Toxicity of Ingested Bismuth Alloy Shot in Game-farm Mallards

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

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Acknowledgments

Abstract

In a 30-day study involving penned game-farm mallards (*Anas platyrhynchos*), no harmful health effects were detected from dosing with either six, No. 4, bismuth/tin (Bi/Sn) alloy shot or six, No. 4, steel (Fe) shot, as compared with sham (0 shot) dosing. Survival, hematocrit (Hct) values, body weight, and mean weights of kidneys, livers, gonads, and gizzards were not affected. Mean concentrations of nutritionally essential elements (calcium [Ca], phosphorous [P], magnesium [Mg], zinc [Zn], copper [Cu], Fe, and Sn) were different among doses and between sexes in kidneys, livers, and gonads. However, concentrations of these elements in these organs and tissues in Bi-dosed ducks were not different from both 0- and Fe-dosed ducks. Bi/Sn alloy shot, as tested in this study, elicited no indications of toxicity in game-farm mallard ducks.

Introduction

To protect waterfowl from poisoning caused by ingested lead (Pb) shot, nontoxic shot regulations were implemented for waterfowl hunting on areas with severe problems with Pb poisoning ("hotspots") in the United States beginning in the early 1970s (Anderson 1992). Federal regulations became nationwide in 1991. Several European countries have converted or are planning to convert to nontoxic shot for waterfowl hunting (Moser 1992) and Canada will implement a nationwide ban on lead shot for all migratory bird hunting in 1997 (Canadian Wildlife Service [CWS] 1995). From the 1970s to the early 1990s, Fe was the only shot material approved as nontoxic by the U.S. Fish and Wildlife Service (USFWS) (Longcore et al. 1974). Although hunters have generally adapted to using Fe shot, some have urged that a search for alternative shot with greater ballistic capability be continued. Specifically, they wanted a shot that is non-toxic, inexpensive, and ballistically similar to lead shot. There have been numerous evaluations of potential substitute shot. Irby et al. (1967) evaluated three types of plastic-coated Pb, two Pb/Mg alloys, Fe, Cu, Zn-coated Fe, and molybdenum-coated Fe. Longcore et al. (1974) evaluated Pb shot with nickel coatings, Pb/phosphor Sn alloy shot, Pb shot with Sn/ nickel alloy coatings, steel shot with Pb coatings, Pb/Sn alloys, two types of disintegrable Pb shot, and Pb shot with biochemical additives. Haseltine and Sileo (1983) evaluated uranium. No satisfactory alternative was found until 1990, when John E. Brown, St. Catherines, Ontario, was awarded a U.S. patent for Bi shot.

Our primary objective was to determine if Bi/Sn alloy shot caused toxic effects in captive game-farm mallards. Secondly, if toxic effects were manifest, we wanted to associate toxic effects with amounts of Bi and other elements in the tissues. Our study complied with the "Acute Toxicity Test" guidelines of the USFWS and the CWS. Dr. Simon Nadeau, CWS, and Dr. Keith A. Morehouse, USFWS, reviewed our protocol before we initiated our study.

Literature Review

The first known report of metallic Bi dosed in 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 doses that averaged 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 birds (mallards). They followed with tests on ingested Bi/Sn alloy shot (Sanderson et al. 1997b) and the present study. Sanderson et al. (1997b) reported that reproduction of game-farm mallards was not affected after chronic dosing with Bi/Sn alloy shot. Sanderson et al. (1997a) reported no toxic effects of Bi shot embedded in the breast muscles of game-farm mallards.

The International Commission on Radiological Protection (ICRP) (1960) reported that Bi is rapidly excreted by the kidneys except for small amounts retained in these organs. Some Bi is lost in bile. The estimated half-time for elimination in humans was about 5 days. Hamilton et al. (1972/ 1973) reported 0.4 µg/g Bi (wet weight) in kidneys and 0.004 µg/g Bi in livers of autopsy cases (humans) with no known exposure to Bi. Kidneys of 22 individuals who had been given Bi salicylate had 33 µg/g Bi and livers had 6.8 µg/g Bi.

Lee (1981) reported that after treatment was terminated, Bi declined about 2.6% in the urine daily, with half purged from the body in about 20 days. Fowler and Vouk (1979:348) stated, "Ingested bismuth is largely eliminated unabsorbed in feces. Model values for the daily balance of bismuth in reference man are: dietary intake 20 µg/g, fecal elimination 18 µg/g, urinary excretion 1.6 µg/g. . . . Absorbed bismuth is mainly excreted in the urine."

Oehme (1979) reported that soluble and insoluble Bi salts, suspended in oil to maintain levels in blood, were injected to treat syphilis. Other Bi compounds were used to treat malaria and amebiasis. Medicinal use of Bi decreased with the advent of newer treatments. Most human exposure to Bi is in compounds that are insoluble and not readily absorbed whether ingested or applied to the skin. Apparently tissue binding is slight, even when Bi is absorbed. An equilibrium is established among tissues, blood, and urine. Kidneys have the highest amounts of Bi, with the liver generally a poor second. With few exceptions, Bi compounds present no problems whether by ingestion, inhalation, or dermal application. Poisoning from industrial exposure is rare (Oehme 1979).

There are no federal standards for Bi or its compounds and no evidence linking Bi or Bi compounds with industrial poisoning. Also, all episodes of Bi poisoning were from soluble compounds used in medicine, and fatalities and near fatalities were mainly from intravenous or intramuscular injection of soluble salts (Key et al. 1977). Venugopal and Lukey (1978) reported that low solubility limits the toxicity of Bi compounds, which are highly toxic. Locke et al. (1987) reported neurotoxic effects at Bi concentrations of < 0.1 µg/g in blood.

Thomas et al. (1988:124) reported, "Since toxicity resulting from environmental or industrial exposure to bismuth or any of its compounds is not a problem, levels of tolerance have not been identified . . . ." Bi telluride, which is used as a semiconductor in the electronics industry (Oehme 1979), is an exception. Thomas et al. (1988) reported, however, that the French Ministry of Health banned the sale and use of all Bi compounds, and that Australia restricted the use of Bi subgallate. These authors suggested that in humans amounts of Bi in blood > 0.48 µmol/L (0.1 µg/mL) are potentially dangerous, that amounts > 0.05 and < 0.1 µg/mL call for careful monitoring of patients, and that amounts < 0.05 µg/mL are considered safe.

Krigman et al. (1985) reported that levels of Bi in blood of humans differ between individuals who show side effects from chronic use of Bi and those who do not. Those who show no effects usually have < 0.05 µg/g Bi in their blood whereas those who show symptoms have > 0.05 µg/g Bi in their blood. These authors conclude that levels of Bi in blood > 0.05 µg/g indicate a high risk and amounts < 0.05 µg/g indicate a low risk. Other investigators (Hillemond et al. 1977; Serfontein and Mekel 1979) concluded that 0.05 µg/g Bi in blood is potentially neurotoxic. Dipalma (1988) stated that levels of Bi in blood should not be > 0.02 µg/g.

Dipalma (1988) reported that exposure of humans to Bi is not considered a serious industrial hazard. According to him, there are few data on Bi concentrations in blood from either oral or topical applications because of the assumption that absorption of Bi is low. Dipalma (1988) indicated 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 µg per L (20 ppb)."

In their review, Slikkerveer and de Wolff (1989) summarized the effects of Bi in mammals and 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 µg/g 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 µg/L of Bi in their blood. Although the site of Bi absorption in the gastrointestinal tract is unknown, Slikkerveer and de Wolff 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 concentrations of Bi were always in the kidney. After 14 months of dosing with colloidal Bi subcitrate in rats, Bi concentrations ranked from high to low in kidney, lung, spleen, liver, brain, and muscle tissues. When bone concentrations were measured, they were usually 10-20 times lower than in the kidney.

Slikkerveer and de Wolff (1989) reported that 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, concentrations in bone were 1.5-6.7 $\mu g/g$ wet weight compared with < 1 $\mu g/g$ wet weight in nonexposed individuals. Bi encephalopathy is mainly supported by elevated blood Bi. A steady-state Bi concentration of > 100 $\mu g/L$ (ppb) of blood in humans was arbitrarily suggested as an "alarm" level and 50 $\mu g/L$ was considered a "safety" level, but no proof supports these choices. Concentrations of blood Bi from 10 to 4,600 $\mu g/L$ were found in 618 Bi encephalopathy patients.

Abbracchio et al. (1985) administered tri-potassium-dicitrato bismuthate intraperitoneally and by gavage in laboratory rats. After intraperitoneal injection, Bi reached peak concentrations in blood within 30 minutes and declined rapidly. When a dose 10 times higher was given by gastric intubation, much lower blood concentrations 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."

Gregus and Klaassen (1986) reported that feces and urine were equally important for the excretion of injected Bi compounds in rats. Biliary excretion apparently determined the fecal excretion of Bi, but the percentage of dosed Bi excreted in the bile was independent of the amount dosed.

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 $\mu g/g$ of Bi subnitrate intraperitoneally in laboratory mice. Although Bi concentrations in blood and brain tissues of mice that showed signs of neurotoxicity were significantly higher than in dosed mice that showed no signs, they concluded that the concentration of Bi in blood did not predict neurologic signs. They suggested that 6 $\mu g/g$ of Bi in the brain show neurologic symptoms and that a concentration of ≥ 0.5-2.0 $\mu g/g$ 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 $\mu g/g$ Bi in the brain was associated with motor dysfunction in humans and mice and that concentrations above 50 $\mu g/L$ (ppb) are necessary to produce frank encephalopathy in humans.

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.

Methods

The Bi/Sn shot used in this study contained 0.0040% to 0.0186% Pb ($\bar{x}$ = 0.0094%, SD = 0.0054%). Because Pb made up < 0.1% of the test shot, Environment Canada (1992) guidelines did not require that tissues be analyzed for Pb. We recognized, however, that researchers are interested in Pb, so we included this metal, albeit at a somewhat high detection limit, in the analyses for residues.

Seventy-five female and 75 male wild-type game-farm mallards 6 to 8 months of age were purchased from Whistling Wings, Hanover, Illinois. The ducks, reared on a 60-acre lake, were transported from Hanover to Champaign, Illinois, by truck in crates on 22 March 1994.

