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ILLINOIS NATURAL HISTORY SURVEY

TOXICITY OF INGESTED BISMUTH ALLOY SHOT ON GAME-FARM MALLARDS

REVISED FINAL REPORT

TO

PETERSEN PUBLISHING COMPANY
c/o Mr. Robert E. Petersen
Attn: Mr. Ken Elliott
8490 Sunset Blvd.
Los Angeles, CA 90096

FROM

UNIVERSITY OF ILLINOIS
ILLINOIS NATURAL HISTORY SURVEY
607 East Peabody Drive
Champaign, IL 61820

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TOXICITY OF INGESTED BISMUTH ALLOY SHOT ON GAME-FARM MALLARDS
(Revised Final Report for the Toxicity Phase)

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SUMMARY

We detected no effects on game-farm mallards of dosing with 6 No. 4 Bi shot or 6 No. 4 Fe shot, as compared with 0-dosed (control) ducks and with each other, on survival to 30 days post dosing, Hct values, body weight, or in the mean weights of kidneys, livers, gonads, and gizzards. All 240 Bi-dosed shot (6 shot in each of 40 ducks) were probably retained during the study. As many as 10 of the 240 Fe shot were probably dissolved, but they may have been voided. Dissolution rates for both Fe and Bi shot from Day 0 to Day 30 were highly variable among ducks. The average dissolution rates were 70.2% for Bi shot and 61.1% for Fe shot. Females dissolved a higher percentage of the Fe shot than males, and males dissolved a higher percentage of Bi shot than Fe shot. Levels of Bi and Pb were determined in kidneys, livers, and gonads. There was 6.96 ppm, wet weight, Bi in kidneys, 2.23 ppm Bi in livers, and 0.468 ppm Bi in gonads of Bi-dosed ducks. Amounts of Pb in Bi-dosed ducks were 0.313 ppm in kidneys, 0.157 ppm in livers, and 0.096 ppm in gonads. Pb in the Bi shot averaged 0.00945%. Levels of Ca, P, Mg, Zn, Cu, Fe, and Sn were determined in kidneys, livers, gonads, blood plasma, and blood cells. There were several significant differences in the mean amounts of these elements among doses and between sexes; however, amounts of these elements in these organs and tissues in Bi-dosed ducks were not different from both 0- and Fe-dosed ducks. Many of the differences were sex related. Fe was higher in kidneys and livers of Fe-dosed ducks

than in 0- and Bi-dosed ducks and was much higher in the gonads of females than in males. Ca was higher in the livers of 0-dosed ducks than in Fe- and Bi-dosed ducks, was higher in the plasma of females than in males, and was up to 17 times higher in gonads of females than in males. Cu was higher in the livers and kidneys of males than in females. The MDL for Sn in the kidneys was 9.47 ppm; 6 of 120 kidneys had Sn above the MDL. This study detected no toxic effects of dosing 6 No. 4 Bi shot in game-farm mallards.

INTRODUCTION

Environment Canada (1992) provided guidelines for tests that were necessary to approve a candidate shot as nontoxic for waterfowl hunting in Canada. The present study was designed to comply with these guidelines. Dr. Simon Nadeau, Canadian Wildlife Service, and Dr. Keith A. Morehouse, U.S. Fish and Wildlife Service, reviewed the protocol prior to initiation of the study. This report is the Revised Final Report for the Toxicity Phase of the study. Results of the Embedded Shot Phase and the Reproductive Effects Phase will be presented in subsequent reports.

LITERATURE REVIEW

The first known report of metallic bismuth being dosed to birds was by Hanzlik and Presho (1923) who administered metallic Bi, Pb, and other heavy metals to pigeons. The fatal dose of metallic Pb in their studies ranged from 0.6 to 2.28 g/kg. By contrast, none of the four Bi-dosed pigeons died after receiving an average dose of 1.39 g/kg, and the researchers concluded that Pb is more toxic--and mortality is higher with smaller doses--than other heavy metals, including Bi. Sanderson et al. (1992) conducted the first comprehensive study to determine the toxicity of ingested Bi shot (100% Bi) in game-farm mallards. They reported no indication of toxicity.

There is some information on the effects of certain Bi compounds in humans, mice, rats, and dogs. Abbracchio et al. (1985) administered tri-potassium-dicitrato bismuthate intraperitoneally and by gavage in laboratory rats. After intraperitoneal injection, Bi reached peak levels in blood within 30 minutes and declined rapidly. When a dose 10 times higher was given by gastric intubation, much lower blood levels were detected. They found no Bi in the brain after oral administration and concluded that there was apparently little risk of neurotoxicity after dosing with this derivative. They stated (P. 143), "This could also be the reason why no appreciable side effects have ever been described after use of this drug in humans."

Locke et al. (1987) reported neurotoxic effects at Bi levels of < 0.1 ppm in blood. Woods and Fowler (1987) reported that little information was available on the effects of Bi in mammals in general, but noted that toxic effects in the liver, kidneys, and blood have been found in humans and laboratory animals after exposure to Bi compounds. In their studies with rats (P. 276) they found that "bismuth significantly impairs the activities of both hepatic ALA synthetase and heme synthetase at all dose levels."

Ross et al. (1988) injected 2,500 ppm of Bi subnitrate intraperitoneally in laboratory mice. Although Bi levels in blood and brain of mice that showed signs of neurotoxicity were significantly higher than in dosed mice that showed no signs, they concluded that Bi blood level did not predict neurologic signs. They suggested 6 ppm of Bi in the brain show neurologic symptoms and that a level of $\geq$ 0.5-2.0 ppm of Bi in blood had to be maintained for several weeks to accumulate enough Bi in the brain to cause neurotoxicity. They also concluded that 5-10 ppm Bi in the brain was associated with motor dysfunction in humans and mice and that levels above 50 ppb are necessary to produce frank encephalopathy in humans.

Dipalma (1988) said that although exposure to Bi is just as common as exposure to mercury and Pb, Bi is not considered a serious industrial hazard. According to him, studies are rarely done of Bi levels in blood from either oral or topical applications because of the assumption that absorption of Bi is very low. He warned of the possibility that bacteria in the intestine might methylate Bi to form a soluble compound. He reported (P. 244), "In animals, trimethyl bismuth is highly toxic and causes an encephalopathic syndrome similar to that seen in man. Blood levels of bismuth should not exceed 20 *ug* per L." (20 ppb).

In a review, Slikkerveer and de Wolff (1989) summarized current information on the effects of Bi in mammals. They reported a peak of Bi in blood 45 minutes after oral dosing with colloidal Bi in humans. Others had reported peaks between 4.7-21 ppm 15-60 minutes after dosing. With continued dosing, 3-4 weeks were necessary to reach a steady-state of Bi in plasma. Persons who had not received Bi therapy had between 1 and 15 ppb of Bi in their blood. Although the site of Bi absorption in the gastrointestinal tract is unknown, they believed that absorption after oral dosing is dependent on solubility and that cysteine, sorbitol, and lactic acid may promote absorption of Bi. They suggested that colloidal Bi is absorbed in the small bowel and stomach.

Meaningful reference values for Bi levels in tissues are not available because of large variations in experimental and analytical techniques, and the chemical form of Bi in blood is unknown. The highest levels of Bi were always in the kidney, and after 14 months of dosing colloidal Bi subcitrate in rats, Bi concentrations ranked from high to low in kidney, lung, spleen, liver, brain, and muscle, respectively. When bone levels were measured, concentrations were usually 10-20 times lower than in the kidney. Slikkerveer and de Wolff (1989) reported, however, that following oral dosing of trimethyl Bi to dogs, the level of Bi was higher in the liver than in the kidney, probably because of the organic character of the molecule. They reported that early toxic effects of Bi may be related to effects on enzymes of the haem synthesis but that anemia has never been associated with ingestion of Bi.

Bi is found in both urine and feces. The Bi in feces comes from Bi excreted in bile, which concentrates plasma Bi by a factor of 10, and from intestinal secretion. In humans showing symptoms of Bi toxicity after exposure, levels in bone were 1.5-6.7 ppm wet weight compared with <1 ppm wet weight, respectively, in non-exposed individuals. Bi encephalopathy is mainly supported by elevated blood Bi. A steady-state Bi level of > 100 ppb of blood in humans was arbitrarily suggested as an "alarm" level and 50 ppb was considered a "safety" level, but no proof supports these choices. Concentrations of blood Bi from 10 to 4,600 ppb were found in 618 Bi encephalopathy patients.

OBJECTIVES

The primary objective of this study was to determine if Bi shot is toxic to game-farm mallards under the conditions as specified for the study. Thus, our main goal was to look for toxic effects. If toxic effects were identified, then it was important to correlate them with various levels of elements in the tissues. If no toxic effects were demonstrated, the levels of elements, including Bi, were of lesser importance to the objectives of this study.

The Bi/Sn shot used in this dosing study contained an average of 0.0094% Pb. Because Pb comprised <0.1% of the shot dosed, it was not necessary to analyze tissues for Pb. Recognizing, however, the interest there is in Pb, we included it--albeit at a somewhat high detection limit--in our analyses.

We emphasize that for the objectives of this study, it matters little if there was 0.1 ppb or 100.0 ppm Bi in various tissues if no effects of dosing with the Bi shot could be shown on survival, body weight, organ weights, Hct, or by macro and micro examination for lesions in the livers, kidneys, and gonads of the ducks.

METHODS

To conduct the Toxicity Study (also the Embedded Shot Study), 75 female and 75 male wild-type game-farm mallards 6 to 8 months of age were purchased from Whistling Wings, Hanover, Illinois. The ducks were reared on a 60-acre lake. The ducks were transported from Hanover to Champaign, Illinois, by truck in crates on 22 March 1994.

Toxicity Study

Ducks for the toxicity tests were weighed and randomly assigned to pens, one duck per pen. Forty ducks (20 females and 20 males were randomly assigned to one of the three treatments--dosed with Bi shot, dosed with Fe shot, or sham dosed with 0 shot (controls). Five male and five female ducks were selected at random from each dosing group for collection of feces to analyze for excreted Bi, Fe, and Sn.

The pens are consecutively-numbered, elevated, outdoor, 3.3-foot (1-m) square pens constructed with vinyl-coated, 1-inch (25.4-mm) mesh, 14-gauge wire (see Sanderson et al. 1992 for more details). A 10- x 40-yard pole barn without sides covered the pens.

Facilities for holding the ducks were inspected by several members of the Laboratory Animal Care Committee, University of Illinois, shortly after the ducks were placed in the pens. The committee also conducted a scheduled semi-annual inspection of the facilities during the study. Commercial duck pellets (Heinhold 17% Duck Finisher Pellet, Heinhold Feeds, Inc., Kouts, IN) were provided *ad libitum* during the 3-week acclimatization period. The duck pellets contained a minimum of 17.0% protein. On the date of dosing, the pellets were removed and shelled corn was provided *ad libitum* for the duration of the study.

The three groups of ducks were each dosed as follows: control (sham dosed), six Number four Fe shot, or six No. four Bi shot. In the present study, ducks in each of the treatment groups are referred to as follows: 0-dosed (controls), Fe-dosed, and Bi-dosed.

The study was begun on 12 April 1994 (Day 0) when the ducks were weighed, bled for blood samples, and dosed. A small plastic funnel fitted with a plastic tube (3/8 inch [9.5 mm] outside diameter, 9 inches [22.9 cm] long) was inserted down the gullet and into the proventriculus. The tube was kept in a pail of water between dosings. The shot were poured into the funnel and flushed into the proventriculus with approximately 5 ml of water. Controls were treated the same except that no shot was placed in the proventriculus. Prior to the date of dosing, the doses of shot were counted, weighed, and placed in individual vials in the laboratory. The kind, number, and weight of shot were recorded on the top of each vial and on a computer printout for each duck. At dosing, the shot dose was matched with the corresponding duck.

