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Jeffrey M. Levengood
Illinois Natural History Survey

Glen C. Sanderson
Illinois Natural History Survey

William L. Anderson
Illinois Natural History Survey

George L. Foley
University of Illinois and
Illinois Natural History Survey

Loretta M. Skowron
Illinois State Water Survey

Patrick W. Brown
Illinois Natural History Survey

James W. Seets
Illinois Natural History Survey

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Illinois Natural History Survey, David L. Thomas, Chief
A Division of the Illinois Department of Natural Resources

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Acute Toxicity of Ingested Zinc Shot to Game-farm Mallards

Abstract

We conducted a 30-day acute toxicity test of zinc (Zn) shot using 6- to 8-month-old wild-type game-farm Mallards (*Anas platyrhynchos*), 40 of which (20 males and 20 females) were dosed with 6 No. 4 candidate shot pellets containing 98% Zn and 2% tin (Sn); the remaining 40 ducks were dosed with 6 No. 4 steel (Fe) shot and served as controls. The Zn shot resulted in high mortality, with a greater proportion of females dying than males. For the 30-day study, survival averaged 18 and 23 days for female and male Zn-dosed ducks, respectively; all Fe-dosed ducks survived to Day 30. Ataxia/paresis and other signs of intoxication were noted in a large portion of Zn-dosed ducks. For all ducks retaining 6 shot pellets, including those that survived < 30 days, shot retention, percent of the original shot weight dissolved, and dissolution rates were similar for Zn- and Fe-dosed ducks. For those ducks that retained 6 pellets and survived to Day 30, percent loss of the original shot weight and the dissolution rate were higher in Zn-dosed ducks.

Mean body weight in Zn-dosed ducks decreased between Days 0 and 15, and between Days 15 and 30 in Zn-dosed females. Zn-intoxicated ducks lost a considerable proportion of their body weight between dosing and death. The kidneys of Zn-dosed ducks as a group were heavier, and the pancreases, livers, and gizzards lighter, as compared with Fe-dosed ducks. The liver (males) and kidneys of Zn-dosed ducks that died as a result of Zn intoxication were heavier, whereas the gonads (females) and gizzards were lighter, as compared with those that survived. The liver, pancreas, and kidneys increased, whereas the gonads (males) and gizzards decreased, as a proportion of total body weight in Zn-dosed ducks that died prior to Day 30.

Mean packed cell volume (PCV) decreased between Days 0 and 15 and increased between Days 15 and 30 in male and female Zn-dosed ducks. PCV values changed little in Fe-dosed ducks over the course of the study; however, PCV decreased dramatically in Zn-intoxicated ducks.

A variety of gross lesions was observed, most often associated with the gastrointestinal tract. Grossly, cecal lesions were the most consistent and dramatic changes observed. No macroscopic lesions were observed in the Fe-dosed ducks, and histologic lesions in this group were considered within normal limits for game-farm ducks. Histologic lesions observed in Zn-dosed Mallards most often included pancreatic apoptosis, splenic lymphoid depletion and/or lympholysis, necrohemorrhagic typhlitis, and necrosis of the epithelial cells of the renal tubules.

We detected high concentrations of Zn, and alterations in levels of other elements, in the tissues of Zn-dosed ducks relative to Fe-dosed controls. Mean Zn concentrations were greater in Zn-dosed ducks for all tissues examined, and levels in the kidneys, livers, and pancreases of Zn-intoxicated Mallards were similar to levels associated with toxic effects reported in other studies. Mean tissue tin (Sn) concentrations were below the Method Detection Limit (MDL) in all cases; few individual Sn values were above MDLs. Changes in tissue element concentrations tended to be more dramatic in ducks that died as a result of Zn intoxication than in Zn-dosed ducks that survived the experiment. Gender differences in analyte concentrations were detected for some tissues. These results indicated that dosing of 6- to 8-month-old game-farm Mallards with 6 No. 4 shot comprised of 98% Zn and 2% Sn produced toxic effects under the test conditions.

Introduction

Much continued interest exists in the development of nontoxic alternatives to lead (Pb) shot due to expansion of restrictions or bans on the use of Pb shot and dissatisfaction with the ballistic properties and hardness of steel shot. In some areas the use of steel shot has been prohibited amid timber industry concerns regarding damage to equipment used in the production of high-grade lumber, resulting in an additional demand for shot composed of softer materials. Currently, iron or steel (Fe) and bismuth/tin (Bi/Sn) shot are approved as nontoxic in both the U.S. and Canada, and the Canadian Wildlife Service has recently

approved the use of tungsten/polymer shot. Tungsten/iron (W/Fe) shot has been granted temporary conditional approval by the U.S. Fish and Wildlife Service (USFWS).

Zinc (Zn) shot is currently in use as a Pb shot substitute for waterfowl hunting in parts of Europe, and Zn coatings of less than 0.0002-inch thickness or less than 1.0% of the weight of the shot pellet are currently approved in the United States (Cyndi Perry, USFWS, pers. comm.). Although lighter in weight than Fe, the greater malleability of Zn makes it a popular alternative to Fe shot in Europe, where thin-walled barrels and expensive shotguns are popular. In addition, Zn shot shells currently are less costly than Bi and W.