Toxicity Study

The ducks were weighed and one duck was randomly assigned to each 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 randomly selected from each dosing group for collection of feces to be analyzed for excreted Bi, Fe, and Sn. Sanderson et al. (1997b) describe the methods we used to randomize the doses, ducks, and pen assignments for the present study.

The pens were consecutively numbered, elevated, outdoor, 1-m^2 structures. They were covered with vinyl-coated, 25.4-mm mesh, 14-gauge wire. A 9.1-m x 36.6-m pavilion (roof but no sides) covered the pens (see Sanderson et al. 1992 for more details).

Facilities for holding the ducks were inspected and approved by several members of the Laboratory Animal Care Committee, University of Illinois, after the ducks were placed in the pens. The

committee also inspected the facilities once during the study. Commercial duck pellets (Heinhold 17% Duck Finisher Pellet™, Heinhold Feeds, Inc., Kouts, Indiana) 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 replaced with whole shelled corn *ad libitum* for the duration of the study. Protocols of the CWS and the USFWS specified these diets (Environment Canada 1992).

The three groups of ducks were each dosed as follows: sham dosed (controls); six, No. 4 (3.30 mm diameter), Fe shot; or six, No. 4, Bi shot. Ducks in each group are hereafter referred to as 0-dosed (controls), Fe-dosed, and Bi-dosed.

We began the study on 12 April 1994 (Day 0) when we weighed, collected blood samples, and dosed the ducks. A small plastic funnel fitted with a plastic tube (9.5 mm outside diameter, 22.9 cm long) was inserted through the pharynx into the proventriculus. To reduce friction, the tube was kept in a pail of water between dosings. Each dose of shot was poured into the funnel and flushed into the proventriculus with approximately 5 mL of water. Controls were treated the same except that no shot were included. Before dosing, the shot were counted, weighed, and placed in individual vials in the laboratory. The type, number, and weight of each dose 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 (> 1% by wt in shot) and for major nutritionally essential elements (Ca, P, Mg, Zn, and Cu). Twenty-gauge, 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.

As each group of 24 hematocrit samples was collected, the samples were centrifuged at the site in a mobile laboratory (house trailer). The tubes were spun for 5 minutes at 11,500 RPM at 13,000-g force, after which the values were read and recorded.

The whole blood samples also were centrifuged at the site, in groups of 12 samples (capacity of the centrifuge) at a time. The tubes were spun for 5 minutes at 3,000 RPM. The plasma was removed with micropipettes and placed in 5-mL nonheparinized Vacutainer tubes. The cells were retained in the 10-mL lithium heparinized tubes. As the plasma and cells were separated, the tubes were placed in racks and put on ice in a styrofoam cooler. All samples were stored in a freezer (-10°C) until analyzed.

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

The 120 ducks were weighed and blood was collected from the wing veins, as scheduled, on Day 30 (12 May 1994). Following these procedures, the ducks were killed by decapitation and necropsied on the same day (with the exception noted below). The gizzard, liver, kidneys, and gonads were excised from each duck.

Two changes were made in the methods as originally approved by the CWS. First, because voided shot were not found in the feces, 20 dosed ducks were radiographed to obtain a positive record of shot retention in the gizzards. The 20 ducks for which daily fecal samples were being collected were chosen 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 or left lateral view radiograph were made for each duck on Day 23 (5 May 1994) by the College of Veterinary Medicine, University of Illinois.

The other change in the methods involved killing the ducks and performing the necropsies over 2 days (instead of 1) to ensure that tissue samples were obtained from freshly killed birds. The pathologist necropsied 30 ducks on 12 May and the other 30 ducks on 13 May 1994.

After the pathologist had examined, weighed, and fixed representative samples of kidneys, liver, and gonads in 10% formalin for histopathology, the remaining residual tissue from these organs was placed in separate, numbered, plastic bags and stored in a freezer as backup samples. Organs from the remaining 60 ducks, which were not necropsied, were removed and weighed, placed

in individual, numbered, plastic bags, and stored in the freezer as additional backup samples.

We took representative samples, after fixing in 10% formalin, from each of the 60 necropsied ducks and examined the samples histopathologically. Sections of gonad (testis or ovary), liver, kidney, and gizzard were embedded, trimmed, and sectioned at 4 microns. Tissues on glass slides were stained with hematoxylin and eosin by standard methods. All ducks were examined by a veterinary pathologist, who did not know the dose history of the ducks. Later, we associated group assignment and weight data with histologic findings to aid in interpretation.

Chemical Analyses
Storage of Samples
Samples were inventoried when received, stored at -10°C, and monitored daily. Samples were allowed to thaw to room temperature, then prepared for metal analysis by labelling by tissue type and a number for identification. The sex of the duck and the shot dose it received were not disclosed to the individuals who analyzed the samples.

Digestions of Samples
Blood cells, blood plasma, livers, kidneys, gonads, and feces were acid digested before analysis for metals with inductively coupled, argon-plasma emission spectroscopy (ICP) and graphite-furnace, atomic-absorption spectroscopy (GFAA). Because wet weight concentrations of the blood and organs were desired, these samples were not dried before digestion. Feces were dried at 104°C to determine percent moisture. The concentrations of metals measured in fecal samples are on a dry-weight basis. We analyzed for Bi, Sn, Fe, Pb, Ca, Mg, P, Zn, and Cu. ICP was used to measure these metals; beryllium (Be) was used as an internal standard. GFAA was used to measure Pb and Bi when they were at low concentrations.

Digestions for ICP Analysis
We used samples of 0.5 to 1.0 g. A mixed portion of the sample was weighed to 1.0 mg with an electronic, top-loading balance and placed into a tared 50-mL conically tipped polypropylene, centrifuge tube. The tubes were precleaned for 24-hr with a 10% nitric acid (HNO_3) soak then rinsed in deionized water. Samples of feces were weighed to 0.1 mg. Approximately 30 to 50 mL of an acid and internal standard solution were added to the sample after taring. The final acid concentrations were 2% HNO_3 and 10% hydrochloric acid (HCl).

The Be concentration was targeted at 2.00 mg/L. The samples were then homogenized into a slurry using a saw-toothed generator made of titanium and TFE-fluorocarbon (Pro Scientific, Monroe, Connecticut). The internal standard solution was used to rinse excess materials from the generator and the amount was accounted for in the total weight.

Samples were prepared with the SpectrPrep System™, an automated microwave-digestion-system (CEM Corporation, Matthews, North Carolina). A 15-mL sample loop was used. After heating, cooling, and filtering, about 12.5 mL of the sample were collected and deposited by autosampler into 15 mL polypropylene test tubes. This digestate without further treatment was then used for ICP analysis. The automated microwave digestion system was a relatively new technique to prepare samples. A few problems arose in adjusting to the system; most were associated with clogging of the small-diameter tubing. A thorough homogenization followed by a few hours in a warm, ultrasonic bath usually improved the operation.

Digestions for GFAA Analysis
We used samples of 0.5 to 1.0 g. A mixed portion of the sample was weighed to 1.0 mg with an electronic, top-loading balance and placed into a tared TFE-fluorocarbon beaker. Approximately 20 mL of deionized water (DI H_2O), 0.250 mL concentrated HNO_3, and 1 mL of hydrogen peroxide (H_2O_2) were added. The mixture was heated at approximately 95°C until the solution started to clear (about 0.5 hr). Approximately 20 mL of DI H_2O and 2 mL H_2O_2 were added. Upon further heating the mixture cleared and "foamed up." DI H_2O was used to rinse contents from the sides of the beaker. The 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 increased to 50 mL in a volumetric flask, filtered through 0.45-μm nitrocellulose filters, and stored in acid-washed, linear, polyethylene bottles. The ultimate acid concentration was 0.5% HNO_3. High-purity acids and hydrogen peroxide (Baker Ultrex™ and Fisher Optima™) were used for all digestions.

Analytical Methods
ICP
We used a Thermo Jarrell Ash (TJA) AtomComp™, Model 61, vacuum spectrometer. The instrument has a polychromator configured with 44 fixed

channels, including analytical lines for high and low concentrations of Ca and Mg. Although we reported results for only a few elements, we measured 30 analytes to monitor for spectral interferences, which we did not detect, with blank subtraction and background correction.

We used USEPA Method 200.7, Revision 4.4, Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectroscopy for our work. We modified the method and used a different digestion process, and we measured Bi, not a listed analyte. We chose Be as an internal standard because it was not present in the samples, it does not cause spectral or background interferences, and it is precisely detectable.

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 so that samples dried on contact. We used method 3113 of Greenberg et al. (1992). We analyzed samples in triplicate and reported the mean.

Quality Control

We calibrated instruments daily and we verified the standard curve using National Institute of Standards and Technology (NIST) traceable, quality control samples (QCS). Samples (usually 10) were bracketed by calibration blanks, laboratory fortified blanks, and instrument-performance, check solutions during analysis as well as periodic checks on the internal-standard solution. The ICP instrument was programmed to compensate for drift by recalculating the slopes of the calibration curves if any analyte was more than ±5% of the true value while measuring the ICP check standard. If an analyte measured greater than ±10% of the true value for this sample, the instrument was recalibrated and the affected samples reanalyzed. The ICP check standard was formulated for a concentration at the midpoint of the calibration curve. It was traceable to NIST Standard Reference Materials (SRMs). The GFAA QCS initially were required to be within 10% of the true value. Subsequent measurement of the bracketed internals was required to be ±15%. If these limits were exceeded, the instrument was recalibrated and the affected samples reanalyzed.

Ten percent of the samples were digested and analyzed in duplicate, half of them spiked. Additional liver samples were treated as duplicates as part of the process of evaluating the automated, microwave-digestion equipment. Digestion blanks and spiked digestion blanks were prepared at a frequency of 10%. They were processed through the complete digestion and analytical system in the same manner as the samples.