Blood was collected from the wing vein in heparinized microhematocrit capillary tubes for hematocrit determination and in 2.5-ml syringes for separation into cells and plasma. The plasma samples were analyzed for major elements ($\geq$1% by wt in shot) and for major nutritionally essential elements (Ca, P, Mg, Zn, and Cu). Twenty-gauge, 1-inch (25.4-mm) needles were used (Baxter Healthcare

Corporation, Scientific Products Division, McGaw Park, Illinois). The whole blood was injected into 10-ml lithium heparinized Vacutainer tubes and centrifuged to separate cells and plasma. Body weights were recorded and blood samples collected on Days 0, 15, and 30.

When 24 hematocrit samples (capacity of the centrifuge) had been collected, the hematocrit tubes were centrifuged and read at the site in a house trailer used as a laboratory-office. The tubes were spun for 5 minutes at 11,500 RPM at 13,000-g force.

The whole blood samples were also centrifuged at the site when 12 samples (capacity of the centrifuge) were collected. The tubes were spun for 5 minutes at 3,000 RPM. The plasma was removed with micropipettes and placed in 5-ml non-hepranized Vacutainer tubes. The cells were left in the 10-ml lithium hepranized tubes. As the plasma and cells were separated, the tubes were placed in metal racks and put on ice in a styrofoam cooler. When all samples were separated, the cooler was taken some 300 meters to a freezer in a laboratory in the Natural Resources Study Annex. The blood samples were held in this freezer until space was available at the laboratories of the State Water Survey. The blood samples were then moved in a styrofoam cooler to a freezer at the State Water Survey; a trip of some 5 minutes. They were stored at -10^0 F until thawed to be analyzed.

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, prior to dosing the ducks. They averaged 98.35% Bi and 1.90% Sn. Others elements averaged <0.1% each; Pb averaged 0.0094%. Fe shot were removed from commercial 12 gauge shotgun shells.

The 120 ducks used for the toxicity portion of the study were weighed and blood was collected from the wing veins, as scheduled, on 12 May 1994. Following these procedures, the ducks were euthanized by decapitation and necropsied on the same date (with the exception noted below), and the gizzards, livers, kidneys, and gonads were excised.

Two slight variations in the methods were incorporated into the study. First, because voided shot were not found in the feces, we radiographed 20 dosed ducks to obtain a positive record of shot retention in the gizzards. We chose the 20 ducks for which daily fecal samples were being collected so that fecal material could be re-examined if the radiographs indicated dosed shot were missing from the gizzards. A dorsal-ventral and a right-left side view were made for each duck on Day 23 (5 May 1994).

The other slight variation in the methods involved the necropsies by the pathologist, Dr. Foley. He suggested that conducting necropsies on 60 ducks in one day was not a good idea because the tissue samples had to be taken from freshly euthanized birds. We agreed, and Dr. Foley necropsied 30 ducks on 12 May, and the remaining 30 ducks were euthanized and necropsied on the morning of 13 May 1994.

The necropsies were conducted in the Animal Autopsy Room of the Natural Resources Studies Annex on the campus of the University of Illinois. The freezer used for storing the blood samples was in this laboratory. When the pathologist had examined, weighed, and preserved a sample of kidneys, liver, and gonads for histopathology study from 60 randomly selected ducks, the individual organs were placed in separate, numbered, plastic bags and stored in the freezer. The organs from the remaining 60 ducks were removed and weighed by project personnel, placed in individual, numbered, plastic bags and stored in the freezer. The frozen organs were moved to the freezer at the State Water Survey with the blood samples and stored in the same freezer until thawed for analysis.

Methods for the chemical analyses, Data Reports, and Quality Assurance are in Appendix A.

Statistical Methods

Differences in concentrations of various elements in livers, kidneys, and gonads; weights of organs (post-mortem); numbers of shot recovered; and dissolution rates of shot were tested buy one- or two-way analysis of variance using sex and dose (shot type) as grouping factors. Homogeneity of variances among groups was assessed with Levene's test. Brown-Forsythe or Welch statistics were used in cases where variances could not be assumed equal. In cases where the overall test of differences among groups was significant, we performed pairwise comparisons and evaluated significance based on the Bonferroni correction. In cases were comparisons were made with controls, we used Dunnett's procedure.

We evaluated variation in body weights, hematocrit counts and concentrations of elements in plasma and red blood cells (all measured at Days 0, 15, and 30) using repeated-measures ANOVA. As above, sex or dose, or both, were used as between-subject factors. Within-subject tests for variation over time were also performed as were tests for interactions between dose and time. When assumptions of compound symmetry were violated, we used Huynh-Feldt adjusted significance probabilities. $P < 0.05$ was accepted as significant.

Analyses of tissues and Other Materials

The Method Detection Limit (MDL) (Glaser et al. 1981) was used to establish the detection limits for levels of elements in tissues and other materials. The MDL procedure should give a value that averages $\geq$ two times larger than the MDL to be considered a meaningful value (Glaser et al. 1981). For statistical analysis, values <MDL were entered as one-half the MDL value.

Most analyses for elements in the tissues were conducted by Inductively Coupled Argon Emission Plasma Spectroscopy (ICP). Because results of ICP analyses for Bi, Pb, and Sn were generally lower than the MDLs, selected samples of kidneys, livers, and gonads were analyzed for Bi and Pb by Graphite Furnace Atomic Absorption (AA). The amounts of plasma and blood cells remaining after the ICP analyses were inadequate to analyze them by AA. AA is not a satisfactory method to analyze for Sn.

To avoid repetition, in this report when two values are reported as "different" or that they "differ," we mean that they differ significantly (*P* <0.05). Two values that are not significantly different between two means are reported as "not different" or that they "do not differ." For comparison among three or more items, variation that is statistically significant (*P* <0.05) is reported as "difference" or that the values "differ." For comparison among three or more items, difference that is not significantly different is reported as "no difference" or that they "do not differ."

RESULTS

Survival

All 120 ducks used for the Toxicity Study survived to 30 days post dosing, when the ducks were euthanized.

Hematocrit (hct)

Hematocrits are presented as mean values and as percentage changes from Day 0 to Day 30 (Table 1). The percentage changes in Hct values from Day 0 to Day 30 did not differ between the sexes. Mean Hct increased from Day 0 to Day 30--by 6.21% for controls, 8.75% for Bi-dosed ducks, and 11.15% for Fe-dosed ducks. Hcts did not differ among doses.

Table 1. Mean hematocrit (Hct) on Days 0[a], 15, and 30[b] of game-farm mallards each dosed with 0 shot, six No. 4 Fe shot, or six No. 4 Bi shot and percentage change in Hct from Day 0[b] to Day 30 (n = 20 females and 20 males in each group).

Sex	Shot	Mean Hct Day 0	Mean Hct Day 15	Mean Hct Day 30	Mean % change in hct-Day 0 to Day 30
F+M	0	46.70	48.25	49.60	+ 6.21
F+M	Fe	45.75	49.70	50.85	+ 11.15
F+M	Bi	45.70	48.70	49.70	+ 8.75

[a] Ducks were dosed on Day 0.
[b] Ducks were euthanized on Day 30.

Difference among doses: $F_{2, 117} = 0.71$; $P = 0.4961$.
Change over time: $F_{2,234} = 50.10$; $P = <0.00001$.

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Body Weight

All groups of dosed ducks, except Bi-dosed males, lost small amounts of body weight during the 30-day study. All groups lost small amounts of weight from Day 0 to Day 15, perhaps as a result of switching from the diet of duck pellets to shelled corn, but by Day 30 they had regained most of their lost weight. Bi-dosed males gained a slight amount of weight from Day 15 to Day 30 (Table 2).

Males weighed more than females, and there was an interaction in weight between sex and time, with females losing a larger percentage of their body weight from Day 0 to Day 30 than males. Although there was an average weight loss over time, the average weight changes for females from Day 0 to Day 30 were only -3.66% for 0-dosed, -3.79% for Fe-dosed, and -5.12% for Bi-dosed ducks. The average weight changes for males from Day 0 to Day 30 were -1.58% for 0-dosed, -3.22% for Fe-dosed, and +1.05% for Bi-dosed ducks. There was no difference in body weights among doses (Table 2).

Retention and Dissolution of Shot

We found no voided shot in the feces. The 20 dosed ducks for which feces were saved for chemical analysis were radiographed by the College of Veterinary Medicine, University of Illinois, on Day 23 (5 May 1994). The radiographed ducks were 5 female and 5 male Bi-dosed and 5 female and 5 male Fe-dosed ducks. All six shot were readily identified in the gizzard of each of these ducks.

Table 2. Mean body weight (Kg) on Days 0[a], 15, and 30[b] of female and male game-farm mallards each dosed with 0 shot, six No. 4 Fe shot, or six No. 4 Bi shot and mean percentage change in body weight from Day 0 to Day 30 (n = 20 females and 20 males in each group).

Sex	Shot	Mean Body Weight			Mean % change in body wt-Day 0 to Day 306
		Day 0	Day 15	Day 30	
F	0	1.11	1.07	1.06	- 3.66
M	0	1.24	1.19	1.22	- 1.58
F	Fe	1.10	1.04	1.05	- 3.79
M	Fe	1.24	1.18	1.20	- 3.22
F	Bi	1.08	1.02	1.03	- 5.12
M	Bi	1.23	1.21	1.24	+ 1.05

[a] Ducks were dosed on Day 0.
[b] Ducks were euthanized on Day 30.
Mean body weight:
Difference between sexes: $F_{1,114} = 66.42$; $P = <0.00001$.
Interaction between sex and time: $F_{2,228} = 3.21$; $P = 0.0471$.
Difference among doses: $F_{2,114} = 0.11$; $P = 0.8995$.
Change over time: $F_{2,228} = 33.17$; $P = <0.00001$.

After euthanasia on Day 30, the contents of all 120 gizzards were removed and saved, and those from Fe-dosed and Bi-dosed ducks were examined for retained shot. The linings of all gizzards appeared to be unaffected, and in this regard, we saw no pattern of variation among doses.

We recovered six pellets, which were sometimes dissolved to small disks, from each of 38 of the 40 Bi-dosed ducks. One male had only five Bi disks in his gizzard. Because of the small size of these disks, we are confident that the sixth pellet had dissolved. A second male had four tiny Bi particles in his gizzard. The particles of Bi collectively weighed only 0.0421g, and we are confident that the fifth and sixth pellets had dissolved.

We recovered six pellets from each of 35 of the 40 Fe-dosed ducks. One female had five tiny pellets, with a total weight of 0.0777g, in her gizzard. This duck had the second highest (2339 ppm) concentration of Fe in the liver. The mean concentration of Fe in livers of Fe-dosed ducks was 1086 ppm. Thus, we are confident that the sixth pellet had dissolved. One female had no shot in her gizzard, but she had 1782 ppm Fe in her liver. We concluded that she probably dissolved the shot. The remaining three ducks, one male and two females, each had five pellets in their gizzards. All of these pellets were small, collectively weighing from 0.1474 to 0.2829 g for each duck. One of these females had the highest concentration (2412 ppm) of Fe in her liver of any duck. The other two ducks had 645 ppm and 1043 ppm, respectively, Fe in their livers. The sixth pellets in these three ducks may have been voided, but they probably were dissolved. None of the dosed ducks with missing shot in their gizzards was among the ducks that were radiographed and for which feces were saved for analysis. Thus, we could not check radiographs or fecal material for fate of the missing shot.

The retained Bi shot and Fe shot differed in appearance. The Fe shot were generally round, although many of them had pits or empty spaces on their surfaces, whereas the Bi shot were generally disk-shaped or flattened. In several instances, there were five Bi disks plus two, three, or four tiny pieces (not flakes) of Bi in the gizzard. It was obvious that when a Bi disk became thin enough it broke into two or more pieces. A small number of flakes of Bi were found in a few gizzards. This finding for Bi-Sn alloy shot is in contrast to the abundance of tiny grains of metallic Bi we found in the dosing study that used 100% Bi shot (Sanderson et al. 1992).

