ILLINOIS
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SURVEY

# Toxicity of Ingested Bismuth Alloy Shot in Game-farm Mallards

Glen C. Sanderson, William L. Anderson, George L. Foley, Loretta M. Skowron, Jeffrey D. Brawn, James W. Seets, and Karen L. Duncan

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# Toxicity of Ingested Bismuth Alloy Shot in Game-farm Mallards: Chronic Health Effects and Effects on Reproduction

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## Abstract

In a 150-day study, we tested for chronic toxicity and effects on reproduction of bismuth/tin (Bi/Sn) alloy shot dosed in game-farm mallards (*Anas platyrhynchos*). Histopathology of livers, kidneys, gonads, hearts, and lungs showed no significant group-related differences among 0-dosed (controls), iron (Fe)-dosed (8, No. 4, steel shot), and Bi-dosed (8, No. 4, Bi/Sn alloy shot) adult ducks or among ducklings from pairs of these dosed groups. Bi shot, under our test conditions, did not elicit toxicity in mallard ducks or affect their reproduction or offspring.

## Introduction

The present study is sequential to the investigation by Sanderson et al. (1997a). In the present study, our first objective was to determine if Bi/Sn alloy shot (i.e., "Bi shot") is chronically toxic to game-farm mallards. Our second objective was to determine if ingested Bi shot affected the ability of game-farm mallards to reproduce under a test protocol as specified by the Canadian Wildlife Service (CWS) (Environment Canada 1992) and modified by the U.S. Fish and Wildlife Service (USFWS), January 1995. We attempted to associate toxic effects, if they occurred, with concentrations of elements in tissues.

Environment Canada (1992) provided guidelines for chronic toxicity and reproductive tests that were necessary to approve a candidate shot as nontoxic for waterfowl hunting in Canada. The original protocol for the present study was designed to comply with these guidelines. Dr. Simon Nadeau, CWS, and Dr. Keith A. Morehouse, USFWS, reviewed the protocol prior to initiation of the study. The reader is referred to Sanderson et al. (1997a) for additional introductory material and to Sanderson et al. (1992, 1997a) for reviews of Bi literature.

## Methods

We randomly assigned ducks, doses, pens, pairings of ducks, and ducklings in our tests. One hundred twenty individual pens were numbered sequentially. The leg band number became the duck number and determined the duck's random assignment to a pen. Four slips of paper, labeled 0, 8 No. 4 Fe, 8 No. 4 Bi, or 8 No. 4 Pb, were placed individually in gelatin capsules, which were placed in a container. The first capsule removed (8, No. 4, Fe) determined that the first 18 male and female bands drawn were assigned to the Fe-dosed group. The procedure was repeated for the remaining three dose assignments.

All female bands were placed in one container and all male bands in a second container. The bands were removed one at a time to determine dose assignments. Subsequently, all bands for female ducks in each dosing group (e.g., 8, No. 4, Bi shot) were placed in one container and all bands for male ducks for the same dosed group were placed in a second container. One band at a time was selected from each container to determine the female:male pairs. This procedure was repeated to determine which five females and which five males from each dosed group were selected for analyses of elements in blood, liver, kidney, and gonads and for necropsy and histological study. However, female and male bands were selected independently and only five ducks were selected for each sex and dose.

Ten ducklings from each dosed group were randomly selected for necropsy and analysis of blood, liver, and kidneys for nine elements. To insure randomization, the numbers of adult females (those that produced live ducklings) in each dosed group were placed in separate gelatin capsules, which were placed in a container. The first 10 numbers selected from each dosed group of females determined the ducklings chosen for tissue analysis, necropsy, and histopathologic study. Because of their small size, samples from the first two ducklings produced by each pair were combined.

For this report, 0- (sham-) dosed ducks are controls. Fe-dosed ducks are those that were dosed with eight, No. 4, Fe shot on Day 0 and (for the survivors) again on Days 30, 60, and 90. Bi-dosed ducks are those that were similarly dosed with Bi shot. Pb-dosed ducks are those that were dosed with eight, No. 4, Pb shot on Day 0. For ducklings, 0-dosed, Fe-dosed, and Bi-dosed indicate that the ducklings were hatched from eggs laid by a female of 0-, Fe-, or Bi-dosed pairs.

### Toxicity Study

Sixty-five male and 65 female wild-type game-farm mallards, 6 to 8 months old, were purchased from Whistling Wings, Hanover, Illinois, and transported to Champaign, Illinois, in crates in an enclosed van on 4 January 1995. The ducks were reared on a 60-acre lake.

Ducks were weighed and randomly assigned, one to a pen, on 4 January 1995. Males and females were randomly assigned to one of the four groups (18 males and 18 females to each of three groups or 6 males and 6 females to one group).

Pens were consecutively-numbered, elevated, 1-$m^2$, and constructed of vinyl-coated, 25.4-mm mesh, 14-gauge wire (Sanderson et al. 1992). A 9.1- x 36.6-m pole barn (metal roof, sides, and ends covered with heavy-duty polyethylene tarpaulin [black outside, silver inside, brass grommets 0.6 m apart, McMaster-Carr, Chicago]), housed the pens and excluded light. These facilities were inspected by several members of the Laboratory Animal Care Committee, University of Illinois, before ducks were placed in pens. The committee also inspected facilities twice during the study.

Beginning 4 January 1995, the ducks were offered commercial duck pellets (Heinhold™ 14% Duck Developer pellets, Heinhold Feeds, Inc., Kouts, Indiana) and water *ad libitum* and exposed to ambient light. After allowing 3 weeks for ducks to acclimatize, males were moved on 26 January 1995 (Day 0) into pens with previously assigned females. At that time, each duck was given one of the following doses: eight, No. 4, (3.30 mm diameter), Fe shot (18 females and 18 males); eight, No. 4, Bi shot (18 males and 18 females); eight, No. 4, Pb shot (6 females and 6 males); or no shot (controls, 18 males and 18 females). All surviving ducks were redosed on Days 30, 60, and 90 with the original dosing regime. Each dose of eight shot was weighed to the nearest 0.1 mg and stored in a numbered vial before it was placed in the duck.

The Bi shot were provided by William S. Montgomery, Jr., Bismuth Cartridge Co., Dallas, Texas. Seven shot were analyzed in the laboratory of the Illinois State Water Survey, Champaign, Illinois, before dosing the ducks. Concentrations of Bi in the shot ranged from 97.27% to 100.05% ($\bar{x}$ = 98.35%, SD = 0.86%) and Sn ranged from 1.69% to 1.98% ($\bar{x}$ = 1.90%, SD = 0.10%). Other elements averaged <0.1% each; Pb ranged from 0.0040 to 0.0186% ($\bar{x}$ = 0.0094%, SD = 0.0054%). Fe and Pb shot were obtained from commercial 12-gauge shotgun shells and were not analyzed.

Seventeen dosed ducks (four of each sex of Fe-dosed and Bi-dosed, plus the one surviving Pb-dosed duck [a male]) were radiographed on 6 February 1995 (Day 11) and (except for the Pb-dosed duck), again on 6 March (Day 39) and on 6 April (Day 70) to determine the number of shot retained in the gizzards. We made a dorsal-ventral and a right-left view radiograph for each duck. Each duck was placed in a square 1.9-L cardboard milk carton with its top open and a hole cut in the bottom to reduce struggling in order to obtain a dorsal-ventral and a right-left side view. For each duck, the dorsal-ventral and right-left views were recorded on opposite halves of a single sheet of 35.6- x 43.2-cm X-ray film.

When the ducks were initially dosed (Day 0, 26 January 1995), light was restricted to 8 hr per day (0800-1600 hr, CST) for 90 days. Beginning on the 91st day (27 April 1995), the daily illumination was gradually increased over 2 weeks to 18 hr per day. Half the daily increase was added in the a.m. and half in the p.m. (approximately 20 minutes each). An Indoor/Outdoor Digital 7-day Timer (Double Pole Single Throw, Model EZ-701-2, EZ Controls Co.—McMaster-Carr, Chicago) was programmed one week at a time to increase the daily light by the proper amount each morning and evening. When 18 hr of light per day were attained (10 May 1995), the light regime was held constant (0500-2300 hr, CST) for the remainder of the study.

On Day 0 (26 January 1995), we weighed ducks and collected blood samples. On this same date, we removed commercial duck pellets and provided shelled corn *ad libitum* for 60 days, at which time we switched the diet to Mazuri Waterfowl Breeder pellets (PMI™ Feeds, Inc., St. Louis) for the duration of the study.

We used a small plastic funnel fitted with a plastic tube (9.5 mm outside diameter, 22.9 cm long) that was inserted through the pharynx to place the shot in the proventriculus. To reduce friction, we kept the tube in a pail of water when not in use. We poured shot into the funnel and flushed them into the proventriculus with 5 mL of water.

Controls were treated in the same manner except that no shot was placed in the proventriculus. At dosing, each shot dose was matched with its randomly selected duck. On Days 30, 60, and 90, the 8-shot doses for each shot type were randomly selected and placed in the same numbered vials that were used on Day 0.

We collected blood from the wing vein of all ducks in heparinized microhematocrit capillary tubes to determine hematocrits (Hcts). In addition, we collected 4 mL of whole blood with 5.0-mL syringes (20-gauge, 25.4-mm needles) from the wing vein of each of five 0-dosed, five Fe-dosed, five Bi-dosed females, and five ducks of each dose/sex group to determine major elements (>1% by wt in shot—Bi, Sn, Pb, and Fe) and major nutritionally essential elements (Ca, P, Mg, Zn,

and Cu). We selected these ducks at random. Because we expected high mortality of the Pb-dosed ducks, we collected blood from all 12 Pb-dosed ducks. Although Fe and Pb were not present in the candidate shot, we analyzed for these metals because the USFWS (1986:42102) procedures for the approval of nontoxic shot require that "...physiological parameters caused by the candidate shot must be significantly less than those caused by lead shot and must not be significantly greater than those caused by steel shot." Whole blood was injected into 10-mL lithium heparinized Vacutainer tubes and frozen until analyzed. We weighed ducks and collected blood from all survivors on Days 0, 30, 60, 90, 120, and 150.

After we had collected 24 hematocrit samples, we centrifuged the hematocrit tubes and read them on site in a mobile field laboratory/office. We spun the tubes for 5 minutes at 11,500 RPM at 13,000-g force.

As we collected each sample of whole blood for analysis, we placed tubes in metal racks and put them on ice in a styrofoam cooler. After all samples were collected, we stored them in a freezer (-10°C) until thawed for analyses.

After we killed adult ducks, livers, kidneys, gonads, hearts, and lungs from 20 females and 20 males (those chosen for collection of blood for analysis—5 ducks of each sex from each dosed group) were examined by the pathologist for gross and microscopic lesions. Livers, kidneys, and gonads of these 40 adult ducks were analyzed for major elements in candidate shot and essential major and trace elements.

We excised gizzards from all ducks, removed the contents, and weighed the gizzards. The contents of gizzards of all dosed ducks were washed through a series of fine screens to recover shot, which were sorted by size (to identify the date dosed), counted, and weighed. We determined the percent of shot retained at death and the percent of the weight of metal dissolved from each dosing.

When necropsying the 40 randomly selected ducks, the pathologist examined and weighed the kidneys, livers, and gonads; a representative sample of each organ was fixed in 10% formalin for histopathology. Hearts and lungs also were examined and samples preserved for histopathology. The residual tissues of these organs were placed in separate, numbered, plastic bags and stored in a freezer until thawed for analysis. For the remaining 60 ducks, the same organs were removed and weighed, placed in individual, numbered, plastic bags and stored in the freezer to serve as backup samples.

When ducks began laying eggs, pens were visited at least twice daily but usually more often. Eggs were removed, weighed, numbered with a felt-tipped marking pen, held overnight at room temperature, and stored for 1 week at 12.8° to 15.6°C and a relative humidity of 75%. The numbering system included the hen's ID number and the sequential order in which the egg was laid, e.g., the 12th egg laid by hen number 205 was numbered 205-12. For each female, we collected eggs until 21 uncracked eggs were obtained or until Day 150, whichever occurred first. When the 21st egg was collected, the female and her mate were weighed, bled for a blood sample, and killed. We removed organs and weighed them. As previously indicated, organs were excised from 40 ducks and stored for chemical analysis; these ducks were necropsied and tissues were saved for histopathology.

All uncracked eggs collected during each 7 days (except the 11th egg for each pair) were placed in an incubator. The temperature in the incubator was maintained at 37.5°C and the relative humidity was 84-87%. After 6 days of incubation, eggs were candled to determine fertility; we removed infertile eggs. Eggs were transferred to a hatcher 4 days before their expected hatching date. The temperature in the hatcher was maintained at 37.2°C and the relative humidity was 87-93%. Fertile eggs that failed to hatch were opened to determine age of embryos at death.

Each of six trays in the hatcher was separated into nine compartments by thin pieces of Masonite™, each compartment was 18.42 x 18.42 cm. Eggs from each female were placed in separate compartments and each tray was fitted with a 0.6-cm mesh hardware cloth cover to prevent the ducklings from moving among compartments. Thus, individual ducklings were associated with their parents.

We removed ducklings from the hatcher approximately 18 hr after they hatched, then weighed and banded them with Size 8 sequentially numbered, aluminum leg bands (National Band and Tag Co., Newport, Kentucky). (Note: these bands are too small to remain on mallard ducklings past 7 days of age.) We maintained the temperature in the brooders at 37.8°C with thermostat-controlled heat lamps. The brooders were constructed of vinyl-covered, 1.3-cm mesh welded wire. Each brooder compartment was 82.6 x 88.9 cm, providing 245 $cm^2$ of floor space for each of 30 ducklings. The minimum requirement for each duckling <7

days of age is 239 $cm^2$ (personal communication, Laboratory Animal Care Committee, University of Illinois). Thus, the ducklings were free to move about and choose a preferred temperature. Water was provided *ad libitum* via waterers equipped with standard 1.9-L jars, which were refilled at least twice daily. Starter mash (Purina™ Duck Grower, 16% protein, Purina Mills, Inc., St. Louis) was provided *ad libitum* in metal feeders.

When ducklings were 7 days old, we sexed and weighed them, collected blood to determine hematocrits, and killed them by decapitation. Ten ducklings, each of different parentage, were selected at random from each dosing group; samples of blood, liver, and kidneys were collected from each bird. These samples were analyzed for the same elements as the tissues from adults. These ducklings also were necropsied; liver and kidney samples (and several hearts) were preserved for histopathology. Because the amounts of kidney and blood from a single duckling were often inadequate for the required analyses, we augmented our samples by adding kidneys and blood from the next clutch mate of the selected ducklings. Because of their small size, gonads were not collected for analysis.

Thickness of shells of the 11th egg laid by each female was measured with a Digimatic Outside Micrometer™ accurate to 0.001 mm (Metutayo, Japan). Measurements were taken at three locations (two each at the apex, cap, and equator) of each egg and averaged. The shell and contents of the 11th egg from each female were saved and analyzed separately for nine elements. The shells were stored at room temperature, and the egg contents were frozen until analyzed.

Pb-dosed ducks were examined periodically by the institutional veterinarian in the Office of Laboratory Animal Resources, University of Illinois, who at various times reported that four ducks were moribund. These four ducks were euthanized. Five of the remaining eight Pb-dosed ducks died during a night when the temperature inside the test facility fell to -20.6°C.

The last Pb-dosed ducks died 9 February 1995 (14 days after dosing), which was before most gonads began to respond to the approaching breeding season. Because most gonads were too small to analyze for all elements, we analyzed them by GFAA for Pb and Bi.

## Chemical Analyses

### *Storage of Samples*

We inventoried samples (labeled by number and type of tissue) and stored them at -10°C in a freezer, which was monitored daily. Some Vacutainer tubes containing blood broke during freezing. If noticed while still frozen, some samples were transferred to polypropylene test tubes and not lost.

### *Digestions of Samples*

We allowed samples to thaw, then used acid to digest samples of blood, liver, kidney, gonad, egg contents, and eggshell for metal analyses. The analyses were performed with either inductively-coupled, argon plasma emission spectroscopy (ICP) or graphite furnace atomic absorption spectroscopy (GFAA) or both. Because we wanted concentrations expressed on a wet-weight basis for blood and organs, we did not dry these samples before they were digested. Metals we sought were either present in the test shot (Bi, Sn, Fe, and Pb) or were essential elemental nutrients (Ca, Mg, P, Zn, and Cu). We used ICP to measure for these metals, and we analyzed for beryllium (Be) as an internal standard. GFAA was used to measure Pb and Bi when concentrations were low.

### *Digestions for ICP Analysis*

A mixed portion of the sample (0.5 to 1.0 g) was placed in a tared 50-mL conically tipped polypropylene centrifuge tube and weighed to 0.1 mg with an electronic top-loading balance. Centrifuge tubes were precleaned by soaking for 24 hr in a 10% nitric acid ($HNO_3$) bath and rinsing with deionized water. Samples and tubes were tared, then we added 1 to 2 mL of hydrogen peroxide ($H_2O_2$) and reweighed. We then added 30 to 50 mL of 2% $HNO_3$, and 10% hydrochloric acid (HCl) and the internal standard solution of Be (2 mg/L).

We homogenized samples into a slurry with a sawtoothed generator manufactured with titanium and TFE-fluorocarbon (Pro Scientific, Monroe, Connecticut). The internal standard solution was used to rinse excess materials from the generator, with the amount of rinse solution accounted for in the total weight.

Sample preparations were completed using a SpectrPrep™ System automated microwave digestion system (CEM Corporation, Matthews, North Carolina). We used a 15-mL sample loop. After heating, cooling, and filtering, about 12.5 mL of the sample were collected and deposited by autosampler into a 15-mL polypropylene test tube. This digestate was then used for ICP analysis. Eggshells tended to clog the small-diameter tubing of the microwave system, but homogenation of the sample mixture, followed by a few hours in

a warm ultrasonic bath, effectively reduced particle size.

