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Toxicity of Ingested Bismuth Alloy Shot in Game-farm Mallards

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

Illinois Natural History Survey Bulletin
Volume 35, Articles 3 and 4
April 1997

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Citations:
Sanderson, G.C., W.L. Anderson, G.L. Foley, L.M. Skowron, J.D. Brawn, and J.W. Seets. 1997. Acute toxicity of ingested bismuth alloy shot in game-farm mallards. Illinois Natural History Survey Bulletin 35(3):185-216.

Sanderson, G.C., W.L. Anderson, G.L. Foley, K.L. Duncan, L.M. Skowron, J.D. Brawn, and J.W. Seets. 1997. Toxicity of ingested bismuth alloy shot in game-farm mallards: chronic health effects and effects on reproduction. Illinois Natural History Survey Bulletin 35(4):217-252.

Editors: Thomas E. Rice and Charles Warwick

US ISSN 0073-4918

Printed by Authority of the State of Illinois
(MJ412759-1M-4-97)

Printed with soy ink on recycled and recyclable paper.

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

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Illinois Natural History Survey Bulletin
Volume 35, Article 3
April 1997

Acknowledgments

James W. Sergent and Wendy L. Grethen, Illinois Natural History Survey, fed the ducks, cleaned the pens, examined the feces for voided shot, saved fecal samples for chemical analysis, and assisted with other phases of the study. The following people assisted with weighing and dosing ducks and collecting and processing blood: Stephen P. Havera, Michelle M. Georgi, Aaron P. Yetter, and Christopher S. Hine, all with the Waterfowl Research Laboratory, Forbes Biological Station, Illinois Natural History Survey, Havana, Illinois; Edward J. Heske, Linda K. Campbell, and Anne E. Zielske, all with the Illinois Natural History Survey; and volunteers Beverley C. Sanderson and J. William Sanderson. Beverley C. Sanderson also assisted with many tasks in the preparation of this manuscript. William R. Manuel, retired, College of Veterinary Medicine, University of Illinois, provided his expertise in the collection of blood. We thank Jerry L. Longcore, Leader, Patuxent Wildlife Research Center, Orono, Maine; Lawrence J. Blus, Wildlife Research Biologist, Biological Resources Division, U.S. Geological Survey; and Louis N. Locke, Wildlife Pathologist, and Milton Smith, Chemist, National Wildlife Health Center, for their reviews of the manuscript. Petersen Publishing Company, Los Angeles, California, provided financial support for the research and costs of this publication.

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 nontoxic, 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 µg/g wet weight compared with < 1 µg/g wet weight in nonexposed individuals. Bi encephalopathy is mainly supported by elevated blood Bi. A steady-state Bi concentration of > 100 µg/L (ppb) of blood in humans was arbitrarily suggested as an "alarm" level and 50 µg/L was considered a "safety" level, but no proof supports these choices. Concentrations of blood Bi from 10 to 4,600 µg/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 µg/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 µg/g of Bi in the brain show neurologic symptoms and that a concentration of ≥ 0.5-2.0 µg/g of Bi in blood had to be maintained for several weeks to accumulate enough Bi in the brain to cause neurotoxicity. They also concluded that 5-10 µg/g Bi in the brain was associated with motor dysfunction in humans and mice and that concentrations above 50 µg/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 (± SD) percentages of elements in these shot were as follows: Bi = 98.35%, ± 0.86%; and Sn = 1.90%, ± 0.10%. Other elements averaged < 0.1% each; Pb ranged from 0.0040% to 0.0186% ($\bar{x}$=0.0094%, ± 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 homogenation 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_20 and 2 mL H_2O_2 were added. Upon further heating the mixture cleared and "foamed up." DI H_20 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) concentration 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-

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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).

		Mean Body Weight (Kg)			
Sex	Dose	Day 0	Day 15	Day 30	Mean % change in body wt-Day 0 to Day 30[c]
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: $F_{2,114} = 1.80$; $P = 0.1706$.

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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.

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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).

		Dose		
Element	Sex	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	Dose 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: $F_{1,19} = 0.65$; $P = 0.4294$.

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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).

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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).

		Dose		
Element	Sex	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.