The dissolution rates were variable in both Fe-dosed and Bi-dosed ducks. In ducks in which all six dosed shot were accounted for, females dosed with Bi shot dissolved an average of 69.54% and males 72.54% (Table 3) of the metal's original weight in 30 days (dissolution in individual ducks ranged from 38.15% to 96.45%). There was no difference between the sexes for Bi-dosed ducks. Fe-dosed females dissolved an average of 69.16% and Fe-dosed males 55.63% of the metal's original weight in 30 days (range for

Table 3. Percent of dosed shot accounted for and mean percent of weight of dosed shot dissolved in 30 days in the gizzard--six No. 4 Fe shot or six No. 4 Bi shot dosed in female and male game-farm mallards (n = 20 females and 20 males in each dosed group).

Sex	Dose	% of Dosed Shot Accounted for	Mean % Wt of Shot Dissolved[a]
F	Fe	92.50	69.16
M	Fe	99.17	55.63
F	Bi	100.00	69.54
M	Bi	100.00	72.54

[a] Based on the shot accounted for on Day 30, when the ducks were euthanized.

Interaction between sex and dose: $F_{1,60}$ = 4.53; P = 0.0374.
Difference between sexes for percent of Fe shot dissolved in 30 days: $F_{1,37}$ = 14.89; P = 0.0014.
Difference between doses for males: $F_{1,31}$ = 17.42; P = 0.0002.

individual doses was from 37.99% to 89.56%). The differing dissolution rates between sexes for Fe-dosed ducks was not unexpected (Table 3). Females approaching the breeding season in spring consume more food than males and thus produce more acid in their gizzards. As a result, Fe shot, which dissolve readily in the HCl acid environment of the gizzard, dissolve more rapidly in females than in males during this season.

Males dissolved more of the weight (72.54%) of the Bi shot than of the Fe shot (55.63%) in 30 days. Females dissolved no more (69.54%) of the weight of the Bi shot than of the Fe shot (69.16%) in 30 days. There was an interaction between sex and dose, which was caused by the lower rate of dissolution of Fe shot by males as compared with the dissolution rate of Fe shot by females and no difference in the dissolution rates of Fe shot and Bi shot by females (Table 3).

Organ Weights

Gizzard

Mean gizzard weights ranged from 29.28g for Fe-dosed females to 32.18g for Bi-dosed males (Table 4). There was no difference in the weight of gizzards between sexes or among doses.

As a percentage of total body weight, mean gizzard weights ranged from 2.6% for all three groups of males to 3.0% for 0-dosed females (Table 4). Gizzards of females contributed a higher percentage of the total body weight than males. There was no

Table 4. Mean weight of gizzard and the mean percentage it contributed to total body weight in game-farm mallards 30 days after dosing with 0 shot, six No. 4 Fe shot, or six No. 4 Bi shot (n = 20 females and 20 males in each group).

Sex	Dose	Mean Weight(g)	Mean % of body wt
F	0	31.75	3.0
M	0	31.62	2.6
F	Fe	29.28	2.8
M	Fe	30.73	2.6
F	Bi	30.16	2.9
M	Bi	32.18	2.6

Difference among doses in weight of gizzard:
$F_{2,14} = 1.50$; $= 0.2265$.

Difference between sexes in percentage gizzard contributed to total body weight:
$F_{1,114} = 34.43$; $P < 0.00001$.

Difference among doses in percentage gizzard contributed to total body weight:
$F_{2,101} = 1.99$; $P = 0.1422$.

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difference among doses in the percentage gizzards contributed to total body weight.

Liver

The mean weights of livers ranged from 19.3 g for Bi-dosed females to 21.7g for Fe-dosed females. There were no differences between sexes or among doses (Table 5).

When considered as a percentage of total body weight, mean values for livers ranged from 1.6% for Bi-dosed and 0-dosed males to 2.0% for Fe-dosed and 0-dosed females. Livers of females comprised a higher percentage of the total body weight than the livers of males. There was no difference among doses in the mean percentage that livers contributed to the total body weight.

Kidneys

Weights of kidneys, the organ most involved in excretion of Bi, differed the least between sexes and varied the least among doses of the organs weighed. Their mean weights ranged from 6.36g for Bi-dosed females to 6.60g for 0-dosed males (Table 6). There were no differences between sexes or among doses in the weights of kidneys.

As with weights of livers, when kidney weights were expressed as a percentage of total body weight, there were differences. Mean percentages ranged from 0.50% for each group of males to 0.60% for each group of females. Kidneys of females comprised a larger percentage of the total body weight than males, but there were no differences among doses.

Table 5. Mean weight of liver and the mean percentage it contributed to total body weight in game-farm mallards 30 days after dosing with 0 shot, six No. 4 Fe shot, or six No. 4 Bi shot (n = 20 females and 20 males in each group).

Sex	Dose	Mean Weight(g)	Mean % of body wt
F	0	21.1	2.0
M	0	20.0	1.6
F	Fe	21.7	2.0
M	Fe	20.5	1.7
F	Bi	19.3	1.9
M	Bi	19.5	1.6

Difference among doses in weight of liver:
$F_{2,14} = 1.10$; $P = 0.3370$.

Difference between sexes in percentage liver contributed to total body weight: $F_{1,84} = 22.21$; $P = <0.00001$.

Difference among doses in percentage liver contributed to total body weight: $F_{2,114} = 1.80$; $P = 0.1706$.

Table 6. Mean weight of kidneys and the mean percentage they contributed to total body weight in game-farm mallards 30 days after dosing with 0 shot, six No. 4 Fe shot, or six No. 4 Bi shot (n = 20 females and 20 males in each group).

Sex	Dose	Mean Weight(g)	Mean % of body wt
F	0	6.46	0.60
M	0	6.60	0.50
F	Fe	6.40	0.60
M	Fe	6.47	0.50
F	Bi	6.36	0.60
M	Bi	6.39	0.50

Difference among doses in weight of kidneys: $F_{2,14} = 0.22$; $P = 0.8006$.

Difference between sexes in percentage kidneys contributed to total body weight: $F_{1,114}=33.91$;$P = <0.00001$.

Differencen among doses in percentage kidneys contributed to total body weight: $F_{2,114} = 0.13$; $P = 0.8756$.

Gonads

There were no differences among doses in the mean weights of gonads (Table 7). As was expected, mean weights of gonads differed between the sexes: 6.40g for 0-dosed females, 26.38g for 0-dosed males; 10.11g for Fe-dosed females, 28.00g for Fe-dosed males; and 4.32g for Bi-dosed females and 22.47g for Bi-dosed males. These sex differences were also evident in gonad weights expressed as a percentage of total body weight; the means ranged from 0.4% for Bi-dosed females to 2.2% for 0-dosed males (Table 7). There were no differences among doses, but there were differences between sexes for the percentage gonads contributed to total body weight.

Heavy Metals and Essential Elements in Organs and Blood

For consistency in presentation of the data, we have usually listed the mean amounts of elements in each organ or tissue for each sex and for sexes combined. When there were no significant differences between sexes, we usually only provide *P* values for the sexes combined.

Kidneys

The MDL for analysis by Inductively Coupled Argon Plasma Spectrometry (ICP) for Bi in kidneys was 17.8 ppm (wet wt). The mean levels observed were less than the MDL in all but 2 of 120 kidneys analyzed.

Because the MDL for Bi by ICP in the kidneys was unacceptably high, we selected kidneys of 10 0-dosed and 11 Bi-dosed ducks for analysis by Atomic Absorption Graphite Furnace (AA). There were no sex differences in the mean level of Bi in the kidneys of Bi-dosed ducks (Table 8). The mean level of Bi in the kidneys (6.86 ppm) of Bi-dosed ducks, with sexes combined, was higher than the mean level of Bi in 0-dosed ducks (0.334 ppm). The mean level of Bi in the kidneys (6.86 ppm) of Bi-dosed ducks was much higher than the mean levels of Bi in the livers (2.23 ppm) of Bi-dosed ducks (Table 10).

The MDL for Pb in the kidneys was 6.54 ppm (wet wt) by ICP. All mean values for Pb in the kidneys were <MDL by this method. By AA, there were no sex differences in the amount of Pb in the kidneys of 0-dosed or Bi-dosed ducks. There was no difference in the amount of Pb in the kidneys of 10 (sexes combined) 0-dosed ducks (0.440 ppm) compared with 11 Bi-dosed ducks (0.313 ppm) (Table 8).

The MDL for Sn in the kidneys was 9.47 ppm (wet wt). Only six ducks had amounts of Sn >MDL. The mean amount of Sn in the kidneys of these six ducks was 14.3 ppm and ranged from 10.5 to 19.7 ppm. Three of these ducks were Bi-dosed (16.7 ppm), two were 0-dosed (11.0 ppm), and one Fe-dosed (13.7 ppm).

There was no difference between sexes in the mean amount of Cu in the kidneys (Table 9). With sexes combined, there were no differences among doses in the mean level of Cu in the kidneys: 6.31 ppm in 0-dosed, 7.31 ppm in Fe-dosed, and 6.14 ppm in Bi-dosed ducks.

There were no sex differences in the mean levels of P in the kidneys (Table 9). There were no differences among doses in the

Table 7. Mean weight of gonads and the mean percentage they contributed to total body weight in game-farm mallards 30 days after dosing with 0 shot, six No. 4 Fe shot, or six No. 4 Bi shot (n = 20 females and 20 males in each group).

Sex	Dose	Mean Weight(g)	Mean % of body wt
F	0	6.40	0.6
M	0	26.38	2.2
F	Fe	10.11	0.9
M	Fe	28.00	2.4
F	Bi	4.32	0.4
M	Bi	22.47	1.9

Difference between sexes in weight of gonads: $F_{1,14} = 83.04$; $P = <0.00001$.

Difference among doses in weight of gonads: $F_{2,14} = 2.54$; $P = 0.0830$.

Difference between sexes in the percentage gonads contributed to total body weight: $F_{1,114} = 67.57$; $P = <0.00001$.

Difference among doses in the percentage gonads contributed to total body weight: $F_{2,114} = 2.47$; $P = 0.0889$.

Table 8. Mean levels (ppm wet wt) of Bi and Pb in kidneys of game-farm mallards 30 days after dosing with 0 shot (controls) compared with ducks dosed with six No. 4 Bi shot, as measured by AA.

Element	Sex	Kind of Shot Dosed 0[a]	Bi[b]
Bi	F	0.140	8.05
	M	0.528	4.77
	F & M	0.334	6.86
Pb	F	0.138	0.427
	M	0.742	0.112
	F & M	0.440	0.313

MDL = Method Detection Limit (ppm wet wt) by AA for Bi in kidneys = 0.10 ppm for 10 ducks and 0.27 ppm for 11 ducks and Pb = 0.27 ppm for 10 ducks and 0.15 ppm for 11 ducks.

[a] N = 10.

[b] N = 11.

Bi

Difference between sexes in Bi-dosed ducks: $F_{1,9}$ = 3.09; P = 0.1127.

Difference between doses: $F_{1,10}$ = 43.32; P = 0.0001.

Pb

Difference between sexes in 0-dosed ducks: $F_{1,4}$ = 0.88; P = 0.4011.

Difference between sexes in Bi-dosed ducks: $F_{1,9}$ = 1.68; P = 0.2274.

Difference between doses: $F_{1,19}$ = 0.15; P = 0.7035.