Literature Review

Zinc is an essential element found in all living organisms and required for many biological processes and optimal health (Walsh et al. 1994). This element is required for the proper functioning of many enzymes, including those important in regulating the synthesis and catabolic rate of RNA and DNA (Vallee 1959; Prasad 1979).

The primary site of Zn absorption is the small intestine, and absorption is affected by intake level, chemical form ingested, and presence of other elements and compounds in the diet (Underwood 1971). Calcium (Ca), copper (Cu), iron (Fe), phosphorus (P), and phytates may decrease the absorption of dietary Zn (Becker and Hoekstra 1971; Walsh et al. 1994), and cadmium (Cd) may compete for protein-binding sites (Underwood 1971) or prevent excretion of Zn (Gunn et al. 1962). Blood Zn levels are reflective of changes in Zn intake; increased dietary Zn intake results in increased whole blood and plasma Zn concentrations (Underwood 1971).

Excretion by way of the feces is an important means of maintaining Zn homeostasis (Underwood 1971). Metallothioneins are metal-binding proteins that are important in mediating intracellular metal homeostasis by binding excess Zn and other metals (Eisler 1993). Zn induces metallothionein production, a process that may help protect against subsequent exposure. Intestinal metallothionein is thought to play a role in the regulation of Zn absorption across the intestinal mucosa (Starcher et al. 1980).

Underwood (1971) reported that normal Zn concentrations in various mammalian tissues ranged from 12 to 223 mg/kg dry weight (DW), with the highest levels found in the prostate. In another study (Prasad 1979), Zn levels in humans and selected domestic mammals varied from 45 to 2,330 mg/kg DW; the higher value was from hyperplasic prostate tissue. Eisler (1993) concluded that levels in tissues of birds and mammals were typically < 210 mg/kg DW, and that Zn poisoning usually occurs in birds at liver or kidney concentrations > 2,100 mg/kg DW and in mammals when kidney, liver, or pancreas levels exceed 274, 465, or 752 mg/kg DW, respectively. For poultry, concentrations in the liver, kidney, and pancreas of 200–700, 300–800, or 1,000–3,500 mg/kg fresh weight (FW), respectively, were indicative of Zn intake at levels likely to cause subclinical, clinical, or pathological signs of toxicity (Puls 1988).

Pancreatic Zn concentrations are relatively high under normal conditions. Zn is an important component of the pancreatic enzymes carboxypeptidase A and B, and is thought to play an important role in the production and functioning of insulin (Vallee 1959; Kirchgessner and Roth 1980). The pancreas is an important route of Zn excretion, and, as a result, increased pancreatic Zn concentrations and pancreatic dysfunction and histopathology are commonly associated with Zn insult.

Many aquatic organisms are sensitive to Zn in various forms, and the toxicity of Zn is affected by pH, alkalinity, dissolved oxygen level, and temperature (Skidmore 1964; Weatherly et al. 1980). Accounts of Zn poisoning in terrestrial vertebrates have been largely experimental or anecdotal; however, there is growing concern about increased exposure due to anthropogenic sources of Zn. Wild and domestic vertebrates have been impacted by high levels of Zn from smelters (Beyer et al. 1985; Sileo and Beyer 1985) and mining runoff (Chupp and Dalke 1964), and wildlife can be secondarily impacted through reduced and/or contaminated invertebrate prey populations and damage to vegetation (Beyer 1988). Commonly reported effects of intake of excessive levels of Zn in terrestrial vertebrates include depressed growth, lowered reproduction, anemia, ataxia, paresis, reduction or cessation of feeding, weight loss, depression,

enteritis, diarrhea, pancreatic and/or hepatic histopathology and dysfunction, developmental abnormalities, Cu and Fe deficiencies, high tissue Zn levels, lethargy, liver and/or kidney hypertrophy, internal hemorrhaging, and resorption, softening, or abnormal formation of bone (Underwood 1971; Eisler 1993; Walsh et al. 1994).

The interaction between Zn and other metals is complex. Zinc may operate antagonistically to protect an organism from the effects of high levels of other metals or cause deficiencies (e.g., Cu and Fe) and associated maladies, or function synergistically to exacerbate the detrimental effects of high levels of other elements. Depending on the chemical form and other conditions, Zn can promote or inhibit the growth of tumors, can be mutagenic or inhibit the mutagenic action of certain carcinogens, and can produce teratogenic effects or protect against some teratogens (see Eisler 1993). Walsh et al. (1994) concluded that the effects of Zn on immunological systems were unclear.

Poultry are routinely fed excessive levels of Zn to induce molting in order to promote long-term egg production. Although hens can tolerate levels as high as 20,000 mg/kg in their diet (Lu and Combs 1988), 8,000 mg/kg causes high mortality in chicks (Oh et al. 1979). Much lower levels can inhibit growth, suppress immune system function, cause Fe and Cu deficiencies, and impair pancreatic function in chicks. Ingestion of particles of galvanized wire used in the construction of cages, known as new wire disease, poses a hazard to caged psitticines (Reece et al. 1986; Howard 1992).

Irby et al. (1967) tested the toxicity of 10 shot types on 1-year-old male game-farm Mallards (*Anas platyrhyncos*) and found that mortality and weight loss in ducks dosed with 8 No. 6 Zn-coated Fe shot did not differ from that of controls. Two of four surviving Mallards dosed with Zn-coated Fe shot developed hemosiderosis of the liver, whereas the livers of all ducks dosed with uncoated and molybdenum-coated Fe shot contained hemosiderin (Locke et al. 1967).