Calculations

We saved the ICP data during analysis in database files with ThermoSpec (TJA) software utilizing Enable OA. Data were then imported into Enable spreadsheets for tabulations and calculations. The Enable spreadsheets were saved in a Lotus 1-2-3 format on diskette. The GFAA results were recorded on an instrument printer as concentrations in μg/L based upon peak-area measurements. These data were manually entered into spreadsheets for tabulations and calculations.

The Method Detection Limit (MDL) (Glaser et al. 1981) was used to establish the detection limits for concentrations of elements in tissues and other materials. Glaser et al. (1981:1426) describe the MDL as "a new performance criterion for chemical analysis . . . defined as that concentration of the analyte that can be detected at a specific confidence level." Also, "The detection limit should be related to the standard deviation of the measured value at or near zero concentration of the analyte . . . ." They further report (1427), "MDL is considered operationally meaningful only when the method is truly in the detection mode, i.e., analyte must be present. *The method detection limit is defined as the minimum concentration of a substance that can be identified.*" To be considered a meaningful difference, the MDL procedure is required to provide a value that averages ≥ two times the MDL (Glaser et al. 1981). For statistical analysis, values < MDL were entered as one-half the MDL value.

Most values for elements in the tissues were determined by ICP. Results of ICP analyses for Bi, Pb, and Sn were usually lower than the MDLs. Thus, selected samples of kidneys, livers, and gonads were analyzed for Bi and Pb by GFAA. The remaining amounts of plasma and blood cells after analysis by ICP were inadequate for further analysis by GFAA. Graphite-furnace atomic absorption is not a satisfactory method to analyze for Sn.

Statistical Analyses

In this report, when two values are reported as "different" or that they "differ," it means that they differ in a statistical sense at an alpha of ($P \leq 0.05$).

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 by one- or two-way ANOVA 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 instances where variances could not be assumed equal. In instances where the overall test of differences among groups was significant, pairwise comparisons were performed and significance evaluated based on the Bonferroni correction. In instances where comparisons were made with controls, Dunnett's procedure was used.

Variation in body weights, hematocrit counts, and concentrations of elements in plasma and red blood cells (all measured at Days 0, 15, and 30) were evaluated 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, Huynh-Feldt-adjusted significance probabilities were used.

Results

Survival
All 120 ducks (controls, Bi-dosed, and Fe-dosed) survived to the end of the 30-day test period.

Retention and Dissolution of Shot
No voided shot were found in the feces from the 20 dosed ducks (5 female and 5 male Bi-dosed and 5 female and 5 male Fe-dosed ducks) for which feces were saved for chemical analysis. Radiographs on Day 23 readily identified all six shot in the gizzard of each of these ducks.

Six pellets, which were sometimes dissolved to small disks, were recovered from 38 of the 40 Bi-dosed ducks. One male contained only five Bi disks in his gizzard. Because most of the shot were highly dissolved, it is probable that the sixth pellet had dissolved. A second male contained four tiny Bi particles in his gizzard. The combined particles of Bi weighed only 42.1 mg, and the fifth and sixth pellets probably had dissolved.

Six pellets were recovered from 35 of the 40 Fe-dosed ducks. One female had five tiny pellets in her gizzard that weighed 77.7 mg. This duck had the second highest (2,339 µg/g) concentra-

tion of Fe in the liver. The mean concentration of Fe in livers of Fe-dosed ducks was 1086 µg/g. Thus, the sixth pellet undoubtedly had dissolved. One female contained no shot in her gizzard, but she had 1,782 µg/g Fe in the liver. This duck probably also had dissolved the shot. The remaining three ducks, one male and two females, each contained five pellets in their gizzards. All of these pellets were small, collectively weighing from 147.4 to 282.9 mg for each duck. One of these females had the highest concentration (2,412 µg/g) of Fe in her liver of any duck. The other two ducks contained 645 µg/g and 1,043 µg/g Fe respectively, in their livers. The sixth pellet in each of 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.

The retained Bi and Fe shot differed in appearance. The Fe shot were usually round, although many were pitted or had empty spaces on their surfaces, whereas the Bi shot were generally disk-shaped or flattened. In several instances, five Bi disks plus two, three, or four tiny pieces (not flakes) of Bi were recovered from the gizzard. Obviously, when a Bi disk became thin enough, it disintegrated into several 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 flakes of Bi 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. Based on the shot recovered from the gizzards on Day 30, females dosed with Bi shot dissolved a mean of 69.5% and males 72.5% (Table 1) of the metal's original weight in 30 days (dissolution in individual ducks ranged from 38.2% to 96.4%). No difference between the sexes was detected for Bi-dosed ducks. Fe-dosed females dissolved an average of 69.2% and Fe-dosed males 55.6% of the metal's original weight in 30 days (range for individual doses was from 38.0% to 89.6%). The different dissolution rates between sexes for Fe-dosed ducks was expected (Table 1). Females approaching the breeding season in spring eat more food than males and thus produce more acid in their gizzards. As a result, Fe shot, which dissolve readily in the acid (HCl) environment of the gizzard, dissolve more rapidly in females than in males during this season.

Males dissolved more of the weight (72.5%) of the Bi shot than of the Fe shot (55.6%) in 30 days.

Females dissolved no more (69.5%) of the weight of the Bi shot than of the Fe shot (69.2%) in 30 days. An interaction, 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, existed between sex and dose (Table 1).

Body Weight

All groups of dosed ducks, except Bi-dosed males, lost from 1.8 to 5.0% of their body weight during the 30-day study. All groups lost from 4.5 to 9.6% of their body weight from Day 0 to Day 15, probably because of the switch from duck pellets to a whole corn diet. By Day 30, most of the birds had regained weight lost after the change in diet. Bi-dosed males gained only 1.6% in body weight from Day 0 to Day 30 (Table 2).

Males weighed more than females, and an interaction in weight between sex and time existed, with females losing a larger percentage of their weight from Day 0 to Day 30 than males. Although ducks lost weight over time, the average weight losses for females from Day 0 to Day 30 were only -3.8% for 0-dosed, -3.8% for Fe-dosed, and -5.0% for Bi-dosed females. The average weight changes for males from Day 0 to Day 30 were -1.8% for 0-dosed, -3.2% for Fe-dosed, and +1.6% for Bi-dosed ducks. No difference existed in body weights among doses (Table 2).

Organ Weights

Gizzard

Mean gizzard weights ranged from 29.3 g for Fe-dosed females to 32.2 g for Bi-dosed males (Table 3). No difference was detected in the weight of gizzards between sexes or among doses.

As a percentage of total body weight, mean gizzard weights ranged from 2.5% for each of Fe-dosed and Bi-dosed males to 3.0% for 0-dosed females (Table 3). Gizzards of females contributed a higher percentage of the total body weight than males. No difference was recorded among doses in the percentage that gizzards contributed to total body weight.

Liver

Mean weights of livers ranged from 19.3 g for Bi-dosed females to 21.7 g for Fe-dosed females. No differences existed between sexes or among doses (Table 4).

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. No difference was detected 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 least between sexes and varied least among doses of the organs weighed. Mean weights of kidneys ranged from 6.4 g for Bi-dosed females, Bi-dosed males, and Fe-dosed females to 6.6 g for 0-dosed males (Table 5). No differences were found 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, sex differences were detected. Mean percentages ranged from 0.5% for each group of males to 0.6% for each group of females. Kidneys of females comprised a larger percentage of the total body weight than males, but no differences existed among doses.

Gonads

No differences among doses in the mean weights of gonads were found (Table 6). As was expected, mean weights of gonads differed between the sexes: 6.4 g for 0-dosed females, versus 26.4 g for 0-dosed males; 10.1 g for Fe-dosed females, versus 28.0 g for Fe-dosed males; and 4.3 g for Bi-dosed females versus 22.5 g for Bi-dosed males. These sex differences also were evident in gonad weights when expressed as a percentage of total body weight; the means ranged from 0.4% for Bi-dosed females to 2.4% for Fe-dosed males (Table 6). No differences appeared among doses, but male gonads contributed a larger percentage of the total body weight than did the female gonads.

Hematocrit (Hct)

Mean hematocrits were not different among doses for the three sample times: Days 0, 15, and 30 (Table 7). The mean percentage changes in Hct values from Day 0 to Day 30 did not differ between the sexes. With sexes combined the mean percentage change in Hct from Day 0 to Day 30 increased (P< 0.00001) by 6.6% for controls, 11.8% for Bi-dosed ducks, and 12.8% for Fe-dosed ducks.

Heavy Metals and Essential Elements in Organs and Blood

For consistency in presentation of the data, usually the mean concentrations of elements in each organ or tissue for each sex and for sexes com-

Table 1. Percent of the 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——in female and male game-farm mallards (n = 20 females and 20 males in each dosed group).

Sex	Dose	% of Dosed Shot Accounted for[a]	Mean % Wt of Shot Dissolved[a]
F	Fe	93.3	69.2
		5.04[b]	2.84
M	Fe	99.2	55.6
		0.84	2.22
F	Bi	100.0	69.5
		0.00	4.01
M	Bi	97.5	72.5
		1.82	3.59

[a] Based on the shot recovered from the gizzards on Day 30,
   when the ducks were killed.
[b] SE.

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.

Table 2. Mean body weight 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	Dose	Mean Body Weight (Kg)			Mean % change in body wt-Day 0 to Day 30[c]
		Day 0	Day 15	Day 30	
F	0	1.11	1.06	1.06	- 3.8
		0.024[d]	0.027	0.030	1.12
M	0	2.24	1.19	1.22	- 1.8
		0.020	0.025	0.022	1.38
F	Fe	1.10	1.04	1.05	- 3.8
		0.020	0.018	0.021	0.97
M	Fe	1.24	1.18	1.20	- 3.2
		0.023	0.027	0.025	1.12
F	Bi	1.08	1.02	1.03	- 5.0
		0.026	0.025	0.026	1.11
M	Bi	1.23	1.21	1.24	+ 1.6
		0.019	0.025	0.039	2.31

[a] Ducks were dosed on Day 0.
[b] Ducks were killed on Day 30.
[c] Because of rounding error, these means are sometimes slightly different than if
   calculated by differences in the Day 0 and Day 30 columns.
[d] SE.

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.

