EFFECTS OF TRICYCLAZOLE ON SELECTED REPRODUCTIVE PARAMETERS IN MALLARDS AND BOBWHITE FOLLOWING CHRONIC DIETARY EXPOSURE

By

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ABSTRACT

EFFECTS OF TRICYCLAZOLE ON SELECTED REPRODUCTIVE PARAMETERS IN MALLARDS AND BOBWHITE FOLLOWING CHRONIC DIETARY EXPOSURE

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Tricyclazole was administered to mallards (Anas platyrhynchos) and bobwhite (Colinus virginianus) for 21 and 22 weeks, respectively, at dietary concentrations of 0, 30, 100, and 300 ppm to evaluate chronic reproductive toxicity. Food consumption, body weight gain, and selected reproductive parameters were monitored.

Chronic dietary administration of tricyclazole did not significantly affect food consumption, body weight gain, mortality, hatchling survival, or the reproductive parameters measured, including egg production, eggshell thickness, fertility, embryo survival, and hatchability. Based on the parameters measured the no-observed effect level for tricyclazole in the diet of mallards and bobwhites is 300 ppm, the highest level evaluated.

ACKNOWLEDGMENTS

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INTRODUCTION

Rice blast is one of the most serious and difficult to control of all rice diseases (Weber, 1982; Anonymous, 1982) and is gaining a substantial foothold in the rice producing areas of the United States' Gulf Coast. The ubiquitous organism Pyricularia oryzae is the infectious agent, but high soil nitrogen and organic matter levels as well as late planting, compound the problem for unknown reasons. Hot, humid weather also accentuates the problem. P. oryzae more easily infects young, developing plant tissue, but rice is susceptible to the fungus at all stages of growth, so timely fungicide applications and coverage are critically important in achieving optimum disease control (Weber, 1982; Anonymous, 1982).

Tricyclazole is a new class of compound developed to serve as a fungicide for control of rice blast as a result of P. oryzae infestation. Tricyclazole is being developed by Eli Lilly and Company in an effort to effectively provide prolonged control of rice blast with increased efficiency due to reduced frequency of application with the added benefit of less labor and equipment usage.

At the initiation of the study in September, 1980, chronic avian reproduction studies were required by the Environmental Protection

Agency under authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) if any of the following conditions existed:

1) if toxic amounts of a pesticide or its metabolite(s) are expected to accumulate on avian feed under normal use conditions.
2) if storage or accumulation of the pesticide or its metabolite(s) in plant or animal tissues is possible
3) if the pesticide is used where birds may be subjected to continued or repeated exposure to the pesticide or its metabolite(s), especially preceeding or during the breeding season.
4) if any other test information indicates that avian reproduction may be adversely affected.

(EPA, 1978)

The purpose of this study was to determine the effects of chronic dietary exposure of the fungicide tricyclazole on reproduction in two avian species. Mallards (Anas platyrhynchos) and bobwhite (Colinus virginianus) were the indicated species (EPA, 1978) due to their ubiquitous distribution and their representation as wild waterfowl and upland game species, respectively.

LITERATURE REVIEW

Tricyclazole

Tricyclazole (5-methyl-1,2,4,-triazolo [3,4-b] benzothiazole) (Figure 1) is a new systemic fungicide (Anonymous, 1982; Weber, 1982) developed by Elanco Products Company, Eli Lilly and Company for the control of rice blast disease caused by Pyricularia oryzae. It is at this time produced under the trade name Beam® under the authority of an EPA experimental use permit and is for experimental use only.

Tricyclazole is an odorless, crystalline solid with a molecular weight of 189.24 and its molecular formula is $C_9H_7N_3S$. It has a melting point of 187-188°C and is thermally stable up to this point. Tricyclazole has a vapor pressure of 2×10^{-7}mm Hg at 25°C. It is not readily degraded by water or sunlight. The solubility of tricyclazole in water at 25°C is 0.7 mg/ml. It is readily soluble in chloroform (> 500.0 mg/ml) but less soluble in other organic solvents (Appendix A) (Elanco, 1976; 1981).

Gas chromatographic or high-performance liquid chromatography procedures for determination of tricyclazole residues are discussed by Day et al. (1980) along with a discussion of biological, chemical and physical properties. Thin layer chromatography can also be used to identify tricyclazole in rice tissue and soil through a bioautographic assay technique using Cladosporium cucumerinum. In this assay, the fungus and compound interact to form a red zone of coloration (Elanco, 1976). The urinary metabolites of tricyclazole in the rat have been identified by thin-layer chromatography and gas chromatograph-mass spectrometry as the alcohol (1,2,4 triazolo [3,4-5]benzothiazole-5-methanol) and acid (1,2,4-triazolo[3,4-5]benzothiazole-5-carboxylic acid) derivatives of tricyclazole (Pierson and Howard, 1978).

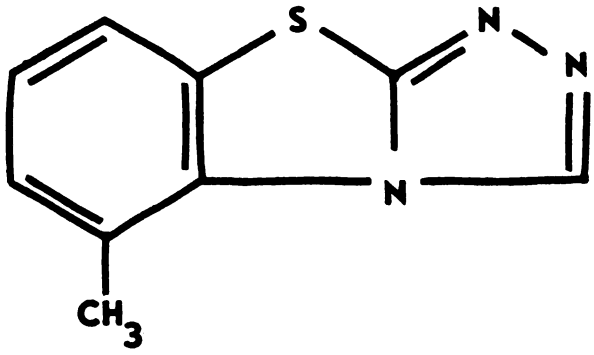


Figure 1. Structure of tricyclazole

Mode of Action and Efficacy

Tricyclazole is a systemic fungicide which is readily absorbed by the plant's roots and leaves, translocated to the leaf tips and provides residual rice blast control after a single soil or foliar application (Weber, 1982, Elanco, 1981, Froyd et al., 1976). This is in contrast to other fungicides which provide protection only for areas exposed to the fungicide (Anonymous, 1982). It also provides control regardless of the weather, cultural practices, or severity of disease.

Tricyclazole does not prevent spore germination nor reduce appressorial formation on the rice plant surface. However, the compound does prevent formation of penetration hyphae from the appressoria which invade the epidermal walls of an untreated plant (Kubo et al., 1982; Shiba and Nagata, 1981; Tokousbalides and Sisler 1978b; Chrysayi, 1976). Tricyclazole inhibits melanization by P. oryzae mycelia and appressoria without affecting fungal growth (Woloshuk, et al., 1982, Yamaguchi, et al., 1982). Melanization in the appressoria appears to be a critical requirement for successful epidermal penetration (Woloshuk et al, 1983). Tokousbalides and Sisler (1978a) have demonstrated that tricyclazole blocks certain aspects of melanin synthesis which leads to a decrease in pigmentation. Several studies have indicated that tricyclazole blocks the reduction of 1,3,8-trihydroxynaphthalene to vermelone through inhibition of 1,3,8-trihydroxynapthalene reductase (Woloshuk et al., 1981; Woloshuk et al, 1980; Tokousbalides and Sisler, 1979; Tokousbalides, 1978) and Tokousbalides (1978) suggest that this reductase may be an unusual type of enzyme peculiar to secondary metabolism in fungi.

This reductase inhibition by tricyclazole has also been observed in other fungal species; Alternaria spp., Cladosporium cucumerinum, Cochliobolus carbonum, Diplodia natalensis, Duechsclera sorokiniana, Macrophomina phaseoli, Rhizoctonia leguminicola, Sclerotinia minor, Thielaviopsis basicola, and Verticillium pp. However, tricyclazole is specific only in preventing rice blast caused by P. oryzae and is not know to control any diseases associated with any of the above listed fungal species (Kubo et al., 1982; Wheeler, 1982; Elanco, 1981; Stipanovic and Wheeler, 1980; Tokousbalides and Sisler, 1979; Wheeler and Stipanovic, 1979a, b).

It has been demonstrated that tricyclazole provides better protection more efficiently (reduced frequency of application) than other fungicides now being used for rice blast control. This is true whether tricyclazole is applied as a soil drench, root treatment or foliar spray (Hasawa et al., 1982; Froyd et al, 1978; Tsai, 1977; Froyd et al., 1976; Hwang et al., 1976). Hwang et al. (1976) have demonstrated that tricyclazole is more efficient when applied before inoculation than after inoculation. Tricyclazole has been shown to result in increased yields when compared to other fungicides (Sakaoka, 1982; Neto et al., 1981; Yang, 1980).