*Digestions for GFAA Analysis*

A mixed portion of the sample (0.5 to 1.0 g) was placed in a tared TFE-fluorocarbon beaker and weighed to 0.1 mg on an electronic top loading balance. We added 20 mL of deionized water (DI $H_2O$), 0.250 mL concentrated $HNO_3$, and 1 mL of hydrogen peroxide ($H_2O_2$). We heated the mixture on a hot plate at 95°C until the solution started to clear (about 0.5 hr). Approximately 20 mL DI $H_2O$ and 2 mL $H_2O_2$ were added. Upon further heating the mixture cleared and "foamed up." We rinsed down contents from the beaker walls with DI $H_2O$. Beakers were then covered with TFE-fluorocarbon watch glasses and allowed to reflux for approximately 1 hr. The resulting solutions were usually clear to yellow. The samples were brought to 50 mL with a volumetric flask, filtered through a 0.45-mm nitrocellulose filter, and stored in acid-washed linear polyethylene bottles. The final acid concentration used was 0.5% $HNO_3$. High purity acids and hydrogen peroxide (Baker Ultrex™ and Fisher Optima™) were used for all digestions.

**Analytical Methods**

Tissues were analyzed "blind" by the chemists—that is, they did not know either the gender of duck or which test shot it had received.

*ICP*

We used a Thermo Jarrell Ash (TJA) AtomComp™, Model 61, vacuum spectrometer, with the polychromator configured with 44 fixed channels, including analytical lines for variable concentrations of Ca and Mg. Although we reported results for only a few elements, we measured for 30 elements to monitor for spectral interferences, which we did not detect. Blank subtraction and background correction were used.

We used USEPA Method 200.7, (Office of Research and Development 1994). We used a different digestion process and we measured for Bi, which was not a listed analyte. We chose Be as an internal standard because it was not in the samples, it caused no spectral or background interference, and it was precisely detectable.

Because samples of eggshells were mostly calcium carbonate, the amounts of Ca were beyond the analytical range of the system. To cope with this situation, we analyzed eggshells by ICP to quantify all the elements except Ca, then we diluted samples with an acid blank solution (10% HCl, 2% $HNO_3$) and reanalyzed for Ca. We could reconstruct the actual Ca values by making comparisons with the internal standard.

*GFAA*

We used a Thermo Jarrell Ash Model 957 Atomic Absorption Spectrophotometer coupled with a Model 188 Furnace Atomizer and FASTAC autosampler. Samples were introduced as a spray and deposited directly into a carbon cuvette at 100°C to obtain drying on contact. Method 3113 of Greenberg et al. (1992) was used. We analyzed samples in triplicate and reported the means.

*Quality Control*

We calibrated instruments daily with the standard curve being verified with traceable, quality-control samples (QCS) from the National Institute of Standards and Technology (NIST). Samples (usually 10) were bracketed by calibration blanks, laboratory fortified blanks, and instrument performance check solutions during analysis, and we performed periodic checks on the internal standard solution. The ICP instrument was programmed to compensate for drift. The calibration was accomplished by recalculating the slopes of the calibration curves when any analyte was more than $\pm$5% of the true value while determining the ICP check standard. When an analyte was $\geq \pm$10% of the true value for a sample, the instrument was recalibrated and the affected sample reanalyzed. The ICP check standard was formulated to equal a concentration at the midpoint of the calibration curve and was traceable to NIST Standard Reference Materials (SRMs). The QCS for the GFAA initially had to be within 10% of the true value. Subsequent measurement of the bracketed internals had to be $\pm$15%; if these limits were exceeded, we recalibrated the instrument and reanalyzed affected samples.

We digested and analyzed in duplicate 10% of the samples, half of them spiked. Also, we prepared digestion blanks and spiked digestion blanks at a frequency of 10%. They underwent the same digestion and analytical process as did the samples.

*Calculations*

Data produced by ICP analysis were transferred to database files with ThermoSpec (TJA) Enable OA software. We then imported these into Enable spreadsheets for tabulations and calculations. We saved the Enable spreadsheets in a Lotus 1-2-3 format on diskette. For the GFAA instrument, results were recorded and data printed on an

instrument printer as concentrations (μg/L) based on measurement of peak area. Data were then manually entered into spreadsheets to tabulate and perform calculations.

### Statistical Analysis

Statistical comparisons among doses for variables measured only once (usually after necropsies) were made with one-way analyses of variance (ANOVA), except two-way ANOVAs were used when there were sex differences. Equality of variances among groups was evaluated with Levene's test (BMDP 1992). In instances where heteroscedasticity ($P < 0.05$) was detected, Brown-Forsyte statistics and approximate degrees of freedom were used. Pairwise differences among groups were evaluated with Bonferroni comparisons.

In instances where variables were measured for two or more periods, dose groups were compared and tested for variation over time with a repeated-measures ANOVA. When necessary, significance levels based on the Huynh-Feldt (BMDP 1992) adjustment were used. Because of unbalanced data sets (caused by animals dying during the experiment), we used Wald statistics in a restricted maximum-likelihood model to estimate parameters to test for differences among doses.

We performed all tests with the BMDP statistical software package, version 7.0 (BMDP 1992). When we report two values as "different" or that they "differ," we mean that they were statistically different at the 95% level of confidence ($P \leq 0.05$).

## Results

### Chronic Toxicity Test

*Survival*

All 12 Pb-dosed ducks died within 14 days after dosing; mean survival was 9.9 days, and no difference in survival existed between sexes. All 0-dosed and Fe-dosed ducks survived until sacrificed; time from Day 0 to sacrifice averaged 115.6 days for 0-dosed ducks and 121.1 days for Fe-dosed ducks. Only one Bi-dosed duck died (on Day 131, after laying 16 eggs). Survival time for Bi-dosed ducks (including the one that died) averaged 120.5 days; mean survival times were not different among the three dosage groups. Both ducks of each pair were sacrificed when the female had laid 21 uncracked eggs. Thus, these survival times only indicate that most ducks survived until sacrificed and that no differences existed among 0-dosed, Fe-dosed, and Bi-dosed ducks in the mean time required to lay 21 uncracked eggs.

*Hematocrit*

Mean Hcts for Pb-dosed ducks declined from 44.6 to 25.2 (Table 1, Figure 1) during their 9.9-day mean survival. However, we obtained Hcts at necropsy for only 4 of the 12 Pb-dosed ducks.

For the other dosage groups, mean Hcts of males did not decline through Day 120 and at necropsy; however, mean Hcts of females declined in all three groups of surviving ducks by Day 90 and at necropsy were lower than mean Hcts of males (Figure 2). Mean Hcts in 0-dosed females declined from 46.3 on Day 0 to 38.2 at necropsy, in Fe-dosed females from 46.2 on Day 0 to 37.9 at necropsy, and in Bi-dosed females from 46.0 on Day 0 to 36.2 at necropsy (Table 1). Except for Pb-dosed ducks, no difference existed among doses in the mean Hcts in the present study (Table 1).

*Body Weight*

All males weighed more than all females from Day 0 through Day 60. By Day 90, the mean weights of males and females did not differ, and on Day 120 and at necropsy females were heavier than males (Figure 3). Changes in weight were caused primarily by gains in females rather than losses in males (Table 2). The gain by females was accompanied by a decline in average Hct (Table 1). Weights of females (and of males) among the three dosed groups were not different at necropsy. Pb-dosed males weighed more than Pb-dosed females on Day 0. At necropsy, Pb-dosed males and Pb-dosed females weighed about one-third of their mean body weights on Day 0, but mean weights of the Pb-dosed ducks were not different between sexes (Table 2).

The only dose-related difference in mean body weights was associated with Pb-dosed ducks, which weighed less at necropsy than 0-dosed, Fe-dosed, or Bi-dosed ducks (Table 2, Figure 4).

*Dissolution of Shot*

Lead—In our study, Pb-dosed females dissolved an average of 31.5% of the weight of eight, No. 4, shot in an average of 9.3 days—3.4% per day. Males dissolved 31.2% of the weight of dosed Pb shot in an average of 10.5 days—3.0% per day. At death, females retained in their gizzards 81.2% and males 87.5% of the number of dosed Pb shot (Table 3). Four of 15 shot not recovered from the

*Continued on page 226*

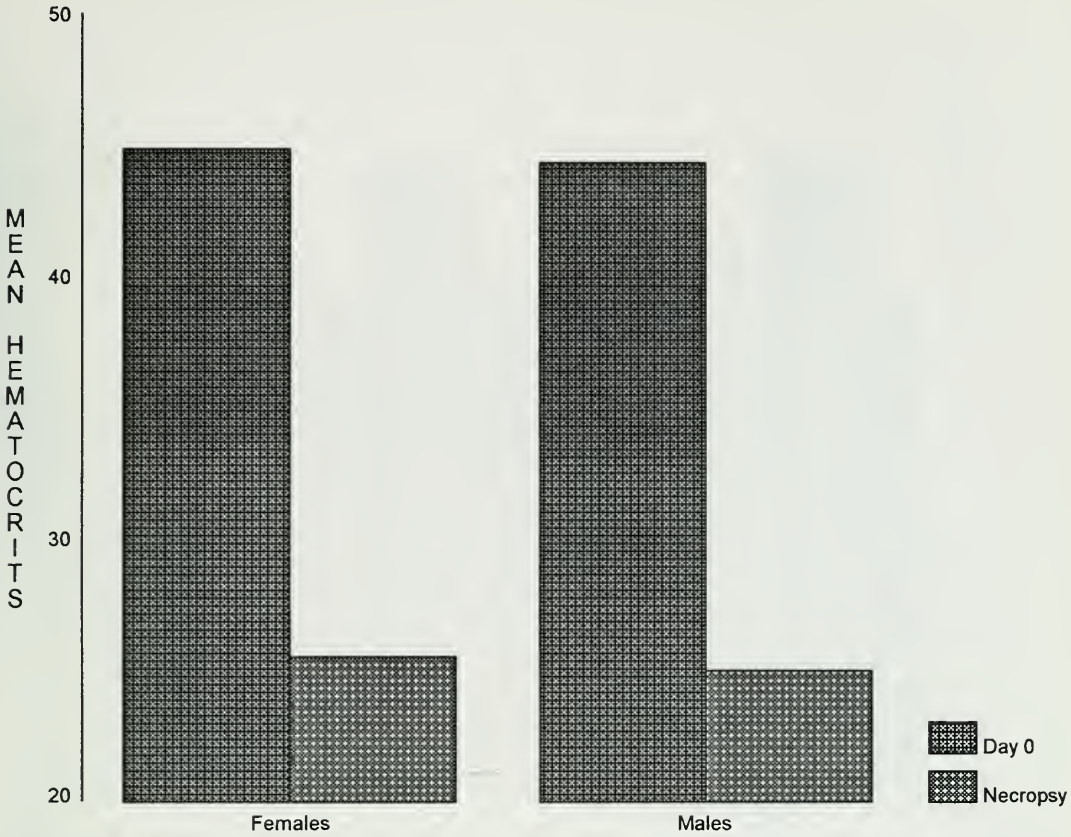


Figure 1. Mean Hcts of game-farm mallard ducks dosed with 8, No. 4, Pb shot on Day 0. n = 12 for Day O and 4 for necropsy.

Table 1. Mean Hcts of adult male and female game-farm mallard ducks dosed with 0 shot (controls); eight, No. 4, Fe shot; eight, No. 4, Bi shot; or eight, No. 4, Pb shot.

| | | Days after first dosing | | | | | |
|---|---|---|---|---|---|---|---|
| Dose | Sex | 0 | 30 | 60 | 90 | 120 | Nec[a] |
| 0 | F | 46.3 | 49.5 | 50.2 | 43.5 | 38.8[c] | 38.2 |
| | | 0.63[b] | 0.72 | 0.55 | 1.52 | 1.12 | 1.08 |
| | M | 46.4 | 48.4 | 48.2 | 45.0 | 45.4[c] | 45.3 |
| | | 0.44 | 0.47 | 0.50 | 0.63 | 0.65 | 1.19 |
| Fe | F | 46.2 | 50.3 | 50.6 | 46.2 | 40.2[d] | 37.9 |
| | | 0.61 | 0.68 | 0.66 | 1.29 | 0.82 | 1.02 |
| | M | 46.7 | 48.4 | 49.5 | 46.9 | 48.4[d] | 44.6 |
| | | 0.40 | 0.54 | 0.49 | 0.56 | 0.61 | 0.84 |
| Bi | F | 46.0 | 47.9 | 47.9 | 43.9 | 37.1[e] | 36.2[f] |
| | | 0.72 | 0.84 | 1.05 | 1.31 | 1.11 | 1.03 |
| | M | 47.3 | 49.1 | 48.8 | 46.0 | 48.0[e] | 47.4[f] |
| | | 0.62 | 0.63 | 0.50 | 0.46 | 0.89 | 0.62 |
| Pb | F & M | 44.6[d] | | | | | 25.2[g] |
| | | 0.61 | | | | | 1.65 |

[a] Ducks were necropsied when one member of a pair died, when 21 uncracked eggs were collected from the pair, or at 150 days post dosing, whichever occurred first. Mean survival was 115.6 days for 0-dosed ducks, 121.6 days for Fe-dosed ducks, and 120.5 days for Bi-dosed ducks. All Pb-dosed ducks died ≤14 days post dosing and only 2 samples from each sex were collected at necropsy.

[b] SE. [e] n = 8.
[c] n = 7 [f] n = 17.
[d] n = 12. [g] n = 4.
n = 18 for all other samples.

Difference in Hcts:
Between sexes:
$F_{1,46}$ = 15.31; $P$ = 0.0003.

Among doses:
$F_{1,46}$ = 1.41; $P$ = 0.2533.

Over time:
$F_{5,230}$ = 101.80; $P$ < 0.00001.

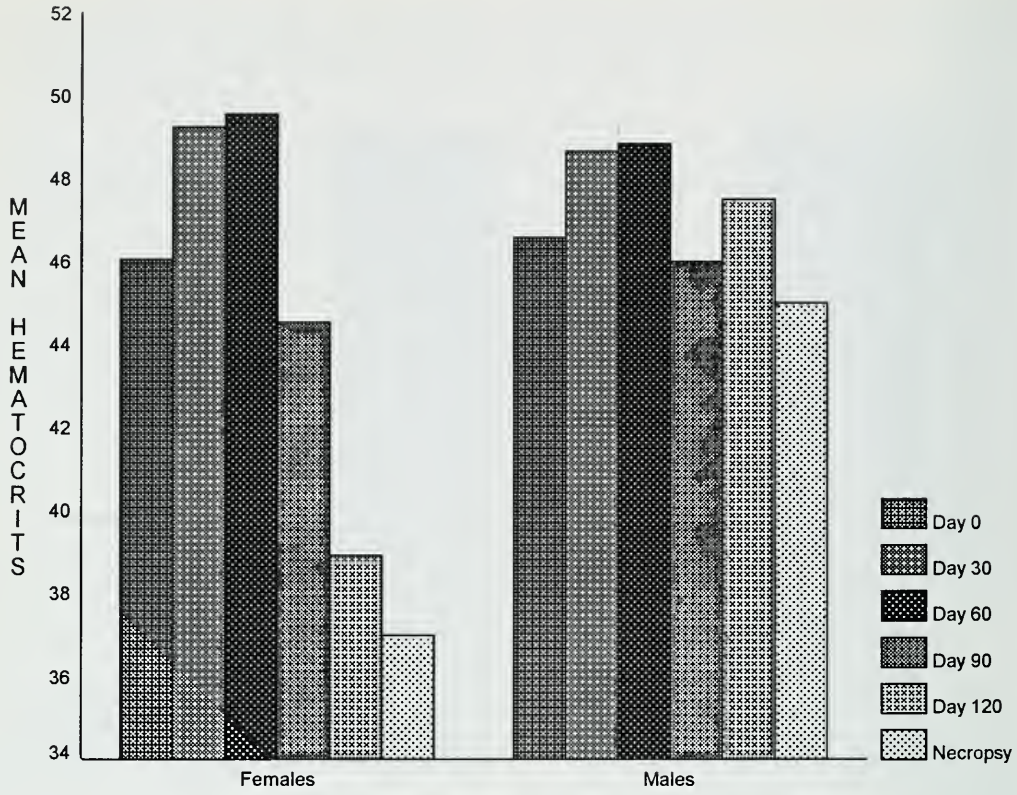


Figure 2. Mean Hcts of game-farm mallards dosed with 0 shot (controls); 8, No. 4, Fe shot; or 8, No. 4, Bi shot on Days 0, 30, 60, and 90. The three groups of ducks were combined for this graph. n = 54 females and 54 males for Days 0, 30, 60, and 90; n = 27 females and 27 males for Day 120; and n = 53 for females and 54 for males at necropsy.

Table 2. Mean body weight (kg) of adult male and female game-farm mallard ducks dosed with 0 (controls) shot; eight, No. 4, Fe shot; eight, No. 4, Bi shot; or eight, No. 4, Pb shot.

| Dose | Sex | Days after first dosing | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 30 | 60 | 90 | 120 | Nec[a] |
| 0 | F | 1.05 | 1.00 | 0.99 | 1.13 | 1.28[b] | 1.25 |
| | | 0.04[c] | 0.02 | 0.02 | 0.03 | 0.03 | 0.03 |
| | M | 1.15 | 1.14 | 1.10 | 1.19 | 1.16[b] | 1.20 |
| | | 0.02 | 0.03 | 0.02 | 0.03 | 0.04 | 0.04 |
| Fe | F | 1.02 | 1.01 | 0.97 | 1.13 | 1.24[d] | 1.25 |
| | | 0.04 | 0.03 | 0.03 | 0.04 | 0.04 | 0.04 |
| | M | 1.20 | 1.14 | 1.11 | 1.20 | 1.20[d] | 1.17 |
| | | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Bi | F | 1.05 | 1.02 | 0.99 | 1.14 | 1.19[e] | 1.22 |
| | | 0.03 | 0.03 | 0.03 | 0.03 | 0.06 | 0.04 |
| | M | 1.18 | 1.17 | 1.12 | 1.20 | 1.16[e] | 1.18 |
| | | 0.02 | 0.03 | 0.03 | 0.02 | 0.04 | 0.03 |
| Pb | F | 1.03[f] | | | | | 0.68[f] |
| | | 0.03 | | | | | 0.03 |
| | M | 1.22[f] | | | | | 0.79[f] |
| | | 0.04 | | | | | 0.05 |

[a] Ducks were necropsied when one member of a pair died, when 21 uncracked eggs were collected from the pair, or at 150 days post dosing, whichever occurred first. Mean survival was 115.6 days for 0-dosed ducks, 121.6 days for Fe-dosed ducks, and 120.5 days for Bi-dosed ducks. All Pb-dosed ducks died ≤14 days post-dosing.