Table 3. Mean weight of gizzard and 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	31.8	3.0
		1.04[a]	0.07
M	0	31.6	2.6
		0.75	0.08
F	Fe	29.3	2.8
		1.07	0.08
M	Fe	30.7	2.5
		0.98	0.08
F	Bi	30.2	2.8
		1.00	0.13
M	Bi	32.2	2.5
		1.08	0.13

[a] SE.

Difference among doses in weight of gizzard:

$F_{2,14}$ = 1.50; P = 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.

Table 4. Mean weight of liver and 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
		1.49[a]	0.10
M	0	20.0	1.6
		0.94	0.06
F	Fe	21.7	2.0
		1.54	0.12
M	Fe	20.5	1.7
		0.94	0.08
F	Bi	19.3	1.9
		0.92	0.08
M	Bi	19.5	1.6
		0.97	0.04

[a] SE.

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.

bined are listed in the tables. When no statistically significant differences existed between sexes, usually only P values for the combined sexes are provided.

Kidneys
The MDL for analysis by ICP for Bi in kidneys was 17.8 μg/g (wet wt). The mean concentrations were < MDL in kidneys of all but 2 of 120 ducks.

Because the MDL for Bi by ICP in the kidneys was unacceptably high, kidneys of 10 0-dosed and 11 Bi-dosed ducks were selected for analysis by GFAA. No sex differences were detected in the mean concentration of Bi in the kidneys of Bi-dosed ducks (Table 8). The mean concentration (6.86 μg/g) of Bi in the kidneys of Bi-dosed ducks, with sexes combined, was higher than the mean concentration of Bi in 0-dosed ducks (0.334 μg/g). The mean concentration of Bi in the kidneys of Bi-dosed ducks was much higher than the mean concentration (2.23 μg/g) of Bi in the livers of Bi-dosed ducks (Table 10).

The MDL for Pb in the kidneys was 6.54 μg/g (wet wt) by ICP. All mean values for Pb in the kidneys were < MDL by this method. By GFAA, no sex differences existed in the concentration of Pb in the kidneys of 0-dosed or Bi-dosed ducks. No difference was found in the concentration of Pb in the kidneys of 10 (sexes combined) 0-dosed ducks (0.440 μg/g) compared with 11 Bi-dosed ducks (0.313 μg/g) (Table 8).

The MDL for Sn in the kidneys was 9.47 μg/g (wet wt). Only six ducks had concentrations of Sn >MDL. The mean concentration of Sn in the kidneys of these six ducks was 14.3 μg/g and ranged from 10.5 to 19.7 μg/g. Three of these ducks were Bi-dosed ($\bar{x}$ = 6.7 μg/g), two were 0-dosed ($\bar{x}$ = 1.0 μg/g), and one was Fe-dosed (13.7 μg/g).

No difference existed between sexes in the mean concentrations of Cu in the kidneys (Table 9). With sexes combined, no differences were detected among doses in the mean concentration of Cu in the kidneys: 6.31 μg/g in 0-dosed, 7.31 μg/g in Fe-dosed, and 6.14 μg/g in Bi-dosed ducks.

No sex differences existed in the mean concentrations of P in the kidneys (Table 9) and no differences existed among doses in the mean concentrations of P in the kidneys. However, an interaction was found between sex and dose.

No difference existed between sexes in the mean concentration of Fe in the kidneys (Table 9), but mean concentrations of Fe in the kidneys differed among doses. Fe-dosed ducks, sexes combined, had higher mean concentrations (145 μg/g) of Fe in their kidneys than 0-dosed ducks (123 μg/g) or Bi-dosed ducks (123 μg/g). No difference was detected in the mean concentrations of Fe in the kidneys of 0-dosed and Bi-dosed females (Table 9).

Females, with doses combined, had higher mean concentrations of Ca in the kidneys than males, but no difference was found in the mean concentrations of Ca in the kidneys among doses. Mean concentrations of Mg in the kidneys did not differ between sexes, and with sexes combined, no difference existed among doses (Table 9).

Mean concentrations of Zn in the kidneys of 0-dosed, Fe-dosed, and Bi-dosed ducks did not differ between sexes within each dose, but with doses combined, males had higher mean concentrations of Zn in the kidneys than females (Table 9). With sexes combined, mean concentrations of Zn in the kidneys varied among doses. Bi-dosed ducks had higher mean concentrations of Zn in their kidneys (28.2 μg/g) than Fe-dosed ducks (25.2 μg/g), but not higher mean concentrations than 0-dosed ducks (26.6 μg/g) (Table 9). No difference was detected in mean concentrations of Zn in the kidneys of 0-dosed and Fe-dosed ducks.

Liver
The MDL (by ICP) for Bi in livers was 18.45 μg/g (wet wt). No concentration of Bi exceeded the MDL in the liver of any duck. Analysis by GFAA produced values of Bi in the livers of 11 Bi-dosed ducks that averaged 2.23 μg/g (0.63 to 5.63 μg/g). Mean amounts of Bi in the liver (Table 10) were not different between sexes. With sexes combined, Bi-dosed ducks contained a higher (2.23 μg/g) mean amount of Bi in the liver than did 0-dosed ducks (0.193 μg/g).

The MDL (by ICP) for Pb in the liver was 7.51 μg/g. The concentrations were all below the MDL. The concentration of Pb in the livers, as determined by GFAA, were from <MDL (0.10 μg/g) to 0.46 μg/g in the 11 Bi-dosed ducks, and the means (sexes combined) were 0.310 μg/g for 10 0-dosed birds and 0.157 μg/g for 11 Bi-dosed ducks. We found no differences among doses or between sexes (doses combined) in the mean concentrations of Pb in the liver (Table 10).

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

Table 5. Mean weight of kidneys and 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	6.5	0.6
		0.27[a]	0.02
M	0	6.6	0.5
		0.23	0.02
F	Fe	6.4	0.6
		0.27	0.02
M	Fe	6.5	0.5
		0.14	0.01
F	Bi	6.4	0.6
		0.22	0.02
M	Bi	6.4	0.5
		0.22	0.01

[a] SE.

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: $F_{1,114} = 33.91; P < 0.00001.$

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

Table 6. Mean weight of gonads and 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	6.4	0.6
		1.84[a]	0.18
M	0	26.4	2.2
		3.02	0.25
F	Fe	10.1	0.9
		2.78	0.25
M	Fe	28.0	2.4
		3.23	0.29
F	Bi	4.3	0.4
		1.46	0.13
M	Bi	22.5	2.0
		2.24	0.19

[a] SE.

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 7. 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 to Day 30 (n = 20 females and 20 males in each group).

Sex	Dose	Mean Hct			Mean % change in Hct—Day 0 to Day 30
		Day 0	Day 15	Day 30	
F+M	0	46.7	48.2	49.6	+ 6.6
		0.49[c]	0.41	0.48	1.25
F+M	Fe	45.8	49.7	50.8	+ 12.8
		0.90	0.58	0.55	2.49
F+M	Bi	45.7	48.7	49.7	+ 11.8
		0.98	0.47	0.52	3.92

[a] Ducks were dosed on Day 0.
[b] Ducks were killed on Day 30.
[c] SE.

Difference among doses: $F_{2,117} = 0.71; P = 0.4961.$

Change over time: $F_{2,234} = 50.10; P < 0.00001.$

Table 8. Mean concentrations (μg/g 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 GFAA.

Element	Sex	Dose	
		0[a]	Bi[b]
Bi	F	0.140	8.05
		0.000[c]	1.14
	M	0.528	4.77
		0.058	1.45
	F & M	0.334	6.86
		0.070	0.99
Pb	F	0.138	0.427
		0.070	0.179
	M	0.742	0.112
		0.640	0.033
	F & M	0.440	0.313
		0.320	0.121

MDL = Method Detection Limit (μg/g wet wt) by GFAA for Bi in kidneys = 0.10 μg/g for 10 ducks and 0.27 μg/g for 11 ducks and Pb = 0.27 μg/g for 10 ducks and 0.15 μg/g for 11 ducks.
[a] N = 10.
[b] N = 11.
[c] SE.

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 concentrations (μg/g 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) compa-red with ducks dosed with six, No. 4, Fe shot or six, No. 4, Bi shot (n = 20 females and 20 males in each group).

Element	Sex	Dose		
		0	Fe	Bi
Cu	F	5.50	5.34	5.74
		0.38[a]	0.26	0.49
	M	7.13	9.08	6.60
		0.45	3.37	0.65
	F & M	6.31	7.31	6.14
		0.34	1.79	0.40
P	F	2758	2937	3050
		53	80	67
	M	3167	2903	3006
		203	30	48
	F & M	2962	2919	3030
		112	40	42
Fe	F	110	152	118
		6.0	8.5	8.4
	M	136	139	129
		7.5	8.2	8.1
	F & M	123	145	123
		5.6	6.0	5.8
Ca	F	84.0	84.4	87.2
		7.1	5.3	4.5
	M	79.9	70.2	72.2
		7.3	1.8	1.6
	F & M	81.9	76.9	80.4
		5.0	3.1	3.0
Mg	F	196	199	206
		3.5	4.2	3.8
	M	216	197	204
		14.8	2.4	3.7
	F & M	206	198	205
		7.7	2.3	2.6
Zn	F	25.3	24.7	27.4
		0.8	0.6	1.2
	M	27.9	25.6	29.3
		1.5	0.8	1.1
	F & M	26.6	25.2	28.2
		0.9	0.5	0.8

[a] SE.

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

P
Difference among doses: $F_{2,55}$ = 0.63; P = 0.5384.

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

Fe
Difference among doses: $F_{2,55}$ = 4.98; P = 0.0103.

Interaction between sex and dose: $F_{2,55}$ = 3.01; P = 0.0574.

Difference between 0-dosed and
Fe-dosed ducks: $P < 0.05$.
Difference between 0-dosed and
Bi-dosed ducks: $P > 0.10$.
Difference between Fe-dosed and
Bi-dosed ducks: $P < 0.05$.

Ca
Difference between sexes: $F_{1,55}$ = 6.99; P = 0.0107.