Table 9. Mean levels (ppm wet wt) of Cu, P, Fe, Ca, Mg, and Zn by (ICP) in kidneys of game farm-mallards 30 days after dosing with 0 shot (controls) compared with ducks dosed with six No. 4 Fe shot or six No. 4 Bi shot (n = 20 females and 20 males in each group).

		Kind of Shot		
Element	Sex	0	Fe	Bi
Cu	F	5.50	5.34	5.74
	M	7.13	9.08	6.60
	F & M	6.31	7.31	6.14
P	F	2757.8	2937.2	3049.8
	M	3166.8	2903.3	3005.7
	F & M	2962.3	2919.4	3029.8
Fe	F	110.3	151.9	117.7
	M	136.5	138.9	129.2
	F & M	123.4	145.0	122.9
Ca	F	83.98	84.39	87.18
	M	79.85	70.23	72.23
	F & M	81.92	76.94	80.39
Mg	F	195.5	198.7	205.8
	M	215.9	196.6	203.5
	F & M	205.7	197.6	204.7
Zn	F	25.26	24.70	27.36
	M	27.90	25.60	29.29
	F & M	26.58	25.17	28.24

Cu

Difference among doses: $F_{2,57}$ = 0.32; P = 0.7280.

P

Difference among doses: $F_{2,58}$ = 0.63; P = 0.5384.

Interaction between sex and dose: $F_{2,17}$ = 3.48; P = 0.0540.

Fe

Difference among doses: $F_{2,58}$ = 4.98; P = 0.0103.

Interaction between sex and dose: $F_{2,58}$ = 3.01; P = 0.0574.

Difference between 0-dosed and Fe-dosed ducks: P = <0.05.

Difference between 0-dosed and Bi-dosed ducks: P = >0.10.

Differfence between Fe-dosed and Bi-dosed ducks: P = <0.05.

Ca

Difference between sexes: $F_{1,58}$ = 6.99; P = 0.0107.

Difference among doses: $F_{2,33}$ = 0.41; P = 0.6680.

Mg

Difference among doses: $F_{2,58}$ = 0.80; P = 0.4536

Zn

Difference between sexes: $F_{1,58}$ = 4.45; P = 0.0395.

Difference among doses: $F_{2,58}$ = 4.56; P = 0.0147.

Difference between Fe-dosed and Bi-dosed ducks: P = <0.05.

mean levels of P in the kidneys, but there was an interaction between sex and dose.

There was no difference between sexes in the mean level of Fe in the kidneys (Table 9). There were differences among doses in the mean levels of Fe in the kidneys. Fe-dosed ducks, sexes combined, had higher mean levels (145.0 ppm) of Fe in their kidneys than 0-dosed ducks (123.4 ppm) or Bi-dosed ducks (122.9 ppm). There was no difference in the mean levels of Fe in the kidneys of 0-dosed and Bi-dosed females (Table 9).

Females, with doses combined, had higher mean levels of Ca in the kidneys than males, but there was no difference in the mean amounts of Ca in the kidneys among doses.

There were no sex differences in the mean levels of Mg in the kidneys, or with sexes combined, no difference among doses (Table 9).

There were no differences between sexes in the mean levels of Zn in the kidneys of 0-dosed, Fe-dosed, or Bi-dosed ducks, but with doses combined, males had higher mean amounts of Zn in the kidneys than females (Table 9). With sexes combined, there was variation in the mean levels of Zn in the kidneys among doses. Bi-dosed ducks (28.24 ppm) had higher mean levels of Zn in their kidneys than Fe-dosed ducks (25.17 ppm), but not higher mean levels than 0-dosed ducks (26.80 ppm) (Table 9). There was no difference in mean levels of Zn in the kidneys of 0-dosed and Fe-dosed ducks.

Table 10. Mean levels (ppm wet wt) of Bi and Pb in livers of game-farm mallards 30 days after dosing with 0 shot (controls) compared with ducks dosed with six No. 4 Bi shot (analyses by AA).

		Kind of Shot Dosed	
Element	Sex	0	Bi
Bi	F	0.140[a]	2.79[b]
	M	0.246[c]	1.25[d]
	F & M	0.193	2.23
Pb	F	0.068	0.184
	M	0.552	0.110
	F & M	0.310	0.157

MDL = Method Detection Limit (ppm wet wt) by AA for Bi = 0.27 for 10 ducks and 0.10 for 11 ducks and for Pb = 0.10 for 10 ducks and 0.15 for 11 ducks.

[a] N = 5.
[b] N = 7.
[c] N = 5.
[d] N = 4.

Bi
Difference between doses: $F_{1,10} = 17.14; P = 0.0020$.

Pb
Difference between doses: $F_{1,19} = 0.65; P = 0.4294$.

PRT-8AA

Liver

The MDL (by ICP) for Bi in livers was 18.45 ppm (wet weight). There was no level of Bi above the MDL in the liver of any duck. Analysis by AA showed that the mean level of Bi in the livers of 11 Bi-dosed ducks was 2.23 ppm and ranged from 0.63 to 5.63 ppm. There was no difference between sexes in the mean amounts of Bi in the liver (Table 10). With sexes combined, Bi-dosed ducks had a higher (2.23 ppm) mean amount of Bi in the liver than 0-dosed ducks (0.193 ppm).

The MDL (by ICP) for Pb in the liver was 7.51 ppm. The amounts were all below the MDL. The amount of Pb in the livers as determined by AA ranged from <MDL (0.10 ppm) to 0.46 ppm in the 11 Bi-dosed ducks and the mean (sexes combined) was 0.310 ppm for 10 0-dosed birds and 0.157 ppm for 11 Bi-dosed ducks. There were no differences among doses or between sexes, doses combined, in the mean amount of Pb in the liver (Table 10).

The MDL for Sn in the liver was 12.8 ppm. All but 3 of 120 livers had <MDL of Sn. The three livers had 13.1 ppm Sn in an Fe-dosed duck, 13.2 ppm in a 0-dosed duck, and 18.8 ppm in a Bi-dosed duck.

The mean level of Cu in the livers of 0-dosed females was 85.48 ppm vs 191.0 ppm in males, 56.26 ppm in the livers of Fe-dosed females vs 172.4 ppm in males, and 78.28 ppm in the livers of Bi-dosed females vs 149.3 ppm in males (Table 11, Fig. 1). Males had higher mean levels of Cu in the liver than females, but there

Table 11. Mean levels (ppm wet wt) of Cu, P, Fe, Ca, Mg, and Zn (by ICP) in livers of game-farm mallards 30 days after dosing with 0 shot (controls) compared with ducks dosed with six No. 4 Fe shot or six No. 4 Bi shot (n = 20 for each sex).

		Kind of Shot Dosed		
Element	Sex	0	Fe	Bi
Cu	F	85.48	56.26	78.28
	M	191.0	172.4	149.3
	F & M	138.2	114.3	113.8
P	F	3163.6	3258.4	3154.2
	M	2998.4	2958.4	2897.2
	F & M	3081.0	3108.4	3025.8
Fe	F	416.3	1157.8	435.0
	M	406.0	1014.6	362.3
	F & M	411.1	1086.2	398.6
Ca	F	66.4	54.0	52.9
	M	59.2	46.8	49.8
	F & M	62.80	50.40	51.36
Mg	F	215.4	224.0	215.6
	M	211.0	214.3	211.6
	F & M	213.2	219.2	213.6
Zn	F	53.3	48.4	50.8
	M	48.9	48.1	45.4
	F & M	51.08	48.25	48.11

Cu

Difference between sexes: $F_{1,76} = 20.64$; $P = < 0.00001$.

Difference among doses: $F_{2,76} = 0.56$; $P = 0.5721$.

Difference between Fe-dosed females and Fe-dosed males: $P = < 0.05$.

Difference between 0-dosed females and 0-dosed males: $P = < 0.05$.

Difference between Bi-dosed females and 0-dosed males: $P = < 0.05$.

P

Difference between sexes: $F_{1,114} = 11.07$; $P = 0.0012$.

Difference among doses: $F_{2,114} = 0.45$; $P = 0.6380$.

Fe

Difference among doses: $F_{2,117} = 67.53$; $P = <0.00001$.
Difference between 0-dosed and Fe-dosed; $P = <0.01$.
Difference between 0-dosed and Bi-dosed; $P = >0.10$.
Difference between Fe-dosed and Bi-dosed; $P = <0.01$.

Ca

Difference among doses: $F_{2,117} = 3.43$; $P = 0.0356$.
Difference between 0-dosed and Fe-dosed; $P = <0.05$.
Difference between 0-dosed and Bi dosed; $P = >0.05$.
Difference between Fe-dosed and Bi dosed; $P = >0.10$.

Mg

Difference among doses: $F_{2,117} = 0.66$; $P = 0.5182$.

Zn

Difference among doses: $F_{2,117} = 0.70$; $P = 0.5005$.

Sn

The mean level was <MDL: = 12.8ppm.

PRT-9A

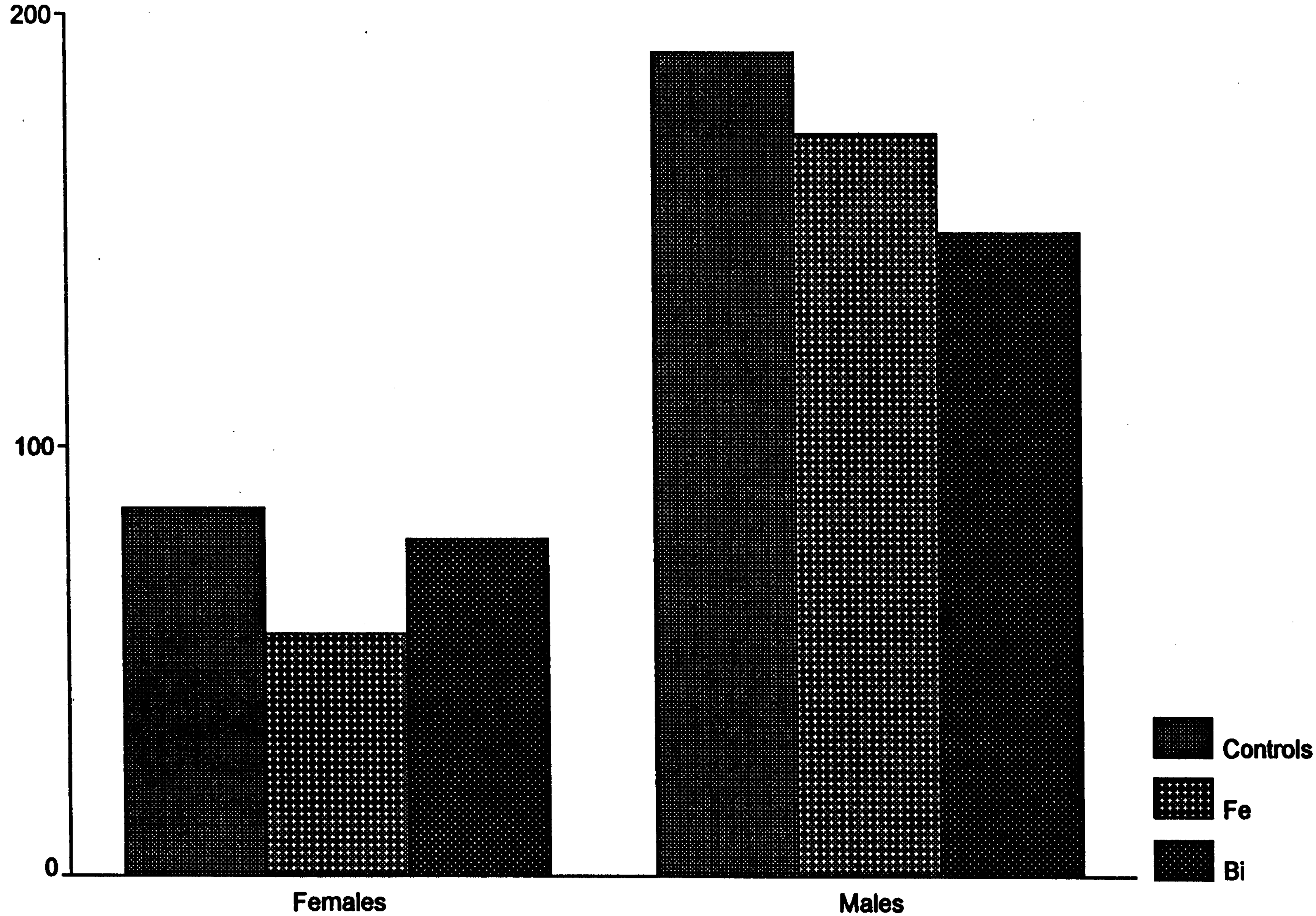

Fig. 1. Cu (ppm) in liver of game-farm mallards 30 days after dosing with 0 or 6 No. 4 Fe or 6 No. 4 Bi shot.

were no differences among doses in the mean amount of Cu in the livers.