[b] n = 7.

[c] SE.

[d] n = 12.

[e] n = 8.

[f] n = 6.

n = 18 for all others.

Difference in body weight:

Between sexes: $F_{1,48} = 7.05$; $P = 0.0107$.

Among doses: $F_{2,48} = 1.28$; $P = 0.2870$.

Over time: $F = 54.88$; $P < 0.00001$.

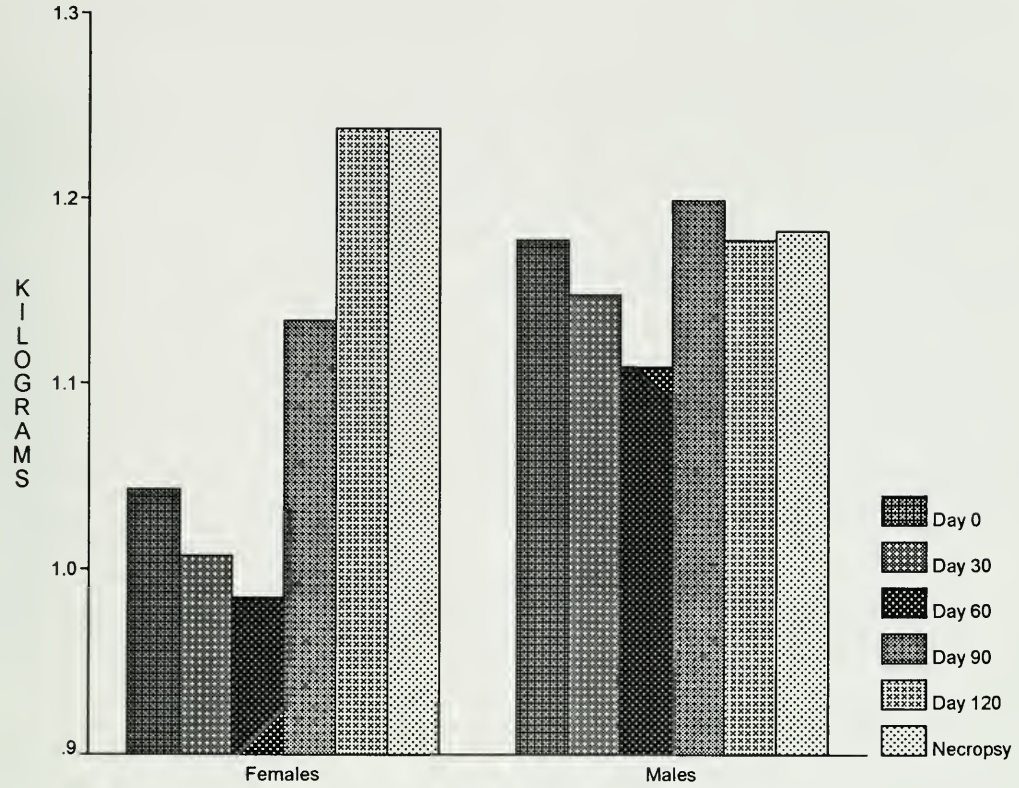


Figure 3. Mean body weight (kg) of game-farm mallards dosed with 0 shot (controls); 8, No. 4, Fe; or 8, No. 4, Bi shot on Days 0, 30, 60, and 90. The doses were combined for this graph. n = 34 for each sex for Days 0, 30, 60, and 90; n = 27 for each sex for Day 120; n = 53 for each sex for necropsy.

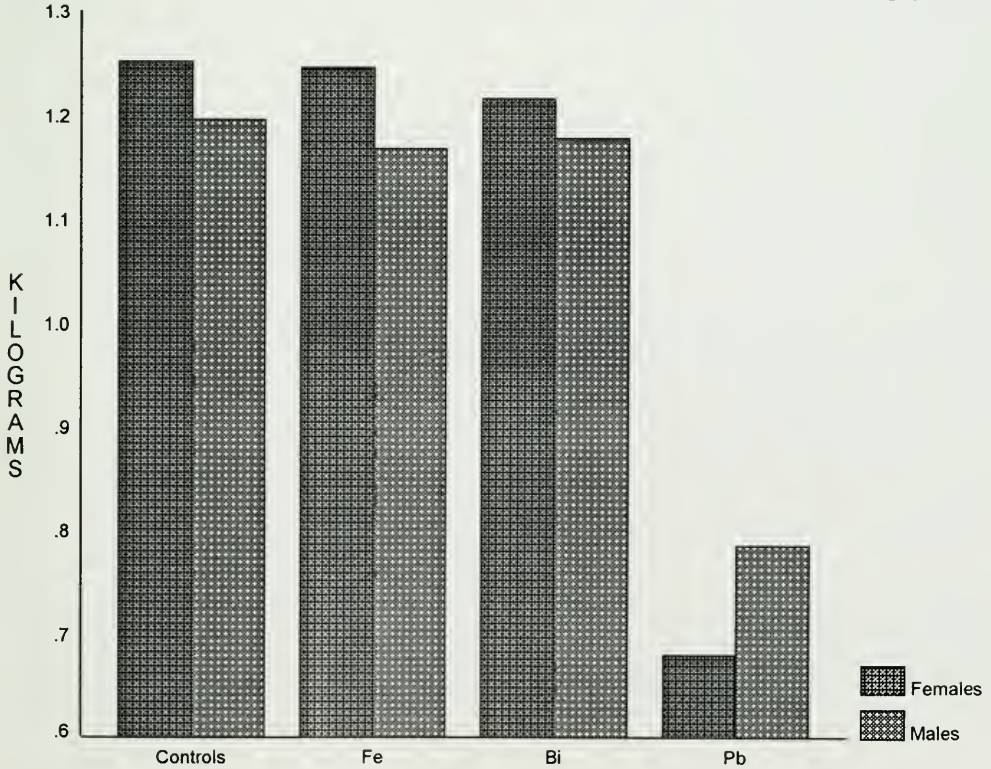


Figure 4. Mean body weight (kg) at necropsy of game-farm mallards dosed with 0 shot (controls); 8, No. 4, Fe shot; or 8, No., 4 Bi shot on Days 0, 30, 60, and 90, or 8 No. 4 Pb shot on Day 0. n = 18 for female

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gizzards were found in the feces of one duck. Because the time between dosing and recovery of the Pb shot was short (Table 4), the 11 missing shot were probably voided, but were not found in the feces. Eight ducks that each retained all eight dosed Pb shot at death dissolved an average of 330.5 mg of Pb from the shot in their gizzards.

To compare the rates at which Pb, Fe, and Bi shot were dissolved in the gizzard, we measured the mean daily rate of dissolution of Pb shot in 9.9 days—the mean survival time for Pb-dosed ducks. We then used multiple regression and estimated that the Fe-dosed ducks dissolved eight, No. 4, Fe shot at a rate of 1.4% per day in 9.9 days, and eight Bi-dosed ducks dissolved Bi shot at a rate of 2.5% per day in 9.9 days. In contrast, 12 ducks dosed with Pb shot on Day 0 dissolved eight, No. 4, Pb shot at a rate of 3.3% per day in an average of 9.9 days (Table 4).

Iron—Fe-dosed females dissolved an average of 99.9% of the weight of the eight, No. 4, Fe shot dosed on Day 0 in a mean of 121.2 days—0.8% per day. They dissolved 48.6% of the weight of Fe shot dosed on Day 90 in a mean of 31.2 days—1.6% per day (Table 3).

Many of the Fe shot dosed on Day 0 were probably completely dissolved in less than 121.2 days as only 1.4% of the number of Fe shot dosed in females on Day 0 were recovered from gizzards. Males dissolved an average of 96.6% of the weight of Fe shot dosed on Day 0 in a mean of 121.2 days—0.8% per day. Males dissolved 27.5% of the weight of Fe shot dosed on Day 90 in 31.2 days—0.9% per day. Each female dissolved an average of 3.9 g of Fe from all Fe shot dosed and each male 3.1 g over a mean period of 121.2 days after the first shot were dosed (Table 3).

In our previous toxicity study, the weight of six, No. 4, Fe shot dosed was 69.2% dissolved in 30 days (Sanderson et al. 1997a). This rate compares with 48.6% of the weight of Fe shot dissolved from eight, No. 4, Fe shot dosed on Day 90 in females in a mean of 31.2 days in the present study. On Day 90, the ducks in the present study retained most or all of the Fe shot dosed on Days 0, 30, and 60. These results suggest that the higher the number of shot in the gizzard, the slower the rate that individual pellets dissolve.

Bismuth—Bi-dosed females dissolved a mean of 98.9% of the weight of eight, No. 4, Bi shot dosed on Day 0 in an average of 120.5 days—0.8% per day. They dissolved 52.5% of the weight of Bi-shot dosed on Day 90 in an average of 30.5 days—1.7% per day. Bi-dosed males dissolved a mean of 99.2% of the weight of Bi shot dosed on Day 0 in an average of 120.5 days—0.8% per day. Bi-dosed males dissolved an average of 55.5% of Bi shot dosed on Day 90 in an average of 30.5 days—1.8% per day. Females dissolved a mean of 5.4 g of metal and males a mean of 5.2 g, from all dosed Bi shot over a mean of 120.5 days (Table 3).

Fe-dosed females dissolved 7.5 times as much metal from shot as did Pb-dosed females, which all died. Fe-dosed males dissolved 5.9 times as much metal from shot as did Pb-dosed males—all also died. Bi-dosed females dissolved 10.4 times as much metal, and males 10.7 times as much metal, as their counterparts dosed with Pb. Similarly, Bi-dosed females dissolved 1.4 times as much metal in their gizzards as did Fe-dosed females. Bi-dosed males dissolved 1.8 times as much metal as was dissolved by Fe-dosed males (Table 3). All Fe-dosed ducks and all but one Bi-dosed duck survived until euthanized at the end of the study, whereas all Pb-dosed ducks died within 14 days after they were dosed.

*Shot Retention*

From the radiographs made on 6 February 1995 (Day 11), the eight pellets that were dosed on Day 0 were identified in the gizzard of each of the 17 ducks selected for examination by radiographs. Usually eight pellets showed in both views (dorsal-ventral and right-left), but sometimes the count was questionable in one view.

From radiographs made on 6 March 1995 (Day 39), the eight pellets dosed on 24 February 1995 in each of the four male and four female Fe-dosed and Bi-dosed ducks were clearly identified. In addition, for the eight Fe-dosed ducks, 16 shot were counted in each of five gizzards, a minimum of 10 shot in one gizzard, and 15 shot in each of two gizzards. In gizzards of the eight Bi-dosed ducks, 16 shot were identified in each of six gizzards and a minimum of 15 shot in each of two gizzards.

Although all shot dosed on 26 January 1995 probably were retained by all ducks on 6 March, this presumption could not be verified by radiographs. With 16 shot compressed in the gizzard, some shot obscured the view of others. Radiographs obtained on 6 April 1995 showed the eight shot dosed on 27 March 1995 in each gizzard of the eight Fe-dosed and eight Bi-dosed ducks. Twenty-four shot were identified in each of two gizzards of Fe-dosed ducks and two Bi-dosed ducks. A mean of 17.8 shot was identified in the gizzards of

*Continued on page 228*

Table 3. Mean weight of eight, No. 4, Fe, Bi, and Pb shot dosed in game-farm mallard ducks, mean weight of shot recovered from the ducks, number and percent of dosed shot recovered, and percent and weight of shot dissolved in the gizzard.

| | Day 0 | | Day 30 | | Day 60 | | Day 90 | |
|---|---|---|---|---|---|---|---|---|
| | Sex | | Sex | | Sex | | Sex | |
| Dose | F | M | F | M | F | M | F | M |
| | | | Mean weight (g) of 8 shot dosed | | | | | |
| Fe | 1.197 | 1.200 | 1.202 | 1.202 | 1.198 | 1.201 | 1.199 | 1.200 |
| | 0.003[a] | 0.002 | 0.001 | 0.002 | 0.002 | 0.002 | 0.003 | 0.003 |
| Bi | 1.649 | 1.663 | 1.654 | 1.661 | 1.644 | 1.654 | 1.646 | 1.653 |
| | 0.005 | 0.003 | 0.004 | 0.005 | 0.006 | 0.004 | 0.010 | 0.005 |
| Pb | 1.658 | 1.666 | | | | | | |
| | 0.008 | 0.009 | | | | | | |
| | | | Mean weight (g) of shot recovered | | | | | |
| Fe | 0.001 | 0.025 | 0.003 | 0.232 | 0.273 | 0.628 | 0.616 | 0.878 |
| | 0.001 | 0.008 | 0.001 | 0.029 | 0.036 | 0.032 | 0.046 | 0.033 |
| Bi | 0.018 | 0.024 | 0.089 | 0.070 | 0.272 | 0.252 | 0.787 | 0.735 |
| | 0.008 | 0.012 | 0.023 | 0.024 | 0.041 | 0.054 | 0.098 | 0.099 |
| Pb | 1.136 | 1.147 | | | | | | |
| | 0.165 | 0.160 | | | | | | |
| | | | Mean % of weight dissolved from shot dosed | | | | | |
| Fe | 99.9 | 96.6 | 99.8 | 80.7 | 76.8 | 47.8 | 48.6 | 27.5 |
| | 0.07 | 1.43 | 0.12 | 2.41 | 2.96 | 2.78 | 3.84 | 2.78 |
| Bi | 98.9 | 99.2 | 93.7 | 95.8 | 83.4 | 82.8 | 52.5 | 55.5 |
| | 0.51 | 0.39 | 1.94 | 1.43 | 2.49 | 3.68 | 5.88 | 5.99 |
| Pb | 31.5 | 31.2 | | | | | | |
| | 9.87 | 9.62 | | | | | | |
| | | | Mean weight (g) dissolved from shot dosed | | | | | |
| Fe | 1.196 | 1.174 | 1.199 | 0.975 | 0.924 | 0.573 | 0.582 | 0.324 |
| | 0.003 | 0.009 | 0.002 | 0.031 | 0.036 | 0.033 | 0.046 | 0.035 |
| Bi | 1.631 | 1.639 | 1.566 | 1.591 | 1.372 | 1.402 | 0.860 | 0.918 |
| | 0.011 | 0.012 | 0.024 | 0.024 | 0.042 | 0.053 | 0.093 | 0.099 |
| Pb | 0.522 | 0.519 | | | | | | |
| | 0.163 | 0.160 | | | | | | |
| | | | Mean number of shot recovered from shot dosed | | | | | |
| Fe | 0.111 | 2.78 | 0.889 | 7.00 | 6.56 | 7.67 | 7.83 | 7.89 |
| | 0.111 | 0.62 | 0.403 | 0.40 | 0.59 | 0.20 | 0.121 | 0.111 |
| Bi | 1.94 | 2.61 | 4.67 | 4.17 | 6.06 | 6.06 | 7.28 | 7.72 |
| | 0.70 | 0.78 | 0.642 | 0.64 | 0.63 | 0.70 | 0.463 | 0.177 |
| Pb | 6.50 | 7.00 | | | | | | |
| | 0.96 | 1.00 | | | | | | |
| | | | Mean % of the number of shot recovered from shot dosed | | | | | |
| Fe | 1.4 | 34.7 | 11.1 | 87.5 | 81.2 | 95.8 | 97.2 | 98.6 |
| | 1.39 | 7.80 | 5.04 | 4.95 | 7.44 | 2.48 | 2.16 | 1.39 |
| Bi | 24.3 | 32.6 | 58.3 | 52.1 | 75.7 | 75.0 | 91.0 | 96.5 |
| | 8.78 | 9.70 | 8.02 | 7.97 | 7.92 | 8.69 | 5.79 | 2.22 |
| Pb | 81.2 | 87.5 | | | | | | |
| | 11.97 | 12.50 | | | | | | |
| | | | Mean No. of days that shot dosed were in the gizzard | | | | | |
| Fe | 121.2 | 121.2 | 91.2 | 91.2 | 61.2 | 61.2 | 31.2 | 31.2 |
| | 2.92 | 2.92 | | | | | | |
| Bi | 120.5 | 120.5 | 90.5 | 90.5 | 60.5 | 60.5 | 30.5 | 30.5 |
| | 4.32 | 4.32 | | | | | | |
| Pb | 9.3 | 10.5 | | | | | | |
| | 0.760 | 0.922 | | | | | | |

SE.

Table 4. Rates at which eight, No. 4, Fe, Bi, and Pb shot dissolved after 10 to 120 days in the gizzards of game-farm mallards (2nd, 3rd, and 4th doses of 8 Fe or 8 Bi shot were dosed on Days 30, 60, and 90).

| Dose | Day Dosed | Mean No. Days Shot in Gizzard | Mean % Wt of Shot Dissolved per Day |
|---|---|---|---|
| Fe | 0 | 9.9 | 1.4[a] |
| | | | 0.62[b] |
| | 0 | 121.1 | 0.8 |
| | | 2.03 | 0.02 |
| | 30 | 91.1 | 0.9 |
| | | 2.03 | 0.06 |
| | 60 | 61.1 | 1.0 |
| | | 2.03 | 0.05 |
| | 90 | 31.1 | 1.3 |
| | | 2.03 | 0.08 |
| Bi | 0 | 9.9 | 2.5[a] |
| | | | 0.26 |
| | 0 | 120.5 | 0.8 |
| | | 3.01 | 0.02 |
| | 30 | 90.5 | 1.0 |
| | | 3.01 | 0.04 |
| | 60 | 60.5 | 1.4 |
| | | 3.01 | 0.06 |
| | 90 | 30.5 | 2.2 |
| | | 3.01 | 0.17 |
| Pb | 0 | 9.9 | 3.3 |
| | | 0.60 | 0.75 |

[a] Estimated by multiple regression.
[b] SE.

Difference in rate shot were dissolved:
Between doses: Dosed Day 60; $P < 0.0001$.
Dosed Day 90; $P < 0.0001$.

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the remaining six Fe-dosed ducks and a mean of 19.7 shot in the remaining six Bi-dosed ducks. All shot dosed on 27 March (Day 60) were readily identified, but it was not possible to distinguish all shot dosed on Day 0 from shot dosed on Day 30.