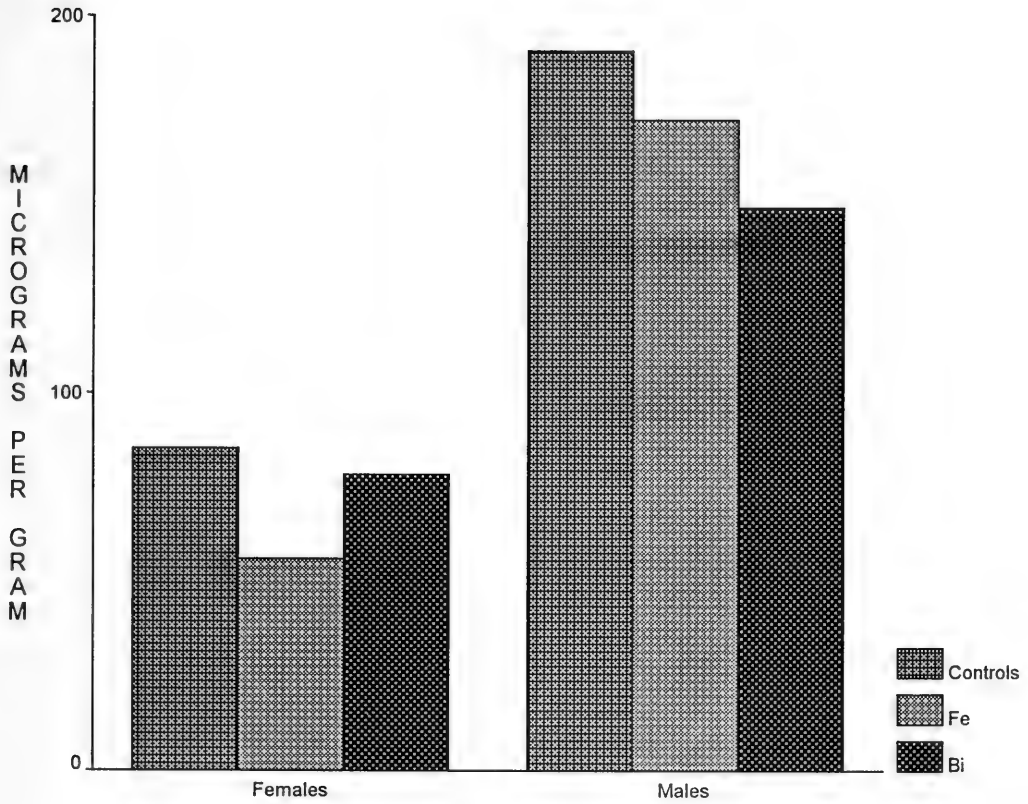

Figure 1. Mean concentrations of copper (µg/g wet weight) in liver of game-farm mallards 30 days after dosing with 0; 6, No. 4, steel (iron); or 6, No. 4, bismuth shot.

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).

		Dose	
Element	Sex	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 (μg/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 μg/g for Bi-dosed males to 53.3 μg/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 μg/g; Fe-dosed ducks, 48.2 μg/g; and Bi-dosed ducks, 48.1 μg/g (Table 11).

Gonads

The MDL for Bi in gonads by ICP was 12.0 μg/g for 33 ducks and 13.2 μg/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 μg/g and averaged 19.9 μg/g.

As determined by GFAA Furnace, the concentrations of Bi in gonads of 0-dosed ducks were all <MDL (0.10 μg/g). All Bi-dosed ducks had a mean of 0.468 μg/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 μg/g (wet wt) for 33 ducks and 7.75 μg/g for 28 ducks. Only one (11.8 μg/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 μg/g) (Table 12).

The MDL for Sn in the gonads by ICP was 15.5 μg/g (wet wt). Only one female had > MDL (17.8 μg/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 μg/g in females versus 12.5 μg/g in males; Fe-dosed, 53.5 μg/g in females versus 10.7 μg/g in males; and Bi-dosed, 40.3 μg/g in females versus 16.8 μg/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

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

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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).

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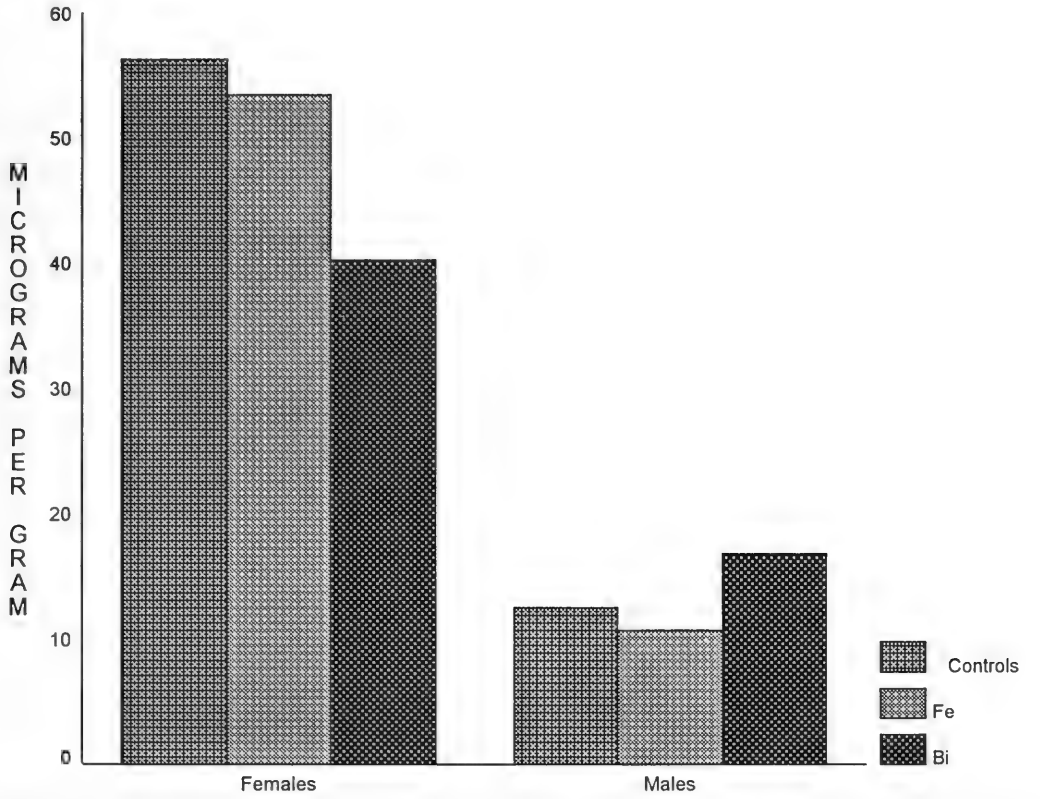

Figure 2. Mean concentrations of iron (µg/g wet weight) in gonads of game-farm mallards 30 days after dosing with 0; 6, No. 4, steel (iron); or 6, No. 4, bismuth shot.