Bioavailability

Weber (1982) discussed some of the factors that affect the bioavailability of tricyclazole. It was determined that aqueous solutions of tricyclazole have a pK_a of 1.6. Tricyclazole is absorbed to a greater extent by clay and sandy loam soils than by soil organic matter. Absorption increases greatly as pH decreases. It is postulated that as pH decreases tricyclazole becomes protonated

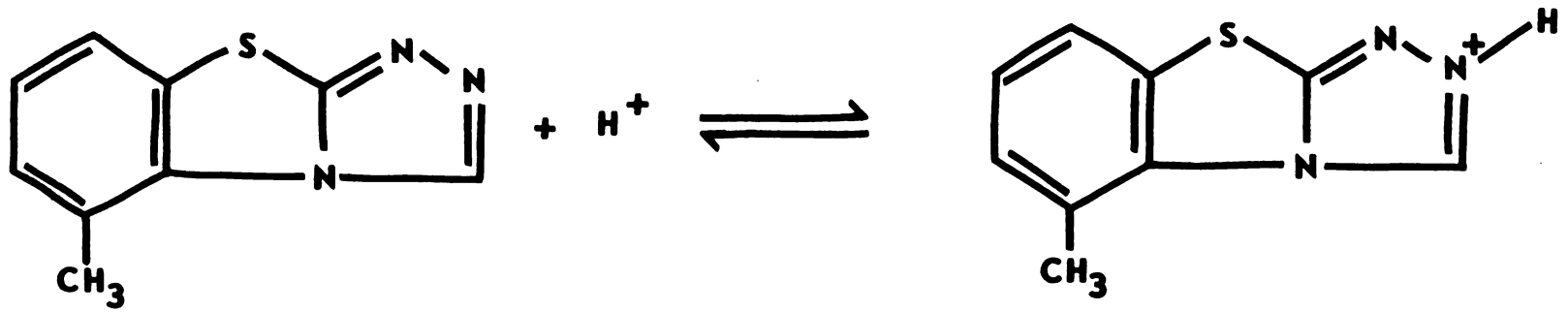
(Figure 2) and at pH levels of 0.5 or lower only protonated species are present while only molecular species are present at pH 3.0 and above (Weber, 1982).

Water is effective in desorbing bound tricyclazole from soil organic matter and sandy loam soils but is relatively ineffective in desorbing the fungicide from clay soils. Desorption is greatly increased with buffered salts (Weber, 1982).

High absorption of tricyclazole by soil organic matter, sandy loam and clay soils, effects of buffered salts and the pH dependence of absorption-desorption mechanisms have a significant impact on the bioavailability of the fungicide in soil systems and in aquatic systems containing soil particulate matter (Weber, 1982).

Toxicology

Pierson and Howard (1978) studied the disposition of tricyclazole in rats by using [14C] tricyclazole (uniformly labeled in the benzene ring) at 60 mg/kg administered by gavage. They found that the disappearance of radioactivity was biphasic, with a primary half-life ($t_{\frac{1}{2}}$) of 2.5 hr was followed by a more prolonged decay. Peak plasma concentration occurred one hour post-oral administration at a level of 5.8 g/ml. Radioactivity was rapidly distributed to all organs with highest concentrations occurring in the liver and kidney. There was no accumulation of radioactivity in any tissue following 7 daily doses of 60 mg/kg. Radioactivity was completely eliminated (98.2%) within 4 days, with urinary excretion being the major route of elimination (65.6); however, of elimination during the first 24 hours, biliary excretion accounted for 75.6% of the orally administered dose of tricyclazole.



tricyclazole

tricyclazole cation

Figure 2. Protonation of tricyclazole

A chronic toxicity-oncogenic study was conducted by Howard et al. (1978) in rats. Results showed a decrease in body weight at 620 and 1600 ppm of tricyclazole in the diet. However, at all dietary levels (up to 1600 ppm) of tricyclazole tested, there was no effect on survival, hematology, serum chemistry or urinalysis determinations. Terminal organ weights indicated a dose-related increase in mean relative weights (grams/100 grams body weight) for liver, kidney, and heart at the 620 and 1600 ppm dietary levels. This relative increase in organ weights parallels the decrease in body weights for these dose levels. Although there was an increase in mean relative weights, mean absolute organ weights were not significantly different from those of the control. Also gross and histopathologic examination of tissues and organs indicate that tricyclazole in the diets of rats, at all dietary levels tested, had no adverse effects (Howard et al., 1978).

In a three month subchronic study in mice, 400 ppm of tricyclazole increased liver weights in female mice. Doses of 1000-3600 ppm resulted in mortality, decreased body weight, increased liver weight and serum glutamic pyruvic transaminase, and caused small bile duct proliferation (Howard et al., 1979). A two year chronic toxicity-oncogenic study in mice (doses of 50, 140, or 400 ppm of tricyclazole) resulted in no significant variation in survival, body weight, water consumption, organ weights, hematology, clinical chemistry, gross and histopathologic observations, or in the incidence of neoplasms. A no effect level of 400 ppm was concluded by Howard and his colleagues (1979).

Other studies have demonstrated that tricyclazole has no effect on survival, growth and reproductive performance in mice and rats when

exposed to dietary concentrations up to 275 ppm for three generations. Also, tricyclazole has been shown not to be teratogenic in rabbits at daily oral doses of 50 mg/kg body weight. Teratogenicity does not occur in the rat or mouse at concentrations of 275 ppm of tricyclazole in the diet (Elanco, 1981). Moriya and his colleagues (1983) performed mutagenicity studies on pesticides by use of bacterial reversion assay systems and concluded that triyclazole is not mutagenic.

Elanco (1981) has compiled information from their own studies and those of others concerning toxicology, environmental toxicity, hazard evaluation, and grade tricyclazole, and subacute and chronic toxicity of tricyclazole. This information is summarized in Appendices B-E, respectively.

MATERIALS AND METHODS

Chronology

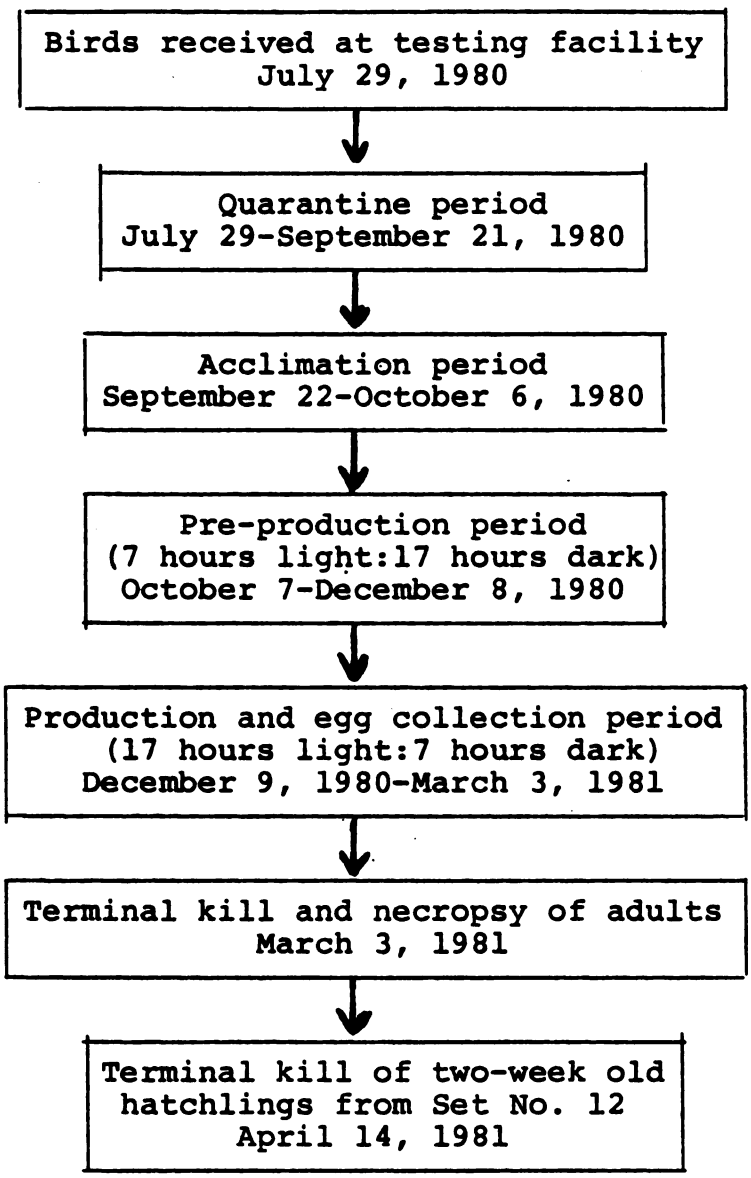
Mallards

The mallards (Anas platyrhynchos) used for the study were received on July 29, 1980 and were immediately placed into quarantine until September 21, 1980. The quarantine period was followed by a 15 day acclimation period, September 22, 1980 through October 6, 1980. During this time the mallards were in their appropriate testing pens and were fed the untreated basal diet. The mallards first received their assigned dietary level of tricyclazole on October 7, 1980, which was the beginning of the pre-production phase. This phase ended on December 9, 1980 when the photoperiod was increased from 7 hours light:17 hours dark per day to 17 hours light:7 hours dark, initiating the egg production phase. The egg production phase ended on March 3, 1981. The last set (set 12) of two-week-old hatchlings was terminated on April 14, 1981 (Figure 3).