Each of the ducks was dosed with 8 shot on 26 January 1995 (Day 0), 24 February 1995 (Day 30), and 27 March 1995 (Day 60); most of the 24 shot were retained on 6 April 1995 (Day 90), the last date that ducks were dosed. At necropsy, remnants of all 32 shot were found in one gizzard of a Fe-dosed duck on Day 99, and all shot were present in each gizzard of three Bi-dosed ducks on Days 109 (2) and 118. In addition, one gizzard contained 30 Bi shot on Day 118, one gizzard contained 31 Bi shot on Day 95, and 30 shot were retained in each gizzard of three Fe-dosed ducks on Days 109, 112, and 132.

In our present study, six females retained an average of 81.2% of the number of dosed Pb shot to an average of 9.3 days. Six males retained an average of 87.5% of the number of the dosed Pb shot to an average of 10.5 days (Table 3). These results show that ducks void ingested Pb shot at a faster rate than they do Fe or Bi shot.

*Organ Weights*

Gizzard—The mean weights of gizzards ranged from 19.2 g for Bi-dosed females to 26.5 g for Pb-dosed males (Table 5). No sex differences existed in any of the four dosed groups. Gizzards of Pb-dosed ducks were heavier than gizzards of 0-, Fe- and Bi-dosed ducks, but no difference was detected among gizzard weights of 0-, Fe-, and Bi-dosed ducks (Table 5).

In our study, ducks were on a diet of commercial duck pellets from Day 61 to necropsy—an average of 58 days. Furthermore, Pb-dosed ducks, all of which died in February 1995, had heavier gizzards than the 0-, Fe-, and Bi-dosed ducks, which were euthanized in April, May, or June 1995. The lower average gizzard weights in our study also may be related to the extended reproductive period of the 0-, Fe-, and Bi-dosed ducks, which was not experienced by the Pb-dosed ducks.

Liver—Mean weights of livers ranged from 17.7 g for Pb-dosed females to 46.6 g for Fe-dosed females (Table 5). Livers of 0-dosed, Fe-dosed, and Bi-dosed females weighed more than twice as much as livers of Pb-dosed females and of males in each dosed group (Figure 5). No difference was

*Continued on page 230*

Table 5. Mean weights (g) of gizzard, liver, kidneys, and gonads of adult male and female game-farm mallard ducks with 0 shot (controls); eight, no. 4 Fe shot; eight, no. 4 Bi shot; and eight, no. 4 Pb shot. n = 18 for each sex for O-, Fe-, and Bi-dosed ducks. n = 6 for each sex for Pb- dosed ducks.

| Dose | Sex | Gizzard | Liver | Kidneys | Gonads |
|---|---|---|---|---|---|
| 0 | F | 21.0 | 42.2 | 9.3 | 43.5 |
| | | 0.83[a] | 2.02 | 0.28 | 2.53 |
| 0 | M | 21.4 | 18.5 | 6.2 | 33.1 |
| | | 0.81 | 0.91 | 0.16 | 2.46 |
| 0 | F & M | 21.2 | 30.4 | 7.8 | 38.3 |
| | | 0.57 | 2.27 | 0.31 | 1.95 |
| Fe | F | 19.7 | 46.6 | 9.1 | 38.9 |
| | | 0.93 | 1.97 | 0.30 | 3.08 |
| Fe | M | 20.1 | 20.0 | 6.3 | 36.8 |
| | | 0.52 | 0.64 | 0.23 | 2.32 |
| Fe | F & M | 19.9 | 33.2 | 7.7 | 37.9 |
| | | 0.53 | 2.47 | 0.30 | 1.91 |
| Bi | F | 19.2 | 43.9 | 9.0 | 45.2 |
| | | 0.77 | 2.36 | 0.36 | 2.37 |
| Bi | M | 21.2 | 18.0 | 6.2 | 36.1 |
| | | 0.76 | 0.94 | 0.19 | 4.26 |
| Bi | F & M | 20.2 | 30.9 | 7.6 | 40.6 |
| | | 0.56 | 2.53 | 0.31 | 2.52 |
| Pb | F | 23.2 | 17.7 | 8.6 | 0.6 |
| | | 1.20 | 1.81 | 0.29 | 0.10 |
| Pb | M | 26.5 | 18.6 | 8.7 | 1.2 |
| | | 2.28 | 2.57 | 0.95 | 0.20 |
| Pb | M & F | 24.8 | 18.1 | 8.7 | 0.8 |
| | | 1.33 | 1.51 | 0.48 | 0.14 |

[a] SE.

Differences between sexes in organ weights. Only significant differences are shown.

Liver: 0-dosed $F_{1,24} = 115.45$; $P < 0.00001$.

Fe-dosed $F_{1,21} = 164.68$; $P < 0.00001$.

Bi-dosed $F_{1,22} = 104.82$; $P < 0.00001$.

Kidneys: 0-dosed $F_{1,27} = 95.44$; $P < 0.00001$.

Fe-dosed $F_{1,34} = 53.48$; $P < 0.00001$.

Bi-dosed $F_{1,34} = 50.49$; $P < 0.00001$.

Gonads: 0-dosed $F_{1,34} = 8.65$; $P = 0.0059$.

Pb-dosed $F_{1,10} = 7.29$; $P = 0.0223$.

Difference among doses in organ weights:

Gizzard: $F_{3,116} = 6.72$; $P = 0.0003$.

Liver: $F_{3,112} = 15.43$; $P < 0.00001$.

Kidneys: $F_{3,112} = 2.70$; $P = 0.0492$.

Gonads: $F_{3,79} = 113.91$; $P < 0.00001$.

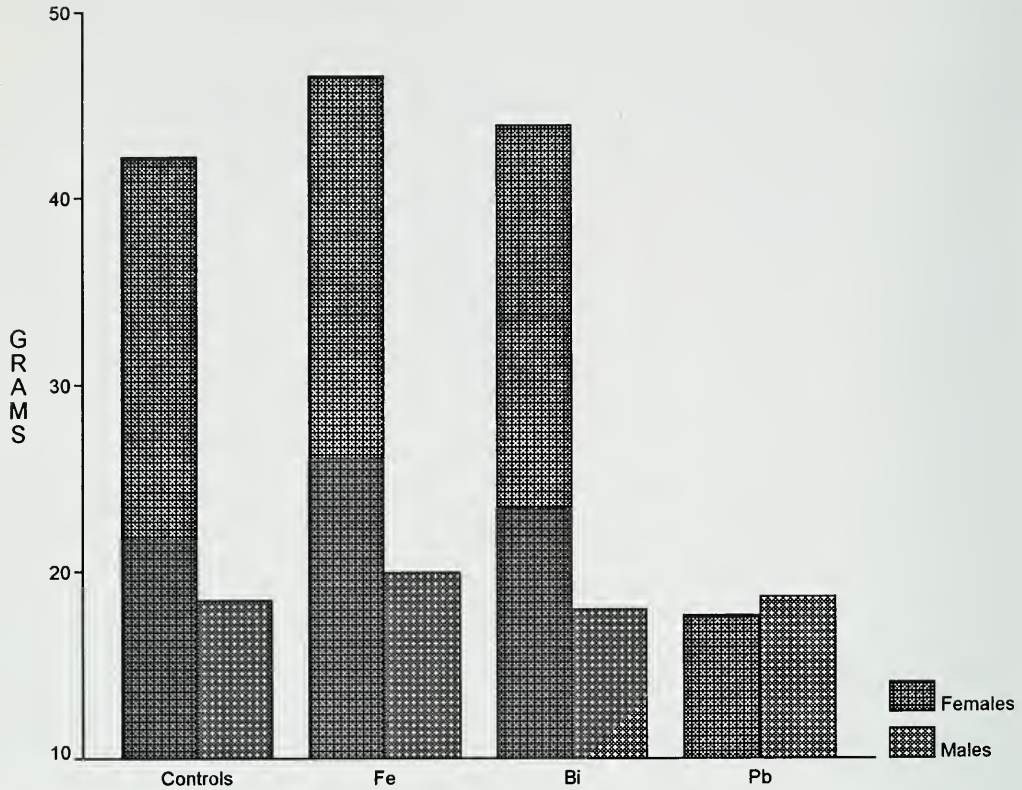


Figure 5. Mean weight (g) of livers of game-farm mallards dosed with 0 shot (controls); 8, No. 4 Fe, shot; or 8, No. 4, Bi shot on Days 0, 30, 60, and 90, or 8, No. 4, Pb shot on Day 0. n = 18 for each sex for 0-, Fe, and Bi-dosed ducks, and n = 6 for each sex for Pb-dosed ducks.

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detected in the mean weights of livers of males among the four dosed groups.

Mean weights of livers of females in our present study were much higher than the mean weights of livers of females in our previous toxicity study (Sanderson et al. 1997a). These differences were manifestations of long-term egg laying. Ducks in the previous study were killed on 12 May 1994, at the start of the reproductive season, whereas ducks in the present study were killed after each female had laid 21 uncracked eggs (most were killed in late May or June 1995).

Kidneys—The mean weights of kidneys ranged from 6.2 g for 0-dosed and Bi-dosed males to 9.3 for 0-dosed females (Table 5). No difference was detected among doses in the mean weights of kidneys of females, or of 0-dosed, Fe-dosed, and Bi-dosed males. The kidneys of male Pb-dosed ducks weighed more than the kidneys in the other dosed groups. Kidneys of female 0-dosed, Fe-dosed, and Bi-dosed ducks weighed more than kidneys of males in the respectively dosed groups. The mean weights of kidneys of female and male Pb-dosed ducks did not differ (Table 5).

As with livers, mean weights of the kidneys of males in the present study (Table 5) were similar to the mean weights of kidneys of males in the earlier study (Sanderson et al. 1997a). Mean weights of kidneys of females in the present study were higher than the mean weights of kidneys of females in the earlier study.

Gonads—No differences were detected among mean weights of gonads for 0-, Fe-, and Bi-dosed ducks. Gonads of 0-dosed females were heavier than gonads of 0-dosed males, and gonads of Pb-dosed males were heavier than gonads of Pb-dosed females. The mean weights of female gonads ranged from 0.6 g for Pb-dosed birds to 45.2 g for Bi-dosed ducks (Table 5). The mean weights of gonads did not differ between sexes for Fe-dosed and Bi-dosed ducks. The mean weights of gonads of both female and male Pb-dosed ducks were lower than the mean weights of gonads of

the respective sexes of 0-dosed, Fe-dosed, and Bi-dosed ducks. These weight differences are, no doubt, the result of the terminal condition of the Pb-dosed ducks; they died on an average date of 5 February, before the gonads had begun their seasonal growth. Thus, effects on weight of gonads from dosing with Pb were not measured in our study.

**Analyses of Tissues and Other Materials**

We used the Method Detection Limit (MDL) (Glaser et al. 1981) to establish the detection limits for levels of elements in tissues and other materials. The MDL procedure must produce a value that averages >two times larger than the MDL value to be considered meaningful (Glaser et al. 1981; see Sanderson et al. 1997a for a definition of MDL).

Because results of ICP analyses for Bi and Pb were usually lower than the MDLs, we usually analyzed the kidneys, livers, gonads, and blood by GFAA for these two elements. Kidneys and livers of all Pb-dosed ducks contained Pb levels several times higher than the MDLs as analyzed by ICP. Concentrations of Pb in the kidneys and livers of Pb-dosed ducks were determined by ICP.

*Kidneys*

At necropsy, with doses combined, females had higher mean concentrations of Ca than males (138.7 vs 109.7 µg/g). Compared with females, males had higher mean concentrations of P (3706 vs 3542 µg/g), Mg (234.5 vs 221.4 µg/g), Zn (34.02 vs 30.51 µg/g), and Cu (9.62 vs 7.07 µg/g). No other sex differences were detected in the concentration of the nine elements of interest in the current study.

With sexes combined, a higher mean concentration of Pb was detected in the kidneys of Pb-dosed ducks (213 µg/g) compared with the kidneys of 0- (0.448 µg/g), Fe- (0.198 µg/g), and Bi-dosed (0.574 µg/g) ducks. A higher mean concentration of Pb was detected in the kidneys of 0-dosed ducks versus Fe-dosed ducks, but no differences existed in the mean concentrations of Pb in the kidneys of 0- and Fe- versus Bi-dosed ducks (Table 6). In spite of the high mean concentrations of Pb in the kidneys of Pb-dosed ducks, we detected no dose-related histopathologic differences in the kidneys.

In our study, Bi-dosed ducks had higher mean concentrations of Bi in their kidneys (1.54 µg/g) than in their livers (0.637 µg/g). Our Bi-dosed ducks were exposed to Bi dissolved from Bi shot in the gizzard from Day 0 to necropsy—an average of 120.5 days.

A higher mean concentration of Bi (1.54 µg/g) was detected in the kidneys of Bi-dosed ducks than in the kidneys of 0-, Fe-, or Pb-dosed ducks; all three of the latter dosed groups had ≤the MDL (0.054 µg/g) of Bi.

A higher mean concentration of Fe was detected in the kidneys of Fe-dosed ducks than in the kidneys of 0-, Bi-, and Pb-dosed ducks, but there was no difference in the mean amounts of Fe in the kidneys of 0-, Bi-, and Pb-dosed ducks (Table 6).

Sn was <MDL (2.25 µg/g) in the kidneys of all dosed groups. A higher mean concentration of P was found in the kidneys of 0-dosed ducks and Bi-dosed ducks than in the kidneys of Pb-dosed ducks. A higher mean concentration of Ca was detected in the kidneys of Pb-dosed ducks than in the kidneys of 0-dosed and Bi-dosed ducks. A higher mean concentration of Mg was found in the kidneys of Fe-dosed ducks than in the kidneys of Pb-dosed ducks, and a higher mean concentration of Cu occurred in the kidneys of Pb-dosed ducks than in the kidneys of the other three dosed groups (Table 6).

*Liver*

Females had higher mean concentrations of Ca in their livers than males ($\bar{x}$= 73.9 vs 52.9 µg/g), and males had higher mean concentrations of Cu in their livers than females ($\bar{x}$= 174.09 vs 37.28 µg/g). No other sex differences existed in mean concentrations of the nine elements of interest in the present study.

With sexes combined, Pb-dosed ducks had higher mean concentrations of Pb in their livers compared with 0-, Fe-, and Bi-dosed ducks. The mean values for Pb in the livers of 0-, Fe-, and Bi-dosed ducks were all <2 X the MDL (MDL = 0.079 µg/g) for Pb in the liver (Table 7).

With sexes combined, Bi-dosed ducks had higher mean concentrations of Bi (0.637 µg/g) in their livers compared with 0-, Fe-, and Pb-dosed ducks (Table 7). The mean values for Bi in the livers of 0-, Fe-, and Pb-dosed ducks were all <MDL (0.054 µg/g). As with the kidneys, no histologic differences were detected among doses in the livers.

Differences among doses existed in the mean concentrations of Fe in the liver. Pb-dosed ducks had more Fe ($\bar{x}$= 2680 µg/g) in their livers than Fe-dosed ducks ($\bar{x}$= 1936 µg/g), and both Pb-dosed and Fe-dosed ducks had higher mean concentrations of Fe in their livers than Bi-dosed ducks (415 µg/g) or 0-dosed ducks (392 µg/g). No difference in the mean concentrations of Fe was detected in the livers of 0-dosed and Bi-dosed ducks (Table 7).

*Continued on page 234*

Table 6. Mean concentrations (µg/g, wet wt) of nine elements at necropsy[a] in kidneys of game-farm mallards dosed with 0 (controls); eight, No. 4 Fe; eight, No. 4 Bi; or eight, No. 4 Pb[b] shot on Days 0, 30, 60, and 90. n = 10 each for 0- and Fe-dosed ducks, n = 11 for Bi-dosed ducks, and n = 12 for Pb-dosed ducks.

| Dose | Sex | Pb | Fe | Bi | Sn | P | Ca | Mg | Zn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | F | 0.532 | 133 | 0.037 | 1.12 | 3663 | 140 | 230 | 29.7 | 6.10 |
| | | 0.135[c] | 19.9 | 0.010 | 0.00 | 28 | 10.1 | 3.31 | 0.82 | 0.28 |
| | M | 0.365 | 96 | 0.027 | 1.40 | 3736 | 79 | 236 | 31.3 | 8.82 |
| | | 0.900 | 5.7 | 0.000 | 0.28 | 80 | 4.78 | 3.12 | 0.38 | 0.79 |
| | F&M | 0.448 | 115 | 0.032 | 1.26 | 3700 | 110 | 233 | 30.5 | 7.46 |
| | | 0.081 | 11.5 | 0.005 | 0.14 | 42 | 11.4 | 2.36 | 0.51 | 0.60 |
| Fe | F | 0.229 | 269 | 0.034 | 1.12 | 3548 | 137 | 226 | 29.0 | 5.65 |
| | | 0.042 | 25.2 | 0.007 | 0.00 | 76 | 14.1 | 5.82 | 1.22 | 0.44 |
| | M | 0.166 | 205 | 0.027 | 1.12 | 3752 | 111 | 238 | 33.0 | 9.62 |
| | | 0.057 | 13.5 | 0.000 | 0.00 | 166 | 29.9 | 5.16 | 0.99 | 0.24 |
| | F&M | 0.198 | 237 | 0.031 | 1.12 | 3650 | 124 | 232 | 31.0 | 7.63 |
| | | 0.035 | 17.2 | 0.004 | 0.00 | 93 | 16.2 | 4.18 | 1.00 | 0.70 |
| Bi | F | 0.860 | 126 | 1.095 | 1.00 | 3054 | 106 | 184 | 25.1 | 5.76 |
| | | 0.297 | 36.8 | 0.465 | 0.38 | 518 | 20.4 | 31.3 | 4.45 | 1.24 |
| | M | 0.231 | 102 | 1.659 | 1.12 | 3907 | 81 | 242 | 33.6 | 9.26 |
| | | 0.047 | 16.9 | 0.478 | 0.00 | 129 | 4.04 | 6.79 | 0.96 | 0.58 |
| | F&M | 0.574 | 126 | 1.54 | 1.23 | 3719 | 104 | 228 | 31.4 | 7.96 |
| | | 0.185 | 21.2 | 0.280 | 0.11 | 97 | 9.3 | 5.99 | 0.95 | 0.55 |
| Pb | F | 220.0 | 108 | 0.027 | 1.12 | 3416 | 154 | 217 | 33.5 | 9.24 |
| | | 28.3 | 12.4 | 0.000 | 0.00 | 48 | 16.7 | 5.43 | 2.54 | 0.47 |
| | M | 206.7 | 103 | 0.069 | 1.12 | 3474 | 158 | 224 | 37.5 | 10.60 |
| | | 49.2 | 8.2 | 0.042 | 0.00 | 40 | 29.4 | 3.98 | 4.00 | 1.26 |
| | F&M | 213 | 106 | 0.048 | 1.12 | 3445 | 156 | 220 | 35.5 | 9.92 |
| | | 27.1 | 7.1 | 0.021 | 0.00 | 31 | 16.2 | 3.40 | 2.34 | 0.67 |

[a] Ducks were necropsied when the female had laid 21 uncracked eggs.
[b] All Pb-dosed ducks died ≤ 14 days after first dosing.
[c] SE.