Eighteen-month-old male Mallards dosed with 8 No. 6 Zn shot comprised of 92% Zn, 0.16% Pb, trace Fe, and 7% undetermined components exhibited weight loss, ataxia and paresis, kidney histopathology (one duck), increased liver Fe concentrations, and increased mortality (Grandy et al. 1968). The authors suggested that consideration of Zn as a nontoxic shot should be discontinued.

Gasaway and Buss (1972) fed Zn carbonate at levels ranging from 3,000 to 12,000 mg/kg to 7-week-old domestic Mallard ducks. Reductions in food consumption and body weight were associated with high dietary Zn levels. Compared with controls, Zn-fed ducks exhibited reductions or increases in organ weights, increased tissue Zn concentrations, lowered PCV and hemoglobin levels, and increased mortality, along with diarrhea and paralysis. Yellowish-red kidneys were the only gross lesions reported.

In contrast, French et al. (1987) dosed 1-year-old Mallards with either 5 or 10 nearly pure (99.9%) No. 6 Zn shot. After 28 days the authors reported no gross lesions or abnormalities in either dosing group, and no histopathological changes in liver or kidneys of the lower dosing group were noted. The dosed ducks gained weight, exhibited normal liver Fe levels, and increased liver Zn concentrations. The authors stated that Zn-dosed ducks had "markedly better conditioned plumage" than controls, and concluded that Zn shot was an acceptable substitute for Pb shot for hunting waterfowl.

The present study was designed to conduct the acute toxicity test of Zn shot as specified in Canadian Wildlife Service (CWS) guidelines (Environment Canada 1993). Thus, the primary objective was to determine if Zn shot is toxic to game-farm Mallards under the prescribed test conditions.

Methods

Toxicity Study

The acute toxicity test was conducted using 40 female and 40 male wild-type game-farm Mallards, 6 to 8 months of age. Upon arrival at the Illinois Natural History Survey's (INHS) facility in Champaign, the ducks were weighed and randomly assigned to pens, with 1 duck per pen. Forty ducks (20 females and 20 males) were randomly assigned to one of the two treatments (dosed with 6 No. 4 Zn or 6 No. 4 Fe shot).

The consecutively numbered, elevated, outdoor 1-m^3 pens were constructed of vinyl-coated 1-inch (25.4-mm) mesh, 14-gauge wire. A 10x40-yard (9.1x35.6-m) pole barn without sides provided a roof over the pens. Individual

metal trays were provided under each pen to catch droppings and facilitate cleaning.

Facilities for holding the ducks were inspected by several members of the Laboratory Animal Care Advisory Committee, University of Illinois, prior to and during the study. A commercial duck ration (Purina Duck Grower W/O, Purina Mills, Inc., St. Louis) was provided *ad libitum* during the 20-day acclimatization period. This ration contained ≥ 16% protein, 5.0% crude fiber, 4,000 to 9,000 ppm Ca, ≥ 5,500 ppm P, and 80 ppm Zn. On the date of dosing, the pellets were removed and whole-kernel corn was provided *ad libitum* for the duration of the study. The corn contained 9.5% protein, 3.7% acid-detergent fiber, 12.5 ppm Ca, 4,390 ppm P, 25.6 ppm Fe, and 14.3 ppm Zn.

The study was initiated on 27 August 1996 (Day 0) when the ducks were weighed, bled, and dosed. A small plastic funnel fitted with a plastic tube (3/8 inch [9.5 mm] outside diameter, 9 inches [22.9 cm] long) was inserted down the gullet and into the proventriculus. The tube was kept in a pail of water between dosings to facilitate insertion into the alimentary canal. The shot were poured into the funnel and flushed into the proventriculus with approximately 5 mL of water. Before dosing, the doses (6 No. 4 [0.13-inch dia.]) of Zn or Fe shot were counted, weighed, and placed in individual vials in the laboratory. The type and weight of the shot dose and a randomly assigned pen number were recorded on the top of each vial and on a field data sheet. At dosing, the shot dose was matched with the corresponding duck (pen).

Blood was collected by venipuncture in heparinized microhematocrit capillary tubes for packed cell volume (PCV) determination and in 2.5-mL syringes fitted with 20-gauge, 1-inch (25.4-mm) needles to obtain samples to ascertain concentrations of selected elements. 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. Microhematocrit tubes were centrifuged for 5 minutes at 13,000-g force, and the PCV read using a Micro-Capillary Reader (Damon/IEC).

Detailed behavioral observations were recorded each morning (without reference to dosing) and a cursory visit was made in the afternoon to note any changes in severity of signs, process any dead ducks, and ascertain whether any ducks might be candidates for euthanasia. We modified the observations of Grandy et al. (1968) to rank affected individuals as exhibiting mild, moderate, or severe signs as follows: *mild*— signs may not be readily apparent; close observation reveals abnormal gait, with bird at least occasionally lifting its feet higher than normal when walking or running; may appear slightly lethargic; otherwise may appear normal; *moderate*— signs readily apparent; bird high-stepping as if walking on hot surface; easily noted difficulty in walking or running; trouble maintaining balance when disturbed and regaining it after a fall; may exhibit difficulty folding wings; may appear normal if undisturbed; will attempt to evade on approach; *severe*— bird cannot stand or maintain balance, or can do so only with great difficulty; no or only feeble attempt to evade on approach.