Bobwhite

The bobwhite (Colinus virginianus) used for the study were received on July 17, 1980 and were immediately placed into quarantine until September 22, 1980. The quarantine period was followed by a 15 day acclimation period, September 23, 1980 through October 7, 1980. During this time the bobwhite were in their appropriate testing pens and were fed the untreated basal diet. The bobwhite first received their assigned dietary level of tricyclazole on October 8, 1980, which was the beginning of the pre-production phase. This phase ended on December 10, 1980 when the photoperiod was increased from 7 hours

Figure 3. Chronology of study for mallards fed tricyclazole.



light:17 hours dark per day to 17 hours light:7 hours dark, initiating the egg production phase. The egg production phase ended on March 11, 1981. The last set (set 10) of two-week-old hatchlings was terminated on April 24, 1981 (Figure 4).

Animals

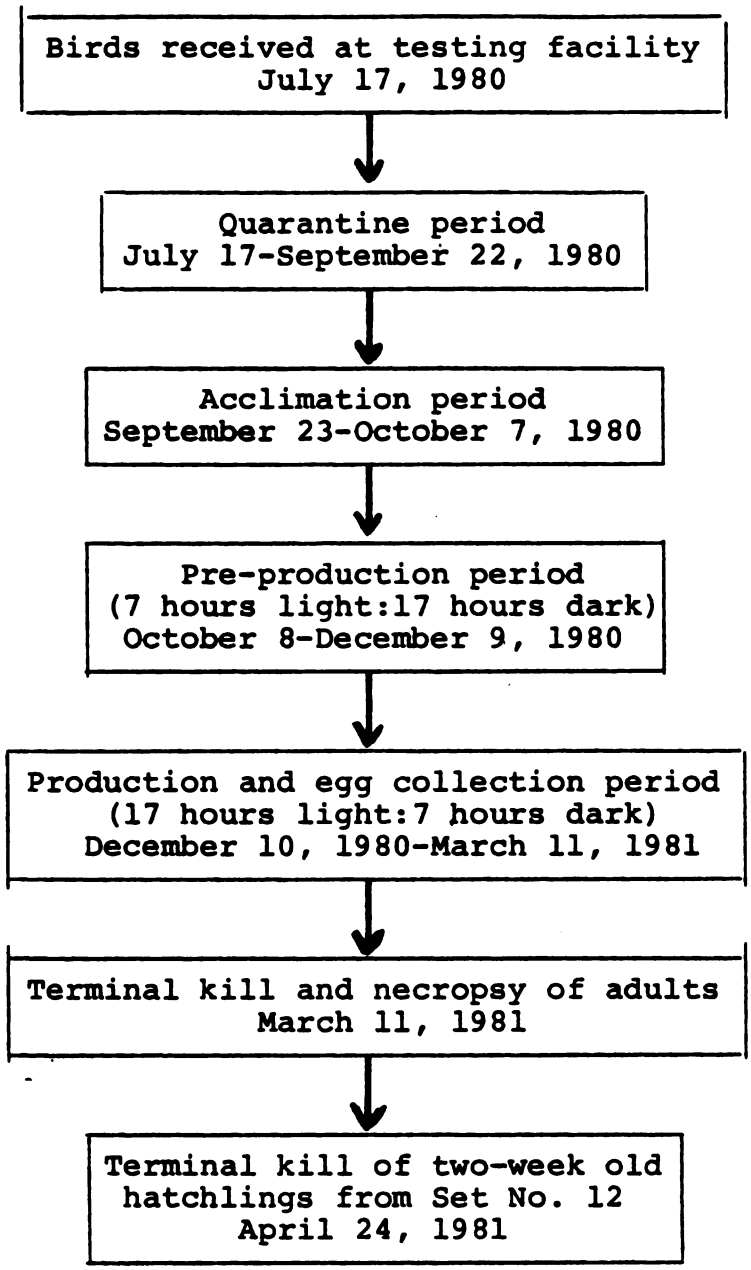
Mallards

Mallards were purchased from Whistling Wings, Hanover, IL. The mallards were hatched on June 16, 1980 and arrived at the test facility on July 29, 1980. The mallards were 14 weeks old at the start of the acclimation phase of the study, and the mean body weights ($\pm$ S.D.) were 1242 $\pm$ 83 g and 1014 $\pm$ 93 g for males and females, respectively. At the start of the acclimation period, all mallards appeared to be in good health. Each mallard was banded with its own unique leg band and was randomly assigned to a pen (1 male and 3 females per pen). Random assignment of dietary level to pens was accomplished by the use of a random numbers table. Two pens per room were randomly eliminated at the end of the acclimation period to maintain equal replication of doses of four pens per treatment per room.

Bobwhite

Bobwhite were purchased from Illinois State Game Farm, Mt. Vernon, IL. The bobwhite were hatched on July 1, 1980 and arrived at the test facility on July 17, 1980. The bobwhite were 12 weeks old at the start of the acclimation phase of the study, and the mean body weights ($\pm$ S.D.) were 177 $\pm$ 12 g and 172 $\pm$ 13 g for males and females, respectively. At the start of the acclimation period, all bobwhite appeared to be in good health. Each bobwhite was banded with its own

Figure 4. Chronology of study for bobwhite fed tricyclazole.



unique leg band and was randomly assigned to a pen (1 male and 1 female per pen). Random assignment of dietary levels to pens was accomplished by the use of a random numbers table.

Treatment Levels

The test compound used in both the mallard and bobwhite studies was tricyclazole (5-methyl-1,2,4,-triazolo [3,4-b] benzothiazole). Selection of dietary concentrations were based on previously conducted 5-day LC_{50} studies, mammalian reproduction studies, mallard and bobwhite palatability studies and environmental residue studies. The four dietary levels selected for both mallard and bobwhite studies were 0.0% (control), 0.003% (30 ppm), 0.01% (100 ppm), and 0.03% (300 ppm) tricyclazole. The 300 ppm treatment level exceeds by 60 fold the maximum field residue values of 5 ppm in rough rice (Cochrane, 1980). For the mallard study there were 8 pens in each of the control and treatment groups. The bobwhite study had 15 cages in the control and 0.003 ppm treatment group, 13 in the 0.01 ppm treatment group, and 14 in the 0.03 ppm treatment group.

Diets

The test diets were prepared by Eli Lilly and Company, Greenfield, Indiana using Lilly Mash. Homogeneity of mix and 8-week stability in feed were determined prior to the initiation of the study. Before the treated diets were shipped to the test facility, samples were removed for determination of the test chemical. Additionally, each dietary concentration was sampled when it was replaced with a freshly prepared diet to provide information on stability of tricyclazole during the 4-5 week period each lot was fed

to the mallards and bobwhites. Hatchlings were fed a starter diet for the two-week period they were raised. Both adults and hatchlings were given food and water ad libitum throughout the study. (See Appendices F through R for composition, analysis, dietary requirements, stability, homogeneity, and assays for tricyclazole.)

Facilities

Mallards

The mallards were housed on the Michigan State University Poultry Research and Teaching Center in House 4, Rooms 4A and 4B. The rooms were entirely enclosed and measured 10.97 m x 12.19 m x 2.44 m (W x L x H). Three rows of six pens were suspended from the ceiling by chains. Each pen measured 1.47 m x 1.55 m x 0.70 m with no top. The pens were constructed of 1" x 1" plastic coated wire mesh. The floor of each pen was sloped from the center downward to the two outer sides allowing the eggs to roll toward the aisles facilitating the collection of and the prevention of damage to the eggs. The pens were labeled with pen number, study number, compound number, dietary level, and individual bird numbers. Each pen had its own individually identified feed container and scoop so that feed consumption could be accurately measured. Photoperiod was controlled by an automatic timer that was appropriately set for the current phase of the study. Light was provided by four 100 watt light bulbs equally spaced throughout the room. A thermometer was located on the wall inside the testing rooms. Room temperature was checked daily and, when necessary, gas heaters were lit to maintain temperatures at 10°C (50°F).

Bobwhites

The bobwhite were housed on the Michigan State University Poultry Research and Teaching Center in House 4, Room E. The room was entirely enclosed and measured 5 m x 9.3 m x 2.4 m. (W x L x H). Two groups of pens were suspended from the ceiling, each consisting of two rows of 15 pens aligned back to back. Each pen was labeled with a cage number, compound number, study number, dietary level, and bird identification number. The dimensions of each cage were 40 cm wide x 43 cm long x 44.5 cm deep. The wire mesh dimensions were 2.6 x 9.8 cm, with the floor being 1.27 x 1.27 cm and plastic coated to protect the birds' feet. The floor of all the pens sloped toward the aisles which allowed the eggs to roll into the collecting area. Each pen was provided with an individual feed hopper (5.1 cm x 12.5 cm x 5.1 cm) and water cup. Also, each pen had its own individually identified feed container and scoop so that feed consumption could be accurately measured. Photoperiod was controlled by an automatic timer that was appropriately set for the current phase of the study. Light was provided by four 100 watt light bulbs equally spaced throughout the room. A thermometer was located on the wall inside the testing room. Room temperature was checked daily and, when necessary, the gas heaters were lit to maintain temperatures at 15.6°C (60°F).