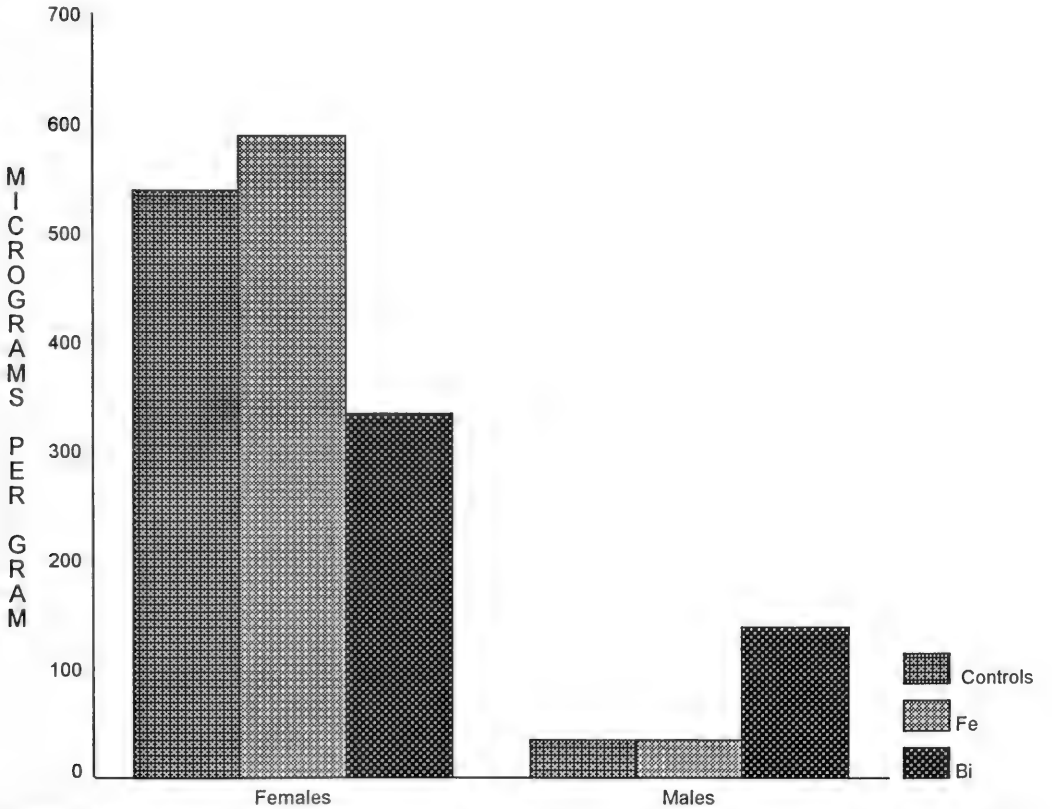

Figure 3. Mean concentrations of calcium (µg/g wet weight) in gonads of game-farm mallards 30 days after dosing with 0; 6, No. 4, steel (iron); or 6, No. 4, bismuth shot.

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
			1.3	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

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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

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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.

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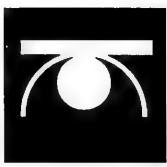
Toxicity of Ingested Bismuth Alloy Shot in Game-farm Mallards: Chronic Health Effects and Effects on Reproduction



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Illinois Natural History Survey Bulletin
Volume 35, Article 4
April 1997

Acknowledgments

Bradley W. Zercher and James W. Sergent, Illinois Natural History Survey, fed the ducks, cleaned the pens, collected and weighed the eggs, and assisted with other phases of the study. Stephen P. Havera, Michelle M. Georgi, Aaron P. Yetter, and Christopher S. Hine, all with the Waterfowl Research Laboratory, Forbes Biological Station, Illinois Natural History Survey, Havana, Illinois, assisted with weighing, dosing, and collecting blood from the ducks. Patrick W. Brown, Brian W. Wilm, Trina H. Simpson, Angela M. Young, Anne E. Zielske, and Linda K, Campbell, all with the Illinois Natural History Survey, and Beverley C. Sanderson and J. William Sanderson, volunteers, assisted with weighing, dosing the ducks, and collecting and processing blood. Beverley C. Sanderson also assisted with many tasks in the preparation of this report. William R. Manuel, retired, College of Veterinary Medicine, University of Illinois, provided his expertise in the collection of blood. Judy K. Holding, Jenny Huffington, and Karen Bischoff, DVM, of the College of Veterinary Medicine, University of Illinois, assisted with the collection of blood. Helen M. Parker, College of Veterinary Medicine, University of Illinois, assisted with the necropsies. Veronica Lasovsky, Illinois State Water Survey, prepared the samples for analysis by ICP and by graphite furnace AA. Saada E. Hamdy, Illinois State Water Survey, conducted the analyses by graphite furnace AA. Gale D. Taylor, Head, Program of Laboratory Animal Medicine, University of Illinois, inspected Pb-dosed sick ducks when we called him and authorized euthanasia when it was apparent that ducks would not survive. Without the enthusiastic assistance and support of all these individuals, the study would have been much more difficult. Their assistance is gratefully acknowledged. We thank Jerry L. Longcore, Leader, Patuxent Wildlife Research Center, Orono, Maine; Lawrence J. Blus, Wildlife Research Biologist, Biological Resources Division, U.S. Geological Survey; and Louis N. Locke, Wildlife Pathologist and Milton Smith, Chemist, National Wildlife Health Center, for their reviews of the manuscript. Petersen Publishing Company, Los Angeles, California, provided financial support for the research and costs of this publication.

