS	PERCENT	LBS./TON
Corn, Yellow Ground	62.42	1248.4
Soybean Meal, Solvent Extracted, Dehulled (49%)	19.58	391.6
Fish Meal	2.00	40.0
Meat and Bone Meal	4.00	80.0
Oat Groats, Rolled	2.50	50.0
Beef Tallow	0.93	18.6
Dicalcium Phosphate, Feed Grade	0.65	13.0
Limestone	6.82	136.4
Trace Mineral Premix TK-01 [2] (1.02)	0.10	2.0
Salt	0.30	6.0
Vitamin Premix TK-01 (1.03) [3]	0.50	10.0
Methionine Hydroxy Analog	0.20	4.0
	100.00	2000.0

[1] Eli Lilly and Company.

[2] Trace mineral premix provides 75 mg of manganese, 50 mg of zinc, 25 mg of iron and 1 mg of iodine per kg of complete food.

[3] Vitamin premix provides 3000 IU of vitamin A, 900 ICU of vitamin D, 40 mg of vitamin E, 0.7 mg of vitamin K, 1000 mg of choline, 70 mg of niacin, 4 mg of pantothenic acid, 4 mg of riboflavin, 100 mcg of vitamin B_{12}, 100 mcg of biotin and 125 mg of ethoxyquin per kg of complete feed.

APPENDIX E

CALCULATED ANALYSIS OF ADULT DUCK MASH

Protein, %	18.5	Vitamin K, mg/kg	0.7
Met. Energy, KCal/Kg	2893	Choline, mg/kg	1804
ME/P Ratio	156.4(71.1)	Niacin, mg/kg	85.2
Fat, %	3 80	Pantothenic Acid, mg/kg	10.9
Fiber, %	2 58	Vitamin B_6, mg/kg	7.0
Ash, %	10.97	Riboflavin, mg/kg	5.6
Calcium, %	3.25	Thiamine, mg/kg	2.9
Phosphorus, %	0.66	Folic Acid, mcg/kg	1802
Available Phosphorus, %	0.46	Vitamin B_{12}, mcg/kg	107
Manganese, mg/kg	89.0	Biotin, mcg/kg	228
Iron, mg/kg	105.7	Arginine, %	1.29(6.97)
Copper, mg/kg	9.7	Lysine, %	0.97(5.24)
Zinc, mg/kg	76.6	Glycine, %	1.10(5.94)
Selenium, mcg/kg	107.	Methionine, %	0.48(2.59)
Magnesium, mg/kg	2172	Cystine, %	0.26(1.41)
Potassium, mg/kg	6706	Total Sulfur Amino Acids	0.74(4.00)
Sodium, mg/kg	1821	Tryptophan, %	0.23(1.24)
Iodine, mg/kg	1	Linoleic Acid, %	1.24
Vitamin A, IU/kg	5060		
Vitamin E, mg/kg	56.2		
Vitamin D, ICU/kg	900		

[1]Eli Lilly and Company.

[2]As supplied from vitamin premix; content of other dietary ingredients not included.

[3]Values in parenthesis represent the amino acids expressed as a percent of dietary protein.

APPENDIX H

COMPOSITION OF ADULT BOBWHITE MASH[1]

INGREDIENTS	PERCENT	LBS./750 lbs.
Corn, Yellow Ground	49.37	370.28
Soybean Meal, Solvent Extracted, Dehulled (49%)	35.67	267.52
Fish Meal, Menhaden	2.00	15.00
Corn Distillers Dried Solubles	4.00	30.00
Beef Tallow	1.00	7.50
Dicalcium Phosphate, Feed Grade	2.88	21.60
Calcium Carbonate (Limestone)	3.88	29.10
Vitamin Premix TK-01 (1.03)[2]	0.50	3.75
Trace Mineral Premix TK-01 (1.02)[3]	0.10	0.75
Salt	0.30	2.25
Methionine Hydroxy Analog	0.30	2.25
	100.00	750.00

[1]Eli Lilly and Company.

[2]Vitamin premix provides 3000 IU of vitamin A, 900 ICU of vitamin D, 40 mg of vitamin E, 0.7 mg of vitamin K, 1000 mg of choline, 70 mg of niacin, 4 mg of pantothenic acid, 4 mg of riboflavin, 100 mcg of vitamin B_{12}, 100 mcg of biotin and 125 mg of ethoxyquin per kg of complete feed.

[3]Trace mineral premix provides 75 mg of manganese, 50 mg of zinc, 25 mg of iron and 1 mg of iodine per kg of complete feed.