Testing

Food consumption was measured biweekly during the acclimation and dietary exposure periods for both studies. Adult body weights were measured on weeks 0, 2, 4, 6, 7, 9, 11, and at termination of the adult exposure phase of both studies. Body weights were not measured

during the egg production phase to avoid adverse effects on egg production due to handling stress.

Egg production, mortality, morbidity, behavior, and any observable clinical signs of intoxication were recorded daily. All birds that died during the study were subjected to gross necropsy. If any abnormalities were noted, samples were taken for histopathology. All birds surviving to the planned termination date were killed by CO_2 asphyxiation and necropsied. At termination, tissues were collected for histopathology from at least 5 animals of each sex per treatment level.

Egg Collection, Storage, and Incubation

Mallards

Egg production began on December 12, 1980. Eggs were collected daily and each egg was marked in pencil with its corresponding pen number and date. Eggs that were soft-shelled, lacking shells, or extremely undersized were recorded as abnormal and discarded. Eggs were stored at 15.6°C (14.4-16.8°C). At weekly intervals, all eggs were candled and those with cracks were recorded and discarded. The remaining eggs were placed in an incubator (Jamesway, single stage, Model 252) for 24 days. The temperature and relative humidity settings were 37.8°C (37.2-38.2°C) and 57% (56-57.2%), respectively. The eggs were candled on day 14 of incubation and the numbers of fertile eggs, infertile eggs, and early embryonic deaths were recorded. On day 21, the eggs were candled again to determine embryo mortality that had occurred after day 14. On day 24, the live eggs were transferred to pedigree baskets and separated according to pen

number. The eggs were placed in the hatchery incubator (Jamesway, single stage, Model 252) which had temperature and relative humidity settings of 37.3°C (36.9-38.2°C) and 66% (63.5-68%), respectively. On day 27 (or on day 28 if the hatch was poor), all pipped (dead and live), unpipped (dead and live), and hatchlings (dead and live), were recorded. All hatchlings were individually weighed, wing banded, and recorded by parental pen number. The hatchlings were placed in cardboard chick boxes for the 5 mile transfer to the MSU Poultry Research and Teaching Center. Precautions were taken to prevent exposure of the hatchlings to cold temperatures. The hatchlings were transferred quickly from the incubation room to a pre-warmed transporting vehicle, and from the vehicle to House 3 of the research facility. The hatchlings were immediately placed in a Petersime 250-24 battery brooder according to dietary level of parental stock. Temperatures were maintained at 37.5°C and the hatchlings were given both starter diet and water ad libitum. The hatchlings were observed daily for two weeks and all mortalities were recorded. At the end of the two-week period, all surviving hatchlings were killed by exposure to chloroform and individual weights recorded. Necropsies were not performed on hatchlings that died during the 2 week survival period, or on those that were killed at termination of the survival period.

Bobwhite

Egg production began on January 6, 1980. Eggs were collected daily and each egg was marked in pencil with its corresponding pen number and date. Eggs that were soft-shelled, lacking shells, or extremely undersized were recorded as abnormal and discarded. Eggs were stored at 15.6°C (14.4-16.8°C). At weekly intervals all eggs

were candled and those with cracks were recorded and discarded. The remaining eggs were placed in an incubator (Jamesway, single stage, Model 252) for 21 days. The temperature and relative humidity settings were 37.8°C (37.2-38.2°C) and 57% (56-57.2%), respectively. The eggs were candled on day 11 of incubation and the numbers of fertile eggs, infertile eggs, and early embryonic deaths were recorded. On day 18, the eggs were candled again to determine embryo mortality that had occurred after day 11. On day 21, the live eggs were transferred to pedigree baskets, and separated according to pen number. The eggs were placed in the hatchery incubator (Jamesway, single stage, Model 252) which had temperature and relative humidity settings of 37.3°C (36.9-38.2°C) and 66% (63.5-68%), respectively. On day 24 (or on day 25 if the hatch was poor), all pipped (dead and live), unpipped (dead and live), and hatchlings (dead and live) were recorded. All hatchlings were individually weighed, wing banded, and recorded by parental pen number. The hatchlings were placed in cardboard chick-boxes and transported to the brooder room. Precautions were taken to prevent exposure of the hatchlings to cold temperatures. They were immediately placed in a Petersime 250-24 battery brooder according to dietary level of parental stock. Temperatures were maintained at 37.5°C and the hatchlings were given both starter diet and water ad libitum. The hatchlings were observed daily for two weeks and all mortalities were recorded. At the end of the two-week period, all surviving hatchlings were killed by exposure to chloroform and individual weights recorded. Necropsies were not performed on hatchlings that died during the 2 week survival period, or on those that were killed at termination of the survival period.

Eggshell Thickness

For the mallard study, eggs were collected on days 2, 16, 30, 44, and 58 from each pen (day 1 was the first day an egg was laid in each individual pen). In the bobwhite study, the 7th, 14th, and 21st egg laid from each pen was collected. Every egg was marked with collection date and pen number. The eggs were cracked open at the girth, contents removed, and shells washed with tap water to remove the albumen. The shells were left to air dry on paper at room temperature for at least 48 hours. Eggshell thickness was measured using an Ames Thickness Measure (Model 25ME).

The shell thickness (including shell membranes) was measured to the nearest 0.01 mm at four approximately equidistant points around the circumference of the shell. An average shell thickness was calculated using these four data points.

Statistical Analysis

Treatment means of the following parameters were compared using Dunnett's two-tailed "t"-test; average number eggs laid/hen/day, % egg fertile, % eggs hatched, % hatchling survival and average eggshell thickness (Gill, 1978). Sample units were the individual pens within each experimental group. Adjustments were made in survivability when live embryos or hatchlings were determined to have suffered an accidental death unrelated to the nature of the study. Prior to conducting Dunnett's two-tailed "t"-test for the mallard study, which was conducted using two separate rooms, a Student's "t"-test was used to compare room treatment means within a parameter to determine if room diffences existed (Gill, 1978).

Pathology

All animals were necropsied following death. The necropsy was a systematic examination of the animal's general physical condition, body orifices, external and internal organs and tissues. The following organs and tissues were collected from at least five mallards and five bobwhite of each sex from each dose, placed in a fixative and processed for histopathologic evaluation: kidney, liver, heart, lung, spleen, thymus, pancreas, proventriculus, gizzard, duodenum, jejunum, ileum, cecum, colon, ovary, magnum, shell gland, testis, skin, pectoral muscle and leg muscle. Selected tissues were also collected from birds with grossly observed lesions, but no tissues were collected from the remaining grossly normal birds.

Histologic preparations of the tissue specimens collected at necropsy were examined microscopically by a certified veterinary pathologist, with experience in evaluating avian tissues, at the Lilly Research Laboratories, Greenfield, Indiana. Particular attention was directed to the identification of treatment-related lesions.

RESULTS

Room Variation-Mallard Study

There were no differences in the means (Student t, $P \leq 0.05$) between Rooms 4A and 4B in the mallard study for the reproductive parameters measured. Thus, control and treatment group data from Rooms 4A and 4B were combined for comparison by Dunnett's "t"-test.

Feed Consumption

There was no apparent effect in either study on food consumption between the birds in the control groups and the birds fed dietary concentrations of tricyclazole (Tables 1 and 2). The biweekly measurements varied, but this variation was consistent across the control and treatment groups. Food consumption plateaued until the two-week period prior to the increase in photoperiod. During this two week period, the control and treatment groups uniformly dropped in food consumption. Following the increase in photoperiod the mallards' food consumption returned to the earlier levels and continued to increase as reproduction commenced. However, the bobwhite maintained this decreased food consumption for an additional two week period after-which they increased to levels similar to those prior to the decrease. The bobwhite then continued increasing food consumption throughout the duration of the study, unlike the mallards which experienced a decline in food consumption during the final two-week period of the production phase. Mallard food consumption decline paralleled the decline in mallard egg production during the same final two-week period.

Table 1. Food consumption of adult mallards fed tricyclazole.

Dietary level	Acclimation period	Treatment period										
	Week 2	Week 4	Week 6	Week 7	Week 9	Week 11[3]	Week 13	Week 15	Week 17	Week 19	Week 21	Week 23
Control[1]												
$\bar{X}$	97[2]	128	130	132	128	74	123	166	183	195	204	168
SE	3	8	14	9	5	7	8	9	5	13	13	13
30 ppm[1]												
$\bar{X}$	94	149	127	130	131	75	127	161	183	199	190	176
SE	5	7	12	10	7	9	9	10	10	15	13	12
100 ppm[1]												
$\bar{X}$	97	142	136	142	139	93	130	172	199	200	212	185
SE	3	9	8	11	10	19	4	6	14	13	17	18
300 ppm[1]												
$\bar{X}$	92	142	129	133	138	82	120	167	201	215	229	217
SE	4	7	10	7	8	4	6	9	6	15	14	17

[1] N = 8.

[2] Grams/bird/day.

[3] Photoperiod increased.

Table 2. Food consumption of adult bobwhite fed tricyclazole.