Abstract

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

Introduction

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

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

Methods

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

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

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

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

Toxicity Study

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Chemical Analyses

Storage of Samples

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

Digestions of Samples

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

Digestions for ICP Analysis

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

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

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

a warm ultrasonic bath, effectively reduced particle size.

Digestions for GFAA Analysis

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

Analytical Methods

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

ICP

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

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

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

GFAA

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

Quality Control

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

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

Calculations

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

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

Statistical Analysis

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

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

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

Results

Chronic Toxicity Test

Survival

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

Hematocrit

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

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

Body Weight

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

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

Dissolution of Shot

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

Continued on page 226

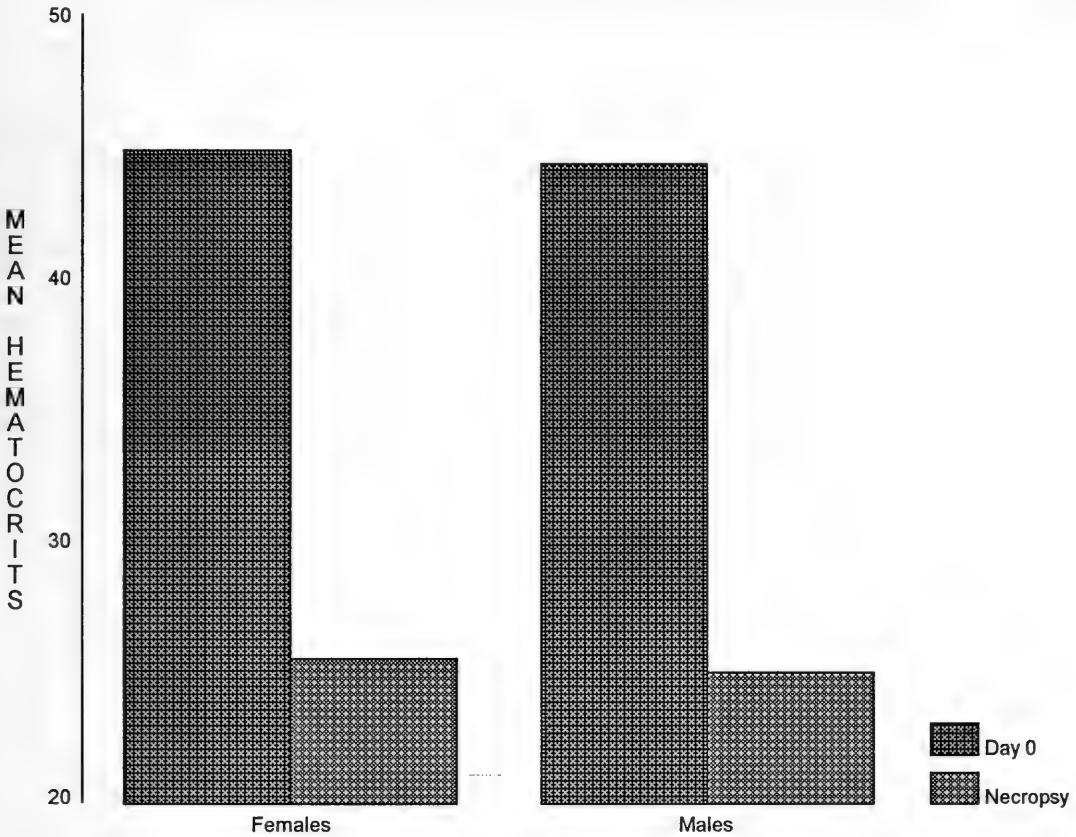

Figure 1. Mean Hcts of game-farm mallard ducks dosed with 8, No. 4, Pb shot on Day 0. n = 12 for Day O and 4 for necropsy.