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

Mg
Difference among doses: $F_{2,55}$ = 0.80; P = 0.4536

Zn
Difference between sexes: $F_{1,55}$ = 4.45; P = 0.0395.

Difference among doses: $F_{2,55}$ = 4.56; P = 0.0147.

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

Table 10. Mean concentrations (µg/g 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 GFAA).

Element	Sex	Dose	
		0	Bi
Bi	F	0.140[a]	2.79[c]
		0.000[b]	0.675
	M	0.246[d]	1.25[e]
		0.033	0.347
	F & M	0.193	2.23
		0.024	0.492
Pb	F	0.068	0.184
		0.018	0.053
	M	0.552	0.110
		0.376	0.030
	F & M	0.310	0.157
		0.195	0.036

MDL = Method Detection Limit (µg/g wet wt) by GFAA 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] SE.
[c] N = 7.
[d] N = 5.
[e] 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.

The mean concentration of Cu in the livers of 0-dosed females was 85.5 µg/g versus 191 µg/g in males, 56.3 µg/g in the livers of Fe-dosed females versus 172 µg/g in males, and 78.3 µg/g in the livers of Bi-dosed females versus 149 µg/g in males (Table 11, Figure 1). Males had higher mean concentrations of Cu in the liver than females, but we found no differences among doses in the mean concentration of Cu in the livers.

Females consistently had more P in their livers than males (Table 11): 0-dosed, 3,164 µg/g in females versus 2,998 P µg/g in males; Fe-dosed, 3,258 µg/g in females versus 2,958 µg/g in males; and Bi-dosed, 3,154 µg/g in females versus 2,897 µg/g in males. We found no differences among doses in the mean concentrations of P in the livers (Table 11).

We detected no difference between sexes in the mean concentrations of Fe in the livers, but the mean concentrations of Fe differed among doses: 411 µg/g in 0-dosed ducks versus 1086 µg/g in Fe-dosed ducks versus 399 µg/g in Bi-dosed ducks,

sexes combined. Differences were detected in the mean concentrations of Fe in 0-dosed versus Fe-dosed ducks and in Fe-dosed versus Bi-dosed, but not in 0-dosed versus Bi-dosed ducks (Table 11).

The mean concentrations of Ca in livers did not differ between sexes, but with sexes combined, the mean concentrations of Ca in the liver were different among doses. The mean concentration of Ca in the livers was higher in 0-dosed ducks (62.8 µg/g) than in Fe-dosed ducks (50.4 µg/g), but was not higher in 0-dosed than in Bi-dosed ducks (51.4 µg/g) (Table 11). We detected no difference in the mean concentrations of Ca in the livers of Fe-dosed and Bi-dosed ducks.

The mean concentration of Mg in the livers ranged from 211 µg/g in 0-dosed males to 224 µg/g in Fe-dosed females, but no difference existed between sexes. With sexes combined, no differences were detected among doses in the mean concentrations of Mg in the livers: 0-dosed ducks, 213 µg/g; Fe-dosed ducks, 219 mg/g; and Bi-dosed ducks, 214 µg/g (Table 11).

Table 11. Mean concentrations (μg/g 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).

| Element | Sex | Dose | | |
		0	Fe	Bi
Cu	F	85.5	56.3	78.3
		16.4[a]	12.1	15.6
	M	191	172	149
		37.6	31.8	32.6
	F & M	138	114	114
		21.9	19.2	18.7
P	F	3164	3258	3154
		126	77	93
	M	2998	2958	2897
		71	88	63
	F & M	3081	3108	3026
		72	62	59
Fe	F	416	1158	435
		37.8	91	43.6
	M	406	1015	362
		58.8	111	24.9
	F & M	411	1086	399
		34.1	72	161.0
Ca	F	66.4	54.0	52.9
		7.0	2.5	3.6
	M	59.2	46.8	49.8
		7.9	3.8	4.6
	F & M	62.8	50.4	51.4
		5.3	2.3	2.9
Mg	F	215	224	216
		8.4	4.6	4.5
	M	211	214	212
		4.9	6.5	5.0
	F & M	213	219	214
		4.8	4.0	3.2
Zn	F	53.3	48.4	50.8
		3.5	2.6	3.2
	M	48.9	48.1	45.4
		2.8	2.8	1.9
	F & M	51.1	48.2	48.1
		2.2	1.9	1.9

a SE.

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: $P < 0.05.$

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

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

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: $F_{2,117} = 0.70;$ $P = 0.5005.$
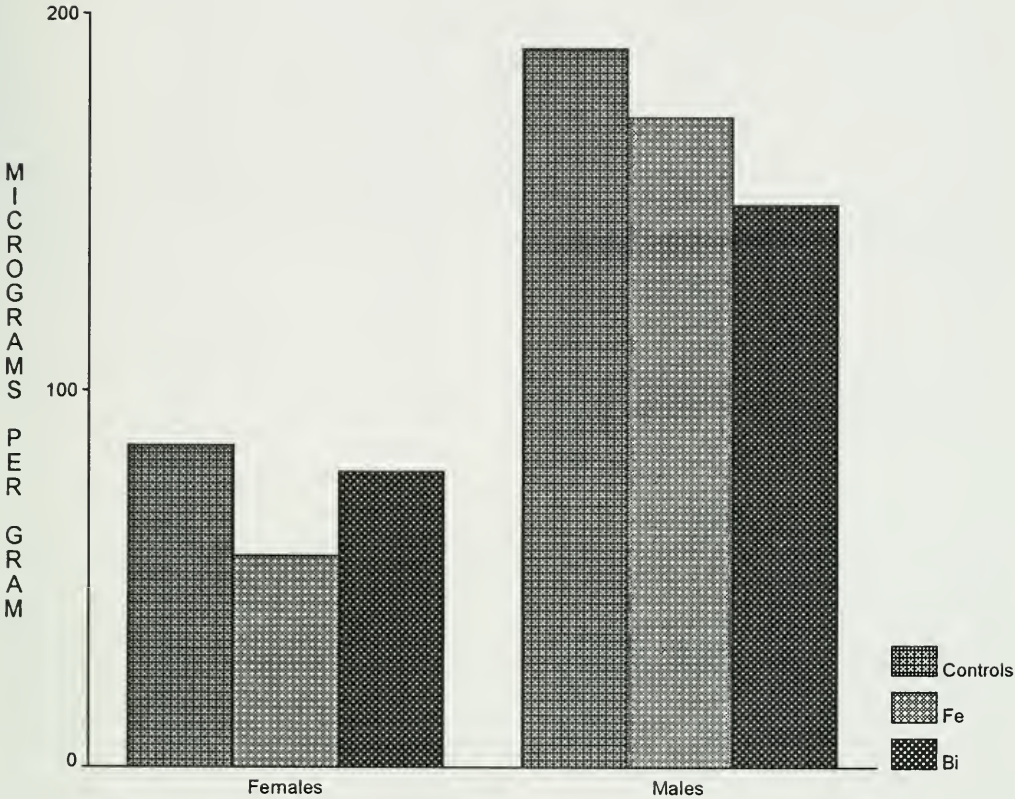Sn
The mean level was < MDL: = 12.8 ppm.

Figure 1. Mean concentrations of copper (μg/g wet weight) in liver of game-farm mallards 30 days after dosing with 0; 6, No. 4, steel (iron); or 6, No. 4, bismuth shot.

Table 12. Mean concentrations (μ/g 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 GFAA Furnace).

Element	Sex	Dose	
		0[a]	Bi[a]
Bi	F	0.050	0.677
		0.000[b]	0.455
	M	0.050	0.155
		0.000	0.048
	F & M	0.050	0.468
		0.000	0.277
Pb	F	0.080	0.093
		0.000	0.013
	M	0.080	0.100
		0.000	0.020
	F & M	0.080	0.096
		0.000	0.011

MDL = Method Detection Limit for gonads ($\mu g/g$ wet wt)
         by GFAA = 0.15 for Pb and 0.10 for Bi.
[a] N = 20.
[b] SE.

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

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The mean concentration of Zn in the livers ranged from 45.4 $\mu g/g$ for Bi-dosed males to 53.3 $\mu g/g$ for 0-dosed females, and values were not different between sexes. With sexes combined, no differences were found among the mean concentrations of Zn in livers of 0-dosed ducks, 51.1 $\mu g/g$; Fe-dosed ducks, 48.2 $\mu g/g$; and Bi-dosed ducks, 48.1 $\mu g/g$ (Table 11).

Gonads
The MDL for Bi in gonads by ICP was 12.0 $\mu g/g$ for 33 ducks and 13.2 $\mu g/g$ for 28 ducks. All but five values for Bi in gonads were <MDL. The concentration of Bi in the five gonads with concentrations >MDL ranged from 15.2 to 27.8 $\mu g/g$ and averaged 19.9 $\mu g/g$.

As determined by GFAA Furnace, the concentrations of Bi in gonads of 0-dosed ducks were all <MDL (0.10 $\mu g/g$). All Bi-dosed ducks had a mean of 0.468 $\mu g/g$ Bi in their gonads compared with <MDL in 0-dosed ducks (Table 12).

The MDL for Pb in the gonads by ICP was 7.10 $\mu g/g$ (wet wt) for 33 ducks and 7.75 $\mu g/g$ for 28 ducks. Only one (11.8 $\mu g/g$) mean concentration of Pb in the gonads was >MDL. The mean concentrations of Pb, as determined by GFAA furnace, in gonads of 0-dosed and Bi-dosed ducks were all <MDL (0.15 $\mu g/g$) (Table 12).

The MDL for Sn in the gonads by ICP was 15.5 $\mu g/g$ (wet wt). Only one female had > MDL (17.8 $\mu g/g$) of Sn in the liver. Mean concentrations of Cu in the gonads differed by sex in 0-dosed ducks, but not in Fe-dosed and Bi-dosed ducks. With doses combined, mean concentrations of Cu differed by sex with males having lower concentrations than females (Table 13). No differences were found for the mean concentrations of Cu in the gonads of mallards among doses.

Mean concentrations of P in the gonads were not different by sex within doses. With sexes combined, no difference was detected in the concentrations of P in the gonads among doses (Table 13).