For reporting of observational data, ducks that exhibited mild signs for only 1 day during the course of the study were not recorded as exhibiting signs of toxicosis. This precaution reduced the possibility that the typical gait of ducks walking on the wire pens was confused with gait abnormalities indicative of Zn toxicosis. Also noted were the condition of feces (if remarkable), whether each individual had apparently fed (noted by the presence or absence of spilled corn on the duck's tray), and any other noteworthy observations.

On 3 September 1996 (Day 7) all dosed ducks were fluoroscoped by a radiologist at the University of Illinois College of Veterinary Medicine's Large Animal Radiology Laboratory to confirm retention of 6 shot pellets. Ducks were transported in poultry crates a distance of 1.6 km to the radiology lab. For fluoroscopy, each bird was restrained in a half-gallon paper milk carton with a hole cut in the bottom, which allowed the head and neck to protrude. The four-sided carton was turned 90° to provide dorsal, ventral, and lateral views, which facilitated determination of the number of pellets present. Ducks retaining fewer than 6 pellets were re-dosed to replace the missing pellets.

All surviving ducks used in the study were weighed and blood was collected from the ulnar vein as scheduled on 11 September (Day 15) and 26 September 1996 (Day 30). Subse-

quently, the ducks were euthanized by decapitation and necropsied on Day 30 (with the exception noted below), and the gizzards, livers, kidneys, pancreases, and gonads were excised, weighed, and frozen.

A total of 59 (20 Fe-dosed controls, 39 Zn-dosed) ducks were necropsied by a board-certified veterinary pathologist (GLF) over the course of the study. These included ducks that died or were euthanized prior to Day 30 and representative numbers of surviving ducks from each treatment group. Gross lesions, total body weight, and weights of liver, kidney, pancreas, gonad, and spleen were recorded at the time of necropsy. Representative samples of major organs (liver, kidney, pancreas, gonad, spleen, proventriculus, ventriculus, small intestine, large intestine, ceca, adrenal, heart, lung, sciatic nerve, and bone marrow) were fixed in 10% neutral buffered formalin. The tissues were subsequently submitted for routine paraffin embedding and sections were stained with hematoxylin and eosin. One duck (#41) was not examined histologically due to a prolonged postmortem interval (died 9/10 and necropsied on 9/20), the carcass having been left in a refrigerator for this period. All tissues were reviewed by a board-certified anatomic veterinary pathologist. Tissues were not identified as to the treatment group until after all histopathologic assessments were completed.

The necropsies on the ducks surviving to Day 30 were conducted in the Animal Autopsy Room of the Natural Resources Studies Annex on the campus of the University of Illinois. On Days 30 and 31 the pathologist examined, weighed, and preserved a sample of kidney, liver, pancreas, and gonad for histopathological examination from 10 randomly selected ducks from each group (sex by dose), with the exception of Zn-dosed females of which < 10 individuals survived. These ducks were euthanized over a period of 2 days to insure that fresh specimens would be examined. The organs from the remaining ducks were examined, removed, and weighed by project personnel on Days 30 and 31. All tissue samples where placed in individual, numbered, plastic bags and stored in the freezer. The frozen organs were moved to the freezer at the Illinois State Water Survey and stored with the blood samples until thawed for analysis.

Statistical Methods

Normality was assessed with the Kolmogorov-Smirnov Statistic with Lilliefors Significance Correction, the Skewness Statistic, and visually using frequency histograms. Levene's test was used to assess homogeneity of variances between groups. Log_e and arcsin transformations were used to improve distributions and reduce heterogeneity. We examined variation in whole body and organ weights, PCVs, and concentrations of selected elements in tissues by a randomized, 2 x 2 factorial, fixed effects ANOVA, using sex and dose as grouping factors. Student's *t* test was used to compare differences between Zn-dosed ducks that survived and those that did not except when sample size for either group was ≤ 5 cases, in which case we employed Mann-Whitney U testing; these tests were available through the T TEST and NPAR TESTS procedures of SPSS (SPSS, Inc. 1996a). Pooled variances and adjusted degrees of freedom were used for *t* tests when significant heteroscedasticity was detected using Levene's test (SPSS, Inc. 1996b). For one-tailed nonparametric testing, $U^1 = n_1 n_2 - U$. (Zar 1984).

Sexes were treated separately except in comparisons of shot retention and dissolution. Kaplan-Meier survival functions were calculated using the KM procedure of SPSS (SPSS, Inc. 1996c); all cases were specified as uncensored and functions were compared using the Breslow, or generalized Wilcoxon, test. Coefficient of variation was calculated as standard deviation/mean (Zar 1984). Change in body weight was expressed as $\{(\text{body weight}_{tj} - \text{body weight}_{ti})/\text{body weight}_{ti}\} * 100$, and change in PCV as $\{(PCV_{tj} - PCV_{ti})/PCV_{ti}\} * 100$. The high mortality observed in Zn-dosed ducks precluded a meaningful repeated measures analysis; changes in variables examined over time were compared descriptively. A probability level of $P \leq 0.05$ was accepted as significant.

Chemical Analyses

Sample Storage

Samples were inventoried upon receipt, stored frozen at –10°C, and monitored daily. The samples were allowed to thaw to room temperature and were labeled by tissue type and specimen number prior to preparation for metals analysis. The gender and shot dose for each specimen were not disclosed to individu-

als analyzing samples. Occasionally glass test tubes broke during the freezing and thawing of blood samples; affected samples were transferred to polypropylene test tubes if breakage occurred.