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

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

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

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

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

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

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

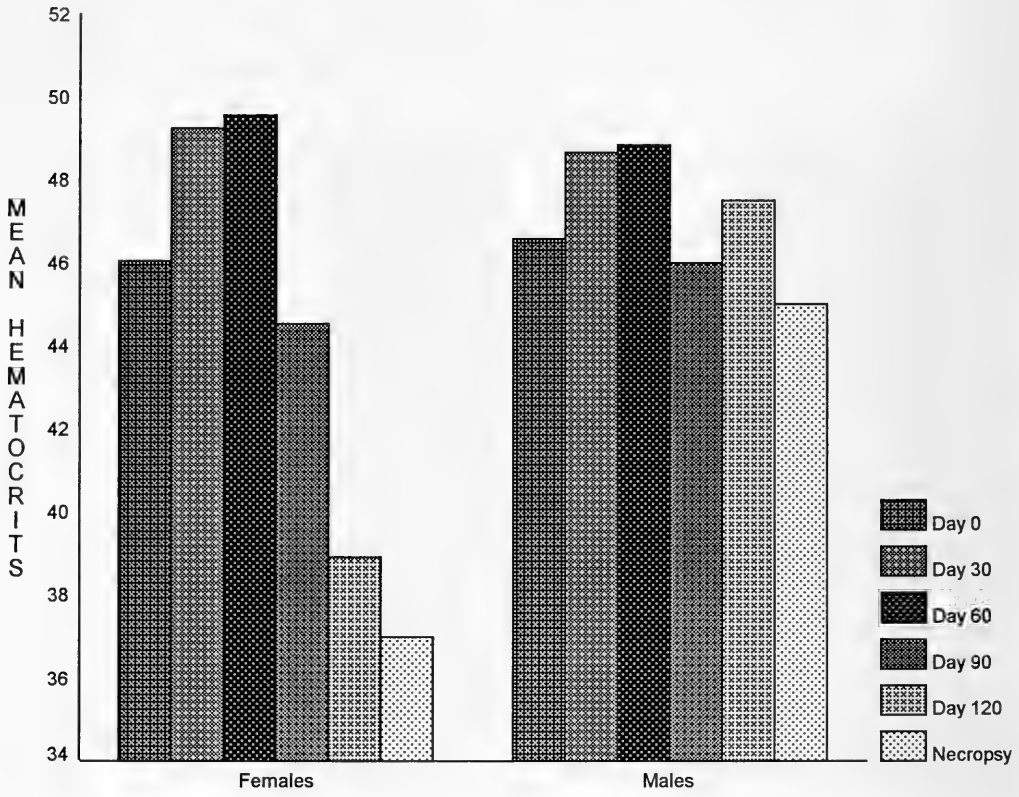

Figure 2. Mean Hcts of game-farm mallards dosed with 0 shot (controls); 8, No. 4, Fe shot; or 8, No. 4, Bi shot on Days 0, 30, 60, and 90. The three groups of ducks were combined for this graph. n = 54 females and 54 males for Days 0, 30, 60, and 90; n = 27 females and 27 males for Day 120; and n = 53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

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

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

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

[b] n = 7.

[c] SE.

[d] n = 12.

[e] n = 8.

[f] n = 6.

n = 18 for all others.

Difference in body weight:

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

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

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

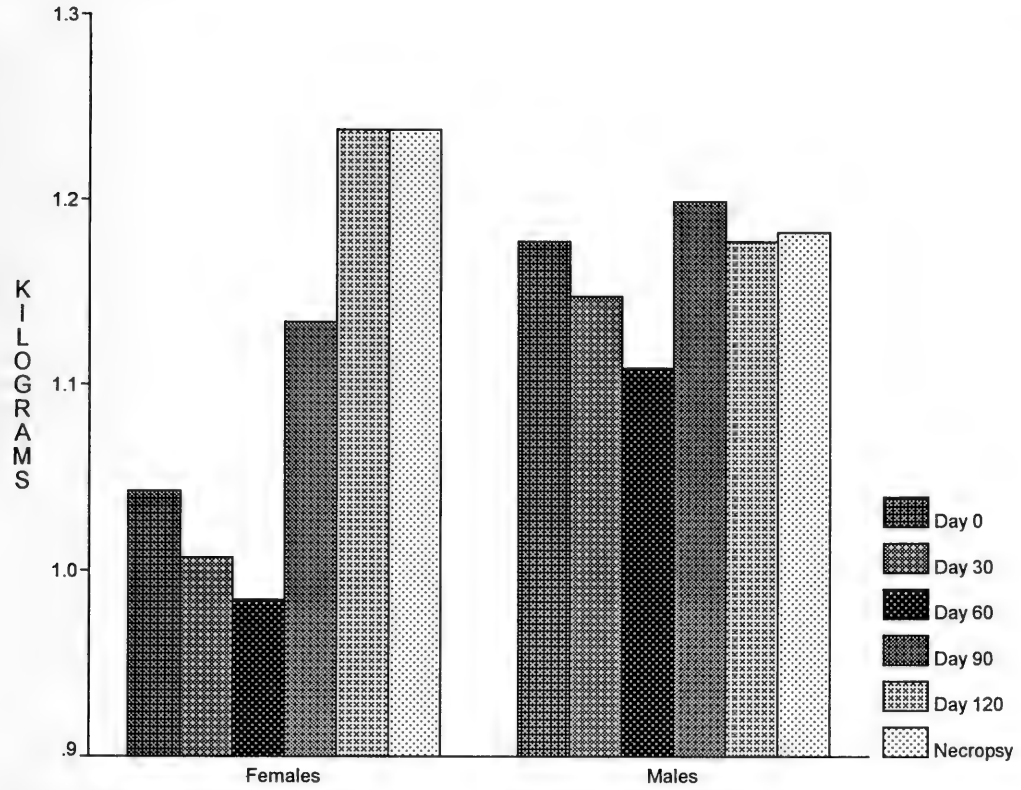

Figure 3. Mean body weight (kg) of game-farm mallards dosed with 0 shot (controls); 8, No. 4, Fe; or 8, No. 4, Bi shot on Days 0, 30, 60, and 90. The doses were combined for this graph. n = 34 for each sex for Days 0, 30, 60, and 90; n = 27 for each sex for Day 120; n = 53 for each sex for necropsy.