Differences in the mean concentrations of Fe in the gonads of males and females were substantial (Figure 2) in all dosed groups: 0-dosed, 56.4 $\mu g/g$ in females versus 12.5 $\mu g/g$ in males; Fe-dosed, 53.5 $\mu g/g$ in females versus 10.7 $\mu g/g$ in males; and Bi-dosed, 40.3 $\mu g/g$ in females versus 16.8 $\mu g/g$ in males. Mean concentrations of Fe in the gonads of ducks within doses were not different (Table 13).

Females contained up to 17 times more Ca in their gonads than males (Figure 3): 0-dosed

Table 13. Mean concentrations (µg/g 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).

Element	Sex	Dose		
		0	Fe	Bi
Cu	F	1.76	1.70	1.48
		0.215[a]	0.152	0.183
	M	0.985	1.12	1.34
		0.149	0.113	0.174
	F & M	1.37	1.41	1.41
		0.155	0.114	0.125
P	F	3132	3102	2566
		388	395	342
	M	2662	2717	2917
		41	91	128
	F & M	2897	2910	2726
		197	202	195
Fe	F	56.4	53.5	40.3
		7.5	8.0	5.4
	M	12.5	10.7	16.8
		2.8	1.2	5.4
	F & M	34.4	32.1	29.1
		6.4	6.3	4.6
Ca	F	540.0	590.1	334.4
		165.4	179.2	102.5
	M	34.5	34.4	139.6
		1.7	1.6	104.5
	F & M	287.2	312.2	241.7
		99.2	108.0	74.6
Mg	F	113	127	126
		15.9	14.6	16.2
	M	203	206	201
		2.5	6.3	11.8
	F & M	158	166	162
		13.0	11.9	13.0
Zn	F	23.7	24.6	19.3
		4.0	3.6	2.9
	M	13.9	14.3	16.4
		0.4	0.6	2.0
	F & M	18.8	19.4	18.0
		2.2	2.1	1.8

[a] SE.

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: $F_{1,39} = 65.70$; $P < 0.00001$.

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: $F_{2,56} = 0.17$; $P = 0.8402$.

females (540.0 mg/g) versus 0-dosed males (34.5 mg/g); Fe-dosed females (590.1 mg/g) versus Fe-dosed males (34.4 mg/g); and Bi-dosed females (334.4 mg/g) versus Bi-dosed males (139.6 mg/g). With doses combined, females also contained higher mean concentrations of Ca in their gonads than males. No differences existed, however, in the mean concentrations of Ca in the gonads among doses within each sex (Table 13).

Males had higher mean concentrations of Mg in their gonads than females (Table 13), but no differences were detected among doses within each sex. With doses combined, females had higher mean concentrations of Zn in their gonads than males (Table 13). With sexes combined, Zn values in gonads varied little among doses.

Plasma and Blood Cells
The MDLs (by ICP) for Bi in plasma were 7.38 µg/g (wet wt) for Day 0, 21.8 µg/g for Day 15, and 11.8 µg/g for Day 30. The MDLs for Bi in blood cells were 8.72 µg/g for Day 0, 9.35 µg/g for Day 15, and 16.3 µg/g for Day 30. All mean levels were <MDLs for Bi in plasma and blood cells. After the analyses were completed by ICP, the amounts of plasma and blood cells remaining were insufficient to analyze for Bi by GFAA.

The MDLs for Pb in the plasma were 2.20 µg/g for Day 0, 4.42 µg/g for Day 15, and 3.34 µg/g for Day 30. MDLs for Pb in blood cells were 2.78 µg/g for Day 0, 2.68 µg/g for Day 15, and 4.69 for Day 30. All of the mean values for Pb concentrations in the plasma and in the cells were <MDLs.

The MDLs for Sn in the plasma were 5.81 µg/g for Day 0, 7.96 µg/g for Day 15, and 10.2 µg/g for Day 30. The MDLs for Sn in blood cells were 8.06 µg/g for Day 0, 5.75 for Day 15, and 6.37 for Day 30. All mean concentrations of Sn in plasma and cells were <MDLs on all three days.

The MDLs for Cu in plasma were 0.17 µg/g for Day 0, 0.483 µg/g for Day 15, and 0.541 µg/g for Day 30. The mean concentrations of Cu in the plasma were <MDL for Days 15 and 30. No differences were detected between sexes or among doses for Day 0 (Table 14). Data for Day 0 are considered to be baseline.

Mean concentrations of Cu in blood cells on Days 0, 15, and 30 were not different between sexes. With sexes combined, no changes were found in concentrations of Cu in the cells from Day 0 to Day 15 to Day 30. With days combined, we detected no differences in the mean concentrations of Cu in blood cells among doses (Table 15).

Mean concentrations of P in the plasma did not differ by sex or among doses for Days 0, 15, and 30 (Table 14). Mean concentrations of P in the plasma did increase from Day 0 to Day 15 to Day 30.

Mean concentrations of P in blood cells did not differ by sex or among doses. Within sexes and doses combined, the mean concentrations of P in cells did not change from Day 0 to Day 15 to Day 30 (Table 15).

Concentrations of Fe in the plasma did not differ between sexes or among doses (Table 14); however, with sexes and doses combined, Fe in the plasma declined from Day 0 to Day 15 to Day 30. This decline probably resulted from switching on Day 0 from a diet of duck pellets to corn, which is low in Fe.

No differences existed between sexes from Day 0 to Day 15 to Day 30 for mean concentrations of Fe in blood cells (Table 15). With sexes combined, no differences were found in mean concentrations of Fe in blood cells among doses on Day 0, Day 15, or Day 30.

Females had higher mean concentrations of Ca in plasma than males (Table 14). Within each sex, differences among doses did not exist in the mean concentrations of Ca in the plasma. However, mean concentrations of Ca in plasma increased from Day 0 to Day 15 to Day 30 in both sexes within each dose.

Mean concentrations of Ca in the blood cells were not different between sexes or among doses. The amount of Ca, however, did change over time and an interaction was detected between sex and dose. Ca in cells increased more consistently and in larger amounts from Day 0 to Day 15 to Day 30 in females than in males.

Females had higher mean concentrations of Mg in the plasma than males. Within sexes, no differences were detected among doses in the mean concentrations of Mg in the plasma, but Mg increased over time in ducks within each dose.

In contrast to plasma, mean concentrations of Mg in red blood cells of males were higher than those of females (Table 15). No differences in the concentrations of Mg in blood cells were detected, and no changes from Day 0 to Day 15 to Day 30 were noted among doses.

The MDLs for Zn in plasma were 3.57 µg/g for Day 0, 4.87 µg/g for Day 15, and 0.613 µg/g for Day 30 (Table 14). The mean concentrations were >MDL only for Day 30, the only data included in this report. Females had higher mean concentrations of Zn in their plasma than males (Table 14).
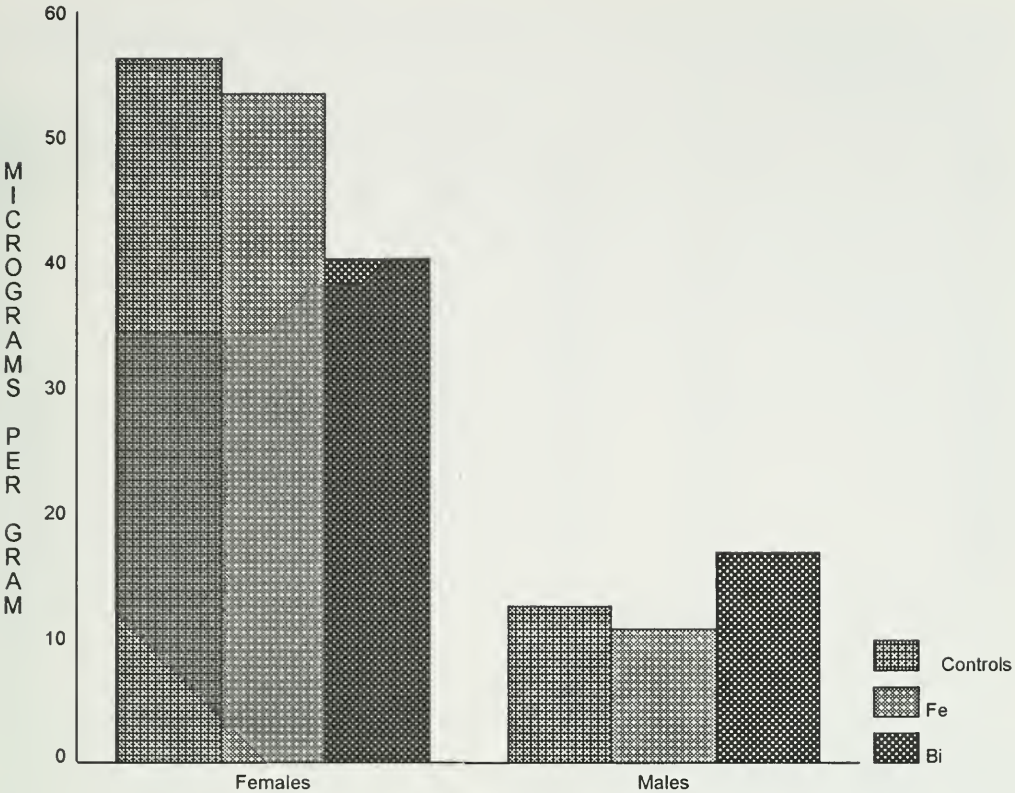
Figure 2. Mean concentrations of iron (µg/g wet weight) in gonads of game-farm mallards 30 days after dosing with 0; 6, No. 4, steel (iron); or 6, No. 4, bismuth shot.
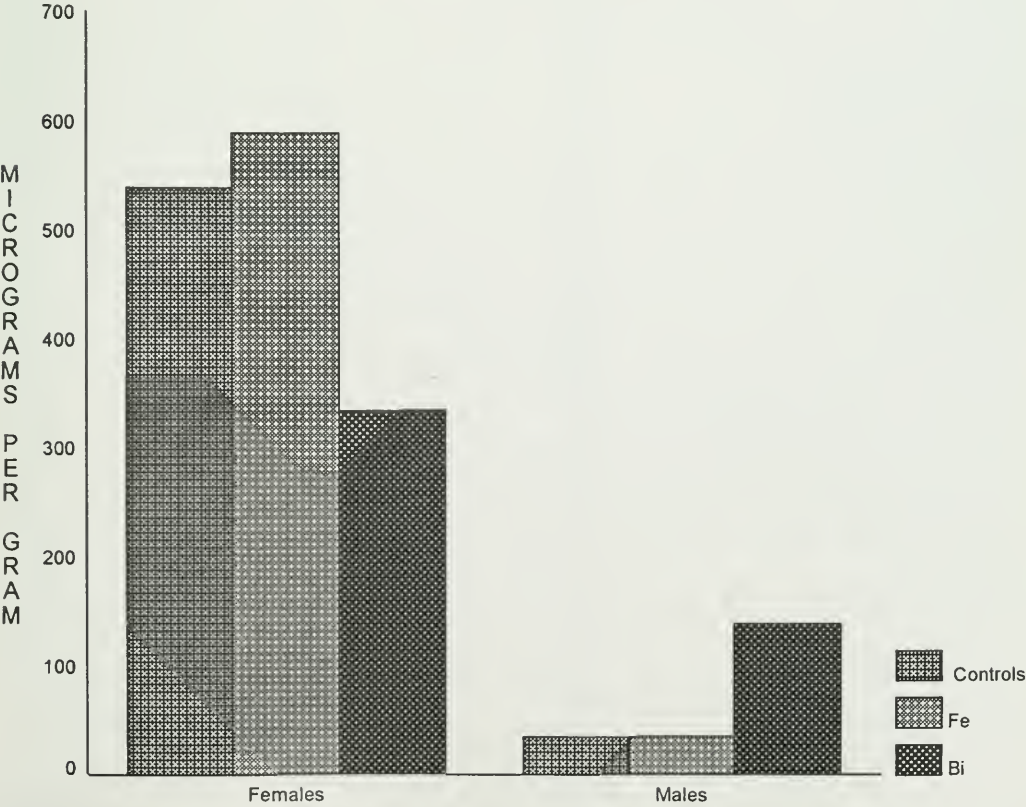
Figure 3. Mean concentrations of calcium (µg/g wet weight) in gonads of game-farm mallards 30 days after dosing with 0; 6, No. 4, steel (iron); or 6, No. 4, bismuth shot.