Females consistently had more P in their livers than males (Table 11): 0-dosed females, 3163.6 ppm vs 2998.4 ppm in males; Fe-dosed females, 3258.4 ppm vs 2958.4 ppm in males; and Bi-dosed females, 3154.2 ppm vs 2897.2 ppm in males. There were no differences among doses in the mean amounts of P in the livers (Table 11).

There was no difference between sexes in the mean levels of Fe in the livers. The mean levels of Fe in the livers differed among doses: 0-dosed ducks was 411.1 ppm vs 1086.2 ppm in Fe-dosed ducks vs 398.6 ppm in Bi-dosed ducks. There were differences in the mean amounts of Fe in 0-dosed vs Fe-dosed ducks and Fe-dosed vs Bi-dosed, but not between 0-dosed vs Bi-dosed ducks (Table 11).

There were no differences between sexes in the mean levels of Ca in livers. With sexes combined, there were differences among doses in the mean levels of Ca in the liver. The mean level of Ca in the livers was higher in 0-dosed ducks (62.80 ppm) than in Fe-dosed ducks (50.40 ppm), but was not higher in 0-dosed than in Bi-dosed ducks (51.36 ppm) (Table 11). There was no difference in the mean levels of Ca in the livers of Fe-dosed and Bi-dosed ducks.

The mean level of Mg in the livers ranged from 211.0 ppm in 0-dosed males to 224.0 ppm in Fe-dosed females. There was no difference between sexes. With sexes combined, there were no differences among doses in the mean levels of Mg in the livers: 0-

dosed ducks, 213.2 ppm; Fe-dosed ducks, 219.2 ppm; and Bi-dosed ducks, 213.6 ppm (Table 11).

The mean level of Zn in the livers ranged from 45.4 ppm for Bi-dosed males to 53.3 ppm for 0-dosed females. There was no difference between sexes. With sexes combined, there were no differences among the mean levels of Zn in livers of 0-dosed ducks, 51.08 ppm; Fe-dosed ducks, 48.25 ppm; and Bi-dosed ducks, 48.11 ppm (Table 11).

Gonads

The MDL for Bi in gonads by ICP was 12.0 ppm for 33 ducks and 13.2 ppm for 28 ducks. All but five mean values for Bi in gonads were <MDL. The amount of Bi in the five gonads with amounts >MDL ranged from 15.2 to 27.8 ppm and averaged 19.88 ppm.

As determined by AA Furnace, the amounts of Bi in gonads of 0-dosed ducks were all <MDL (0.10 ppm). Because there was no variance in the 0-dosed ducks , ANOVAs could not be run. All Bi-dosed ducks had a mean of 0.468 ppm Bi in their gonads compared with half the MDL (0.05 ppm) in 0-dosed ducks (Table 12).

The MDL for Pb in the gonads by ICP was 7.10 ppm (wet wt) for 33 ducks and 7.75 ppm for 28 ducks. Only one (11.8 ppm) mean amount of Pb in the gonads was >MDL.

The amounts of Pb, as determined by AA furnace, in gonads of 0-dosed ducks were all <MDL (0.15 ppm). Although statistical analyses could not be run on the data, the mean amounts of Pb

(0.096 ppm) in gonads of Bi-dosed ducks were not appreciably different from one-half of the MDL (0.080 ppm) (Table 12).

The MDL for Sn in the gonads by ICP was 15.5 ppm (wet wt). Only one female had >MDL (17.8 ppm) of Sn in the liver.

There were sex differences in the mean levels of Cu in the gonads of 0-dosed ducks, but no sex differences in mean levels of Cu in Fe-dosed and in Bi-dosed ducks. With doses combined, there were sex differences, with males having lower amounts of Cu than females (Table 13). There were no differences in the mean amounts of Cu in the gonads among doses.

There were no sex differences in the mean levels of P in the gonads among doses. With sexes combined, there was no dixfference in the amount of P in the gonads among doses (Table 13).

Sex differences in the mean levels of Fe in the gonads were striking (Fig. 2) in all dosed groups: 0-dosed, 56.36 ppm in females vs 12.49 ppm in males, Fe-dosed, 53.49 ppm in females vs 10.66 ppm in males, and Bi-dosed, 40.28 ppm in females vs 16.78 ppm in males. There was no difference in the mean levels of Fe in the gonads among doses (Table 13).

Females had up to 17 times more Ca in their gonads than males (Fig. 3): 0-dosed females (540.0 ppm) vs 0-dosed males (34.46 ppm), Fe-dosed females (590.1 ppm) vs Fe-dosed males (34.39 ppm), and Bi-dosed females (334.4 ppm) vs Bi-dosed males (139.6 ppm). With doses combined, females had higher mean amounts of Ca in their

Table 12. Mean levels (ppm wet wt) of Bi and Pb in gonads of game-farm mallards 30 days after dosing with 0 shot (controls) compared with ducks dosed with six No. 4 Bi shot (by AA Furnace).

		Kind of Shot Dosed	
Element	Sex	0[a]	Bi[a]
Bi	F	0.050	0.677
	M	0.050	0.155
	F & M	0.050	0.468
Pb	F	0.080	0.093
	M	0.080	0.100
	F & M	0.080	0.096

MDL = Method Detection Limit for gonads (ppm wet wt) by AA = 0.15 for Pb and 0.10 for Bi.
[a] N = 20.

Bi
Difference between 0-dosed males and Bi-dosed females: $F_{1,7} = 6.2821$; $P = 0.0406$.

PRT-12A

Table 13. Mean levels (ppm wet wt) of Cu, P, Fe, Ca, Mg, and Zn in gonads of game-farm mallards 30 days after dosing with 0 shot (controls) compared with ducks dosed with six No. 4 Fe shot or six No. 4 Bi shot (N = 10, 11, or 12 for each sex).

		Kind of Shot Dosed		
Element	Sex	0	Fe	Bi
Cu	F	1.76	1.70	1.48
	M	0.985	1.12	1.34
	F & M	1.37	1.41	1.41
P	F	3132.0	3101.7	2566.0
	M	2662.4	2717.4	2917.3
	F & M	2897.2	2909.6	2725.7
Fe	F	56.36	53.49	40.28
	M	12.49	10.66	16.78
	F & M	34.4	32.1	29.1
Ca	F	540.0	590.1	334.4
	M	34.46	34.39	139.6
	F & M	287.2	312.2	241.7
Mg	F	112.8	126.8	125.6
	M	203.4	205.8	201.0
	F & M	158.1	166.3	161.5
Zn	F	23.72	24.56	19.32
	M	13.86	14.32	16.45
	F & M	18.79	19.44	18.01

Cu
Difference between sexes: $F_{1,55} = 13.20$; $P = 0.0006$.
Difference between sexes in 0-dosed ducks: $P = <0.05$.
Difference among doses: $F_{2,55} = 0.03$; $P = 0.9664$.

P
Difference among doses: $F_{2,56} = 0.23$; P 0.7965.

Fe
Difference between sexes: $F_{1,35} = 64.51$; $P = 0.00001$.
Difference between sexes in 0-dosed ducks: $P = <0.01$.
Difference between sexes in Fe-dosed ducks: $P = <0.01$.
Difference between sexes in Bi-dosed ducks: $P = <0.05$.
Difference among doses: $F_{2,55} = 0.57$; $P = 0.5704$.

Ca

Difference between sexes: $F_{1,29} = 19.50$; $P = 0.00001$.
Difference among doses: $F_{2,55} = 0.22$; $P = 0.8021$.

Mg

Difference between sexes: $F_{1,39} = 65.70$; $P \leq 0.00001$.
Difference between sexes in 0-dosed ducks: $P = <0.01$.
Difference between sexes in Fe-dosed ducks: $P = <0.01$.
Difference between sexes in Bi-dosed ducks: $P = <0.01$.
Difference among doses: $F_{2,55} = 0.22$; $P = 0.8051$.

Zn

Difference between sexes: $F_{1,31} = 12.66$; $P = 0.0012$.
Difference among doses: $F_{2,56} = 0.17$; $P = 0.8402$.

PRT-11A

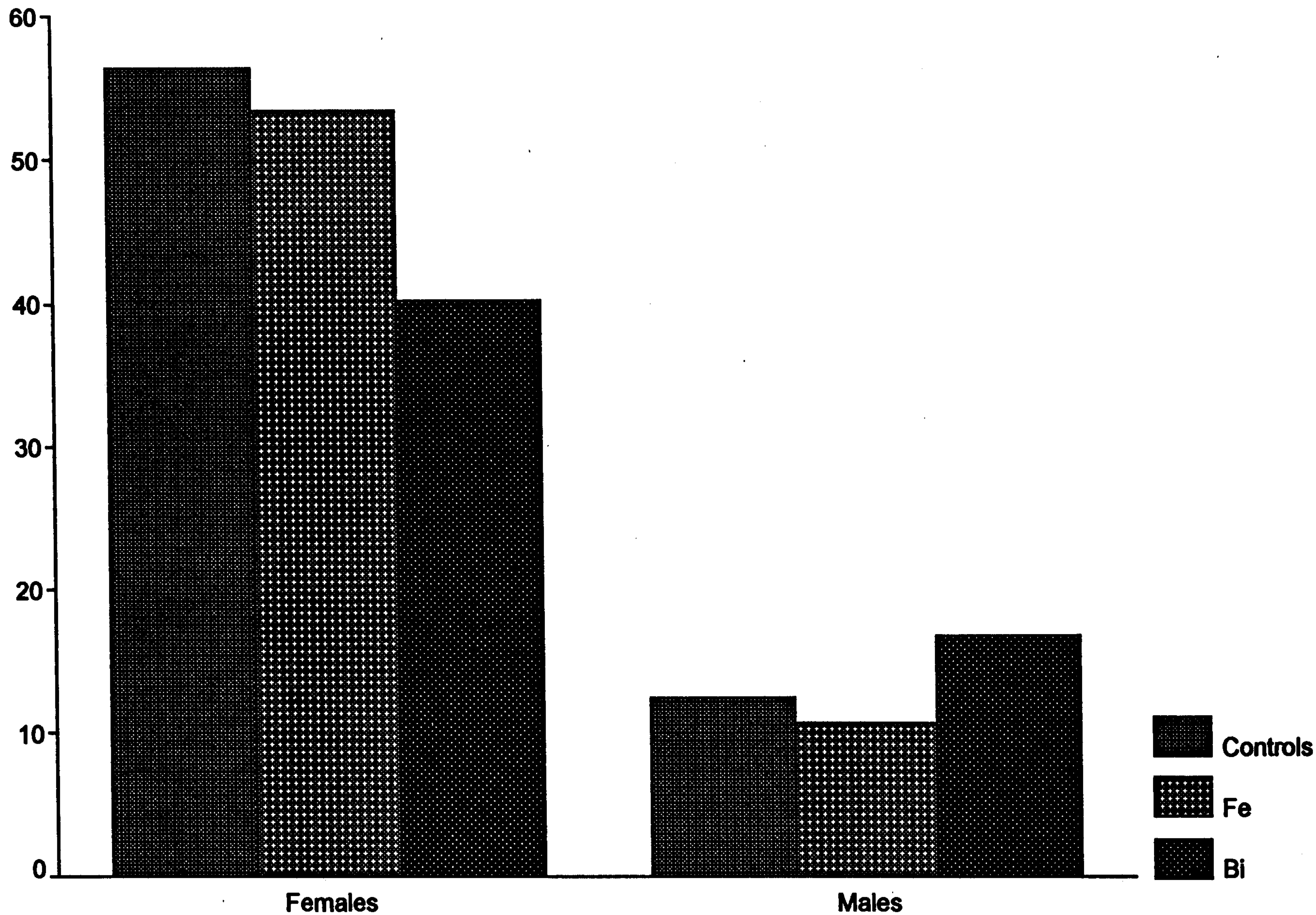

Fig. 2. Fe (ppm wet wt) in gonads of game-farm mallards 30 days after dosing with 0 or 6 No. 4 Fe or 6 No. 4 Bi shot.