Sample Digestions

Blood plasma, blood cells, livers, kidneys, and pancreases were acid digested prior to analysis using inductively coupled argon plasma emission spectroscopy (ICP). Because wet weight concentrations of the blood and organs were desired, samples were not dried prior to digestion. The percent dry weights of the sample types were determined separately. The elements of interest were calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), phosphorous (P), tin (Sn), and zinc (Zn). Beryllium (Be) was used as an internal standard.

Digestions for ICP analysis

We used samples of 0.5 to 1.0 g. A mixed portion of the sample was weighed to 0.1 mg using an electronic top-loading balance and placed into a tared 50 mL conically tipped polypropylene centrifuge tube. The tubes were precleaned for 24 hours with a 10% nitric acid (HNO_3) soak, then rinsed in deionized water. The samples and tubes were tared, 1.0 mL of hydrogen peroxide (H_2O_2) was added, and the weights recorded. Approximately 20 to 30 mL of a 2% HNO_3 and 10% hydrochloric acid (HCl) and internal standard solution were then added to the sample after taring. The Be concentration was targeted at 2.00 mg/L. The samples were then homogenized into a slurry using a saw-toothed generator manufactured with titanium and TFE-fluorocarbon by Pro Scientific, Monroe, CT. The internal standard solution was used to rinse excess materials from the generator, and the amount was accounted for in the total weight.

Sample preparations were accomplished using a SpectroPrep System automated microwave digestion system manufactured by CEM Corporation, Matthews, NC. A 15-mL sample loop was used. After heating, cooling, and filtering, about 9.0 mL of each sample was collected and deposited by autosampler into 15-mL polypropylene test tubes. This digestate was then used for ICP analysis without any further treatment. High purity acids and hydrogen peroxide (Baker Ultrex and Fisher Optima brands) were used for all digestions.

Analytical Methods

ICP

We used a Thermo Jarrell Ash (TJA) AtomComp Model 61 vacuum spectrometer with a polychromator configured with 44 fixed channels, including analytical lines for high and low concentrations of Ca and Mg. Although we reported results for a limited number of elements, measurements were actually made for 30 analytes to monitor for spectral interferences, 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 analyses. We modified the method, using Be as an internal standard because it was not a listed analyte, causes no spectral or background interferences, and it is very precisely detectable.

Quality Control

We calibrated instruments daily and verified the standard curve using NIST traceable quality control samples (QCS). Samples (usually 10) were bracketed by calibration blanks, laboratory fortified blanks (LFB), and instrument performance check solutions (ERA3410, IPC, ERA9959, Check Standards) during analysis, as well as periodic checks of 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 LFB was formulated as 0.5% of the concentrations of the high calibration standard. It was prepared with the same stock standard solutions. The ICP check standard was formulated for a concentration at the midpoint of the calibration curve. It was traceable to National Institute of Standards & Technology (NIST) Standard Reference Materials (SRMs).

Ten percent of the samples were digested and analyzed in duplicate, half of them spiked. Spike solutions were traceable to NIST SRMs. Digestion blanks and spiked digestion blanks were prepared at a frequency of 10%, and were

processed in the same manner as the samples.

We saved the resulting ICP data into database files using ThermoSpec (TJA) software utilizing Enable OA. These data were then imported into Enable spreadsheets for tabulations and calculations. We saved the Enable spreadsheets in a Lotus 1-2-3 format on diskette.

The Method Detection Limit (MDL), defined as the minimum concentration of a substance that can be identified, was employed to establish the limits of detection for tissue element concentrations (Glaser et al. 1981). To be meaningful, values should average ≥ 2 times the MDL. For statistical analysis of chemical concentrations, values that were less than the Method Detection Limit (< MDL) were entered as one-half the MDL value. Concentrations of elements in tissues are expressed as µg/g wet weight. Tissue water content was determined to allow conversion to dry weight for comparisons with published information. Three sets of 10 randomly selected samples each were dried overnight at 104°C; the grand means for each tissue expressed as mean % dry weight were as follows: red blood cells (RBC) Day 0, 31.98%; RBC Day 15, 33.33%; RBC Day 30, 33.51%; blood plasma (BP) Day 0, 6.18%; BP Day 15, 5.77%; BP Day 30, 5.72%; liver, 32.23%; kidney, 25.70%; pancreas, 33.57%.

Results

Method Detection Limits and Quality Assurance

MDLs (mg/g) for Ca, Cu, Fe, Mg, P, Sn, and Zn, respectively, in tissues were as follows: erythrocytes, 1.35, 0.37, 1.69, 0.26, 17.8, 4.32, 0.43; plasma, 0.73, 0.20, 0.92, 0.14, 9.67, 2.35, 0.23; kidneys, 1.04, 0.28, 1.30, 0.20, 13.7, 3.34, 0.33; liver, 1.09, 0.30, 1.36, 0.21, 14.4, 3.50, 0.35; pancreas, 1.08, 0.29, 1.36, 0.21, 14.3, 3.48, 0.35. Results of analyses of duplicates, spiked samples, and blanks were satisfactory and are presented in the Appendix.