Table 14. Mean concentrations (µg/g 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 (N = 18, 19, or 20 for each sex).

Sex	Day	Elements Detected	Dose		
			0	Fe	Bi
F & M	0	Cu	0.334	0.304	0.370
			0.036[a]	0.031	0.048
F	0	P	179	204	196
			18.0	20.9	13.9
	15		245	268	225
			14.1	12.5	8.2
	30		291	303	270
			20.4	14.3	13.3
M	0		220	202	215
			19.4	11.8	17.0
	15		257	262	282
			15.2	6.3	22.3
	30		259	252	251
			9.6	7.3	14.0
F & M	0		199	203	206
			13.5	11.8	11.0
	15		251	265	252
			10.2	6.9	12.4
	30		275	277	260
			11.5	9.0	9.8
F	0	Fe	8.40	14.5	10.7
			1.2	3.5	1.7
	15		6.31	7.55	5.08
			0.8	0.6	0.8
	30		7.47	9.07	6.85
			0.9	1.0	0.7
M	0		15.4	7.62	13.8
			4.5	1.0	3.4
	15		5.94	5.67	8.19
			0.8	0.5	1.2
	30		7.71	6.08	5.17
			1.1	0.6	0.5
F & M	0		11.8	11.1	12.2
			2.3	1.9	1.9
	15		6.14	6.61	6.59
			0.5	0.4	0.7
	30		7.48	7.58	6.03
			0.7	0.7	0.4
F	0	Ca	88.3	87.9	87.0
			6.1	5.7	5.8
	15		144	140	120
			12.2	7.8	3.8
	30		176	169	168
			17.3	12.6	12.4
M	0		83.0	80.1	80.4
			6.3	5.8	6.2
	15		110	117	115
			5.4	1.7	1.5
	30		107	111	109
			1.7	1.3	0.5
F & M	0		85.7	84.0	84.1
			3.9	4.0	4.2
	15		127	129	118
			7.2	4.3	2.1
	30		141	140	139
			10.2	7.8	8.2
F	0	Mg	17.4	19.2	17.8
			1.2	1.4	1.2
	15		23.3	24.5	21.7
			1.0	0.8	0.5
	30		26.6	27.0	26.2
			1.2	1.0	0.8

Table 14 continued

Sex	Day	Elements Detected	Dose		
			0	Fe	Bi
M	0		18.0	16.3	17.1
			1.5	1.1	1.3
	15		22.5	22.7	23.8
			1.2	0.5	1.0
	30		24.4	24.3	24.4
			0.4	0.4	0.6
F & M	0		17.7	17.8	17.5
			0.8	0.9	0.9
	15		22.8	23.6	22.6
			0.8	0.5	0.6
	30		25.5	25.7	25.3
			0.6	0.6	0.5
F	30	Zn	4.56	4.27	4.63
			0.39	0.34	0.30
M	30		2.83	2.77	2.72
			0.13	0.08	0.17
F & M	30		3.69	3.52	3.64
			0.25	0.21	0.23

[a] SE.

Cu

Difference among doses; Day 0: $F_{2,112}$ = 0.74: $P = 0.4789$.

Mean concentrations for Days 15 and 30 were <MDLs: 0.483 µg/g and 0.541 µg/g, 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 µg/g and 41.87 µg/g, respectively.

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Within sexes, no differences were found among doses in the mean concentrations of Zn in the plasma. We found no differences between sexes, among doses, or over time in the mean concentrations of Zn in blood cells.

Feces

All concentrations of Bi in the feces were <MDL (103 µg/g, dry wt) on Day 0. We found little change in the concentrations of Bi in the feces of 0-dosed and Fe-dosed ducks from Day 1 to Day 2. We did not perform statistical tests because most concentrations were <MDL. For Bi-dosed ducks, Bi in the feces increased from <MDL on Day 0 to 2,275 µg/g on Day 1 to 3,689 µg/g on Day 2. These high concentrations were maintained to the end of the 30-day study—3,636 µg/g for Days 1-10 combined and 3,485 µg/g for Days 11-30 combined (Table 16).

The main purpose of collecting and analyzing the feces was to provide data for calculating the percentage of dissolved Bi (from Bi shot in the gizzard) that was excreted in the feces. An estimated 90% of all fecal matter was analyzed. The remaining 10% fell on the ground under the pens, adhered to the pans when feces were collected, was 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. The Bi in the estimated 10% of the feces lost was included in our estimates of the percentage of Bi excreted.

All fecal samples that were analyzed were dried to constant weight and then weighed. By using the concentration of Bi in each sample—daily concentrations for Days 1-10 and one concentration for the combined sample for Days 11-30—the amount of Bi excreted in the feces was calculated for each Bi-dosed duck. The results were compared with the amount of Bi dissolved from the six Bi shot dosed in the same duck. By this process, we estimated a mean of 88% of the Bi dissolved from the shot in the gizzards was excreted in the feces. The percentages ranged from 61% to 103% in the 10 ducks.

Sn in the feces of all ducks was <MDL (19.5 µg/g, 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, mean concentrations of Sn increased to 185 µg/g on Day 1, was 111 µg/g on Day 2, and declined to 67 µg/g for Days 11-30 combined (Table 16).

Sn made up only 2% of the dosed Bi shot compared with 98% for Bi. In the feces analyzed

from 10 Bi-dosed ducks, Sn increased from <MDL (19.5 µg/g, dry wt) on Day 0 to a range of 48 to 321 µg/g on Day 1. By making calculations similar to those described for Bi, we estimated that a mean of 85% of the Sn dissolved from the Bi shot was excreted in the feces. The calculated percentages of Sn dissolved from the shot that were excreted in the feces of each of the 10 ducks ranged from 58 to 127%.

The mean concentrations of Fe in the feces of 0-dosed ducks declined from 1174 µg/g on Day 0 to 809 µg/g on Day 1 to 358 µg/g on Day 2, but increased to 520 µg/g for Days 11-30 combined. The mean concentrations of Fe in feces of Fe-dosed ducks increased from 1055 µg/g on Day 0 to 2442 µg/g on Day 1 to 2611 mg/g on Day 2. In Bi-dosed ducks, the mean concentrations of Fe decreased from 1,010 µg/g on Day 0 to 720 µg/g on Day 1 to 514 µg/g on Day 2 to 349 µg/g for Days 11-30. Although Bi-dosed ducks were associated with temporal decreases of Fe in the feces, a similar relationship was evident for 0-dosed ducks. We assumed that the sharp declines in the amount of Fe in the feces over time were a result of the switch in diets on Day 0 from commercial duck pellets to corn, which is known to be low in Fe.

Histopathology

Lesions were identified in the testes, ovaries, kidneys, and livers in control and dosed ducks, but presence of a specific lesion and dosage were not correlated. The mild inflammatory changes seen histologically were within the range of normal tissues of birds.

Gonadal Lesions

Female

Tissue sections of many of the females documented a mild to moderate lymphocytic oophoritis, but without any evidence of etiology. This lesion typically consisted of scattered lymphocytes and plasma cells in the interstitium of the ovary. Severe necrotizing oophoritis was observed in the ovary of one 0-dosed female, which is consistent with an egg yolk peritonitis. Milder lesions with necrotic areas were detected in two other Bi-dosed females. These lesions may represent resolving cases of egg yolk peritonitis. Weights of ovaries ranged widely.

Male

No inflammatory lesions were detected in gonads of male ducks except for one Fe-dosed duck

Table 15. Mean concentrations (µg/g 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 with six, No. 4, Fe shot or six, No. 4 Bi, shot (N = 18, 19, or 20 for each sex).