Dietary level	Acclimation period Week 2	Treatment period Week 4	Week 6	Week 7	Week 9	Week 11	Week 13	Week 15	Week 17	Week 19	Week 21	Week 23	Week 24
Control[1]													
$\bar{X}$	16[3]	17	21	22	22	19	19	21	24	27	29	29	33
SE	0.3	0.4	0.4	0.9	0.6	0.8	0.7	1.0	1.0	1.7	1.0	1.0	1.4
30 ppm[1]													
$\bar{X}$	15	18	20	20	20	19	18	20	22	25	26	26	26
SE	0.2	0.5	0.5	0.7	0.6	1.1	0.8	0.8	1.1	1.4	1.1	0.9	1.4
100 ppm[2]													
$\bar{X}$	17	20	21[5]	23	22	18	18	20	23	27	29	28	30
SE	0.4	0.7	1.1	0.7	0.7	1.3	0.6	0.7	0.7	1.3	1.3	1.2	1.3
300 ppm[2]													
$\bar{X}$	16	19	22	22	21	19	18	20	21	25	26	27	29
SE	0.4	0.6	0.8	0.8	0.7	0.7	0.7	0.6	0.7	1.0	1.1	1.2	1.6

[1] N = 15.

[2] N = 13.

[3] Grams/bird/day.

[4] Photoperiod increased.

[5] N = 12.

Body Weight

There was no apparent effect on mean body weights in either study between birds in the control groups and the birds fed dietary concentrations of tricyclazole (Tables 3 and 4). Mean body weights increased slightly in all treatment groups prior to the onset of the production phase, however, a slight decrease occurred during the two-week period prior to the increase in the photoperiod. This decrease in mean body weights corresponded to the decrease in food consumption during the same period. At the termination of the studies, mean body weights were slightly greater than the pre-egg production levels.

Reproduction

Chronic dietary administration of tricyclazole had no effect on the reproduction in mallards and bobwhite (Tables 5 and 6). There were no significant differences between the means of the control groups and the means from the treatment groups in the following parameters: number of eggs/hen/day, mean eggshell thickness, mean % fertility, mean % hatchability, and mean % survivability. Also, tricyclazole in the diets of mallard and bobwhite breeders had no effect on eggshell thickness of either species.

Clinical Observations

No clinical signs could be attributed to feeding dietary concentrations of tricyclazole to either mallards or bobwhite. In mallards, the clinical signs observed were the result of increasing the photoperiod which resulted in excessive aggression or treading. The limping observed in some birds could also be attributed to injuries caused by the pen flooring. Hyperpnea was observed in one female

Table 3. Body weights of adult mallards fed tricyclazole.

Sex	Dietary level							
	Control		30 ppm		100 ppm		300 ppm	
	M[5]	F[6]	M[5]	F[6]	M[5]	F[6]	M[5]	F[6]
Week 0[1]	1221 ± 23[2]	1034 ± 22	1249 ± 19	1005 ± 17	1237 ± 36	1006 ± 17	1261 ± 39	1011 ± 21
Week 2[2]	1165 ± 21	1028 ± 24	1247 ± 20	992 ± 13	1234 ± 32	993 ± 13	1233 ± 38	996 ± 21
Week 4	1183 ± 21	1069 ± 24	1286 ± 19	1031 ± 14	1250 ± 26	1034 ± 17	1257 ± 42	1036 ± 23
Week 6	1209 ± 23	1097 ± 26	1309 ± 26	1066 ± 18	1297 ± 29	1077 ± 21	1309 ± 46	1068 ± 26
Week 7	1234 ± 27	1128 ± 31	1332 ± 29	1099 ± 19	1328 ± 36	1114 ± 23	1348 ± 44	1101 ± 27
Week 9	1280 ± 27	1171 ± 33	1344 ± 33	1132 ± 20	1365 ± 37	1155 ± 28	1387 ± 47	1136 ± 31
Week 11[3]	1170 ± 27	1078 ± 28	1222 ± 27	1061 ± 19	1249 ± 33	1058 ± 21	1256 ± 46	1058 ± 26
Week 23[4]	1254 ± 35	1211 ± 41	1358 ± 62	1240 ± 28	1432 ± 52	1208 ± 24	1400 ± 41	1210 ± 22

[1] Acclimation period begins.
[2] Treatment begins.
[3] Photoperiod increased.
[4] Termination
[5] N = 8.
[6] N = 24.
[7] $\bar{X}$ ± SE (grams).

Table 4. Body weights of adult bobwhite fed tricyclazole.

Sex	Dietary level							
	Control		30 ppm		100 ppm		300 ppm	
	M[5]	F[6]	M[5]	F[6]	M[5]	F[6]	M[5]	F[6]
Week 0[1]	181 ± 3[7]	176 ± 3	174 ± 4	174 ± 4	177 ± 3	173 ± 4	172 ± 4	167 ± 3
Week 2[2]	194 ± 3	187 ± 3	188 ± 3	187 ± 4	187 ± 3	186 ± 4	187 ± 3	179 ± 3
Week 4	194 ± 3	188 ± 3	190 ± 3	189 ± 4	188 ± 4	189 ± 3	189 ± 3	184 ± 3
Week 6	198 ± 3	193 ± 3	194 ± 3	194 ± 4	192 ± 4	193 ± 4	192 ± 3	189 ± 3
Week 7	199 ± 3	195 ± 3	195 ± 3	195 ± 4	193 ± 4	196 ± 4	192 ± 3	189 ± 3
Week 9	202 ± 4	201 ± 3	202 ± 3	201 ± 4	199 ± 5	202 ± 4	198 ± 3	198 ± 3
Week 11[3]	202 ± 3	198 ± 3	201 ± 3	198 ± 4	197 ± 4	199 ± 4	197 ± 3	194 ± 3
Week 24[4]	204 ± 5	227 ± 6	207 ± 4	226 ± 5	203 ± 4	234 ± 6	200 ± 4	225 ± 5

[1] Acclimation period begins.
[2] Treatment period begins.
[3] Photoperiod increased.
[4] Termination.
[5] N = 15.
[6] N = 13.
[7] $\bar{X}$ ± SE (grams).

Table 5. Effect of feeding tricyclazole on reproductive parameters in mallards.

Dietary level	Egg/ hen/day	Percent activity	Percent live embryos	Percent hatch-ability	Percent survi-vability	Percent eggshell thickness (mm)
Control[1]						
$\bar{X}$	0.56	80	97	57	93	0.39
SE	0.04	9	2	3	2	0.00
30 ppm[1]						
$\bar{X}$	0.53	80	97	58	94	0.39
SE	0.05	7	1	5	2	0.00
100 ppm[1]						
$\bar{X}$	0.57	77	97	60	95	0.39
SE	0.03	9	1	7	2	0.01
300 ppm[1]						
$\bar{X}$	0.57	77	94	51	96	0.39
SE	0.03	10	2	5	1	0.00

[1] N = 8.

Table 6. Effect of feeding tricyclazole on reproductive parameters in bobwhite.

Dietary level	Egg/ hen/day	Percent fertility	Percent live embryos	Percent hatch-ability	Percent survi-vability	Percent eggshell thickness (mm)
Control						
$\bar{X}$	0.37	80	98	76	86	0.20
SE	0.04	5	1	4	4	0.00
n	15	15	15	15	15	15
30 ppm						
$\bar{X}$	0.40	87	96	76	78	0.21
SE	0.30	4	1	4	6	0.00
n	15	15	15	15	15	15
100 ppm						
$\bar{X}$	0.40	89	99	74	87	0.21
SE	0.06	2	0	8	3	0.00
n	13	12	12	12	11	11
300 ppm						
$\bar{X}$	0.39	89	96	74	79	0.20
SE	0.04	3	1	5	4	0.00
n	13	13	13	13	13	13

NOTE: Unequal replication within dietary levels due to elimination as a result of disqualification (i.e. 0 live embryos because 0 fertile).

mallard in the control group, but she returned to normal within one day. Also, one female in the 0.03% treatment group suffered a prolapsed oviduct (Table 7).

In the bobwhite, the clinical signs observed were feather loss and head and leg lacerations. These lesions appeared to be caused by the wire caging, however, feather loss could have been the result of excessive treading.

Intest Mortality

Six female mallards died during the course of the study (Table 8). No mortalities occurred prior to increasing the photoperiod. Two females from the 30 ppm treatment level and 3 females from the 100 ppm treatment level died as a result of excessive treading or aggression between females. One female from the control group died from bacteriemia. In the bobwhite study, only one male, from the 300 ppm treatment group, died during the course of the study. The cause of death was not apparent and histopathology showed no substantive tissue alteration. No mortality could be attributed to the chronic administration of dietary tricyclazole to either mallards or bobwhite.

Pathology

Pathological findings provided by the Eli Lilly Company are provided in the Appendix (Appendices S and T). Alterations that were present included gross and microscopic lesions of nodular and ulcerative dermatitis present on the feet and heads of some control and treated bobwhite. A few mild inflammatory and degenerative lesions were observed in ducks that died on test as well as in ducks killed at the termination of the study. Some birds experienced a

Table 7. Clinical observations of mallards fed tricyclazole.