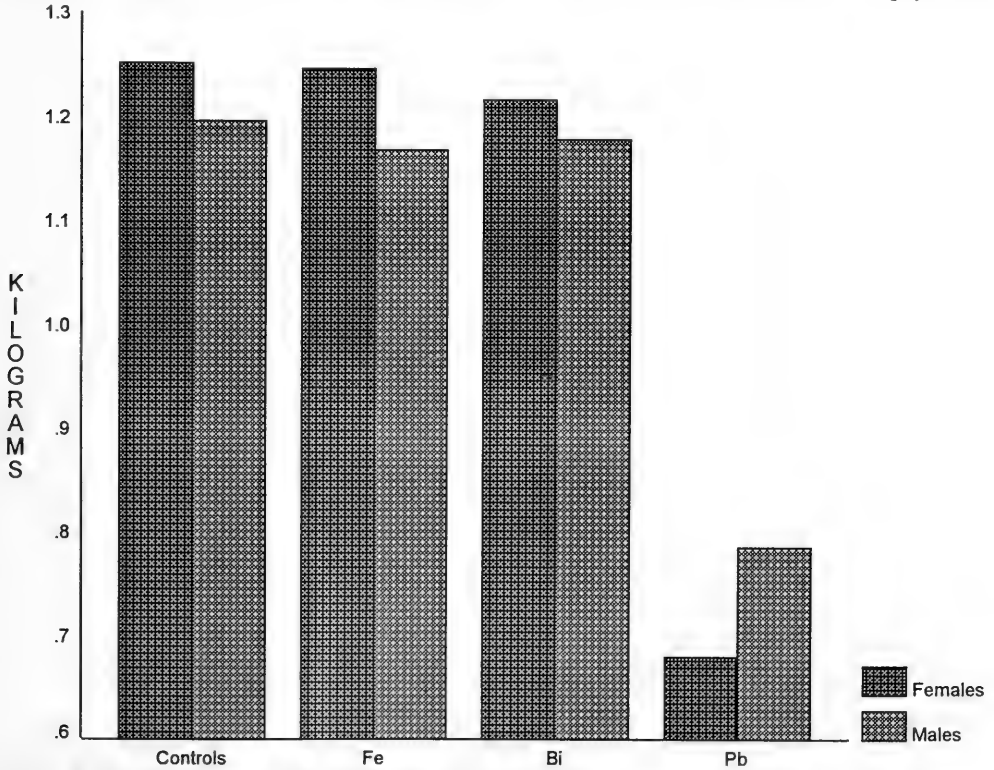

Figure 4. Mean body weight (kg) at necropsy of game-farm mallards dosed with 0 shot (controls); 8, No. 4, Fe shot; or 8, No., 4 Bi shot on Days 0, 30, 60, and 90, or 8 No. 4 Pb shot on Day 0. n = 18 for female

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

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

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

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

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

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

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

Shot Retention

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

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

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

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

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

SE.

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

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

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

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

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

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

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

Organ Weights

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

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

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

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

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

[a] SE.

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

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

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

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

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

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

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

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

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

Difference among doses in organ weights:

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

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

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

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

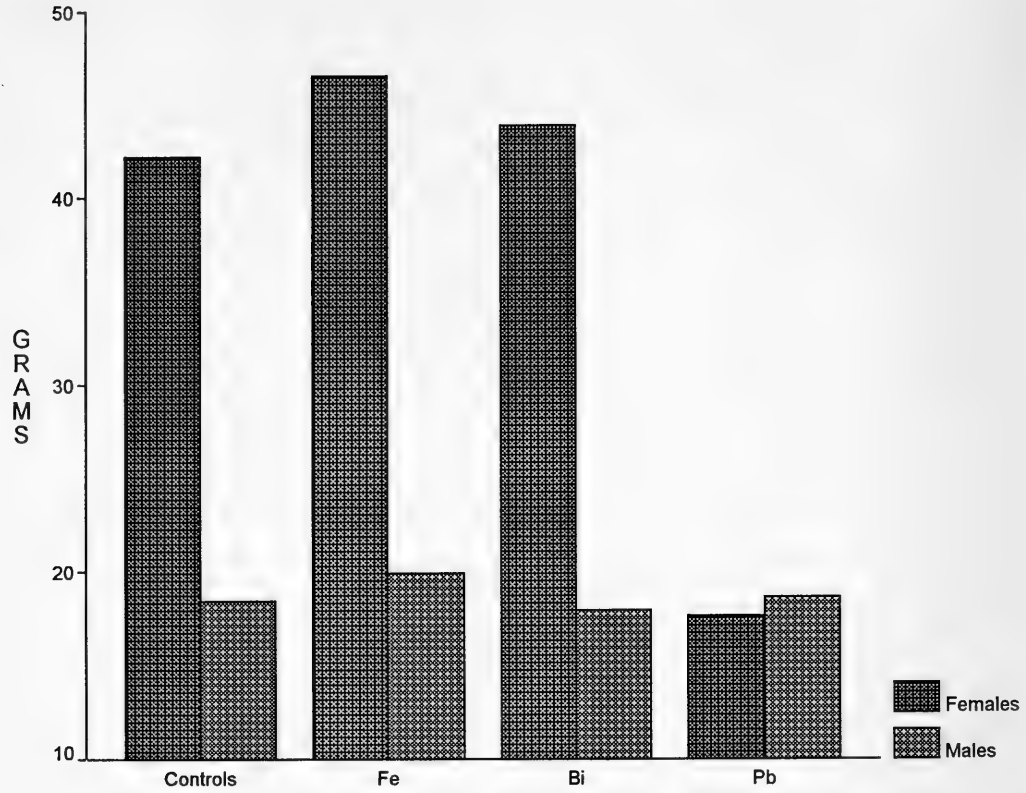

Figure 5. Mean weight (g) of livers of game-farm mallards dosed with 0 shot (controls); 8, No. 4 Fe, shot; or 8, No. 4, Bi shot on Days 0, 30, 60, and 90, or 8, No. 4, Pb shot on Day 0. n = 18 for each sex for 0-, Fe, and Bi-dosed ducks, and n = 6 for each sex for Pb-dosed ducks.