Shot Composition

Ten randomly selected Zn shot pellets were composed of an average (± SD) of 98.03% (± 0.51%) Zn and 1.97% (± 0.03%) Sn; other elements averaged < 0.1% each. The steel (Fe) shot were commercially available pellets sold for reloading shotshells and were not analyzed.

Survival

All Fe-dosed ducks survived to Day 30/31, when the ducks were euthanized. Sixteen of 20 (80%) female and 9 of 20 (45%) male, for a total of 25 of the 40 (62.5%) Zn-dosed ducks, died prior to Day 30 (3 dorsally recumbent individuals were euthanized). In the present study, survival in male Zn-dosed ducks averaged 23 days, and ranged from 9 to 30 days. Survival in female Zn-dosed ducks averaged 18 days, and ranged from 5 to 30 days. Survival rates did not differ between male and female Zn-dosed ducks ($X^2 = 3.21$; $P = 0.07$).

Retention and Dissolution of Shot

All of the Fe-dosed ducks and 36 of 40 (90%) Zn-dosed ducks retained all 6 shot to 3 September 1996 (Day 7) when they were fluoroscoped. Of the Zn-dosed ducks, 1 male and 1 female each had 2, 1 female had 3, and 1 male had 5 shot in their gizzards. These ducks were re-dosed to replace the missing pellets.

After necropsy, the contents of the gizzard, proventriculus, and a short length of intestine were examined for shot. The gizzards of 8 Zn-dosed ducks contained fewer than 6 shot, however, pellets recovered from 4 of the individuals that survived to Day 30 were worn to tiny pellets. These ducks retained 2, 3, 4, and 5 pellets, respectively. Because of the small size of the remaining shot and the high overall retention rate observed, we assumed that the other pellets had completely dissolved. Given this assumption, we accounted for 97.9% (235/240) of the original dose of Fe shot, and 96.3% of the original Zn shot dose. The difference in mean number of shot recovered between Fe- and Zn-dosed ducks was not significant ($t_{0.05(2),78} = -0.71$, $P = 0.48$).

For all ducks that retained 6 shot, including those Zn-dosed birds that survived < 30 days, the mean percentage of the original shot weight that had dissolved appeared slightly higher in Fe-dosed (56.3 %) than in Zn-dosed (53.3 %) ducks, although this difference was not significant ($t_{0.05(2),41} = -0.567$, $P = 0.57$). The percent of the original shot weight dissolved ranged from 9% to 100% (< 1.0% remained) in Zn-dosed ducks and from 36% to 76% in Fe-dosed ducks. Dissolution rates (0.017 g/day) were identical in these two groups.

In ducks that retained 6 shot and survived to Day 30, the mean percentage of the original

shot weight dissolved was significantly higher in Zn-dosed (88.1%) than in Fe-dosed (53.3%) ducks ($t_{0.05(2),49} = 5.59$, $P = < 0.001$). The percentage of shot weight dissolved ranged from 71% to 100% (< 1.0% remained) in Zn-dosed ducks. The dissolution rate was also higher in Zn-dosed (0.022 g/day) compared with Fe-dosed (0.017 g/day) ducks ($t_{0.05(2),49} = 11.10$, $P < 0.001$). The dissolution rate ranged from 0.02 to 0.03 g/day in Zn-dosed and from 0.01 to 0.02 g/day in Fe-dosed ducks. We detected no differences ($P > 0.05$) between the sexes for the mean number of shot retained, percent of original shot weight dissolved, or dissolution rate.

Signs of Intoxication

Behavioral signs of intoxication included ataxia, paresis, and reduction or cessation of food intake. Signs of Zn toxicosis were first noted in 9 ducks (7 females) on Day 4 of the experiment. By Day 15, 32 of 40 (80%) Zn-dosed ducks had exhibited at least mild (> 1 observation day) signs.

Thirty-five of 40 (87.5%) Zn-dosed ducks exhibited behavioral signs during the course of the experiment. Of these, 26 (74%) were ranked as moderate or severe at some point over the 30 days. Zinc-dosed ducks typically exhibited a reduction and ultimately cessation of feeding several days before showing moderate signs of toxicosis. Five Zn-dosed ducks (3 males and 2 females) survived the experiment without displaying any behavioral signs. All animals that exhibited only mild behavioral signs survived to Day 30, whereas only 2 ranked with moderate signs at some point survived to the end of the experiment. Some Zn-dosed ducks went through periods of improvement and deterioration over the course of a few days or even several hours; however, no animals showing behavioral abnormalities completely recovered from the effects of Zn-intoxication over the course of the experiment.

The severely anemic condition of Zn-dosed animals became apparent through pallor of the oral cavity. Dark or bright green feces were noted within 1 or 2 days of dosing. Five Zn-dosed ducks passed blood with their feces; however, this condition persisted to death in only one female. Another female passed blood once, but exhibited no other signs throughout the experiment. Other signs of Zn toxicosis noted included diarrhea, excreta with a foul odor, a drooping or tucked tail, clacking of the bill and associated uncontrolled movements of the head, and evasive behavior often associated with diseased waterfowl (e.g., would try to hide under the water pipe in their pen).