Observation	Cause
Feather loss	Aggression
Weight loss, lameness	Injured leg
Lameness	Injured leg
Ataxia, weight loss	Injured leg
Hyperpnea (dose 0.00%)	Unknown
Lameness	Injured foot
Prolapse	Parturition

Table 8. Interim deaths of Mallards fed tricyclazole

Pen #/Dietary Level	Bird #	Sex	Date of Death	Cause of Death[1]
20 / 0.00%	23	♀	2-19-81	Bacteriemia
16 / 0.003%	70	♀	1- 9-81	Trauma, Excessive treading
19 / 0.003%	64	♀	2-16-81	"
17 / 0.01%	93	♀	2-19-81	"
23 / 0.01%	75	♀	1-24-81	"
23 / 0.01%	51	♀	2-27-81	"

[1]Diagnosis provided by Lilly Research Laboratories.

slight degree of amyloidosis, however, this condition and the other pathological alterations that occurred were not considered to be related to the compound tricyclazole.

DISCUSSION

The Environmental Protection Agency (EPA) (1978) suggests, for mallard reproduction studies, that a minimum of 2 males and 5 females per pen replicated by four or more pens be used per treatment group. A minimum of ten pens per treatment group should be used if only one male and one female are to be used. The EPA (1978) suggests a minimum of a 12 pen replication for bobwhite studies, if one male and two females per pen are used. If only one male and one female per pen are used, then additional pens should be utilized.

In mallard chronic reproduction studies where there was a 2 male:5 female ratio per pen, a majority of the deaths that occurred were due to trauma associated with excessive aggression and rape (Jones, 1977; Breslin, 1981; Flaga, 1981). In an effort to reduce this cause of death, the present study used only one male and three females per pen. As a result, there was a reduction in number of interim deaths. Only six female mallards died during the study and of these, five were attributed to excessive treading, rape, and aggression between females. The absence of male deaths in this study can be attributed to the lack of male - male aggression. Single males in each pen were unable to physically express their aggressive breeding behavior toward other males due to cage separation.

In bobwhite chronic reproduction studies with similar design to the present study (1 male - 1 female per cage with a replication of 15), there was a comparatively higher mortality rate than in the present study in which only one male bobwhite died from the 300 ppm treatment group (Breslin, 1981; Flaga, 1981; Howell, 1981). Bobwhite

mortality, in all studies mentioned, were not attributed to any common cause and none were attributed to the test compounds.

Of the deaths that were not attributed to aggression, one control female mallard died of bacteriemia. The death of the single male bobwhite was due to unknown causes. Histopathology revealed no substantive tissue alteration and death was not considered to be treatment related.

Bobwhite food consumption was similar to consumption rates seen by Breslin (1981), Flaga (1981), and Howell (1981). There was a continuous increase in food consumption throughout the entire study period. There was a slight decrease and then plateau in consumption during the 2 two-week periods prior to and following the increase in the photoperiod. After this, food consumption returned to normal levels and then continued to increase throughout the remainder of the study.

Mallard food consumption continuously increased from the onset of the study until the two-week period prior to the increase in the photoperiod, when a decrease in consumption occurred. A decrease in consumption would not be expected until after the increase in the photoperiod when hormonal flucuations stimulating reproduction would also result in increased aggressiveness which is characteristic of reproductively active birds (Sturkie, 1976; Welty, 1979). Decreased food consumption following an increased photoperiod was observed by Breslin (1981), Flaga (1981), and Jones (1977). Following the decline in food consumption during the two-week period prior to the increase in photoperiod, there was an increase throughout the remainder of the study. During the final two-week period, a slight decline in food

consumption again occurred. This decline paralleled a similar decline in egg production for the same period, which itself corresponds to normal decline in reproductive performance following prolonged reproductive activity (Ringer, 1980). A breeding season for mallards normally lasts ten weeks (EPA, 1978) therefore the decline in food consumption and egg production was expected during the final weeks of the present study, especially since the study had a 12 week breeding season. Dietary tricyclazole did not adversely affect food consumption in either mallards or bobwhite.

Body weight changes generally parallelled the fluctuations in food consumption. Body weights consistently increased during the preproduction phase until food consumption decreased at the time of photoperiod increase. Mallards experienced a decline in body weight in the two weeks prior to the increase in the photoperiod, while bobwhite showed an insignificant decline after the change in the photoperiod. The flucuations in body weights corresponded well with food consumption characteristics. This pattern of body weight fluctuations was also seen by Breslin (1981), Flaga (1981), Howell (1981), and Jones (1977).

During the production phase, the birds were not weighed until termination in an effort to eliminate stress on reproduction due to handling. Thus, trends in body weights could not be thoroughly documented during this phase of the study, but it can be assumed that body weights continued to increase (perhaps at decreased rates), possibly with a slight decrease when food consumption declined during the final two-week period of the production phase. This decline occurred only with the mallards, Bobwhite continued to increase food consumption up

to termination of the study. A decline in mallard body weights did not neccessarily occur during the final weeks of the studies even though mallard food consumption declined. During this same period, reproductive activity was declining, allowing a higher percentage of energy intake to be utilized for body maintenance and growth. Chronic administration of tricyclazole in the diet of mallards and bobwhite did not affect body weight gain in either species.

Normal values for mallard and bobwhite egg production during a ten week breeding season are 28 to 38 eggs per female (EPA, 1978). Egg production in the present studies agree well with the expected values, with a range of 45 to 48 (12 week production period) and 34 to 37 eggs per female for mallards and bobwhite, respectively. These values are similar to those obtained by Flaga (1981), Howell (1981), and Jones (1977). Bobwhite values are similar to those of Breslin (1981), but mallard values tended to be greater in the present study.

The range of values for eggs cracked are 2.5 (0.03 ppm) to 4.0% (0.003%) for mallards and 7.0 (0.01 ppm) to 13.5% (control) for bobwhite. The values for the mallards are in good agreement with expected values (EPA, 1978), but are lower than those reported by Jones (1977). Bobwhite values are much greater than expected (EPA, 1978), with the control group having the highest percentage of cracked eggs. However, these values for cracked eggs in bobwhite are only slightly greater than those of Howell (1981). These extreme values are not considered to be dose related and may have been a result of cage design. The floor of the cages were slanted to allow the eggs to roll freely into a collection area, thus facilitating collection and preventing damage by the adult birds. Some floors developed upward

bows that sometimes prevented eggs from rolling into the collection area or trapped the eggs between the front wall of the cage and the floor itself. The eggs could then be trampled by the adult birds or cracked by the cage due to the slight springing action of the cage floor during bird activity.

Eggshell thickness for bobwhite correspond well with the expected values listed by the EPA (1978), ranging from 0.20 to 0.21 mm. Bobwhite eggshell thickness in this study was similar to those of Howell (1981), but tend to be slightly less than those of Flaga (1981). In contrast, mallard eggshell thickness, for all treatment levels, averaged 0.39 mm, which is greater than expected (EPA, 1978), but are similar to Jones (1977). Breslin (1981) and Flaga (1981) both obtained greater values for shell thickness in mallards than the present study.

Values for the reproductive parameters; fertility, embryo survival (days 18 and 21 of incubation for bobwhite and mallards, respectively), and hatchability are within the normal ranges expected (EPA, 1978) for the bobwhite. The bobwhite did have lower embryo survival in the 30 and 300 ppm treatment levels. The mallards, however, have values below the normal range for fertility and embryo survival, with hatchability being low but still within the expected range. Studies by Breslin (1981) and Flaga (1981) reported lower values in fertility, embryo survival, and hatchability for bobwhite while Howell (1981) reported similar hatchability but greater fertility than did the present study. For the same reproductive parameters in mallards, Breslin (1981), Flaga (1981), and Jones (1977)

reported higher values than did this study, except Breslin (1981) reported a decrease in embryo survival.

Though the results for the reproductive parameters discussed exhibited a tendency for values below what is to be expected, none of the treatment groups, from either the mallard or the bobwhite studies, differed significantly from their respective controls for any of the reproductive parameters evaluated.

Survivability of mallard and bobwhite hatchlings were within the normal range (EPA, 1978). Breslin (1981) and Flaga (1981) experienced high mortality rates in their studies and suspect it was a result of exposure to cold environmental temperatures. When young animals become chilled, the lack of ability to adequately control body temperature through thermoregulation greatly decreases the chance of survival. To prevent inadequate thermoregulation, attempts were initiated to avoid adverse environmental conditions. Bobwhite hatchlings were reared in the same building they were hatched in and mallard hatchlings were transported to their respective brooding batteries in prewarmed vehicles only. These precautions seemed successful as survival rates were much greater than those of Breslin (1981) and Flaga (1981).

Pathologically, no mortality or lesion in either the mallard or the bobwhite studies could be attributed to the fungicide, tricyclazole. Mortality, feather loss, dermititis, and other lesions about the head and feet were considered due to aggression, rape, and caging. The single case of bacteriemia suffered by a control female mallard was due to the presence of microorganisms or their poisonous products in the blood stream (Thomas, 1981). Though some birds

experienced a slight degree of amyloidosis the condition was not considered to be dose related.