MDL:

Pb - 0.079 µg/g.
Bi - 0.054 µg/g.
Sn - 2.25 µg/g.

Differences among doses with sexes combined:

Pb - $F_{3,7} = 56.33$; $P < 0.00001$.

Fe - $F_{3,17} = 19.66$; $P < 0.00001$.

Bi - $F_{3,8} = 25.54$; $P = 0.0002$.

P - $F_{3,17} = 3.61$; $P = 0.0351$.

Ca - $F_{3,16} = 3.50$; $P = 0.0399$.

Cu - $F_{3,17} = 5.90$; $P = 0.0060$.

Table 7. Mean concentrations (µg/g, wet wt) of nine elements at necropsy[a] in livers of game-farm mallards dosed with 0 (controls); eight, No. 4, Fe; eight, No. 4 Bi; or eight, No. 4, Pb[b] shot on Days 0, 30, 60, and 90. n = 10 each for 0- and Fe-dosed ducks, n = 11 for Bi-dosed ducks, and n = 12 for Pb-dosed ducks.

| Dose | Pb | Fe | Bi | Sn | P | Ca | Mg | Zn | Cu |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0.133 | 392 | 0.053 | 1.12 | 3740 | 59.8 | 251.5 | 41.5 | 102 |
| | 0.030[c] | 53.0 | 0.014 | 0.00 | 121 | 5.68 | 4.93 | 3.47 | 60.1 |
| Fe | 0.096 | 1936 | 0.053 | 1.12 | 3253 | 51.6 | 219.8 | 32.8 | 62.9 |
| | 0.016 | 233 | 0.009 | 0.00 | 83 | 5.82 | 5.18 | 3.20 | 32.0 |
| Bi | 0.132 | 415 | 0.637 | 1.12 | 3306 | 50.7 | 226.2 | 39.0 | 79.5 |
| | 0.031 | 69.3 | 0.134 | 0.00 | 174 | 7.58 | 10.2 | 3.36 | 36.5 |
| Pb | 91.1 | 2680 | 0.052 | 1.12 | 3604 | 88.9 | 239.2 | 98.4 | 163 |
| | 4.55 | 249 | 0.018 | 0.00 | 85 | 9.18 | 5.6 | 9.24 | 81.8 |

[a] Ducks were necropsied when the female had laid 21 uncracked eggs.
[b] All Pb-dosed ducks died ≤14 days after first dosing.
[c] SE.

MDL:
Pb - 0.079 µg/g.
Bi - 0.054 µg/g.
Sn - 2.23 µg/g.

Differences among doses:

Pb - $F_{3,9} = 392.69$; $P < 0.00001$.

Fe - $F_{3,16} = 47.12$; $P < 0.00001$.

Bi - $F_{3,5} = 22.71$; $P = 0.0024$.

P - $F_{3,35} = 3.70$; $P = 0.0207$.

Ca - $F_{3,19} = 8.98$; $P = 0.0006$.

Mg - $F_{3,35} = 3.73$; $P = 0.0198$.

Zn - $F_{3,9} = 30.89$; $P < 0.00001$.

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The Fe pigment in the hepatocyte cytoplasm corresponds to the anemia (low Hct) observed in the Pb-dosed ducks. The mean Hct of the Pb-poisoned ducks at necropsy was 25.2 compared with 41.8 for 0-dosed ducks (Table 1). With recycling of hemoglobin, Fe content of the liver tends to increase.

Sanderson et al. (1997a) found a mean of 1086 µg/g Fe in the liver on Day 30 for game-farm mallards dosed with six, No. 4, Fe shot. This amount is compared with a mean of 1936 µg/g of Fe in the livers after repeated dosing with eight No. 4 Fe shot in the present study: on Days 0, 30, 60, and 90. The mean concentrations of Fe in the previous study for 0-dosed ducks (411 µg/g) for Fe-dosed ducks and for Bi-dosed ducks (399 µg/g) are similar to those found in our study (392 µg/g in 0-dosed and 415 µg/g in Bi-dosed ducks). The repeated dosing with eight shot versus one dosing with six shot apparently resulted in the higher concentrations of Fe in the livers in the present study.

Tin was <MDL (2.23 µg/g) in all livers analyzed. In our study, Cu ranged from 62.9 to 163 µg/g in the liver compared with 7.46-9.92 µg/g in the kidneys and means of < 1.0 µg/g in blood and 1.40 µg/g in gonads. Underwood (1971) reported that a few species, including ducks, have consistently high concentrations of Cu in the livers—a range of 100-400 µg/g is normal. In our study, no differences were found among doses in the mean concentrations of Cu in the liver (Table 7).

Differences existed in mean concentrations of P, Ca, Mg, and Zn in the livers of ducks from the three doses. Phosphorous was highest in 0-dosed ducks, followed by Pb-dosed, Bi-dosed, and Fe-dosed ducks, in that order. No difference existed between the mean concentrations of P in the livers of Bi-dosed ducks and the mean concentration of P in the livers of 0-, Fe-, and Pb-dosed ducks. The mean concentration of Ca was higher in the livers of Pb-dosed ducks than in 0-dosed, Fe-dosed, and Bi-dosed ducks. No difference was detected in the mean concentrations of Ca in the livers of 0-dosed, Fe-dosed, and Bi-dosed ducks. The mean concentration of Zn was higher in the livers of Pb-dosed ducks than in the livers of 0-dosed, Bi-dosed, and Fe-dosed ducks. As with Ca, no difference was found in the mean concentrations of Zn in the livers of 0-dosed, Fe-dosed, and Bi-dosed ducks. The mean concentration of Mg was higher in the livers of 0-dosed ducks than in the livers of Bi-dosed and Fe-dosed ducks. No difference was detected in the mean concentrations of Mg in the livers of Bi-dosed ducks versus Fe-dosed ducks and Pb-dosed ducks.

Thus, the mean concentrations of seven elements in the livers of Bi-dosed ducks were not different from the mean concentrations of these elements in 0-dosed and Fe-dosed ducks. The mean concentrations of Fe and Mg in the livers of Bi-dosed ducks differed in only two instances: Fe-dosed ducks had a higher value for Fe and 0-dosed ducks had a higher value for Mg.

The mean concentrations of P in the liver were highest in 0-dosed (3,740 µg/g) and Pb-dosed ducks (3,604 µg/g) versus Fe-dosed (3,253 µg/g) and Bi-dosed (3,306 µg/g) ducks in our study. A difference was found among doses in the mean concentrations of P in the livers.

*Gonads*

Gonads exhibited more sex- and dose-related differences in the mean concentrations of elements than livers, kidneys, or blood. Most of the differences involved Pb-dosed ducks versus 0-dosed, Fe-dosed, and Bi-dosed ducks. Major sex-related differences were detected in the mean concentrations of Ca and P in the gonads of 0-, Fe-, and Bi-dosed ducks, with females always having the higher concentrations.

Females also had higher mean concentrations of Pb, Fe, and Zn in their gonads than males for all groups except Pb-dosed ducks, which revealed no sex-related differences for Fe, P, Ca, and Zn. Males had a higher mean concentration of Mg in their gonads than females for all groups except Pb-dosed ducks. No sex-related differences were found in the mean concentrations of Bi, Sn, and Cu in the gonads (Table 8).

The sex-related differences for Ca, P, Pb, Fe, Zn, and Mg are no doubt related to physiological changes in the female in preparation for the egg-laying season. For example, Underwood (1971) reported a fivefold increase in Fe in the serum of ducks during the egg-laying season. Many of the female gonads in the present study contained large follicles.

Differences were detected among doses in the mean concentrations of Pb in the gonads. Both female and male Pb-dosed ducks had higher mean concentrations of Pb (females - $\bar{x}$ = 9.84 µg/g and males - $\bar{x}$= 3.49 µg/g) in their gonads than 0-, Fe- and Bi-dosed (all <0.250 µ/g) ducks. No differences existed among 0-, Fe-, and Bi-dosed ducks in the mean concentrations of Pb in the gonads.

Differences were detected among doses in the mean concentrations of Fe in the gonads. Fe-dosed females had a higher mean concentration

*Continued on page 236*

Table 8. Mean concentrations (µg/g, wet wt) of nine elements at necropsy[a] in gonads of game-farm mallards dosed with 0 (controls); eight, No. 4, Fe; eight, No. 4, Bi; or eight, No. 4, Pb[b] shot on Days 0, 30, 60, and 90, by sex. n = 5 for each sex for 0- and Fe-dosed and male Bi- and Pb-dosed, 6 for female Bi-dosed and female and male Pb-dosed; except n = 1 for Sn for female Pb-dosed ducks.

| Dose | Sex | Pb | Fe | Bi | Sn | P | Ca | Mg | Zn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | F | 0.129 | 65.3 | 0.036 | 1.38 | 4827 | 1492 | 113 | 36.1 | 1.77 |
| | | 0.035[c] | 5.26 | 0.000 | 0.31 | 594 | 206 | 5.3 | 1.42 | 0.07 |
| | M | 0.121 | 10.0 | 0.036 | 1.70 | 2788 | 41.9 | 223 | 14.7 | 1.03 |
| | | 0.037 | 1.14 | 0.000 | 0.40 | 46 | 2.21 | 4.8 | 0.48 | 0.09 |
| Fe | F | 0.206 | 90.6 | 0.036 | 1.07 | 5577 | 1660 | 126 | 44.6 | 1.86 |
| | | 0.044 | 6.85 | 0.000 | 0.000 | 122 | 110 | 7.0 | 2.13 | 0.17 |
| | M | 0.086 | 13.1 | 0.036 | 1.07 | 2585 | 45.7 | 221 | 14.1 | 0.86 |
| | | 0.021 | 1.29 | 0.000 | 0.000 | 56 | 2.69 | 5.2 | 0.20 | 0.03 |
| Bi | F | 0.191 | 57.7 | 0.042 | 1.28 | 4931 | 1405 | 115 | 38.7 | 1.87 |
| | | 0.040 | 5.59 | 0.007 | 0.210 | 130 | 70 | 6.4 | 2.10 | 0.20 |
| | M | 0.195 | 9.68 | 0.098 | 1.07 | 2576 | 41.9 | 213 | 13.6 | 0.85 |
| | | 0.111 | 0.77 | 0.042 | 0.000 | 59 | 2.28 | 2.5 | 0.15 | 0.07 |
| Pb | F | 9.84 | 74.8 | 0.247 | 1.07 | 2811 | 88.3 | 190 | 24.8 | 4.55 |
| | | 3.22 | 6.88 | 0.074 | 0.000 | 162 | 6.30 | 6.6 | 1.90 | 1.30 |
| | M | 3.49 | 50.5 | 0.117 | 1.48 | 2910 | 63.0 | 203 | 25.4 | 7.20 |
| | | 0.61 | 13.0 | 0.051 | 0.410 | 149 | 4.26 | 10.0 | 1.43 | 0.93 |

[a] Ducks were necropsied when the hen had laid 21 uncracked eggs.
[b] All Pb-dosed ducks died ≤ 14 days after the first dosing.
[c] SE.

MDL:
Pb - 0.073 µg/g.
Bi - 0.073 µg/g for 0-, Fe-, and Bi-dosed ducks and 0.204 µg/g for Pb-dosed ducks because of low gonad weights.
Sn - 2.14 µg/g.

Differences among doses:
Pb - $F_{3,5} = 15.82$; $P = 0.0055$.
Fe - $F_{3,35} = 7.79$; $P = 0.0044$.
Bi - $F_{3,12} = 7.71$; $P = 0.0039$.
Ca - $F_{3,7} = 34.27$; $P = 0.0001$.
P - $F_{3,6} = 10.22$; $P = 0.0090$.
Mg - $F_{3,26} = 11.00$; $P = 0.0001$.
Zn - $F_{3,35} = 3.07$; $P = 0.0402$.
Cu - $F_{3,9} = 30.62$; $P = 0.00001$.

Differences between sexes:
Fe - $F_{1,35} = 109.41$; $P < 0.00001$.
Ca - $F_{1,7} = 337.16$; $P < 0.00001$.
P = $F_{1,6} = 121.32$; $P < 0.00001$.
Mg = $F_{1,25} = 307.34$; $P < 0.00001$.
Zn = $F_{1,21} = 337.88$; $P < 0.00001$.

Differences between sexes for Pb:
Pb-dosed - $P < 0.05$.

Differences between doses for Fe:
Fe-dosed males vs Pb-dosed males - $P < 0.05$.
0-dosed males vs Pb-dosed males - $P < 0.01$.
Bi-dosed males vs Pb-dosed males - $P < 0.01$.

Differences between doses for Mg:
0-dosed females vs Pb-dosed females - $P < 0.01$.
Fe-dosed females vs Pb-dosed females - $P < 0.01$.
Bi-dosed females vs Pb-dosed females - $P < 0.01$.

Differences between doses for Zn:
0-dosed females vs Fe-dosed females - $P < 0.05$.
0-dosed females vs Pb-dosed females - $P < 0.01$.
0-dosed males vs Pb-dosed males - $P < 0.01$.
Fe-dosed females vs Pb-dosed females - $P < 0.01$.
Fe-dosed males vs Pb-dosed males - $P < 0.01$.
Bi-dosed females vs Pb-dosed females - $P < 0.01$.
Bi-dosed males vs Pb dosed males - $P < 0.01$.

Differences between doses for Cu:
0-dosed males vs Pb-dosed males - $P < 0.01$.
Fe-dosed males vs Pb-dosed males - $P < 0.01$.
Bi-dosed males vs Pb-dosed males - $P < 0.01$.

*continued from page 234*

of Fe in their gonads than 0- or Bi-dosed females but not more than Pb-dosed females. Pb-dosed males had a higher mean concentration of Fe in their gonads than 0-, Fe-, or Bi-dosed males, but there was no difference in the mean concentrations of Fe in the gonads of 0-, Fe-, and Bi-dosed males (Table 8).

The mean concentrations of Bi in the gonads of 0-, Fe-, and Bi-dosed ducks and male Pb-dosed ducks were all <2xMDL. The mean concentration of Bi in the gonads of female Pb-dosed ducks was >2xMDL. The apparent higher mean concentration of Bi in the gonads of the Pb-dosed females probably resulted from the low gonad weights of Pb-dosed females, which died before the seasonal increase in gonad size. The high dilution ratio associated with the small samples probably caused the apparent higher levels of Bi. Thus, we concluded that no differences existed among doses in the mean concentration of Bi in the gonads.

All mean concentrations of Sn were below MDL for Sn in gonads. The mean concentrations of Ca and P were lower in gonads of Pb-dosed females than in gonads of 0-, Fe-, and Bi-dosed females, but no difference was found among the latter three dosed groups. Although Pb-dosed males had higher mean concentrations of Ca and P in their gonads than 0-, Fe-, and Bi-dosed males, these differences were not significant.

Pb-dosed females had a higher mean concentration of Mg in their gonads than 0-, Fe-, or Bi-dosed females. No differences were detected in the mean concentrations of Mg in the female gonads of 0-, Fe-, and Bi-dosed ducks, or among doses in the male ducks (Table 8).

Pb-dosed females had a lower mean concentration of Zn in their gonads than 0-, Fe-, or Bi-dosed females. Fe-dosed females had a higher mean concentration of Zn in their gonads than 0-dosed females. No differences were found in the mean concentrations of Zn in the gonads of Fe-dosed and Bi-dosed females, or between Bi and 0-dosed females. Lead-dosed males had a higher mean concentration of Zn in their gonads than 0-, Fe-, or Bi-dosed males (Table 8).

*Blood*

The mean values for Pb, Bi, and Sn were all <2xMDL and all but 6 of the 57 means for these three elements were <MDL (Table 9). Thus, we concluded that four oral doses (at 30-day intervals) of eight, No. 4, Fe or eight, No. 4, Bi shot had no effect on the amount of Pb, Bi, or Sn in the blood from Day 30 to Day 150. Pb-dosed ducks did not survive to Day 30, when the first post-dosing blood samples were taken.

The mean concentrations of P (Figure 6) and Mg did not differ among doses from Day 0 to Day 150 in the blood of 0-dosed, Fe-dosed, and Bi-dosed ducks (Table 9). Irving (1973) reported that feeding excess amounts of Fe to humans had no effect on the amount of P in the blood. The mean concentrations of Ca, Zn, and Cu increased over time in the blood of females, but there was no dose effect. The number of samples was insufficient to test for differences in males. With sexes combined, there were no differences in the mean concentrations of Ca and P in the blood between Days 0 and 150. Fe-dosed ducks had higher mean concentrations of Fe in their blood than 0-dosed or Bi-dosed ducks, but there was no difference between the mean concentrations of Fe in the blood of 0-dosed and Bi-dosed ducks (Figure 7, Table 9). There was no increase in the mean concentrations of Fe in the blood of Fe-dosed ducks from Day 0 to samples taken between 120 and 150 days. The ducks were last dosed with Fe shot on Day 90.

**Reproduction**

*Eggs*

The first egg was laid by a Fe-dosed female on 11 March 1995. This date was 47 days before the increase in daily illumination was begun on 27 April and 55 days before the daily light reached the maximum of 18 hours per day on 11 May. Before 27 April, 0-dosed females had laid 145 eggs, Fe-dosed females had laid 70 eggs, and Bi-dosed females had laid 83 eggs. From 27 April through 11 May, 0-dosed females laid 133 eggs, Fe-dosed females laid 98 eggs, and Bi-dosed females laid 126 eggs. For all groups combined, females laid 298 eggs while under a constant day length of 8 hours, and 357 eggs when the day length was being increased to 18 hours.