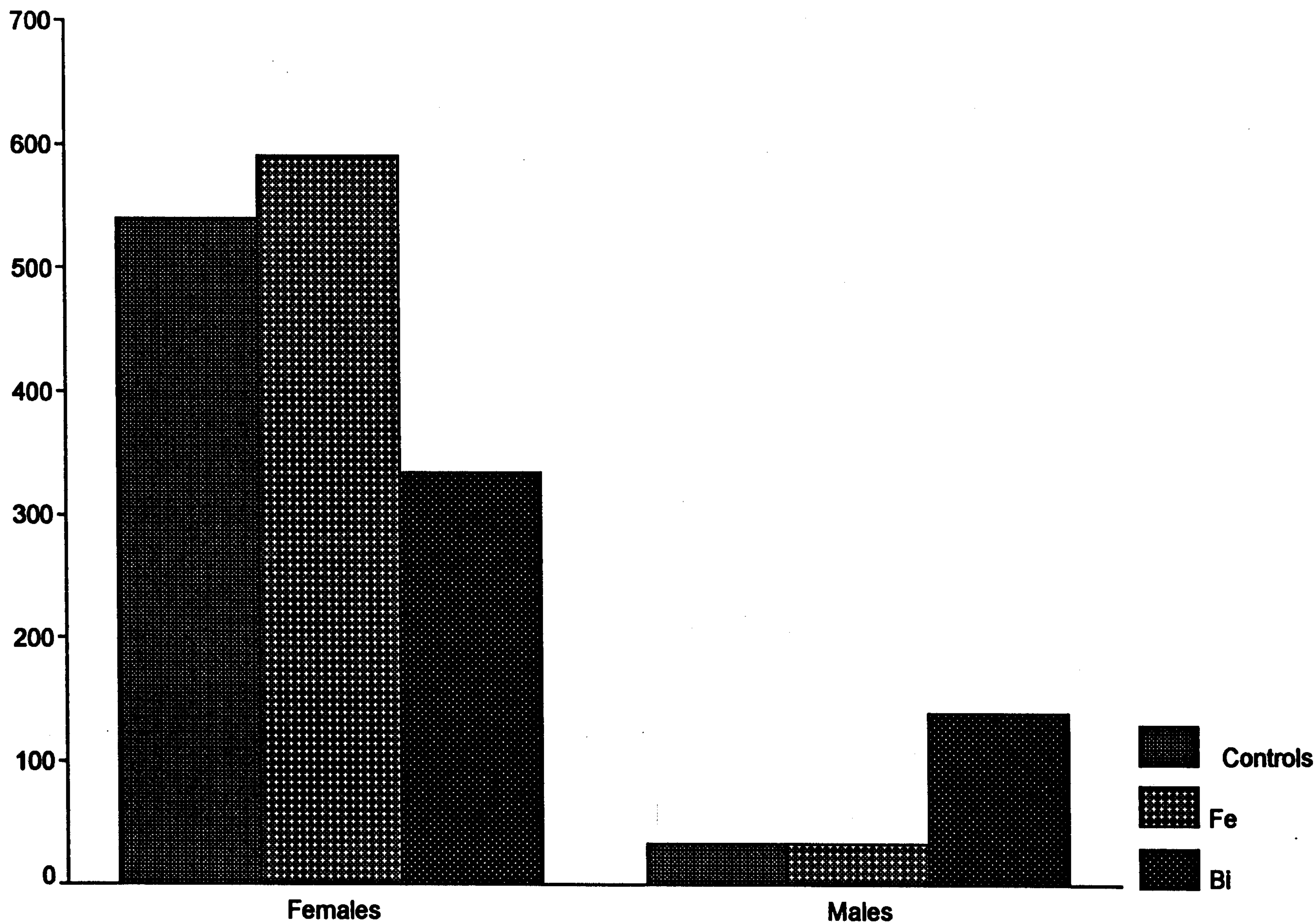

Fig. 3. Ca (ppm wet wt) in gonads of game-farm mallards 30 days after dosing with 0 or 6 No. 4 Fe or 6 No. 4 Bi shot.

gonads than males. There were no differences in the mean levels of Ca in the gonads among doses (Table 13).

Males had higher mean levels of Mg in their gonads than females (Table 13). There were no differences among doses in the mean levels of Mg in the gonads.

Females had higher mean levels of Zn in their gonads than males (Table 13). There was no variation among doses in the mean levels of Zn in the gonads.

Plasma and Blood Cells

The MDL (by ICP) for Bi in plasma was 7.38 ppm (wet wt) for Day 0, 21.8 ppm for Day 15, and 11.8 ppm for Day 30. The MDL for Bi in blood cells was 8.72 ppm for Day 0, 9.35 ppm for Day 15, and 16.3 ppm for Day 30. All mean levels were <MDLs for Bi in plasma and blood cells. After the analyses were run by ICP, the amounts of plasma and blood cells remaining were insufficient to analyze for Bi by AA.

The MDLs for Pb in the plasma were 2.20 ppm for Day 0, 4.42 ppm for Day 15, and 3.34 ppm for Day 30. MDLs for Pb in blood cells were 2.78 ppm for Day 0, 2.68 ppm for Day 15, and 4.69 for Day 30. None of the mean values for Pb residues in the plasma and in the cells were above the MDLs.

The MDLs for Sn in the plasma were 5.81 ppm for Day 0, 7.96 ppm for Day 15, and 10.2 ppm for Day 30. The MDL for Sn in blood cells for Day 0 was 8.06 ppm, for Day 15 was 5.75 ppm, and for Day

30 was 6.37 ppm. All mean amounts were below the detection limit for Sn in plasma and cells on all three days.

The MDLs for Cu in plasma were 0.17 ppm for Day 0, 0.483 ppm for Day 15, and 0.541 for Day 30. The mean amounts of Cu in the plasma were <MDL for Days 15 and 30. There were no differences between sexes or among doses for Day 0 (Table 14). Data for Day 0 are included as baseline data.

There were no sex differences in mean levels of Cu in blood cells on Days 0, 15, and 30. With sexes combined, there were no changes in levels of Cu in the cells Day 0 to Day 15 to Day 30. With days combined, there were no dixfferences in mean levels of Cu in blood cells among doses (Table 15).

There were no sex differences in the mean amount of P in the plasma. There were no differences among doses in the mean amounts of P in the plasma for Days 0, 15, and 30 (Table 14). There were increases in the mean amounts of P in the plasma from Day 0 to Day 15 to Day 30.

There were no sex differences or differences among doses in mean levels of P in blood cells. The mean levels of P in cells did not change from Day 0 to Day 15 to Day 30 (Table 15).

There were no differences between sexes in the amount of Fe in the plasma or in the amount of Fe among doses (Table 14). There was, however, a decline from Day 0 to Day 15 to Day 30 in the amount of Fe in the plasma. No doubt this decline was a result of

Table 14. Mean levels (ppm wet wt) of Cu, P, Fe, Ca, Mg, and Zn in plasma of game-farm mallards dosed with 0 shot (controls) compared with ducks dosed with six No. 4 Fe shot or six No. 4 Bi shot and males compared with females (N = 18, 19, or 20 for each sex).

			Kind of Shot Dosed		
Sex	Day	Element	0	Fe	Bi
F & M	0	Cu	0.334	0.304	0.370
F	0	P	179.0	204.1	196.4
	15		245.1	268.2	225.3
	30		291.1	303.1	270.2
M	0		220.3	201.6	214.6
	15		257.4	261.6	281.7
	30		258.9	251.6	251.3
F & M	0		199.1	202.8	205.5
	15		251.1	264.9	252.3
	30		275.4	277.4	260.1
F	0	Fe	8.40	14.53	10.66
	15		6.31	7.55	5.08
	30		7.47	9.07	6.85
M	0		15.43	7.62	13.85
	15		5.94	5.67	8.19
	30		7.71	6.08	5.17
F & M	0		11.82	11.07	12.21
	15		6.14	6.61	6.59
	30		7.48	7.58	6.03
F	0	Ca	88.34	87.88	86.95
	15		144.4	140.4	119.8
	30		175.7	168.6	167.5
M	0		82.97	80.14	80.40
	15		110.1	116.9	114.8
	30		106.6	110.9	108.8
F & M	0	Ca	85.72	84.01	84.12

	15		127.3	128.6	117.5
	30		141.3	139.8	139.3
F	0	Mg	17.36	19.20	17.76
	15		23.28	24.48	21.67
	30		26.56	27.04	26.21
M	0		17.96	16.31	17.13
	15		22.47	22.72	23.79
	30		24.44	24.28	24.38
F & M	0		17.66	17.76	17.49
	15		22.83	23.60	22.61
	30		25.51	25.66	25.31
F	30	Zn	4.56	4.27	4.63
M			2.83	2.77	2.72
F & M			3.69	3.52	3.64

Cu
Difference among doses; Day 0: $F_{2,112}$ = 0.74: P = 0.4789.
Mean amounts for Days 15 and 30 were <MDLs: 0.483 ppm and 0.541 ppm, respectively. Data for Day 0 are recorded as baseline information.

P
Difference among doses: $F_{2,111}$ = 0.39: P = 0.6747.
Change over time: $F_{2,222}$ = 42.24: P <0.00001.
Interaction between sex and time: $F_{2,222}$ 8.07; P = 0.0005.

Fe
Difference among doses: $F_{2,110}$ = 0.03; P = 0.9747.
Interaction between sex and dose: $F_{2,110}$ = 4.98; P = 0.0085.
Change over time: $F_{2,220}$ = 16.23; P <0.00001.

Ca
Difference between sexes: $F_{1,111}$ = 50.56; P = 0.00001.
Difference among doses: $F_{2,111}$ = 0.56; P = 0.5712.
Change over time: $F_{2,222}$ 87.32; P <0.00001.
Interaction between sex and time: $F_{2,222}$ = 22.08; P <0.00001.

Mg
Difference between sexes: $F_{1,111}$ = 4.40; P = 0.0383.
Difference among doses: $F_{2,111}$ = 0.43; P = 0.6489.
Change over time: $F_{2,222}$ = 102.43; P = 0.00001.

Zn
Difference between sexes; Day 30: $F_{1,114}$ = 63.84; P <0.00001.
Difference among doses; Day 30: $F_{1,114}$ = 0.23; P = 0.7928.
Means for Days 0 and 14 were <MDLs: 3.57 ppm and 41.87 ppm, respectively.

Table 15. Mean levels (ppm wet wt) of Cu, P, Fe, Ca, Mg, and Zn in red blood cells of game-farm mallards dosed with 0 shot (controls) compared with ducks dosed with six No. 4 Fe shot or six No. 4 Bi shot and males compared with females (N = 18, 19, or 20 for each sex).