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

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

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

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

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

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

Analyses of Tissues and Other Materials

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

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

Kidneys

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

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

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

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

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

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

Liver

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

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

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

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

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

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

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

MDL:

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

Differences among doses with sexes combined:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Gonads

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Blood

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

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

Reproduction

Eggs

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

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

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

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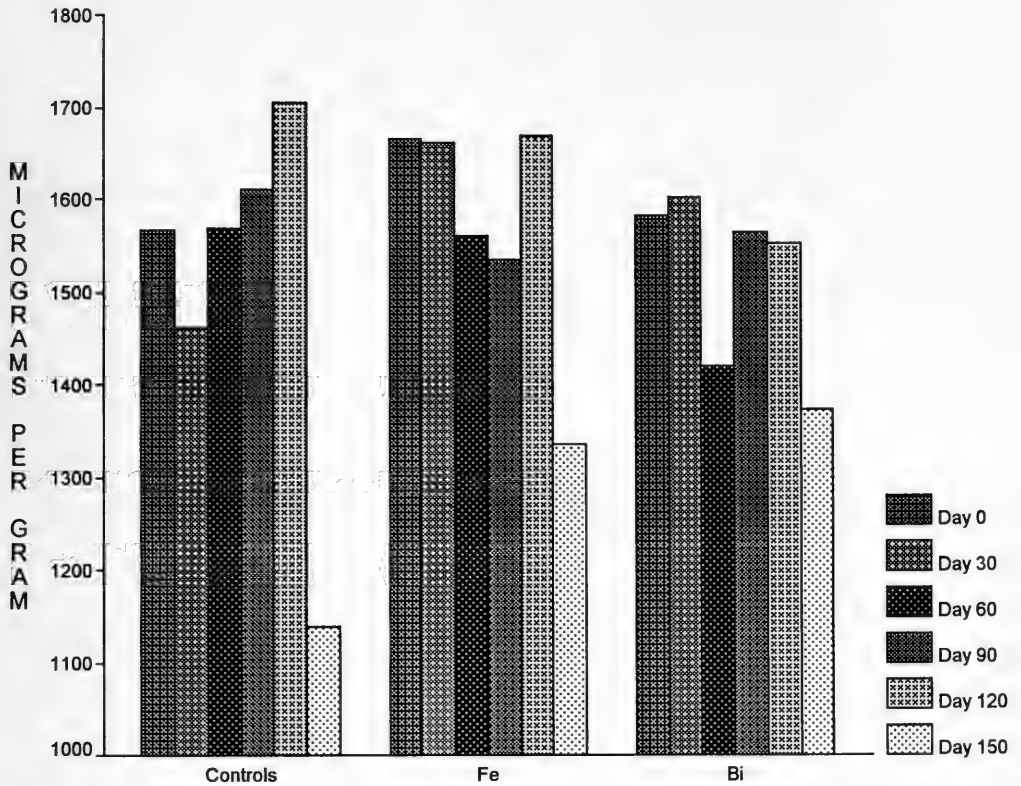

Figure 6. Mean concentration (µg/g) of P in the blood of game-farm mallards dosed with 0 shot (controls); 8, No. 4, Fe shot; or 8, No. 4, Bi shot on Days 0, 30, 60, and 90. The sexes were combined for this graph. See Table 7 for sample sizes.