After laying began, 0-dosed females laid 21 hard-shelled eggs in a mean of 27.4 days, Fe-dosed females laid 21 eggs in a mean of 25.7 days, and Bi-dosed females laid 21 eggs in a mean of 25.9 days. The few soft-shelled eggs produced were not counted, but hard-shelled eggs that were cracked were counted. The latter eggs usually sustained their cracks as a result of activities by the ducks when approached by the egg collector. No differences were found among surviving ducks in the time required to lay 21 eggs (Table 10). All Pb-dosed ducks died before laying.

The average date that egg laying began was Day 84 (19 April) for control (0-dosed) females, Day 94 (29 April) for Fe-dosed females, and Day

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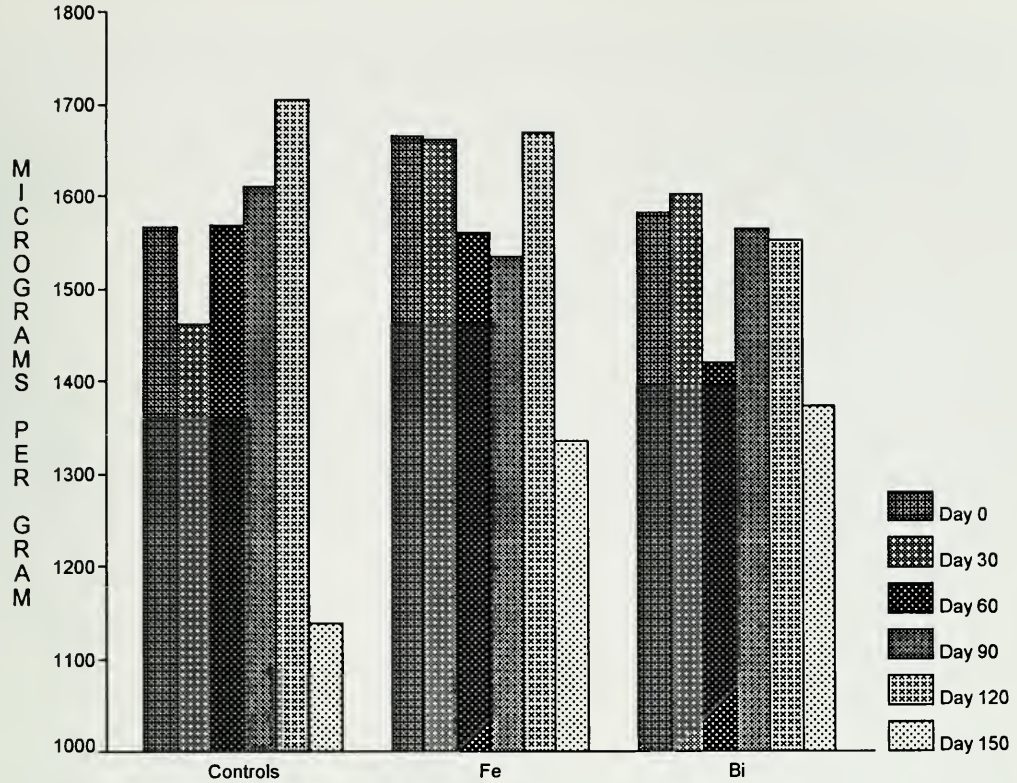


Figure 6. Mean concentration (µg/g) of P in the blood of game-farm mallards dosed with 0 shot (controls); 8, No. 4, Fe shot; or 8, No. 4, Bi shot on Days 0, 30, 60, and 90. The sexes were combined for this graph. See Table 7 for sample sizes.

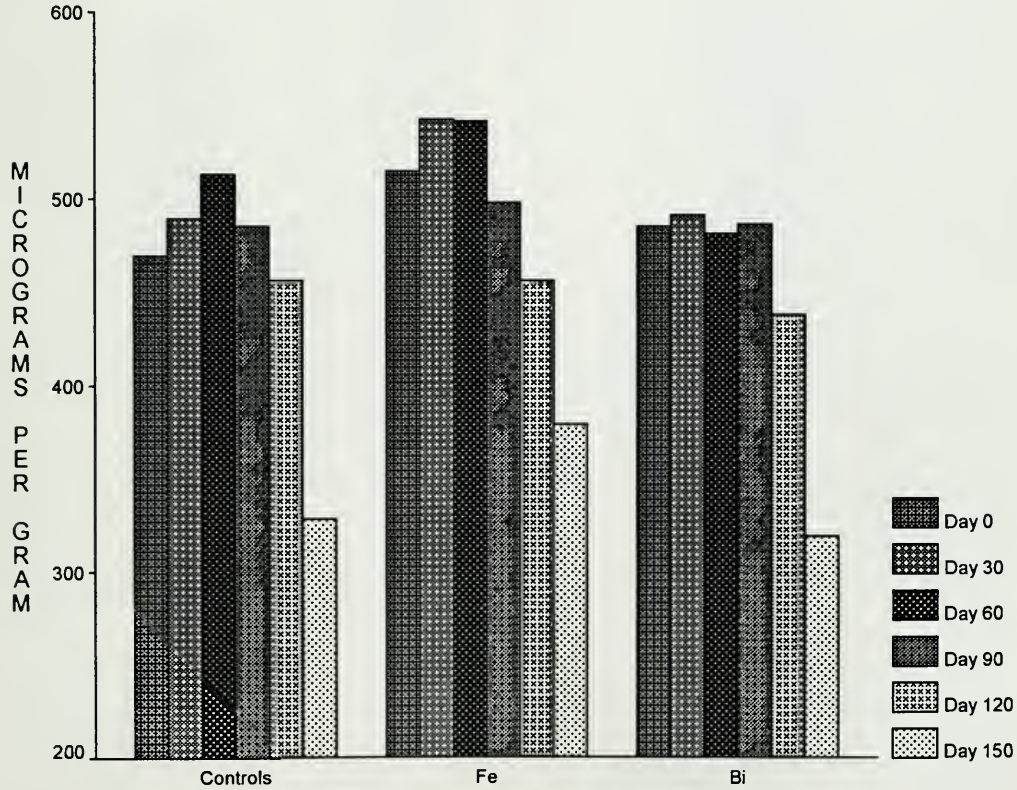


Figure 7. Mean concentration (µg/g) of Fe in the blood of game-farm mallards dosed with 0 shot (controls); 8, No. 4, Fe shot; or 8, No. 4, Bi shot on Days 0, 30, 60, and 90. The sexes were combined for this graph. See Table 9 for sample sizes.

Table 9. Mean concentrations (µg/g, wet wt) of nine elements in blood of game-farm mallards dosed with 0 (controls); eight, No. 4, Fe; eight, No. 4, Bi; or eight, No. 4, Pb shot on Days 0, 30, 60, and 90[a].

| Dose | Day | Pb | Fe | Bi | Sn | P | Ca | Mg | Zn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|
| 0[b] | 0 | 0.066 | 470 | 0.068 | 1.61 | 1568 | 61.0 | 78.2 | 5.67 | 0.385 |
| | | 0.000[c] | 15.0 | 0.023 | 0.30 | 55 | 1.03 | 1.79 | 0.10 | 0.077 |
| Fe[d] | | 0.081 | 515 | 0.068 | 1.07 | 1666 | 68.8 | 83.2 | 5.96 | 0.420 |
| | | 0.010 | 2.64 | 0.020 | 0.00 | 66 | 6.60 | 4.04 | 0.27 | 0.055 |
| Bi[e] | | 0.066 | 486 | 0.055 | 1.07 | 1582 | 62.8 | 78.4 | 6.02 | 0.478 |
| | | 0.000 | 19.3 | 0.007 | 0.00 | 76 | 1.60 | 2.00 | 0.14 | 0.058 |
| Pb[f] | | 0.094 | 489 | 0.070 | 1.47 | 1621 | 65.3 | 78.8 | 6.02 | 0.566 |
| | | 0.016 | 9.2 | 0.016 | 0.18 | 30 | 1.47 | 1.05 | 0.24 | 0.061 |
| 0[e] | 30 | 0.066 | 489 | 0.105 | 2.08 | 1461 | 63.4 | 78.8 | 5.91 | 0.460 |
| | | 0.000 | 24.8 | 0.029 | 0.36 | 89 | 5.55 | 1.33 | 0.20 | 0.083 |
| Fe[d] | | 0.082 | 543 | 0.074 | 2.28 | 1662 | 64.7 | 83.6 | 5.95 | 0.525 |
| | | 0.011 | 14.2 | 0.020 | 0.52 | 54 | 6.81 | 1.87 | 0.22 | 0.045 |
| Bi[d] | | 0.078 | 491 | 0.093 | 2.05 | 1603 | 60.2 | 80.2 | 6.15 | 0.418 |
| | | 0.012 | 32.6 | 0.018 | 0.80 | 85 | 2.25 | 2.34 | 0.13 | 0.053 |
| 0[e] | 60 | 0.075 | 514 | 0.045 | 2.06 | 1570 | 71.2 | 83.9 | 6.25 | 0.550 |
| | | 0.009 | 12.9 | 0.000 | 0.42 | 50 | 8.61 | 1.00 | 0.30 | 0.030 |
| Fe[d] | | 0.125 | 542 | 0.045 | 2.00 | 1561 | 57.2 | 84.8 | 5.93 | 0.477 |
| | | 0.022 | 18.8 | 0.000 | 0.37 | 79 | 1.74 | 1.59 | 0.14 | 0.047 |
| Bi[g] | | 0.110 | 481 | 0.055 | 2.13 | 1421 | 62.1 | 79.2 | 6.01 | 0.508 |
| | | 0.019 | 23.9 | 0.010 | 0.38 | 113 | 2.98 | 2.63 | 0.16 | 0.063 |
| 0[e] | 90 | 0.147 | 485 | 0.045 | 1.26 | 1611 | 125.7 | 80.1 | 6.78 | 0.518 |
| | | 0.038 | 13.5 | 0.000 | 0.19 | 39 | 32.1 | 1.57 | 0.60 | 0.041 |
| Fe[d] | | 0.087 | 498 | 0.089 | 1.07 | 1535 | 92.4 | 84.8 | 6.32 | 0.509 |
| | | 0.014 | 13.1 | 0.029 | 0.00 | 8 | 18.49 | 1.14 | 0.28 | 0.036 |
| Bi[e] | | 0.168 | 487 | 0.045 | 1.39 | 1566 | 104.4 | 81.1 | 6.49 | 0.425 |
| | | 0.029 | 13.7 | 0.000 | 0.318 | 48 | 22.4 | 1.44 | 0.35 | 0.037 |
| 0[e] | 120[h] | 0.086 | 456 | 0.082 | 2.46 | 1706 | 173.4 | 80.5 | 7.55 | 0.493 |
| | | 0.013 | 22.3 | 0.025 | 0.437 | 45 | 37.4 | 1.25 | 0.70 | 0.087 |
| Fe[d] | | 0.066 | 456 | 0.070 | 1.61 | 1670 | 164.8 | 82.0 | 7.54 | 0.487 |
| | | 0.000 | 20.9 | 0.025 | 0.290 | 68 | 37.3 | 2.78 | 0.84 | 0.074 |
| Bi[e] | | 0.081 | 438 | 0.059 | 1.26 | 1553 | 149.4 | 79.9 | 7.78 | 0.490 |
| | | 0.010 | 28.1 | 0.014 | 0.184 | 93 | 30.2 | 1.77 | 0.80 | 0.059 |
| 0[i] | 150[j] | 0.096 | 329 | 0.044 | 1.07 | 1139 | 207.0 | 75.6 | 7.70 | 0.602 |
| | | 0.030 | 39.4 | 0.000 | 0.000 | 90 | 89.1 | 6.75 | 1.65 | 0.201 |
| Fe[k] | | 0.066 | 379 | 0.044 | 1.29 | 1334 | 154.2 | 82.2 | 7.51 | 0.645 |
| | | 0.000 | 22.2 | 0.000 | 0.216 | 76 | 38.6 | 1.39 | 1.08 | 0.095 |
| Bi[l] | | 0.124 | 319 | 0.044 | 1.07 | 1374 | 278.5 | 81.8 | 9.21 | 0.632 |
| | | 0.059 | 90.0 | 0.000 | 0.000 | 522 | 75.5 | 12.65 | 1.29 | 0.032 |

[a] All Pb-dosed ducks died < 14 days after dosing.
[b] n = 9, except Pb = 10.
[c] SE.
[d] n = 9.
[e] n = 10.
[f] n = 12.
[g] n = 10, except Fe = 9.
[h] Blood samples taken from > 90 to 120 days.
[i] n = 5.
[j] Blood samples taken from >120 to 150 days.
[k] n = 4.
[l] n = 2.

MDL:
Bi - 0.081 µg/g.
Sn - 2.14 µg/g.
Pb - 0.132 µg/g.
Cu - 0.180 µg/g.

Difference over time in females:
Ca: DF5, Chi-square 313.8533; $P < 0.00001$.
Zn: DF5, Chi-square 183.6148; $P < 0.00001$.
Cu: DF5, Chi-square 20.4004; $P = 0.0011$.

Table 10. Mean number of days required for 0-dosed (controls), Fe-dosed, and Bi-dosed game-farm mallard female ducks to lay 21 eggs and mean number of days after Day 0 the first egg was laid. Sample sizes are in parentheses.

| Dose | Mean days to lay 21 eggs | Mean Days after Day 0 first egg was laid |
|---|---|---|
| 0[a] | 27.4(17) | 83.8(17) |
| | 2.04[b] | 4.30 |
| Fe | 25.7(18) | 94.0(18) |
| | 1.56 | 4.12 |
| Bi[ac] | 25.9(15) | 91.6(17) |
| | 1.26 | 3.66 |

[a] One 0-dosed and one Bi-dosed female laid no eggs; they suffered from egg yolk peritonitis.
[b] SE.
[c] One Bi-dosed hen died 24 days (and 16 eggs) after laying her first egg and one Bi-dosed female was sacrificed on Day 150 when she had laid 17 eggs in 35 days after laying her first egg. These two females are not included.

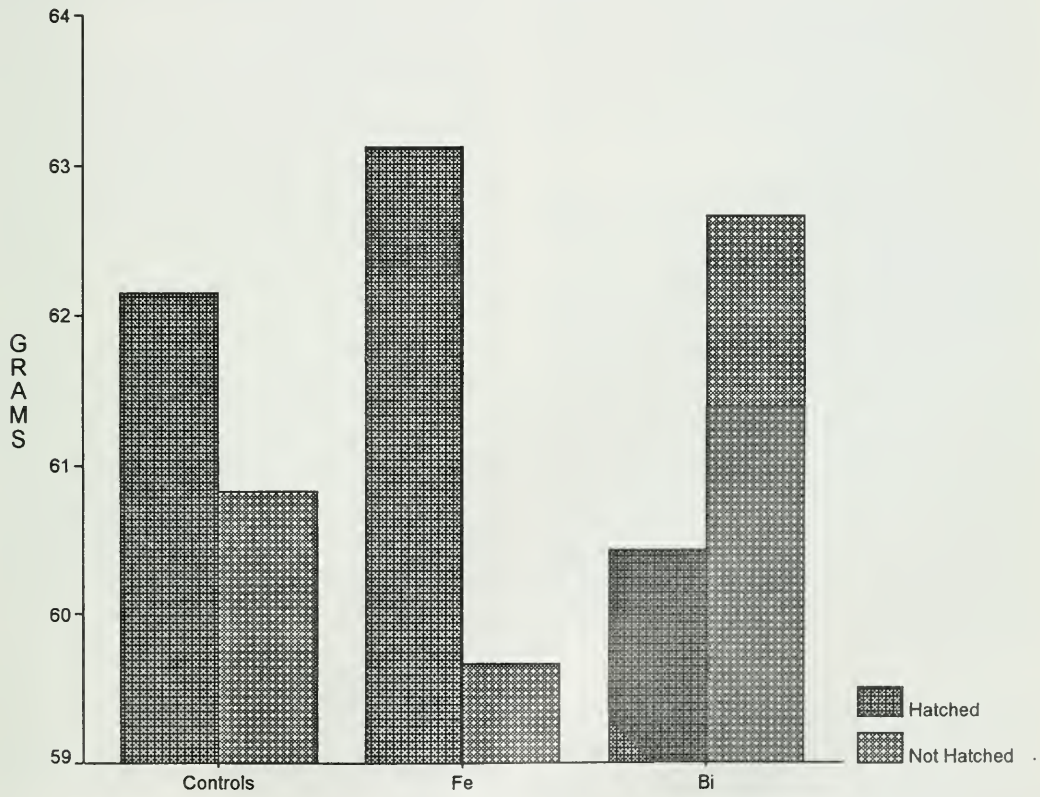


Figure 8. Mean weight (g) of hatched and not hatched fertile eggs from 0-dosed (controls), Fe-dosed, and Bi-dosed game-farm mallard pairs. See Table 11 for sample sizes.

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92 (27 April) for Bi-dosed females (Table 10). These dates do not differ statistically.

Two 0-dosed females each laid 21 eggs in 21 days, and one 0-dosed female required 54 days to lay 21 eggs. Three Fe-dosed females each laid 21 eggs in 21 days, and one Fe-dosed female required 46 days to lay 21 eggs. Three Bi-dosed females each laid 21 eggs in 21 days, and one Bi-dosed female required 45 days to lay 21 eggs.

One 0-dosed and one Bi-dosed female laid no eggs by Day 150, and both hens had egg yolk peritonitis. Probably activities associated with catching, weighing, bleeding, and dosing these ducks when egg yolks were about to be released into the infundibula resulted in the yolks being discharged into the body cavity, causing peritonitis. One Bi-dosed female died of unknown causes 24 days after laying her first egg on Day 113 and after she had laid 16 eggs. One Bi-dosed female laid 17 eggs in 35 days after laying her first egg and by the time she was sacrificed on Day 150. We detected no differences among doses in 0-dosed, Fe-dosed, and Bi-dosed females in the mean date laying was initiated or the mean number of days required to lay 21 eggs.