			Kind of Shot Dosed		
Sex	Day	Element	0	Fe	Bi
F	0	Cu	0.612	0.557	0.681
	15		0.709	0.668	0.624
	30		0.626	0.688	0.699
M	0		0.762	0.956	0.605
	15		0.539	0.590	0.645
	30		0.675	0.626	0.577
F & M	0		0.687	0.757	0.644
	15		0.624	0.629	0.630
	30		0.651	0.657	0.643
F	0	P	2544.2	2310.6	2303.4
	15		2369.4	2394.7	2342.6
	30		2304.8	2211.9	2384.6
M	0		2336.1	2359.0	2451.5
	15		2444.6	2396.2	2282.4
	30		2604.8	2556.0	2554.6
F & M	0		2440.1	2334.8	2375.6
	15		2407.0	2395.4	2322.0
	30		2454.8	2384.0	2462.6
F	0	Fe	803.7	727.1	738.8
	15		769.3	789.2	775.6
	30		732.0	704.9	762.4
M	0		758.2	751.8	776.3
	15		803.2	786.8	747.2
	30		832.0	804.8	830.9
F & M	0		780.9	739.4	757.1
	15		786.2	788.0	761.4

	30		782.0	754.8	793.9
F	0	Ca	32.04	30.98	29.29
	15		42.08	33.22	32.78
	30		45.73	47.84	35.56
M	0		30.22	29.63	34.17
	15		33.91	28.06	42.42
	30		32.32	31.40	39.78
F & M	0		31.13	30.30	31.67
	15		38.00	30.64	37.36
	30		39.03	39.62	37.41
F	0	Mg	123.3	112.8	111.8
	15		117.2	115.6	114.2
	30		110.0	107.6	111.1
M	0		114.7	117.8	116.4
	15		122.3	121.8	115.4
	30		127.1	120.6	122.8
F & M	0		119.0	115.3	114.0
	15		119.8	118.7	115.0
	30		118.6	114.1	116.8
F	0	Zn	7.76	7.22	7.20
	15		7.39	7.27	7.02
	30		7.46	7.15	7.42
M	0		7.19	6.99	7.22
	15		6.99	6.87	6.61
	30		7.64	7.30	7.33
F & M	0		7.48	7.10	7.21
	15		7.19	7.07	6.81
	30		7.55	7.22	7.37

Cu
Difference among doses: $F_{2,113} = 0.11$; $P = 0.8938$.
P
Difference among doses: $F_{2,113} = 0.47$; $P = 0.6293$.
Fe
Difference among doses: $F_{2,113} = 0.48$; $P = 0.6193$.

Ca

Difference among doses: $F_{2,113} = 0.64$; $P = 0.5306$.

Interaction between sex and dose: $F_{2,113} = 5.49$; $P = 0.0053$.

Change over time: $F_{2,226} = 7.03$; $P = 0.0011$.

Mg

Difference between sexes: $F_{1,113} = 5.85$; $P = 0.0171$.

Difference among doses: $F_{2,113} = 0.85$; $P = 0.4282$.

Zn

Difference among doses: $F_{2,113} = 1.18$; $P = 0.3115$.

PRT-15B

switching from a diet of duck pellets to corn, which is low in Fe, on Day 0.

There were no differences between sexes or in changes in the mean levels from Day 0 to Day 15 to Day 30 of Fe in blood cells (Table 15). With sexes combined, there were no differences in mean levels of Fe in blood cells among doses on Day 0 vs Day 15 vs Day 30.

Females had higher amounts of Ca in their plasma than males (Table 14). There were no differences among doses in the mean amounts of Ca in the plasma, but there were increases in mean levels of Ca in plasma from Day 0 to Day 15 to Day 30 in both sexes for all doses.

There were no sex differences in the mean levels of Ca in the blood cells and no differences among doses. There was, however, a change over time and an interaction between sex and dose, with females showing consistent and larger increases over time than males, which showed smaller and less consistent increases from Day 0 to Day 15 to Day 30.

Females had higher mean amounts of Mg in the plasma than males. There were no differences among doses in the mean amounts of Mg in the plasma, but there were increases over time for all three doses.

In contrast to plasma, red blood cells of males had higher mean amounts of Mg than females (Table 15). There were no differences among doses in the amount of Mg in blood cells, and no changes in the mean levels of Mg in blood cells from Day 0 to Day 15 to Day 30.

The MDLs for Zn in plasma were 3.57 ppm for Day 0, 4.87 ppm for Day 15, and 0.613 ppm for Day 30 (Table 14). Only for Day 30 were the mean values above the MDL. Only data for Day 30 are included in this report. Females on Day 30 had higher mean levels of Zn in their plasma than males (Table 14, Fig. 4). There were no differences among doses in the mean amount of Zn in the plasma on Day 30.

There were no sex differences, differences among doses, or changes over time in the mean amounts of Zn in blood cells.

Feces

All amounts of Bi in the feces were <MDL (103 ppm, dry wt) on Day 0. There was little change in the amount of Bi in the feces of 0-dosed and Fe-dosed ducks on Days 1 and 2. Statistical analyses were not run because several or all of the amounts were <MDL. For Bi-dosed ducks, Bi in the feces increased from <MDL on Day 0 to 2275.4 ppm on Day 1 to 3689.0 ppm on Day 2. These high levels were maintained to the end of the 30-day study--3636.0 ppm for Days 1-10 combined and 3485.4 ppm for Days 22-30 combined (Table 16).

The main purpose of collecting and analyzing the feces was to calculate the percentage of the Bi shot dissolved in the gizzard that was excreted in the feces. We estimated that 90% of the feces was recovered. The remaining 10% fell outside the pans and onto the ground under the pens, adhered to pans when feces were

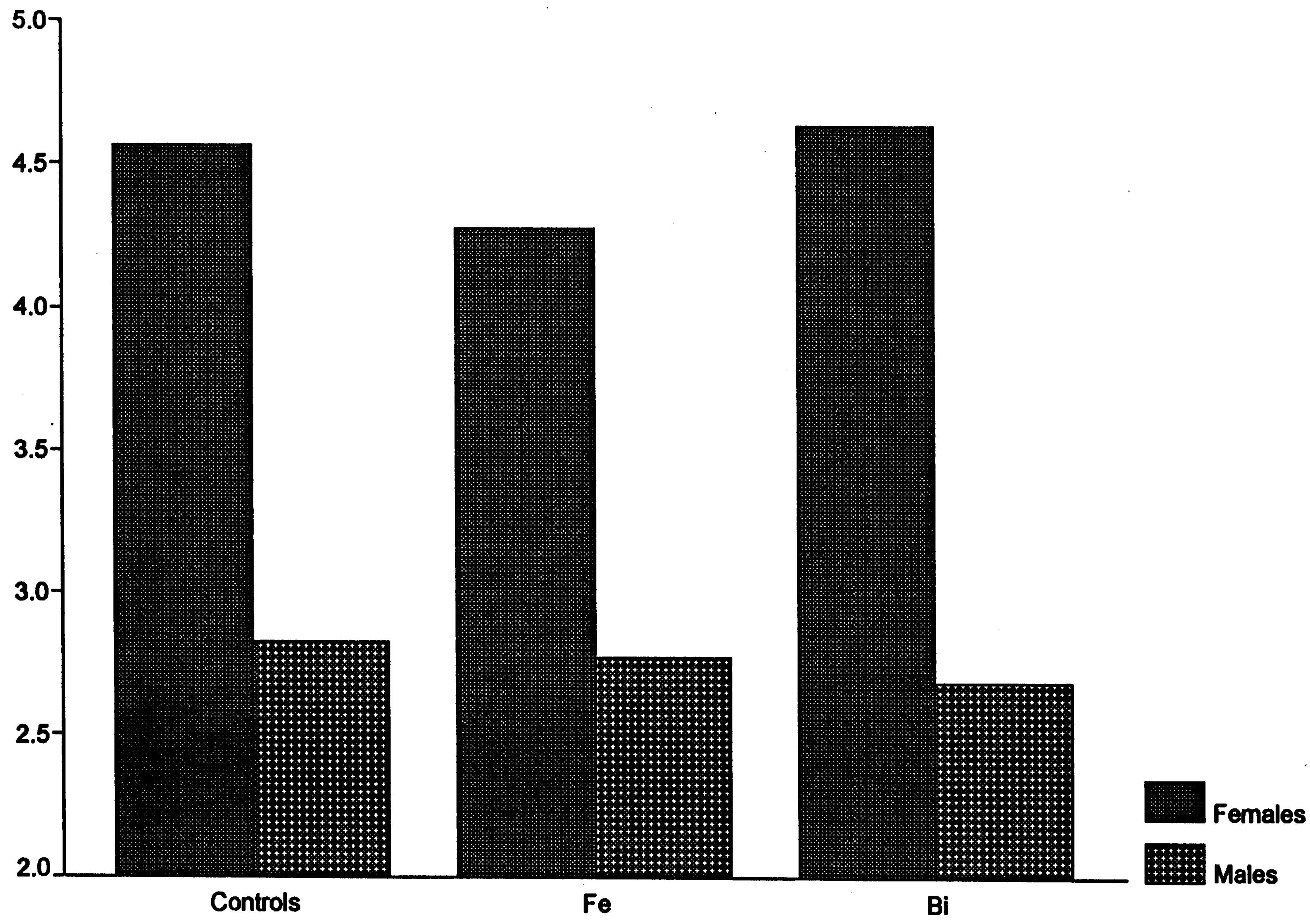

Fig. 4. Zn (ppm) in plasma on Day 30 in game-farm mallards 30 days after dosing with 0 or 6 No. 4 Fe or 6 No. 4 Bi shot.

Table 16. Mean levels (ppm dry wt) of Bi, Sn, and Fe in feces of game-farm mallards (sexes combined) dosed with 0 shot (controls) compared with ducks dosed with six No. 4 Fe shot or six No. 4 Bi shot.

		Kind of Shot Dosed		
Element	Days[a]	0	Fe	Bi
Bi	0	<MDL[b]	<MDL[c]	<MDL[d]
	1	<MDL[b]	<MDL[b]	2275.4[e]
	2	<MDL[b]	<MDL[c]	3689.0[e]
	1-10	<MDL[b]	<MDL[a]	3636.0[f]
	11-30	<MDL[a]	<MDL[a]	3485.4[f]
Sn	0	<MDL[b]	<MDL[c]	<MDL[d]
	1	<MDL[b]	<MDL[b]	184.6[e]
	2	<MDL[b]	<MDL[c]	111.1[e]
	11-30	<MDL[b]	<MDL[b]	67.29[e]
Fe	0	1174.0[b]	1054.8[c]	955.2[e]
	1	809.0[b]	2241.7[b]	719.7[e]
	2	357.7[b]	2611.0[c]	513.8[e]
	11-30	519.7[b]	2288.3[b]	348.8[f]

[a] Days after dosing; 0 = day of dosing.
[b] N = 3.
[c] N = 4.
[d] N = 5.
[e] N = 10.
[f] N = 9.

MDL = Method Detection Limit by ICP:
MDL for Bi = 103 ppm (dry wt) for Days 0, 1, 2, and 1-10 and 58.9 ppm for Days 11-30.
MDL for Sn = 19.5 ppm (dry wt) for Days 0, 1, and 2 and 14.9 ppm for Days 11-30.
MDL for Fe = 31.8 ppm (dry wt) for Days 0, 1, and 2 and 19.2 ppm for Days 11-30.

collected, were blown out of the pans by high winds accompanied by rain, or adhered to the plastic bags when the samples were removed for weighing and analysis.