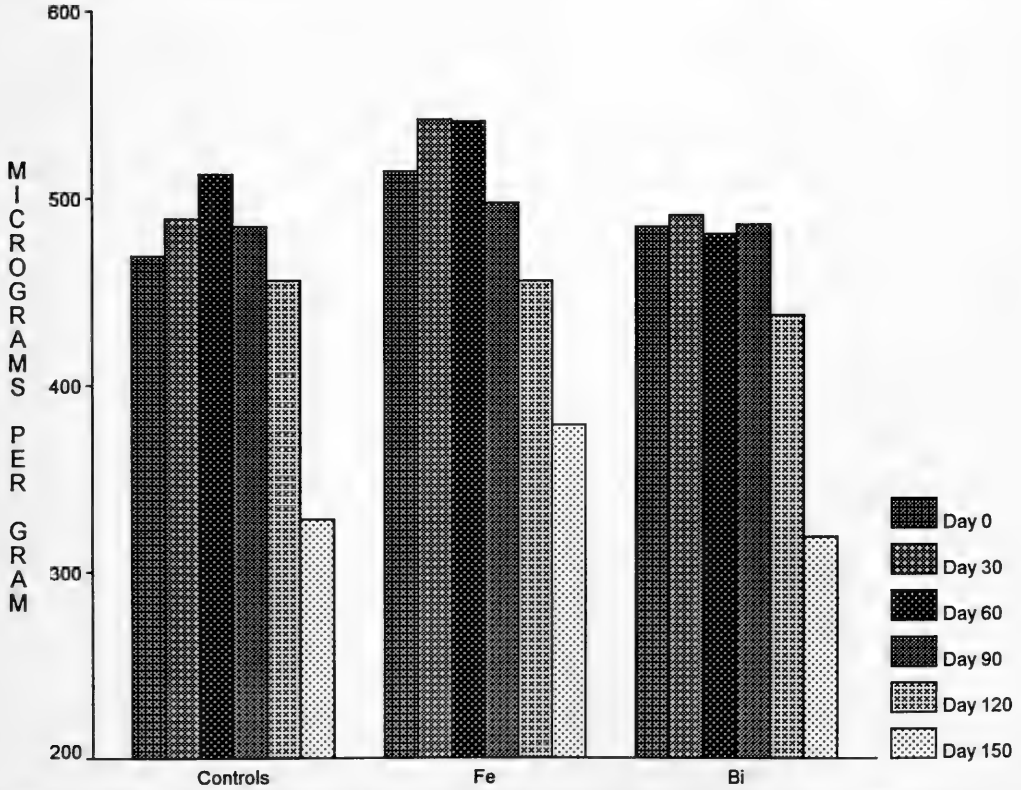

Figure 7. Mean concentration (µg/g) of Fe in the blood of game-farm mallards dosed with 0 shot (controls); 8, No. 4, Fe shot; or 8, No. 4, Bi shot on Days 0, 30, 60, and 90. The sexes were combined for this graph. See Table 9 for sample sizes.

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

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

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

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

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

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

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

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

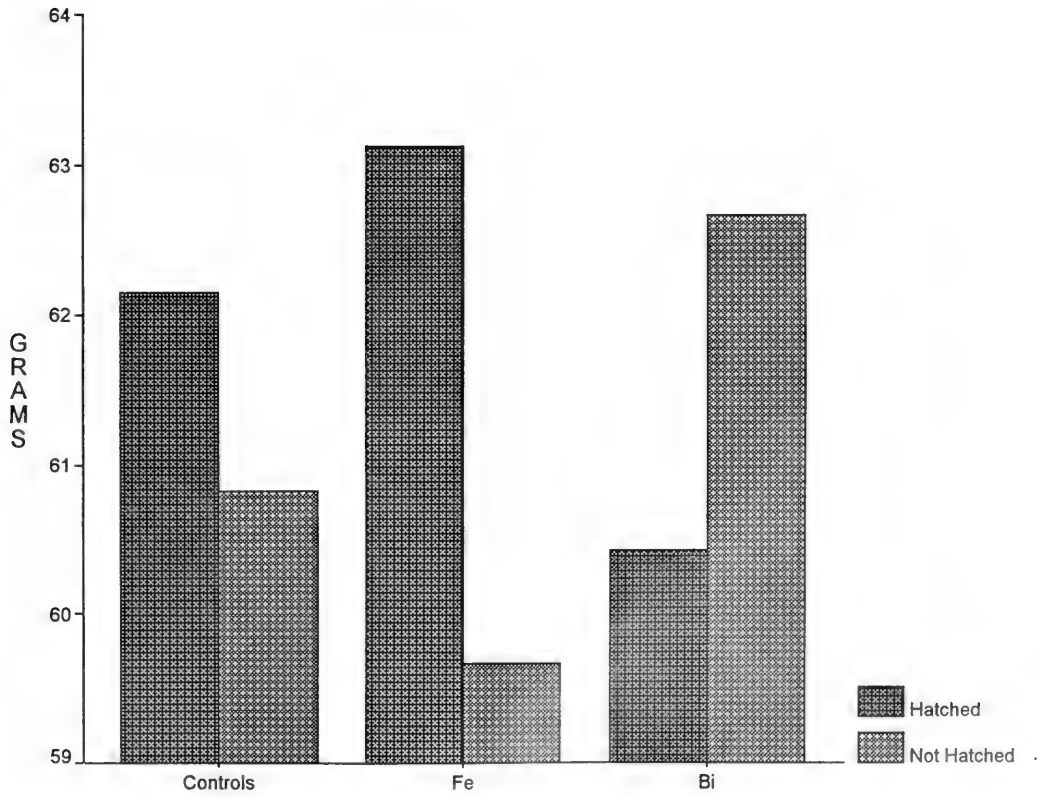

Figure 8. Mean weight (g) of hatched and not hatched fertile eggs from 0-dosed (controls), Fe-dosed, and Bi-dosed game-farm mallard pairs. See Table 11 for sample sizes.

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

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

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

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

Ducklings

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

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

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

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

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

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

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

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

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

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

[a] SE.

Difference in weight of hatched and unhatched fertile eggs:

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

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

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

[a] SE.

Differences among doses:

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

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

Differences between sexes:

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

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

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

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

[a] SE.

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

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

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

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

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

[a] SE.

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

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

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

[a] SE.

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

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

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

[a] SE.

MDL:

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

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

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

[a] SE.

Difference among doses:

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

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

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

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

[a] SE.

Difference among doses:

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

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

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

Egg Weights

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

Egg Shell Thickness

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

Fertility Rates

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

Hatchability Rates

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

Egg Shell Analysis

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

Egg Content Analysis

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

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

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

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

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

[a] SE.

MDL:

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

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

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

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

[a] SE.

MDL:

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

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

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

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

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

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

a=SE.

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

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

[a] SE.

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

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Age of Embryo at Time of Death

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

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

Histopathology

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

Adults

Kidneys

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

Liver

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

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

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

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

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Conclusions

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

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

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

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