Differences were found between the weights of fertile eggs that hatched and those that did not hatch in all dosed classes. For 0-dosed and Fe-dosed pairs, fertile eggs that hatched were heavier than eggs that did not hatch. For Bi-dosed pairs, fertile eggs that did not hatch weighed more than eggs that hatched (Figure 8). Differences existed among doses in the weights of both hatched and unhatched fertile eggs. Hatched eggs from Fe-dosed pairs were heaviest followed by hatched eggs from 0-dosed pairs and Bi-dosed pairs. Fertile eggs that did not hatch from Bi-dosed pairs weighed more than nonhatched fertile eggs from 0-dosed and Fe-dosed pairs, in that order. With doses combined, fertile eggs that hatched weighed more (61.9 g) than fertile eggs that did not hatch (60.9 g) (Table 11).

*Ducklings*

Body Weight—All ducklings were weighed at the time of hatching, and a difference existed in mean body weights among dose groups. Ducklings from Bi-dosed pairs weighed approximately 2 grams less, on the average, than either the 0-dosed or the Fe-dosed ducklings. However, by day 7, we found no difference in body weights of ducklings among the dosed groups. Body weights did not differ between sexes at hatching or at Day 7 (Table 12).

Survivability—All but two ducklings survived the first 7 days after hatching. These deaths resulted from the ducklings entangling their legs in the wire floor of the brooder. One of the ducklings experienced neurologic deficits in the affected leg and the other duckling suffered a fractured leg. Both ducklings, offspring of Fe-dosed pairs, stopped eating and were emaciated at death.

Hematocrit—Mean Hcts for ducklings at 7 days of age ranged from 35.0 for Fe-dosed ducklings to 35.8 for Bi-dosed ducklings (Table 13). Hcts were not different among doses. Mean Hcts for adult (parent) ducks ranged from 44.6 to 47.3 prior to dosing (Table 1).

Sex Ratios—Of 399 ducklings hatched, 382 were identified as to sex: 189 females and 193 males. We found no differences among doses in the sex ratios of ducklings (Table 14).

Organ Weights—The mean weights of kidneys of ducklings were: 0-dosed ducklings—1.76 g, Fe-dosed ducklings 1.64 g, and Bi-dosed ducklings—1.56 g (Table 13). The mean weights of livers of ducklings were: 0-dosed—5.70 g, Bi-dosed—5.15 g, and Fe-dosed—5.20 g. Neither mean kidney weights nor mean liver weights differed among doses. Because of their small sizes, gonads of ducklings were not weighed.

Elements in Kidneys—No differences were detected among doses in the mean concentrations of the elements studied in the kidneys of 7-day-old ducklings. The mean concentrations of Bi in the kidneys were <MDL for Bi, and the mean concentrations of Sn were <2xMDL for Sn. The mean concentration of Pb (0.203 µg/g) in the kidneys of 0-dosed ducks equalled 2xMDL for Pb, but the mean concentrations of Pb in Fe- and Bi-dosed ducks were <2xMDL for Pb (Table 15).

Elements in Liver—We detected no differences among doses in the mean concentrations of the nine elements studied in the livers of 7-day-old ducklings. The mean concentrations of Pb, Bi, and Sn were <MDLs for these elements in the liver (Table 16).

Elements in Blood—No differences were found among doses in the mean concentrations of the nine elements studied in the blood of 7-day-old ducklings (Table 17). The mean concentrations of

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Table 11. Mean weight (g) of hatched and non-hatched fertile eggs from 0-dosed (controls), Fe-dosed, and Bi-dosed pairs of game-farm mallard ducks. Sample sizes are in parentheses.

| | Mean Weight of Fertile Eggs | |
|---|---|---|
| Dose | Hatched | Not Hatched |
| 0 | 62.1(114) | 60.8(192) |
| | 0.49[a] | 0.46 |
| Fe | 63.1(155) | 59.7(211) |
| | 0.39 | 0.35 |
| Bi | 60.4(137) | 62.7(148) |
| | 0.49 | 0.45 |

[a] SE.

Difference in weight of hatched and unhatched fertile eggs:

$$F_{1,955} = 8.30;\ P = 0.0040.$$

Table 12. Mean body weight (g) of ducklings (sexes combined) from 0-dosed (controls), Fe-dosed, and Bi-dosed game-farm mallard pairs and mean body weight of female and male ducklings (doses combined) on Days 0 and 7. Sample sizes are in parentheses.

| Dose | Body Weight - Day 0 | Body Weight - Day 7 |
|---|---|---|
| 0 | 42.0(108) | 120.3(106) |
| | 0.39[a] | 1.76 |
| Fe | 42.8(156) | 123.0(149) |
| | 0.33 | 2.27 |
| Bi | 40.8(135) | 118.9(130) |
| | 0.38 | 2.11 |
| Sex | | |
| Female | 42.1(188) | 120.8(188) |
| | 0.31 | 1.53 |
| Male | 41.9(193) | 122.6(193) |
| | 0.31 | 1.62 |

[a] SE.

Differences among doses:

Body weight : Day 0 - $F_{2,396} = 8.54;\ P = 0.0002.$

: Day 7 - $F_{2,382} = 1.01;\ P = 0.3640.$

Differences between sexes:

Body weight : Day 0 - $F_{1,379} = 0.22;\ P = 0.6357.$

: Day 7 - $F_{1,379} = 0.4203;\ P = 0.4203.$

Table 13. Mean Hcts, kidney weights, and liver weights for 7-day-old ducklings from 0-dosed (controls), Fe-dosed, and Bi-dosed game-farm mallard duck pairs. Sample sizes are in parentheses.

| Dose | Sex | Hct | Kid Wt(g) | Liv Wt(g) |
|---|---|---|---|---|
| 0 | F&M | 35.6(105) | 1.76(14) | 5.70(14) |
| | | 0.40[a] | 0.11 | 0.26 |
| Fe | F&M | 35.0(143) | 1.64(13) | 5.15(14) |
| | | 0.29 | 0.11 | 0.46 |
| Bi | F&M | 35.8(126) | 1.56(14) | 5.20(13) |
| | | 0.33 | 0.09 | 0.28 |

[a] SE.

Table 14. Sex ratios of ducklings from 0-dosed (controls), Fe-dosed, and Bi-dosed game-farm mallards.

| | Females | | Males | |
|---|---|---|---|---|
| Dose | No. | Percent | No. | Percent |
| 0 | 45 | 42.4 | 61 | 57.5 |
| Fe | 71 | 47.6 | 78 | 52.3 |
| Bi | 73 | 57.5 | 54 | 42.5 |

Difference among doses in sex ratios of ducklings:
Likelihood Ratio, 2df, $P = 0.0641$.

Table 15. Mean concentrations (µg/g, wet wt) of nine elements in kidneys of 7-day-old ducklings hatched from pairs of game-farm mallards dosed with 0 (controls); eight, No. 4, Fe; or eight, No. 4, Bi shot on Days 0, 30, 60, and 90. n = 9 for 0-dosed and Bi-dosed and 7 for Fe-dosed ducklings.

| Dose | Pb | Fe | Bi | Sn | P | Ca | Mg | Zn | Cu |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0.203 | 62.7 | 0.036 | 1.26 | 3813 | 95.6 | 250 | 22.8 | 3.95 |
| | 0.042[a] | 3.66 | 0.000 | 0.28 | 72 | 5.62 | 3.6 | 0.64 | 0.12 |
| Fe | 0.140 | 57.6 | 0.036 | 2.47 | 3782 | 95.6 | 254 | 23.0 | 4.72 |
| | 0.044 | 2.09 | 0.000 | 1.08 | 76 | 3.95 | 5.3 | 0.49 | 0.78 |
| Bi | 0.114 | 59.4 | 0.036 | 1.09 | 3793 | 113 | 251 | 23.4 | 4.70 |
| | 0.019 | 3.12 | 0.000 | 0.11 | 70 | 10.89 | 4.7 | 0.42 | 0.82 |

[a] SE.

MDL:
Pb - 0.10 µg/g.
Sn - 1.98 µg/g.
Bi - 0.07 µg/g.

Table 16. Mean concentrations (µg/g, wet wt) of nine elements in livers of 7-day-old ducklings hatched from pairs of game-farm mallards dosed with 0 (controls); eight, No. 4, Fe; or eight, No. 4, Bi shot on Days 0, 30, 60, and 90. n = 10 for 0-dosed and 8 each for Fe- and Bi-dosed ducklings.

| Dose | Pb | Fe | Bi | Sn | P | Ca | Mg | Zn | Cu |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0.061 | 99.0 | 0.034 | 1.27 | 3514 | 56.1 | 234 | 30.4 | 34.7 |
| | 0.016[a] | 20.3 | 0.008 | 0.151 | 69 | 3.1 | 4.4 | 1.53 | 3.15 |
| Fe | 0.038 | 167 | 0.027 | 1.54 | 3533 | 56.0 | 232 | 31.3 | 32.1 |
| | 0.000 | 32.4 | 0.000 | 0.280 | 122 | 3.0 | 7.0 | 1.87 | 3.09 |
| Bi | 0.061 | 117 | 0.027 | 1.12 | 3630 | 55.9 | 241 | 35.8 | 29.1 |
| | 0.012 | 31.2 | 0.000 | 0.000 | 91 | 1.9 | 5.4 | 3.71 | 2.77 |

[a] SE.

MDL:
Pb - 0.077 µg/g.
Sn - 2.23 µg/g.
Bi - 0.054 µg/g.

Table 17. Mean concentrations (µg/g, wet wt) of nine elements in blood of 7-day-old ducklings hatched from pairs of game-farm mallards dosed on Days 0, 30, 60, and 90 with 0 (controls); eight, No. 4, Fe; or eight, No. 4, Bi shot. n = 9, except n = 10 each for 0-dosed ducklings.

| Dose | Pb | Fe | Bi | Sn | P | Ca | Mg | Zn | Cu |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0.036 | 336 | 0.027 | 1.22 | 1324 | 77.6 | 110 | 6.61 | 0.388 |
| | 0.005[a] | 15.1 | 0.006 | 0.155 | 85 | 3.97 | 3.84 | 0.17 | 0.049 |
| Fe | 0.028 | 296 | 0.019 | 1.45 | 1148 | 80.1 | 96 | 6.24 | 0.364 |
| | 0.000 | 19.6 | 0.000 | 0.377 | 117 | 3.51 | 6.56 | 0.28 | 0.075 |
| Bi | 0.031 | 293 | 0.019 | 1.07 | 1215 | 87.8 | 105 | 7.28 | 0.407 |
| | 0.003 | 16.0 | 0.000 | 0.000 | 58 | 6.54 | 4.62 | 0.73 | 0.050 |

[a] SE.

MDL:

Pb - 0.055 µg/g.
Bi - 0.038 µg/g.
Sn - 2.14 µg/g.

Table 18. Mean egg weight and shell thickness of eggs from 0-dosed (controls), Fe-dosed, and Bi-dosed game-farm mallard ducks. Sample sizes are in parentheses.

| Dose | Egg Weight(g) | Egg Shell Thickness(mm) |
|---|---|---|
| 0 | 61.2(411) | 0.335(17) |
| | 0.28[a] | 0.005 |
| Fe | 61.2(425) | 0.338(18) |
| | 0.26 | 0.006 |
| Bi | 61.3(378) | 0.335(17) |
| | 0.29 | 0.006 |

[a] SE.

Difference among doses:

Egg weights: $F_{2,1211} = 0.10$; $P = 0.9035$.

Egg shell thickness: $F_{2,49} = 0.14$; $P = 0.8707$.

Table 19. Mean fertility rates (%) of uncracked eggs and hatchability rates of fertile eggs from 0-dosed (controls), Fe-dosed, and Bi-dosed game-farm mallard ducks. Sample sizes (female mallards) are in parentheses.

| Dose | Fertility Rate | Hatchability Rate |
|---|---|---|
| 0 | 86.4 (17) | 37.4 (17) |
| | 7.15[a] | 6.73 |
| Fe | 96.8 (18) | 42.2 (18) |
| | 1.10 | 7.10 |
| Bi | 86.4 (17) | 50.3 (17) |
| | 7.29 | 6.20 |

[a] SE.

Difference among doses:

Fertility rates: $F_{2,49} = 1.24$; $P > 0.05$.

Hatchability rates: $F_{2,48} = 1.07$; $P > 0.05$.

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Pb, Bi, and Sn in the blood were all <MDLs for these elements.

*Egg Weights*

As the ambient temperature increased with the onset of spring, the females began to lay. The first egg was laid on the 44th day of the study, well before the increase in daily illumination was initiated on the 91st day. Eggs were collected and weighed from each pair of ducks in each dosed group until 21 uncracked eggs were obtained from each pair. No eggs were collected from the Pb-dosed ducks because this entire dosed group died before egg laying ensued. One 0-dosed and one Bi-dosed hen failed to lay, and at necropsy both were found to have egg yolk peritonitis. The statistical analysis was applied to the weights of all hard-shelled eggs rather than being limited to the 21 uncracked eggs. Table 18 includes the total number of hard-shelled eggs collected from each dosed group and the mean egg weight for each group. We found no difference among doses in the mean weights of eggs collected from the 0-dosed, Fe-dosed, and Bi-dosed ducks.

*Egg Shell Thickness*

The 11th egg laid by each duck was analyzed for the thickness of its shell. No difference was detected in the thickness of egg shells among the dosed groups (Table 18).

*Fertility Rates*

Fertility rates were measured as a ratio of the number of fertile eggs to the total number of uncracked eggs collected for analysis, with 20 as the maximum. The fertility rates among pairs were generally high (> 85%), and the few pairs of ducks with low fertility rates had pathology of the male reproductive tracts. The mean fertility rates of the 0-dosed and Bi-dosed pairs were equal and the mean fertility rates of the Fe-dosed pairs were higher (Table 19). However, we found no statistical difference in the fertility rates among the dosed groups.

*Hatchability Rates*

The normal incubation period for mallard duck eggs is reported to be 28 days, but a majority of eggs that hatched during our study did so in 25 or 26 days. Most eggs that had not hatched by the 27th day were found to contain dead embryos. Hatchability rates were measured as a ratio of number of hatched eggs to the total number of fertile eggs. The hatchability rates varied widely for unknown reasons and were low for each dosed group. The hatchability rates for the Fe-dosed and Bi-dosed groups exceeded the hatchability rate for the 0-dosed group (Table 19), but we detected no difference in the hatchability rates among the dosed groups.

*Egg Shell Analysis*

The only differences among doses for the nine elements studied were higher mean concentrations of Pb in shells of eggs from 0-dosed ducks ($\bar{x}$= 0.300 µg/g) and Bi-dosed ducks ($\bar{x}$= 0.261 µg/g) than in shells of eggs from Fe-dosed ducks ($\bar{x}$= 0.145 µg/g). No difference existed in the mean concentrations of Pb in the egg shells from eggs of 0-dosed and Bi-dosed ducks (Table 20). As with other organs and tissues in this study, high concentrations of Fe in the diet resulted in lower concentrations of Pb in egg shells.

*Egg Content Analysis*

The contents of the 11th egg from each female were saved and analyzed for the nine elements. No differences were detected in the mean concentrations of seven elements—Bi, Sn, Ca, P, Mg, Zn, and Cu—among the three dosed groups of ducks (Table 21).

Mean concentrations of Pb were higher in contents of eggs from 0-dosed and Bi-dosed ducks than in contents of eggs from Fe-dosed ducks. These differences were manifested by a reduction in the concentration of Pb in the Fe-dosed eggs because no difference was found between 0-dosed and Bi-dosed ducks. Yip et al. (1981) found increased mean concentrations of Pb in children as Fe deficiency increased.

The contents of eggs from Fe-dosed ducks contained higher mean concentrations of Fe ($\bar{x}$ = 40.3 µg/g) than contents of eggs from Bi-dosed ducks ($\bar{x}$= 33.0 µg/g), but no other differences were found among the dosed groups. However, we found suggested differences ($P < 0.10$) between contents of eggs from the Fe-dosed group and contents of eggs from the 0-dosed group ($\bar{x}$ = 34.4 µg/g). Underwood (1971) reported that, in ducks, iron in serum was elevated by a factor of almost five during the laying season. Also, suggested differences ($P < 0.10$) were found between Cu in contents of eggs from Fe-dosed ducks ($\bar{x}$= 1.30 µg/g) and contents of eggs from 0-dosed ducks ($\bar{x}$ = 1.37 µg/g), and between Cu in contents of eggs from Fe-dosed ducks and contents of eggs from Bi-dosed ducks ($\bar{x}$= 1.43 µg/g).

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Table 20. Mean concentrations (µg/g) of nine elements in egg shells from 0-dosed (controls), Fe-dosed, and Bi-dosed game-farm mallards. n = 17 each for all except n = 18 each for Pb and Bi from Fe-dosed ducks.

| Dose | Pb | Fe | Bi | Sn | Ca | P | Mg | Zn | Cu |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0.300 | 5.32 | 0.232 | 1.40 | 377106 | 1732 | 1364 | 0.936 | 29.2 |
| | 0.042[a] | 1.31 | 0.079 | 0.184 | 11342 | 56 | 39 | 0.137 | 0.856 |
| Fe | 0.145 | 6.29 | 0.305 | 1.17 | 392059 | 1695 | 1397 | 0.960 | 29.8 |
| | 0.020 | 2.70 | 0.079 | 0.146 | 3877 | 47 | 28 | 0.181 | 1.771 |
| Bi | 0.261 | 7.87 | 0.353 | 1.45 | 381559 | 1658 | 1381 | 0.871 | 29.4 |
| | 0.027 | 3.60 | 0.092 | 0.222 | 12960 | 57 | 32 | 0.142 | 1.166 |

[a] SE.

MDL:

Pb - 0.072 µg/g.
Bi - 0.050 µg/g.
Sn - 1.93 µg/g.

Differences among doses:
Pb in egg shells: $F_{2,49} = 7.0404$; $P = 0.002$.