Sex	Day	Elements Detected	Dose		
			0	Fe	Bi
F	0	Cu	0.612	0.557	0.681
			0.076[a]	0.034	0.099
	15		0.709	0.668	0.624
			0.073	0.042	0.095
	30		0.626	0.688	0.699
			0.094	0.128	0.039
M	0		0.762	0.956	0.605
			0.192	0.456	0.107
	15		0.539	0.590	0.645
			0.072	0.159	0.120
	30		0.675	0.626	0.577
			0.118	0.148	0.081
F & M	0		0.687	0.757	0.644
			0.103	0.288	0.072
	15		0.624	0.629	0.630
			0.053	0.081	0.075
	30		0.651	0.657	0.643
			0.074	0.097	0.045
F	0	P	2544	2311	2303
			240	41	70
	15		2369	2395	2343
			56	72	48
	30		2305	2212	2385
			55	101	40
M	0		2336	2359	2452
			42	49	133
	15		2445	2396	2282
			44	36	77
	30		2605	2556	2555
			276	134	160
F & M	0	P	2440	2335	2376
			121	32	74
	15		2407	2395	2322
			36	40	45
	30		2455	2384	2463
			141	87	82
F	0	Fe	804	727	739
			82.0	13.4	21.9
	15		769	789	776
			18.2	25.3	19.1
	30		732	705	762
			19.5	31.9	13.1
M	0		758	752	776
			16.4	19.5	49.6
	15		803	787	747
			14.3	14.3	26.3
	30		832	805	831
			97.8	33.7	45.3
F & M	0	Fe	781	739	757
			41.4	11.8	26.5
	15		786	788	761
			11.8	14.3	16.2
	30		782	755	794
			49.8	24.3	23.8
F	0	Ca	32.0	31.0	29.3
			1.4	1.8	1.4
	15		42.1	33.2	32.8

Table 15 continued

Sex	Day	Elements Detected	Dose		
			0	Fe	Bi
			4.2	2.9	2.2
	30		45.7	47.8	35.6
			4.6	5.6	3.5
M	0		30.2	29.6	34.2
			3.0	2.9	3.7
	15		33.9	28.1	42.4
			3.4	2.0	5.7
	30		32.3	31.4	39.8
			1.6	4.2	7.3
F & M	0		31.1	30.3	31.7
			1.6	1.7	2.0
F & M	15	Ca	38.0	30.6	37.4
			2.7	1.8	3.1
	30		39.0	39.6	37.4
			2.6	3.7	4.0
F	0	Mg	123	113	112
			11.6	2.0	3.5
	15		117	116	114
			2.5	3.9	1.9
	30		110	108	111
			1.9	4.0	1.4
M	0		115	118	116
			2.3	2.3	6.6
	15		122	122	115
			2.0	1.5	3.2
	30		127	121	123
			13.6	6.1	7.6
F & M	0		119	115	114
			5.9	1.6	3.6
	15		120	119	115
			1.6	2.1	1.8
	30		119	114	117
			6.9	3.7	3.9
F	0	Zn	7.76	7.22	7.20
			0.64	0.15	0.18
	15		7.39	7.27	7.02
			0.14	0.21	0.12
	30		7.46	7.15	7.42
			0.19	0.28	0.12
M	0		7.19	6.99	7.22
			0.25	0.08	0.48
	15		6.99	6.87	6.61
			0.13	0.08	0.17
	30		7.64	7.30	7.33
			0.80	0.36	0.44
F & M	0		7.48	7.10	7.21
			0.34	0.09	0.25
	15		7.19	7.07	6.81
			0.10	0.12	0.11
	30		7.55	7.22	7.37
			0.40	0.23	0.29

[a] SE.

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

Table 16. Mean concentrations (µg/g 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.

Elements Detected	Days[a]	Dose		
		0	Fe	Bi
Bi	0	<MDL[b]	<MDL[c]	<MDL[d]
	1	<MDL[b]	<MDL[b]	2275[e]
				414[f]
	2	<MDL[b]	<MDL[c]	3689[e]
				588
	1-10	<MDL[b]	<MDL[a]	3636[g]
				183
	11-30	<MDL[a]	<MDL[a]	3485[g]
				382
Sn	0	<MDL[b]	<MDL[c]	<MDL[d]
	1	<MDL[b]	<MDL[b]	185[e]
				29.2
	2	<MDL[b]	<MDL[c]	111[e]
				23.9
	11-30	<MDL[b]	<MDL[b]	67[e]
				5.7
Fe	0	1174[b]	1055[c]	1010[e]
		174	38	38
	1	809[b]	2242[b]	720[e]
		18.2	409	70.6
	2	358[b]	2611[c]	514[e]
		21.4	563	66.6
	11-30	520[b]	2288[b]	349[g]
		76.5	410	23.0

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

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

and one Bi-dosed duck. In the Fe-dosed duck, a small-sperm granuloma was found, but the residual parenchyma was normal. The 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 with no evidence of inflammation.

The testis of the Bi-dosed duck contained scattered aggregates of lymphocytes and plasma cells, and multinucleated giant cells (presumably sloughed spermatocytes) were observed within scattered tubules. Evidence of normal production of spermatozoa was present on the slide.

A small 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 number 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 probably represented an acute bacterial infection. The hepatic lipidosis seen histologically seemed to correlate with livers that were heavier.

Kidneys

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

Gizzard

On Day 30, the contents of the gizzards from all 120 ducks were removed and saved, and those from Fe-dosed and Bi-dosed ducks were exam-

ined for retained shot. The linings of all gizzards appeared to be unaffected, and there was no pattern of variation among doses. No lesions were detected in any of the gizzard sections examined.

Discussion

Copper
Approximately twice the concentration of Cu was detected in the livers of male ducks as in the livers of female ducks in all dosed groups (Table 11). Hanson and Jones (1974) found significantly higher concentrations of Cu in the feathers of female Ross' geese (*Anser rossii*) than in males. They presumed estrogen was responsible for the difference. Underwood (1971:61, 63), discussing Cu in the liver, stated, "There is no effect of sex, except in the Australian salmon (*Arripis trutta*) in which the female carried higher concentrations than the male."

Van Campen (1971:214) reported that "Administration of estrogens induces large increases in serum copper in humans, rats, and swine." He also reported that androgens increased serum Cu concentrations 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. In the present study, the diet (corn) of the ducks (both females and males) was 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) stated, "The factors that are most influential in determining the tissue levels of copper are age, hormones, disease and diet. . . . calcium apparently can either increase or decrease copper absorption, depending on the composition of the diet to which they are added."

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

Phosphorous
The lower concentrations of P in livers of Fe-dosed males, as compared with females, resulted from decreases of P in the livers of Fe-dosed males. Concentrations of P 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 concentration of P in livers of males, which resulted in a significant difference between the sexes. Concentrations of P were essentially the same in 0-dosed females and Bi-dosed females (Table 11).

Iron
Dosing with Fe shot resulted in large concentrations of Fe deposits in the livers, but dosing with Bi shot did not significantly affect the concentrations of Fe in the liver (Table 11). Although females dissolved a higher percentage of the dosed Fe shot than males (Table 1), the Fe-dosed females did not have significantly higher concentrations of Fe in their livers than the Fe-dosed males.

Females had significantly higher concentrations of Fe in their gonads than males. These sex differences in the concentrations of Fe in the gonads may be related to the preparation of the ovaries for egg laying. No differences were found in the mean concentrations 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 concentrations of Ca in the livers and kidneys of ducks as compared with controls (Table 9).

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

Ca increased substantially 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. Ca in the plasma among doses did not vary statistically.

Feces
Both Bi and Sn greatly increased in the feces of Bi-dosed ducks the day after dosing. Birds excreted Bi in the feces at high concentrations to the end of the 30-day study. Mean concentrations 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. It appears that almost all of the Bi dissolved from Bi

shot in the gizzards is excreted in the feces of ducks.

The mean concentration of Sn in the feces of Bi-dosed ducks was higher at the end of the 30-day study than the background level found the day prior to dosing. However, the mean concentration of Sn in the feces of Bi-dosed ducks declined substantially after Day 10.

These findings for Sn in the feces seem to support Underwood (1971), who reported that the available evidence for humans shows that Sn is poorly absorbed, poorly retained, and excreted primarily in the feces. In humans, 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 it is absorbed slowly and is excreted rapidly in feces.

The mean concentration of Fe in feces declined sharply for 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 feces of 0-dosed and Bi-dosed ducks was probably a result of switching on Day 0 from a diet of commercial duck food to corn, which is low in Fe.

Conclusions

We detected no toxic effects in game-farm mallards dosed with six Bi/Sn alloy shot and observed for 30 days. Survival, body weight, Hct, and weights of organs were not affected. Gross and microscopic examination of the kidneys, liver, and gonads of the ducks also revealed only slight tissue changes. Our data support the conclusions of Sanderson et al. 1992, who reported no toxic effects in game-farm mallards dosed with 100% Bi shot.

A number of differences in weights of organs and in mean concentrations of individual elements were detected between females and males. These differences appear related to physiological changes associated with the onset of breeding, especially in egg-laying females.

Although a few "anomalies" were linked to dosing with Fe shot or with Bi shot, no toxic effects were detected with either. For example, livers and kidneys of both Bi-dosed and Fe-dosed ducks had lower mean concentrations of Ca than livers and kidneys of 0-dosed ducks. The difference in the mean concentrations of Ca in the livers of Fe-dosed ducks versus Bi-dosed ducks was not significant (Table 11).

With two exceptions (kidneys of males, mean = 0.528 µg/g [range 0.36 to 0.72 µg/g], and livers of males, mean = 0.246 µg/g [range 0.18 to 0.37 µg/g]), Bi was not found in the livers, kidneys, or gonads of 0-dosed ducks. The mean concentrations of Bi in kidneys of Bi-dosed ducks were 8.05 µg/g for females (range 4.69 to 12.6 µg/g) and 4.77 µg/g (range 2.02 to 8.82 µg/g) for males. The mean concentration of Bi in livers of Bi-dosed females was 2.79 µg/g (range 1.19 to 5.63 µg/g). The differences for Bi-dosed versus 0-dosed ducks were significant for both kidneys and livers. Both macro and micro histological observations detected no toxic effects of Bi on the kidneys, liver, or gonads.

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