CONCLUSION

Chronic dietary administration of tricyclazole did not affect the food consumption or body weight gain of adult mallards or bobwhite. Similarly, tricyclazole did not adversely influence the manifestation of degenerative alterations in tissue or organ morphology, nor did it increase mortality in either species. Evaluation of the reproductive parameters of egg production, fertility, embryo survival, hatchability, hatchling survival, and eggshell thickness revealed no negative relationship between dietary tricyclazole exposure and the a forementioned parameters.

In conclusion, chronic dietary administration of tricyclazole did not result in toxicity to mallards or bobwhite at concentrations up to 300 ppm, the highest level tested, which is a 60 fold increase over the maximum environmental residue value of 5 ppm in rough rice.

APPENDICES

Appendix A. Solubility of tricyclazole in organic solvents[1].

Solvent	Solubility (mg/ml)[2]
Chloroform	>500.0
Methylene chloride	33.0
Ethyl alcohol	25.0
Methyl alcohol	25.0
Acetone	10.4
Acetonitrile	10.4
Cyclohexanone	10.0
Benzene	4.2
Xylene	2.1
Water	0.7
Hexane	<0.1

[1] Courtesy of Eli Lilly and Co., Greenfield, IN, 1981.

[2] 25°C.

Appendix B. Toxicology of Tricyclazole[1]

Species	Sex	LD_0[2]	LD_{50}[2]
Albino ICR mouse (*Mus musculus*)	M,F		245
Albino Wistar rat (*Rattus noruegicus*)	M,F		314
Beagle dog (*Canis familiaris*)	M,F	> 50	
Bobwhite (*Colinus virginianus*)	M,F	>2000	
Mallard (*Anas platyrhynchos*)	M,F	> 100	

[1]Courtesy of Eli Lilly and Co., Greenfield, Indiana 1981.
[2]mg/kg.

Appendix C. Environmental Toxicity of Tricyclazole[1]

Carp (*Cyprinus carpio*)	LC_{50} 14.6 mg/liter (48 hr)
Goldfish (*Carassius auratus*)	LC_{50} 13.5 mg/liter (96 hr)
Bluegill sunfish (*Lepomis macrochirus*)	LC_{50} 16.0 mg/liter (96 hr)
Rainbow trout (*Salmo gairdneri*)	LC_{50} 7.3 mg/liter (96 hr)
Channel catfish (*Ictalurus punctatus*)	LC_{50} 18.6 mg/liter (96 hr)
Sheepshead minnow (*Cyprinodon variegatus*)	LC_{50} 8.0 mg/liter (96 hr)
Himedaka (*Oryzias latipes*)	LC_{50} 18.8 mg/liter (48 hr)
Pink shrimp (*Penaeus duorarum*)	LC_{50} >18 <32 mg/liter (96 hr)
Eastern oyster (*Crassotrea virginica*)	EC_{50} >10 <32 mg/liter (48 hr)
Fiddler crab (*Uca pugilator*)	EC_{50} >32 <56 mg/liter (96 hr)
Blue crab (*Callinectes sapidus*)	LC_{50} 37 mg/liter (96 hr)
Earthworm (*Lumbricus terrestris*)	No effect from 100 ppm in soil (14 days)
Daphnia (*Daphnia magna*)	EC_{50} 20.4 ppm (48 hr)
Bobwhite quail chick (*Colinus virginianus*)	LC_{50}>0.5% (>5000 ppm) in diet (5 days)
Mallard duckling (*Anas platyrhynchos*)	LC_{50}>0.5% (>5000 ppm) in diet (5 days)

[1]Courtesy of Eli Lilly and Co., Greenfield, Indiana. 1981.

Appendix D. Hazard Evaluation of Tricyclazole[1]

Species	Rate of Exposure	Dose	Results
Rat	Oral	--	LD_{50} = 354 mg/kg[2]
Rat	Inhalation[2]	3.71 mg/liter	No toxicity
	Inhalation[3]	0.146 mg/liter	No toxicity
Rabbit	Dermal[2,3]	2 g/kg	No irritation or systemic toxicity
Rabbit	Ocular[2]	41 mg	Corneal dullness, mild iritis and slight conjunctivitis which cleared in 96 hr
	Ocular[3]	78 mg	Slight iritis and conjunctivitis which cleared in 72 hr
Guinea Pig	Dermal		No sensitization produced during 5 week challenge

[1]Courtesy of Eli Lilly and Co., Greenfield, Indiana 1981.
[2]Beam(R) (tricyclazole formulated as a 75% wettable powder)
[3]Technical tricyclazole.

Appendix E. Subacute and chronic toxicity of tricyclazole[1].

Species	Route	Duration	No-effect level
Dog	Oral	3 months	7.5 mg/kg/day
Chicken	Diet	1 month	600 ppm
Mouse	Diet	2 years	400 ppm
Rat	Diet	2 years	275 ppm

[1] Courtesy of Eli Lilly and Co., Greenfield, IN, 1981.

Appendix F. Nutrient Requirements of Mallards (in Percentage or Amount Per Kilogram of Feed)

Nutrient	Growing Ducks	Breeding Ducks
Metabolizable energy (kcal/kg)	2,900	2,900
Protein (%)	16	15
Lysine (%)	0.9	0.7
Methionine + cystine (%)	0.8	0.55
Vitamin A (IU)	4,000	4,000
Vitamin D (ICU)	220	500
Riboflavin (mg)	4	4
Pantothenic acid (mg)	11	10
Niacin (mg)	55	40
Pyridoxine (mg)	2.6	3
Calcium (%)	0.6	2.75
Phosphorus (%)	0.6	0.6
Sodium (%)	0.15	0.15
Manganese (mg)	40	25
Magnesium (mg)	500	500

From: Nutrient Requirements of Poultry. 1977.

Appendix G. Composition of Duck Breeder-Layer Diet

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 (1.02)[1]	0.10	2.0
Salt	0.30	6.0
Vitamin premix TK-01 (1.03)[2]	0.50	10.0
Methionine hydroxy analog	0.20	4.0
	100.0	2000.0

[1]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.

[2]Vitain 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 H. Calculated Analysis of Duck Breeder-Layer Diet

Protein, %	18.50	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	1082
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		

Values in parenthesis represent the amino acids expressed as a % of dietary protein.

Appendix I. Composition of duck starter diet.

Ingredients	Percent	lbs./ton
Corn, yellow ground	60.43	1208.6
Soybean meal, solvent extracted, dehulled (49%)	27.09	541.8
Fish meal	2.00	40.0
Meat and bone meal	4.00	80.0
Oat groats, rolled	2.50	50.0
Beef tallow	1.90	38.0
Dicalcium phosphate, feed grade	0.64	12.8
Limestone	0.34	6.8
Trace mineral premix TK-0 (1.02)[1]	0.10	2.0
Salt	0.30	6.0
Vitamin premix TK-01 (1.03)[2]	0.50	10.0
Methionine hydroxy analog	0.20	4.0
	100.00	2000.0

[1] 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.

[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.

Appendix J. Calculated analysis of duck starter diet.

Protein, %	22.01	Vitamin K, mg/kg	0.7
Met. energy, Kcal/kg	3079	Choline, mg/kg	1932
ME/P ratio	139.9 (63.6)	Niacin, mg/kg	89.7
Fat, %	4.74	Pantothenic acid, mg/kg	11.8
Fiber, %	2.74	Vitamin B_6, mg/kg	7.5
Ash, %	5.19	Riboflavin, mg/kg	5.8
Calcium, %	0.80	Thiamine, mg/kg	2.8
Phosphorus, %	0.70	Folic acid, mcg/kg	1345
Avail. phosphorus, %	0.46	Vitamin B_{12}, mcg/kg	107
Manganese, mg/kg	87.0	Biotin, mcg/kg	250
Iron, mg/kg	99.7	Arginine, %[1]	1.56 (7.09)
Copper, mg/kg	12.3	Lysine, %	1.21 (5.50)
Zinc, mg/kg	78.9	Glycine, %	1.30 (5.91)
Selenium, mcg/kg	112	Methionine, %	0.53 (2.41)
Magnesium, mg/kg	2149	Cystine, %	0.32 (1.45)
Potassium, mg/kg	8120	Total sulfur amino acids	0.85 (3.86)
Sodium, mg/kg	1820	Tryptophan, %	0.29 (1.32)
Iodine, mg/kg	1	Linoleic acid, %	1.22
Vitamin A, IU/kg	4994	Vitamin E, mg/kg	56.1
Vitamin D, ICU/kg	900		

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

Appendix K. Nutrient requirements of bobwhite (in percentage or amount per kilogram of feed).

Nutrient	Growing	Breeding
Metabolizable energy (kcal/kg)	2,800	2,800
Protein (%)	28	24
Lysine (%)	1.4	0.7
Methionine + cystine (%)	0.9	0.6
Glycine + serine (%)	1.6	0.9
Vitamin A (IU)	3,000	3,000
Vitamin D (ICU)	900	900
Riboflavin (mg)	3.8	4.0
Pantothenic acid (mg)	12.6	15
Niacin (mg)	31	20
Choline (mg)	1,500	1,000
Linoleic acid (%)	1.0	1.0
Calcium (%)	0.65	2.3
Chlorine (%)	0.11	0.15
Phosphorus (%)	0.65	1.0
Sodium (%)	0.085	0.15
Iodine (mg)	0.30	0.30
Magnesium (mg)	600	400
Manganese (mg)	90	70
Zinc (mg)	50	50

FROM: Nutrient Requirements of Poultry (1977).