All fecal samples that were analyzed were dried and weighed. By using the level of Bi in each sample--daily levels for Days 1-10 and one level for the combined sample for Days 11-30--we calculated the amount of Bi excreted in the feces of each Bi-dosed duck and compared the amount with the amount dissolved from the six Bi shot dosed in the same duck. We calculated that 88.4% of the Bi dissolved from the shot in the gizzards was excreted in the feces. The calculated percentages of Bi dissolved from the shot that was excreted in the feces of each of the 10 ducks ranged from 61.0% to 102.6%.

Sn in the feces of all ducks was <MDL (19.5 ppm, dry wt) on Day 0 and remained below the MDL for Day 1 and Day 2 in 0-dosed and Fe-dosed ducks. In the Bi-dosed ducks, Sn increased to 184.6 ppm on Day 1 and 111.1 ppm on Day 2 and declined to 67.29 ppm for Days 11-30 combined (Table 16).

Sn comprised only 2% of the dosed Bi shot compared with 98% Bi. In the feces analyzed from 10 Bi-dosed ducks, Sn increased from <MDL (19.5 ppm, dry wt) on Day 0 to a range of 47.6 to 321 ppm on Day 1. By making calculations similar to those made for Bi, we estimated that 84.9% of the Sn dissolved from the Bi shot was excreted in the feces. The calculated percentages of Sn dissolved

from the shot that was excreted in the feces of each of the 10 ducks ranged from 57.6 to 127.4%.

The mean levels of Fe in the feces of 0-dosed ducks declined from 1174.0 ppm on Day to 809.0 ppm on Day 1 to 357.7 ppm on Day 2, but rose to 520 ppm for Days 11-30 combined. After dosing (Day 0), in Fe-dosed ducks, the mean levels of Fe increased from 1054.8 ppm to 2441.7 ppm on Day 1 to 2611.0 ppm on Day 2. In Bi-dosed ducks the mean levels of Fe decreased from 955.2 ppm on Day 0 to 719.7 ppm on Day 1 to 513.8 ppm on Day 2 to 348.8 ppm average for Days 11-30. Although there was a strong correlation between dosing with Bi shot and the decrease of Fe in the feces during the 30-day study, there was a similar decline in the mean level of Fe in the feces of 0-dosed ducks from Day 0 (1174.0 ppm) to Days 1 (809.0 ppm) and 2 (357.7 ppm) to an average of 519.7 ppm for Days 11-30.

We assume that the sharp declines in the amount of Fe in the feces of 0-dosed and Bi-dosed ducks from Day 0 to Days 1 and 2 were the result of changing from a diet of commercial duck pellets on Day 0 to a diet of corn, which is low in Fe.

DISCUSSION

In most cases where there were no significant differences related to dosing (e.g., survival, body weight, organ weights, amounts of elements in tissues, etc), we do not discuss the results beyond what is included in the RESULTS portion of this report. In a few cases (e.g., Cu in the liver) we discuss sex differences that were not related to dosing because they appear to be different from some results reported in the literature.

Copper

We found approximately twice the amount of Cu in the livers of males as in the livers of females (Table 11). Underwood (1971:61, 63), discussing Cu in the liver, states, "There is no effect of sex, except in the Australian salmon (*Arripis trutta*) in which the female carried higher concentrations than the male." Hanson and Jones (1974) found levels of Cu in the feathers of female Ross' geese (*Anser rossii*) that were significantly higher than in males. They presumed estrogen was responsible for the difference.

Van Campen (1971:214) reported that "Administration of estrogens induces large increases in serum copper in humans, rats, and swine.", and that androgens increased serum Cu levels in humans. Hill and Matrone (1961) found that when both Cu and Fe were low in the diet an increase in one partly compensated for the deficiency of the other. Matrone (1960) concluded that Cu absorption is not directly affected by Fe. Thus, the Fe:Cu interaction is affected by something other than absorption. The diet of our ducks (both females and males), corn, is low in Fe, but dosing with Fe shot did not have a significant effect on the level of Cu in the livers.

Van Campen (1971:221-222) states, (pp. 221-222) that "The factors that are most influential in determining the tissue levels of copper are age, hormones, disease and diet. . . . calcium apparently can either increase of decrease copper absorption, depending on the composition of the diet to which they are added."

In the present study, females had higher mean levels of Cu in their gonads than males, which is in contrast to kidneys and livers where males always had higher mean levels of Cu.

Phosphorous

The lower levels of P in livers of Fe-dosed males, as compared with females, were manifested by decreases of P in the livers of Fe-dosed males. P levels were only slightly higher in the livers of Fe-dosed females than in 0-dosed females. Dosing with Bi shot also caused a decrease in the amount of P in livers of males, which resulted in a significant difference between the sexes. P was essentially the same in 0-dosed females and Bi-dosed females (Table 11).

Iron

Dosing with Fe shot resulted in large amounts of Fe deposits in the livers, but dosing with Bi shot did not significantly affect the levels of Fe in the liver (Table 11). Although females dissolved a higher percentage of the dosed Fe shot than males (Table 3), the Fe-dosed females did not have significantly higher levels of Fe in their livers than the Fe-dosed males.

Females had significantly higher amounts of Fe in their gonads than males. These sex differences in the amount of Fe in the gonads may be related to the preparation of the ovaries for egg laying. There were no differences in the mean levels of Fe in the gonads attributed to dosing with either Fe shot or Bi shot.

Calcium

It appears that dosing with Fe or Bi shot is associated with lower amounts of Ca in the livers and kidneys of ducks as compared with controls (Table 9).

Forth and Rummel (1971:182) reported that the transfer of Fe through the small intestine of the rat was inhibited by an increased amount of Ca and that the transfer of Ca was inhibited by an increased amount of Fe. They concluded that it was possible there is "a common transport mechanism for iron and calcium . . .." Perhaps Bi results in a similar reduction in the transfer of Ca, although Bi has apparently not been studied in this context.

There were significant increases in the Ca in the plasma of both males and females for all dosed groups from Day 0 to Day 15 to Day 30 (Table 14). The increase cannot be related to increase in Ca in the diet because after dosing, all ducks were on a corn diet, and corn is low in Ca. There was no variation in the Ca in the plasma among doses.

Feces

Both Bi and Sn greatly increased in the feces of Bi-dosed ducks the day after dosing. The excretion of Bi at high levels in the feces continued to the end of the 30-day study. Mean levels of Bi were not substantially different between 0-dosed and Fe-dosed ducks on Days 0, 1, and 2. Bi was much higher in feces of Bi-dosed ducks than in either 0-dosed or Fe-dosed ducks on Days 1 and 2.

Although the mean level of Sn in the feces was higher at the end of the 30-day study than the background level found the day prior to dosing, the mean level of Sn in the feces declined significantly after Day 10.

Our findings for Sn in the feces appear to support Underwood (1971:451-452) who reported that the available evidence shows that in man Sn is poorly absorbed, poorly retained, and excreted primarily in the feces. In man, the amount of Sn ingested with food was approximately the same as the amount excreted in the feces. Underwood's conclusion was that Sn shows little toxicity, probably because of its slow absorption rate and high excretion rate in the feces.

The mean level of Fe declined sharply in the feces of both 0-dosed and Bi-dosed ducks starting on Day 1. The decline continued to the end of the study in Bi-dosed ducks. Feces of 0-dosed and Fe-dosed ducks were not analyzed for the entire study. The decline of Fe in the feces of 0-dosed and Bi-dosed ducks was probably a result of switching from a diet of commercial duck food to corn, which is low in Fe, on Day 0.

HISTOPATHOLOGY REPORT

George L . Foley, DVMS, PhD, Diplomate ACVP

Following formalin fixation, representative samples were obtained from each of the 60 ducks submitted for histopathologic examination. Sections of gonad (testis or ovary), liver, kidney, and gizzard were trimmed and sectioned at 4 microns. Glass slides were stained with hematoxylin and eosin. All ducks were examined without knowledge of group assignment and results were recorded. (The raw data were submitted to G.C. Sanderson). Subsequently, group assignment and weight data were correlated with histologic findings for interpretation.

I have identified lesions in the testes, ovaries, kidneys, and livers in control and dosed ducks. I have not found any correlation between the presence of any specific lesion and dosage. The mild inflammatory changes I have seen histologically would be consistent with the range of normal for these birds.

<u>Gonadal Lesions</u>

Female

Many of the females had a mild to moderate lymphocytic oophoritis without any evidence of etiology in the sections examined. Typically this lesion consisted of scattered lymphocytes and plasma cells in the interstitium of the ovary. One 0-dosed female had severe necrotizing oophoritis, which is consistent with an egg yolk peritonitis. Two other Bi-dosed females had milder lesions with necrotic areas and these may represent resolving cases of egg yolk peritonitis. There was a dramatic range of ovarian weights in all groups examined.

Male

There was no inflammatory changes noted in the male ducks examined with the exception of one Fe-dosed duck and one Bi-dosed duck. There were scattered aggregates of lymphocytes and plasma cells in the testis of the Bi-dosed male. In the Fe-dosed duck there was a small sperm granuloma on one section of the slide with the residual parenchyma within normal limits. One Fe-dosed duck had evidence of a locally extensive tubular atrophy consisting of a decreased height in the seminiferous epithelium in one zone of the examined testis. The adjacent tubules were within normal limits and there was no evidence of inflammation. In one Bi-dosed duck there were multinucleated giant cells (presumably sloughed spermatocytes) within scattered tubules. Evidence of normal production of spermatozoa was present on the slide. A percentage of ducks in all three groups had mild vacuolar changes in the seminiferous epithelium. These ducks had spermatozoa within the genital ducts and in seminiferous epithelium.

Liver

Nearly all ducks had a variable amount of lymphocytes and plasma cells within the liver. The most common pattern was around the portal triads. Occasionally, the inflammatory cells formed small nodules scattered in the parenchyma. One 0-dosed male had abscesses within the liver, which most likely represent an acute bacterial infection. The hepatic lipidosis seen histologically roughly correlates with the heavier liver weights at necropsy.

Kidney

Nearly all ducks had a variable amount of lymphocytes and plasma cells in the wall of the ureter.

Gizzard

No lesions were seen in any of the gizzard sections examined.

CONCLUSIONS

We observed no toxic effects of the dosed Bi shot on survival, body weight, PCVs, and organ weights of ducks in the present study. Gross and microscopic pathological studies of the kidneys, liver, and gonads of the ducks also revealed no indication of toxicity (see below). The results of this study support the conclusions of our earlier study, which was completed in 1992 and involved dosing game-farm mallards with 100 percent Bi shot. Under the conditions of these two studies, we observed no toxic effects of ingested Bi shot in game-farm mallards.

There were a number of significant sex differences in organ weights and mean levels of individual elements in the organs. These differences appear to be related to the physiological changes associated with the approaching breeding season; especially in females, which are preparing for egg laying.

We found a few correlations related to dosing with Fe shot and with Bi shot, but saw no toxic effects of dosing with either. For example, livers and kidneys of both Bi-dosed and Fe-dosed ducks had lower mean levels of Ca than livers and kidneys of 0-dosed ducks, but there was no significant difference in the mean levels of Ca in the livers of Fe-dosed ducks vs Bi-dosed ducks (Table 11).

With two exceptions, (kidneys of males, mean of 0.528 ppm, and livers of males, mean of 0.246 ppm) we found no Bi in the livers, kidneys, or gonads of 0-dosed ducks. The mean level of Bi in kidneys of Bi-dosed females was 8.05 ppm (range 4.69 to 12.6 ppm); in Bi-dosed males the mean level was 4.77 ppm. The mean level of Bi in livers of Bi-dosed females was 2.79 ppm and ranged from 1.19 to 5.63 ppm. The differences for Bi-dosed vs 0-dosed ducks were significant for both kidneys and livers. Both gross observations and histological study by the pathologist detected no toxic effects of these mean levels of Bi on the kidneys, liver, or gonads.

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