Table 21. Mean concentrations (µg/g) of nine elements in the contents of eggs from 0-dosed (controls), Fe-dosed, and Bi-dosed game-farm mallards. n = 52 for all samples.

| Dose | Pb | Fe | Bi | Sn | Ca | P | Mg | Zn | Cu |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0.170 | 34.4 | 0.037 | 1.28 | 1186 | 2696 | 124.7 | 18.3 | 1.37 |
| | 0.024[a] | 1.79 | 0.007 | 0.21 | 48 | 98 | 4.40 | 1.04 | 0.04 |
| Fe | 0.092 | 40.3 | 0.035 | 1.05 | 1113 | 2498 | 122.8 | 16.4 | 1.30 |
| | 0.013 | 2.16 | 0.006 | 0.14 | 45 | 109 | 2.54 | 0.84 | 0.05 |
| Bi | 0.185 | 33.0 | 0.024 | 0.91 | 1161 | 2488 | 120.5 | 16.6 | 1.43 |
| | 0.027 | 1.94 | 0.002 | 0.00 | 31 | 101 | 2.44 | 0.74 | 0.10 |

[a] SE.

MDL:

Pb - 0.064 µg/g.
Bi - 0.045 µg/g.
Sn - 1.82 µg/g.

Differences among doses:
Pb: $F_{2,49} - 5.26$; $P = 0.0086$.

Fe: $F_{2,49} - 3.96$; $P = 0.0255$.

Cu: $F_{2,47} = 2.47$; $P = 0.0950$.

Table 22. Numbers of embryo deaths per day (expressed in percentages of the embryos available to die on a specific day) for embryos from 0-dosed (controls), Fe-dosed, and Bi-dosed pairs of game-farm mallards.

| Day of Incubation | Dose | | |
|---|---|---|---|
| | 0 | Fe | Bi |
| 1 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 |
| 3 | 0 | 3.2 | 0 |
| | | 1.5[a] | |
| 4 | 0.3 | 0.7 | 0 |
| | 0.3 | 0.5 | |
| 5 | 0 | 0 | 0 |
| 6 | 0 | 0 | 0 |
| 7 | 0.6 | 0 | 0 |
| | 0.4 | | |
| 8 | 0 | 0 | 0 |
| 9 | 0 | 0 | 0 |
| 10 | 0.2 | 0 | 0 |
| | 0.2 | | |
| 11 | 0 | 0.6 | 0.6 |
| | | 0.4 | 0.4 |
| 12 | 0.3 | 0.3 | 0.9 |
| | 0.3 | 0.3 | 0.7 |
| 13 | 0.6 | 0 | 0 |
| | 0.4 | | |
| 14 | 0.9 | 0.3 | 0 |
| | 0.5 | 0.3 | |
| 15 | 0.6 | 1.0 | 0.3 |
| | 0.4 | 0.5 | 0.3 |
| 16 | 0.6 | 1.4 | 0.4 |
| | 0.4 | 0.9 | 0.3 |
| 17 | 1.6 | 0.6 | 0.7 |
| | 0.6 | 0.4 | 0.5 |
| 18 | 2.3 | 1.1 | 1.6 |
| | 1.2 | 0.5 | 1.0 |
| 19 | 1.7 | 2.5 | 4.3 |
| | 0.8 | 1.1 | 1.3 |
| 20 | 5.3 | 4.6 | 4.1 |
| | 2.2 | 1.7 | 1.1 |
| 21 | 5.1 | 9.2 | 3.7 |
| | 1.6 | 2.2 | 0.8 |
| 22 | 13.2 | 8.8 | 8.5 |
| | 2.9 | 2.3 | 2.2 |
| 23 | 16.9 | 23.6 | 11.8 |
| | 4.2 | 4.0 | 3.6 |
| 24 | 16.3 | 21.7 | 16.2 |
| | 3.5 | 6.2 | 3.7 |
| 25 | 9.5 | 3.7 | 7.4 |
| | 4.0 | 1.8 | 2.4 |
| 26 | 3.4 | 4.5 | 2.4 |
| | 1.6 | 1.6 | 1.6 |
| 27 | 4.5 | 6.0 | 3.5 |
| | 2.3 | 3.0 | 1.8 |
| 28 | 4.3 | 0.4 | 5.0 |
| | 2.3 | 0.4 | 2.2 |

a=SE.

Table 23. Mean age at death of embryos in fertile, but unhatched, eggs from 0-dosed (controls), Fe-dosed, and Bi-dosed game-farm mallard pairs. Sample sizes are in parentheses.

| Dose | Age at Death (days) |
|---|---|
| 0 | 21.6(189) |
| | 0.31[a] |
| Fe | 20.9(206) |
| | 0.38 |
| Bi | 22.2(147) |
| | 0.27 |
| All doses | 21.5(542) |

[a] SE.

Difference among doses:
Age at death of embryos: $F_{2,539} = 4.43$; $P = 0.0125$.

*continued from page 244*

*Age of Embryo at Time of Death*

A written protocol for determining the age of mallard duck embryos at the time of death was not found. Thus, determining the ages of embryos in the study was accomplished by combining published criteria for wood duck and turkey embryos and by comparing mallard duck embryos extracted from eggs opened at various stages of incubation. The criteria used for aging the embryos relied primarily on overall body length, extent of feathering, and size of the yolk. As with wood duck and turkey embryos, the criteria of eye closure and bill length were inconsistent among the mallard duck embryos, and therefore were not used. The highest rate of embryo deaths (63.2% of the embryos at risk died) occurred from Day 20 through Day 25 of incubation (Table 22).

The embryos from the Fe-dosed pairs experienced low peaks of embryonic death at Days 3 and 4. Embryos from neither of the other two dosed groups experienced similar peaks early in incubation. Differences were found among the dosed groups in the ages at which embryos died, particularly embryos from Fe-dosed ducks and Bi-dosed ducks (Table 23). Embryos from Bi-dosed ducks died at a later age, on the average, than embryos from the other two dosed groups.

## Histopathology

Thomas et al. (1988:120) reported, "One of the commonest toxic effects [of Bi] recorded is that of renal tubular damage, extending to acute tubular necrosis with some renal failure. Nephrotic syndrome as a result of glomerular damage has also been described. The liver can be affected with jaundice, various bleeding disorders, and multifocal hepatic necrosis being described." None of these effects was observed in our Bi-dosed ducks or their offspring.

### Adults

*Kidneys*

All but seven ducks had slight inflammatory changes in the ureters of the kidneys. This change was noted regardless of the dosed group and is considered normal for this group of ducks, based on results from this and previous histologic examinations. Two ducks (one Bi-dosed and one Fe-dosed) had focal granulomas in the kidney parenchyma. These small granulomas were not related to dose. Six of the seven ducks with no significant lesions (NSL) were in the Pb-dosed group. This pattern indicates that the mild inflammatory changes become more common with age (also evident by the lack of inflammatory kidney changes in ducklings) as the Pb-dosed ducks died at an earlier age than the other dosed groups.

*Liver*

Four histologic changes (inflammation, fatty change, hepatocellular swelling, and hemosiderosis) were noted in all dosed groups. Inflammatory changes were mild to moderate in severity and lesions and numbers affected were similar in all groups. Fatty change was most frequently associated with egg production and should be considered within a normal range. Hepatocellular swelling occurred in equal proportion in the Bi-, Fe-, and 0-dosed ducks. Hemosiderosis was most pronounced in the Pb-dosed group (9 of 12 ducks) and had a slightly higher incidence in the Fe-dosed group. No hemosiderosis was detected in the 0-dosed ducks. Pb-dosed ducks did not have any degree of fatty change nor hepatocellular swelling. The lack of these histologic changes,

which reflect fat mobilization and glycogen storage/mobilization, is consistent with the emaciation associated with Pb toxicity.

*Gonads*
Ovaries were morphologically normal in all groups/ducks examined. Three ducks had varying degrees of egg yolk peritonitis that could negatively impact fertility. Of the three ducks, two came from the Bi group and one came from the control group.

Testes from the Bi-, Fe-, and 0-groups were normal. One Fe-dosed duck and one Bi-dosed duck had small areas of inflammation but normal spermatogenesis. One Fe-dosed duck had normal spermatogenesis and mild vacuolization of the seminiferous epithelium. The minimal degree of vacuolization is not judged to be significant to fertility. Of the Pb-dosed ducks, five of the six males did not have spermatogenesis; however, Pb-dosed ducks died shortly after the start of the experiment—before the breeding season.

*Heart*
All hearts examined were normal. Three ducks in the Pb-dosed group had varying degrees of inflammation, most likely related to Pb toxicity and secondary systemic illnesses.

*Lungs*
All lung parenchyma was normal in the ducks examined. All groups had mild degrees of peribronchiolar inflammation and lymphoid hyperplasia. The Pb-dosed ducks had the lowest incidence of inflammatory lesions around bronchi, which is most likely related to their early demise in the experiment. The remaining dosed groups had varied incidence of this mild inflammatory lesion: 12 of 12 in Bi-dosed ducks, 5 of 9 in Fe-dosed ducks, and 7 of 10 in 0-dosed ducks. The inflammatory change is not judged to be significant to the health of the animals and probably represents a range of normal for these ducks.

**Ducklings**
*Liver*
The most common finding was a minimal to mild hepatocellular swelling. Based on the ducklings' young age, this condition is considered normal and due primarily to glycogen storage of the ducklings.

*Kidneys*
The kidneys were free of any histologic lesions with the exception of two Fe-dosed ducklings and one 0-dosed duckling. Both Fe-dosed ducklings had minimal lesions. The 0-dosed duckling had an inflammatory lesion that probably represented a systemic illness as supported by a small granuloma in the heart.

*Heart*
Several hearts of ducklings were examined and no significant lesions were found.

## Discussion

Only one duck (a Bi-dosed female) died of "natural" causes during our study. She died on Day 131, after laying 16 eggs. She weighed 0.97 kg when initially dosed compared with the mean weight of 1.04 kg for all females on Day 0. Although her body weight was lower than the mean weight of all females, she maintained her weight throughout the study and weighed the same (0.97 kg) at the time of death (10 June 1995) as on Day 0. The pathologist necropsied the duck, but postmortem changes prevented histopathological study. He identified no cause of death. This duck was not selected for collection of blood. Thus, no blood samples were available for analysis.

Sanderson et al. (1992) reported a mean Hct of 25.5 in six game-farm mallards 30 days after they were dosed with eight No. 2 Pb shot. In our present study, we found a mean Hct of 25.2 in four Pb-dosed ducks after a mean survival of 9.9 days. In our acute toxicity study (Sanderson et al. 1997a), on Day 30 the mean Hcts were 49.6 for 0-dosed, 50.8 for Fe-dosed, and 49.6 for Bi-dosed ducks, sexes combined. In our present study, mean Hcts for 0-, Fe-, and Bi-dosed males did not decline through Day 120. We did not expect an effect on Hcts by dosing with Bi shot as Slikkerveer and deWolf (1989) stated that anemia had never been associated with ingestion of Bi. Hcts of 0-, Fe-, and Bi-dosed females all declined about 9% from Day 0 to Day 120, perhaps as a result of stresses associated with egg laying.

We found no effect of dosing with eight, No. 4, Bi or Fe shot on body weight compared with 0-dosed ducks. Puls (1988) found that 1,000 ppm of Bi in the diet had no effect on body weight in chickens.

Kimball and Munir (1971:364) "...believe that the effect of the grinding action of the gizzard is to prevent the accumulation of the corrosion products on the surface of the pellet." In our study, females dissolved Fe shot that were in the gizzard for a mean of 31.2 days at a faster rate than they dissolved Fe shot that were in the gizzard for a

mean of 121.2 days. The higher dissolution rate for the former probably resulted from more surface area exposed to dissolution per day, on average, for the shot dosed on Day 90 than for the shot dosed on Day 0.

From radiographs made on Days 11 and 39, we clearly identified all eight, No. 4, Fe or Bi pellets dosed in each of eight female and eight male ducks. Sanderson et al. (1997a) radiographed 20 ducks on Day 23 of their study and identified all shot in the gizzards of five female and five male ducks each dosed with six, No. 4, Bi shot or six, No. 4, Fe shot.

The mean weights of gizzards in our present study ranged from 19.2 g for Bi-dosed females to 26.5 g for Pb-dosed males. Sanderson et al. (1997a) reported gizzard weights ranging from 29.3 g to 32.2 g for 0-, Fe-, and Bi-dosed ducks on 12 May 1994, Day 30 of the acute toxicity study. These latter relatively heavy gizzards may be a seasonal phenomenon or they may be related to diet. In Sanderson et al. (1997a), ducks were on a diet of shelled corn for the 30 days before necropsy, whereas in our current study, ducks were on a diet of breeder pellets before necropsy.

Mean weights of livers of males in the present study (Table 5) were similar to the mean weights of livers of males in the acute toxicity study (Sanderson et al. 1997a), but mean weights of both livers and kidneys of females were higher than mean weights of these organs in the earlier study. These differences may be related to long-term egg laying by females in our present study. Because of season-related increases, gonads were heavier in both sexes in the present study compared with weights reported by Sanderson et al. (1997a).

We found that Bi-dosed ducks had higher mean concentrations of Bi in their kidneys than in their livers, but Gregus and Klaasen (1986) reported that feces and urine were equally important in the excretion of Bi. Krigman et al. (1985:65) estimated a half-time of about 5 days for elimination of Bi from the whole body of humans.

Our Bi-dosed ducks had a mean concentration of 1.54 µg/g of Bi in their kidneys. Hamilton et al. (1972/1973) reported that humans with no known exposure to Bi had the following concentrations of Bi at autopsy (µg/g wet wt): kidney - 0.4, muscle - 0.007, and liver - 0.004. Our 0-, Pb-, and Fe-dosed ducks had $\leq$0.054 µg/g of Bi in their kidneys.

We found a higher mean concentration of Fe in the kidneys of Fe-dosed ducks than in the kidneys of 0-, Bi-, and Pb-dosed ducks. Forth and Rummel (1971) and Skoryna and Waldron-Edward (1971) reported that absorbed Fe differs from other metals by its slow rate of excretion. Sanderson et al. (1997a) found that mean concentrations of Fe were more than double in the liver and feces of Fe-dosed ducks, but not in the kidneys, gonads, plasma, and blood cells, as compared with 0- and Bi-dosed ducks.

The high mean concentration of Fe in the livers of Fe-dosed ducks, as compared with 0- and Bi-dosed ducks in our present study, probably is a result of the low excretion rate of Fe once it is absorbed (Forth and Rummel 1971; Skoryna and Waldron-Edward 1971). Also, Gregus and Klaassen (1986) found that the percentage of Fe in the liver increased as the dose increased, and corresponded to a reduced percentage of the Fe in bone, blood, plasma, heart, lung, and brain.

We found a much higher concentration of Cu in the liver than in the kidneys, blood, and gonads. Copper is reported to concentrate in the livers of domestic ducks (37-555 µg/g) (Underwood 1971:62). Underwood (1971) reported that Cu concentrations in the liver are affected by the levels of Fe and Zn in the diet in rats (an Fe-deficient diet results in high concentrations of Cu in the liver). In our present study, we found no difference among doses in the mean amounts of Cu in the liver. Sanderson et al. (1997a) reported means of 3,081 µg/g P in livers of 0-dosed, 3,108 µg/g in Fe-dosed, and 3,026 µg/g in Bi-dosed game-farm mallards on Day 30 after dosing with 0, six, No. 4 Fe, or six, No. 4, Bi shot. No differences existed in the mean concentrations of P in the livers of ducks on Sanderson et al's (1997a) study. Our current study found higher concentrations of P in the livers of 0- and Pb-dosed ducks versus Fe- and Bi-dosed ducks.

There seems to be little agreement as to the concentrations of Bi in the blood that are diagnostic for intoxication. Krigman et al. (1985) reported that blood Bi concentrations in humans administered oral therapeutics differ between those who exhibit side effects from chronic use and those who do not. Those with no symptoms usually have Bi concentrations <0.05 µg/g in blood and those with neurological symptoms have concentrations >0.05 µg/g. Hillemond et al. (1977) and Serfontein and Mekel (1979) concluded that 0.05 µg/g Bi in blood is an index of potential neurotoxicity in humans.

Dipalma (1988) said that Bi should not exceed 0.02 µg/g in blood of humans, and Locke et al. (1987) reported neurotoxic effects at Bi concentrations of <0.1 µg/g in blood. Ross et al. (1988) suggested that 6 µg/g of Bi in the brain of labora-

tory mice showed neurologic symptoms and that a concentration of ≥0.5-2.0 µg/g of Bi in blood had to be maintained for several weeks to accumulate enough Bi in the brain to cause neurotoxicity. Thomas et al. (1988:124) reported that concentrations of Bi in blood of more than 0.1 µg/g were potentially dangerous in humans and indicated that treatment with Bi should be stopped. Concentrations between 0.05 and 0.1 µg/g indicate that patients should be carefully monitored, and concentrations of less than 0.05 µg/g are considered safe.

In our present study, we found no effect of dosing ducks with Bi shot on egg laying compared with 0- and Fe-dosed ducks. Hermayer et al. (1977) added 1, 10, 100, and 1,000 ppm Bi trioxide to the diet of female chickens and found no effect on feed intake, number of eggs laid, or changes in body weight. Puls (1988) found that 1,000 ppm Bi in the diet had no effect on egg production in chickens.

## Conclusions

We conclude that under the conditions of this study, eight No. 4, Bi shot, repeatedly dosed in game-farm mallards, resulted in no demonstrable toxic effects on adult ducks or the eggs and ducklings they produced.

Survival of game-farm mallards was not affected during a 150-day test in which groups of ducks were dosed with eight, No. 4, Bi shot and compared with survival of 0-dosed and Fe-dosed ducks. All ducks dosed with eight, No. 4, Pb shot died within 2 weeks. No adverse effects on tissues were detected and concentrations of residues of elements in tissues were not different for 0-, Fe-, and Bi-dosed ducks.

No adverse effects were manifest for egg fertility, egg weight, eggshell thickness, egg hatchability, duckling weight at Day 7, and survival of ducklings to Day 7, for ducks dosed with eight, No. 4, Bi shot. Values for these variables were not different from those of 0- and Fe-dosed ducks. The only clear difference between Bi-dosed ducks and 0- and Fe-dosed ducks was in the timing of embryonic mortality, which was later for Bi-dosed ducks than for 0- and Fe-dosed ducks. We believe that the overall low hatchability of eggs, regardless of dose, might be related to repeatedly disturbing the ducks on Days 0, 30, 60, and 90 to weigh, dose, and bleed them and to collect eggs twice daily.

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