Appendix L. Composition of quail breeder-layer diet.

Ingredients	Percent	lbs./750
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)[1]	0.50	3.75
Trace mineral premix TK-01 (1.02)[2]	0.10	0.75
Salt	0.30	2.25
Methionine hydroxy analog	0.30	2.25
	100.00	750.00

[1] 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 of vitamin B_{12}, 100 mcg of biotin, and 125 mg of ethoxyquin per kg of complete feed.

[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 feed.

Appendix M. Calculated analysis of quail breeder-layer diet.

Protein, %	24.00	Vitamin K, mg/kg	0.7
Met. energy, Kcal/kg	2803	Choline, mg/kg	2139
ME/P ratio	116.79 (53.09)	Niacin, mg/kg	97
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
Manganese, mg/kg	93	Biotin, mcg/kg	301
Iron, mg/kg	116	Arginine, %	1.717 (7.15)[1]
Copper, mg/kg	18	Lysine, %	1.380 (5.75)
Zinc, mg/kg	84	Glycine, %	1.248 (5.20)
Selenium, mcg/kg	121	Methionine, %	0.629 (2.62)
Magnesium, mg/kg	2104	Cystine, %	0.353 (1.47)
Potassium, %	0.95	Total sulfur amino acids, %	0.982 (4.09)
Sodium, mg/kg	1626	Tryptophan, %	0.337 (1.40)
Iodine, mg/kg	1	Linoleic acid, %	1.22
Vitamin A, IU/kg	4929	Vitamin E, mg/kg	55.2
Vitamin D, ICU/kg	900		

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

Appendix N. Composition of quail starter.

Ingredients	Percent	lbs./750
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)[1]	0.50	3.75
Trace mineral premix TK-01 (1.02)[2]	0.10	0.75
Salt	0.20	1.50
Methionine hydroxy analog	0.30	2.25
	100.00	100.00

[1] 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 B12, 100 mcg of biotin, and 125 mg of ethoxyquin per kg of complete feed.

[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 feed.

Appendix O. Calculated analysis of quail starter diet.

Protein, %	27.99	Vitamin K, mg/kg	0.7
Met. energy, Kcal/kg	2861	Choline, mg/kg	2330
ME/P ratio	102.22 (46.46)	Niacin, mg/kg	102
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
Manganese, mg/kg	95	Biotin, mcg/kg	324
Iron, mg/kg	123	Arginine, %	1.991 (7.11)
Copper, mg/kg	20	Lysine, %	1.675 (5.98)
Zinc, mg/kg	89	Glycine, %	1.488 (5.32)
Selenium, mcg/kg	168	Methionine, %	0.710 (2.54)
Magnesium, mg/kg	2146	Cystine, %	0.413 (1.48)
Potassium, %	1.13	Total sulfur amino acids, %	1.124 (4.02)
Sodium, mg/kg	1629	Tryptophan, %	0.169 (0.60)
Iodine, mg/kg	1	Linoleic acid, %	1.17
Vitamin A, IU/kg	4535	Vitamin E, mg/kg	54.8
Vitamin D, ICU/kg	900		

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

Appendix P. Stability and homogenicity of tricyclazole in mallard and bobwhite diets[1]. (Concentration (theoritical) tested: 0.001%)

Stability of Tricyclazole						
		Assay result (%)				
Diet	Storage conditions	Week 0	Week 1	Week 2	Week 4	Week 8
Duck	25°C	0.0009	0.0009	0.0009	0.0012	0.0004
Duck	37°C	0.0009	0.0009	0.0009	0.0006	0.0004
Quail	25°C	0.0009	0.0009	0.0010	0.0006	0.0006
Quail	37°C	0.0009	0.0010	0.0009	0.0006	0.0003

Homogenicity of Tricyclazole					
	Assay result (%)				
	Sample				
Diet	1	2	3	4	5
Duck	0.0009	0.0008	0.0010	0.0008	0.0008
Quail	0.0007	0.0007	0.0008	0.0009	0.0008

[1] Courtesy of Eli Lilly and Co., Greenfield, IN, 1981.

Appendix Q. Results of assays of diets that contained tricyclazole fed to mallards[1].

Date prepared	Nominal dietary conc. (%)	Tricyclazole activity			
		% of diet		% of nominal	
		fresh	4-week	fresh	4-week
9/27/80	0.0	Neg	Neg	--	--
	0.003	0.003	0.0029	100	97
	0.01	0.01	0.01	100	100
	0.03	0.028	0.027	93	90
10/23/80	0.0	Neg	Neg	--	--
	0.003	0.0027	0.0029	90	97
	0.01	0.01	0.0092	100	92
	0.03	0.028	0.027	93	90
11/20/80	0.0	Neg	Neg	--	--
	0.003	0.0023	0.0024	77	80
	0.01	0.0096	0.009	96	90
	0.03	0.026	0.027	87	90
12/17/80[a]	0.0	Neg	Neg	--	--
	0.003	0.0032	0.0024	107	80
	0.01	0.011	0.0082	110	82
	0.03	0.032	0.026	107	87
1/14/81	0.0	Neg	Neg	--	--
	0.003	0.0027	0.0023	90	77
	0.01	0.0099	0.0079	99	79
	0.03	0.03	0.026	100	87
2/11/81[3]	0.0	Neg	Neg	--	--
	0.003	0.0035	---	117	--
	0.01	0.011	---	110	--
	0.03	0.026	---	87	--

[1] Courtesy of Eli Lilly and Co., Greenfirld, IN, 1982.

[2] Aged sample was 5-weeks.

[3] No age diet sample was taken.

Appendix R. Results of assays of diets that contained tricyclazole fed to bobwhite[1].

Date prepared	Nominal dietary conc. (%)	Tricyclazole activity			
		% of diet		% of nominal	
		fresh	4-week	fresh	4-week
9/28/80	0.0	Neg[a]	Neg	--	--
	0.003	0.0033	0.0029	110	97
	0.01	0.01	0.01	100	100
	0.03	0.029	0.028	97	93
10/23/80	0.0	Neg	Neg	--	--
	0.003	0.0026	0.0029	87	97
	0.01	0.0092	0.10	92	100
	0.03	0.027	0.027	90	90
11/19/80	0.0	Neg	Neg	--	--
	0.003	0.0026	0.0026	87	87
	0.01	0.009	0.0088	90	88
	0.03	0.031	0.030	103	100
12/18/80[2]	0.0	Neg	Neg	--	--
	0.003	0.0031	0.0029	103	97
	0.01	0.0089	0.0012	89	120
	0.03	0.028	0.031	93	103
1/15/81	0.0	Neg	Neg	--	--
	0.003	0.0031	0.0023	103	77
	0.01	0.0094	0.0091	94	91
	0.03	0.029	0.025	97	83
2/11/81[3]	0.0	Neg	---	--	--
	0.003	0.0033	---	110	--
	0.01	0.01	---	100	--
	0.03	0.027	---	90	--

[1] Courtesy of Eli Lilly and Co., Greenfield, IN, 1982.

[2] Sample aged 5-weeks.

[3] No. aged sample was taken.

Appendix S. Summary of pathologic diagnoses for mallards fed tricyclazole[1].

	0.0[2]		0.003		0.01		0.03	
	♂	♀	♂	♀	♂	♀	♂	♀
	8[3]	24	8	24	8	24	8	24
Died on test, no substantive microscopic tissue alteration		1		2		3		
Kolled terminal, no substantive microscopic tissue alteration	5	6	5	6	5	5	5	5
Killed terminal, no substantive gross tissue alteration	3	17	3	16	3	16	3	19
Systemic								
Bacteremia		1						
Trauma, gross				2		3		

[1] Courtesy of Eli Lilly and Co., Greenfield, IN, 1982: Compound 71491; Study, A024-80; Route, diet; Duration, 21 weeks.

[2] Dose %.

[3] Number of animals.

Appendix T. Summary of pathologic diagnoses for bobwhite fed tricyclazole[1].

	0.0[2]		0.003		0.01		0.03	
	♂	♀	♂	♀	♂	♀	♂	♀
	15[3]	15	15	15	17	13	16	14
Died on test, no substantive microscopic tissue alteration							1	
Kolled terminal, no substantive microscopic tissue alteration	5	5	5	5	4	5	5	4
Killed terminal, no substantive gross tissue alteration	7	9	10	9	12	7	10	9
Skin								
Nodule, gross		1		1				
Ulcerative dermatitis	3				1	1		1

[1] Courtesy of Eli Lilly and Co., Greenfield, IN, 1982: Compound 74191; Study, A025-80; Route, diet; Duration, 22 weeks.

[2] Dose %.

[3] Number of animals.

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