APPENDIX I

CALCULATED ANALYSIS OF ADULT BOBWHITE MASH[1]

Protein, %	24.00	Vitamin K, mg/kg	0.7
met. energy, KCal/kg	2803.00	Choline, mg/kg	2139.0
ME/P Ratio	116.79(53.09)	Niacin, mg/kg	97.0
Fat, %	3.32	Pantothenic Acid, mg/kg	12.6
Fiber, %	2.68	Vitamin B_6, mg/kg	7.5
Ash, %	9.80	Riboflavin, mg/kg	6.2
Calcium, %	2.30	Thiamine, mg/kg	2.7
Phosphorus, %	1.00	Folic Acid, mg/kg	1.6
Avail. Phosphorus, %	0.72	Vitamin B_{12}, mcg/kg	103.0
Manganese, mg/kg	93.0	Biotin, mcg/kg	301.0
Iron, mg/kg	116.0	Arginine, %	1.717(7.15)[2]
Copper, mg/kg	18.0	Lysine, %	1.380(5.75)
Zinc, mg/kg	84.0	Glycine, %	1.248(5.20)
Selenium, mcg/kg	121.0	Methionine, %	0.629(2.62)
Magnesium, mg/kg	2104.0	Cystine, %	0.353(1.47)
Potassium, %	0.95	Total Sulfur Amino Acids, %	0.982(4.09)
Sodium, mg/kg	1626.0	Tryptophan, %	0.337(1.40)
Iodine, mg/kg	1.0	Linoleic Acid, %	1.22
Vitamin A, IU/kg	4629.0		
Vitamin D, ICU/kg	900.0		
Vitamin E, mg/kg	55.2		

[1]Eli Lilly and Company.

[2]Values in parenthesis represent the amino acids expressed as a percent of dietary protein.

APPENDIX J

COMPOSITION OF BOBWHITE CHICK MASH[1]

INGREDIENT	PERCENT	LBS./TON
Corn, Yellow Ground	46.52	348.90
Soybean Meal, Solvent Extracted, Dehulled (49%)	41.26	309.45
Fish Meal, Menhaden	4.00	30.00
Corn distillers Dried Solubles	4.00	30.00
Dried Whey	2.00	15.00
Dicalcium Phosphate, Feed Grade	0.39	2.92
Calcium Carbonate (Limestone)	0.73	5.48
Vitamin Premix TK-01 (1.03)[2]	0.50	3.75
Trace Mineral Premix TK-01 (1.02)[3]	0.10	0.75
Salt	0.20	1.50
Methionine Hydroxy Analog	0.30	2.25
	100.00	750.00

[1]Eli Lilly and Company.

[2]Vitamin premix provides 3000 IU of vitamin A, 900 ICU of vitamin D, 40 mg of vitamin E, 0.7 mg of vitamin K, 1000 mg of choline, 70 mg of niacin, 4 mg of pantothenic acid, 4 mg of riboflavin, 100 mcg of vitamin B_{12}, 100 mcg of biotin and 125 mg of ethoxyquin per kg of complete feed.

[3]Trace mineral premix provides 75 mg of manganese, 50 mg of zinc, 25 mg of iron and 1 mg of iodine per kg of complete feed.

APPENDIX K

CALCULATED ANALYSIS OF BOBWHITE CHICK MASH[1]

Protien, %	27.99	Vitamin K, mg/kg	0.7
Met. Energy, KCal/kg	2861.0	Choline, mg/kg	2330.0
ME/P Ratio	102.22(46.46)	Niacin, mg/kg	102.0
Fat, %	2.46	Pantothenic acid, mg/kg	14.3
Fiber, %	2.79	Vitamin B_6, mg/kg	7.8
Ash, %	5.54	Riboflavin, mg/kg	6.9
Calcium, %	0.70	Thiamine, mg/kg	2.8
Phosphorus, %	0.65	Folic Acid, mg/kg	1.8
Avail. Phosphorus, %	0.34	Vitamin B_{12}, mcg/kg	107.0
Manganese, mg/kg	95.0	Biotin, mcg/kg	324.0
Iron, mg/kg	123.0	Arginine, %	1.991(7.11)[2]
Copper, mg/kg	20.0	Lysine, %	1.675(5.98)
Zinc, mg/kg	89.0	Glycine, %	1.488(5.32)
Selenium, mcg/kg	168.0	Methionine, %	0.710(2.54)
Magnesium, mg/kg	2146.0	Cystine, %	0.413(1.48)
Potassium, %	1.13	Total Sulfur Amino Acids, %	1.124(4.02)
Sodium, mg/kg	1629.0	Tryptophan, %	0.169(0.60)
Iodine, mg/kg	1.0	Linoleic Acid, %	1.17
Vitamin A, IU/kg	4535.0		
Vitamin D, ICU/kg	900.0		
Vitamin E, mg/kg	54.8		

[1]Eli Lilly and Company.

[2]Values in parenthesis represent the amino acids expressed as a percent of dietary protein.

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