Body Weight

All ducks gained weight (for males, $\bar{x} = 60$ g; for females $\bar{x} = 40$ g) during the 20-day acclimatization. Both male and female Zn-dosed ducks lost weight, on average, between Days 0 and 15. Mean body weight in Zn-dosed males increased between Days 15 and 30, whereas mean body weight in Zn-dosed females did not change during this period (Table 1). These data include body weights for ducks that died between Day 0 and 15 and Day 15 and 30, respectively. Variability increased over time, due to weight loss in ducks experiencing moderate to severe Zn-toxicosis, as compared with ducks exhibiting no or mild signs. Male and female Fe-dosed ducks showed little or no change in weight from Day 0 to 15 and from Day 15 to 30.

Males weighed significantly more than females at Days 0, 15, and 30, and Fe-dosed ducks weighed significantly more than Zn-dosed ducks at Days 15 and 30 (Table 1). We also detected an interaction between sex and dose, caused by a combination of sexual dimorphism in body weight and little or no weight change in Fe-dosed ducks compared with greater effects in Zn-dosed ducks. The effects of dosing with Zn shot on body weight were more pronounced in females, perhaps due to a greater dose/unit body weight (at dosing: for males, $\bar{x} = 0.62$ mg Zn/g body weight; for females, $\bar{x} = 0.69$ mg Zn/g body weight).

Male and female Zn-dosed ducks experienced a greater change in body weight between Days 0 and 30 or death (if < 30 days), as compared with Fe-dosed ducks (Fig. 1). Change in body weight was similar between Fe-dosed Mallards and Zn-dosed ducks that survived to Day 30. Male and female Zn-dosed ducks surviving < 30 days experienced a considerable loss of body weight, on average, between Day 0 and death.

Organ Weights

Liver

Liver weight did not differ significantly between male and female Mallards; however,

Table 1. Mean body weight ± SE (CV) of 6- to 8-month-old male and female game-farm Mallards dosed with 6 No. 4 Zn or Fe shot.[a]

		Mean Body Weight (Kg)		
Dose	Sex	Day 0	Day 15[b]	Day 30[c]
Zn	M	1.16 ± 0.02 (0.09)	0.99 ± 0.04 (0.17)	1.02 ± 0.05(0.18)
	F	1.04 ± 0.02 (0.09)	0.71 ± 0.04 (0.25)	0.71 ± 0.05(0.25)
Fe	M	1.15 ± 0.02 (0.07)	1.13 ± 0.02 (0.08)	1.13 ± 0.02(0.07)
	F	1.03 ± 0.02 (0.09)	0.98 ± 0.02 (0.09)	0.97 ± 0.02(0.08)

[a] n = 20 except male Zn-dosed Day 30, n = 15; female Zn-dosed Day 15, n = 18, Day 30, n = 13

[b] includes ducks surviving < 15 days

[c] includes ducks that survived > 15 and < 30 days

Results of ANOVA testing:

Day 0

Sex $F_{1,76} = 38.2$; $P < 0.001$

Day 15

Dose $F_{1,74} = 42.4$; $P < 0.001$

Sex $F_{1,74} = 40.6$; $P < 0.001$

Inter. $F_{1,74} = 6.8$; $P = 0.05$

Day 30

Dose $F_{1,64} = 38.2$; $P < 0.001$

Sex $F_{1,64} = 50.5$; $P < 0.001$

Inter. $F_{1,64} = 9.0$; $P = 0.02$

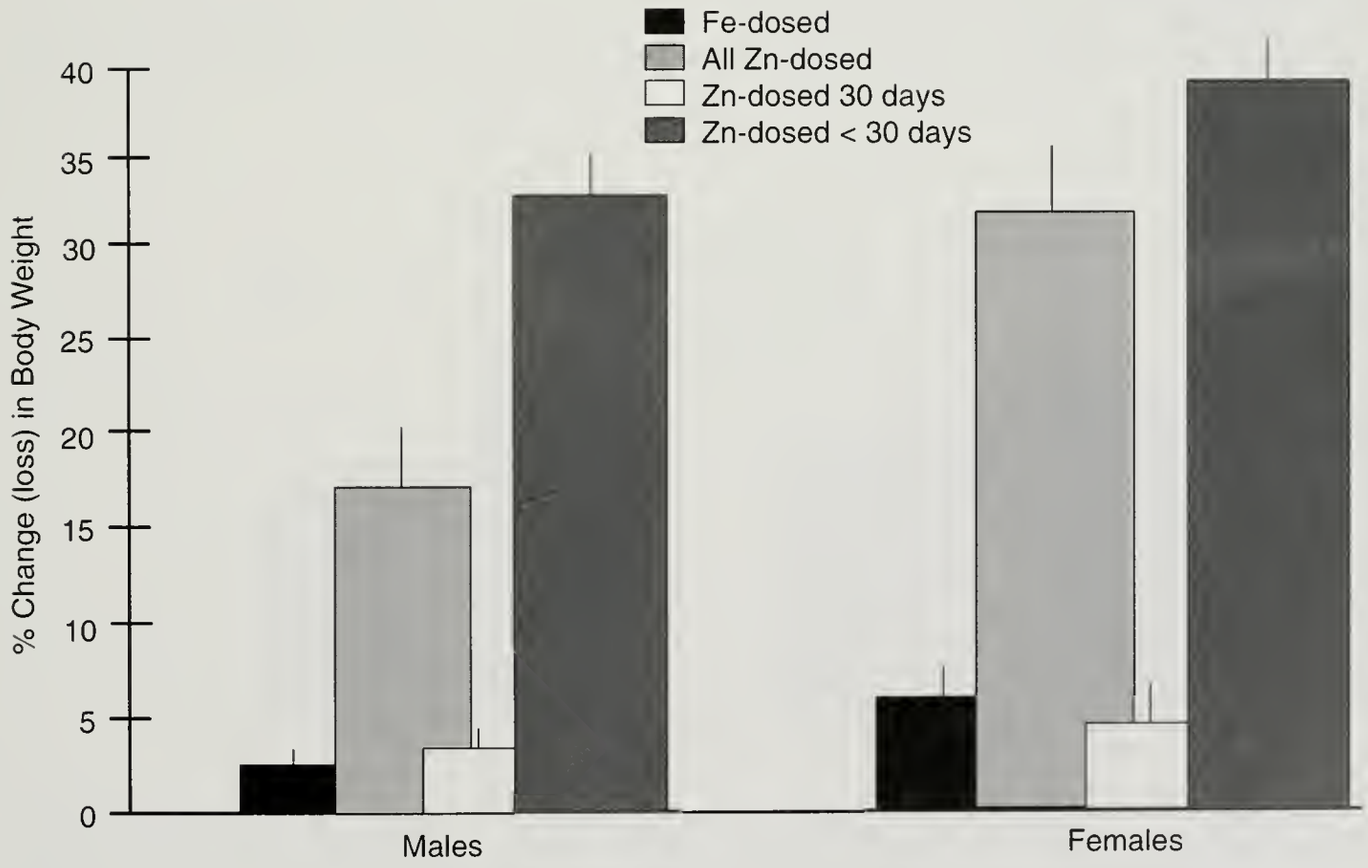

Figure 1. Mean change (loss) in body weight between Days 0 and 30 (or death) in game-farm Mallards dosed with 6 No. 4 Zn or Fe shot. Sample sizes equal 20 except for Zn-dosed males surviving 30 days, n = 11, males surviving < 30 days, n = 9; females surving 30 days, n = 4; females surviving < 30 days, n = 14. Vertical lines represent +S.E.

mean liver weight was significantly greater in Fe-dosed ducks than in Zn-dosed ducks (Table 2). Liver weight in Zn-dosed males that died prior to Day 30 was significantly greater than in those surviving to Day 30 ($t_{0.05(1)18} = -2.79$, $P < 0.05$). Mean liver weight did not differ between female Zn-dosed ducks that survived to Day 30 and those that did not ($U'_{0.05(1)4,16} = 46$, $P > 0.10$).

Table 2. Mean ± SE (CV) weight of selected organs in game-farm Mallards dosed with 6 No. 4 Zn or Fe shot.[a]

	Sex	Dose	Mean Weight (g)
Liver			
	M	Zn[b]	16.8 ± 1.2 (0.3)
	M	Fe	18.0 ± 0.5 (0.1)
	F	Zn[b]	15.3 ± 1.2 (0.3)
	F	Fe	16.9 ± 0.6 (0.2)
	M	Zn30[c]	14.5 ± 0.9 (0.2)
	M	Zn < 30[d]	19.7 ± 2.0 (0.3)
	F	Zn30[c]	12.6 ± 0.2 (0.0)
	F	Zn < 30[d]	16.0 ± 1.4 (0.4)
Pancreas			
	M	Zn[b]	1.8 ± 0.1 (0.3)
	M	Fe	2.7 ± 0.1 (0.1)
	F	Zn[b]	1.3 ± 0.1 (0.3)
	F	Fe	2.4 ± 0.1 (0.2)
	M	Zn30[c]	1.9 ± 0.2 (0.3)
	M	Zn < 30[d]	1.7 ± 0.2 (0.3)
	F	Zn30[c]	1.3 ± 0.0 (0.1)
	F	Zn < 30[d]	1.4 ± 0.1 (0.4)
Kidneys			
	M	Zn[b]	6.7 ± 0.4 (0.3)
	M	Fe	5.4 ± 0.1 (0.1)
	F	Zn[b]	6.5 ± 0.4 (0.3)
	F	Fe	5.1 ± 0.2 (0.2)
	M	Zn30[c]	5.6 ± 0.3 (0.2)
	M	Zn < 30[d]	8.1 ± 0.4 (0.4)
	F	Zn30[c]	4.8 ± 0.2 (0.1)
	F	Zn < 30[d]	6.9 ± 0.4 (0.2)
Gonads			
	M	Zn[b]	4.8 ± 1.5 (1.4)
	M	Fe	5.8 ± 1.2 (0.9)
	F	Zn[b]	0.3 ± 0.0 (0.4)
	F	Fe	0.7 ± 0.2 (0.2)
	M	Zn30[c]	8.1 ± 2.3 (0.9)
	M	Zn < 30[d]	0.7 ± 0.2 (0.7)
	F	Zn30[c]	0.4 ± 0.1 (0.2)
	F	Zn < 30[d]	0.3 ± 0.0 (0.4)
Gizzards			
	M	Zn[b]	23.5 ± 1.6 (0.3)
	M	Fe	29.2 ± 0.5 (0.1)
	F	Zn[b]	17.2 ± 1.3 (0.3)
	F	Fe	27.2 ± 0.8 (0.1)
	M	Zn30[c]	29.0 ± 1.4 (0.2)
	M	Zn < 30[d]	16.9 ± 0.5 (0.1)
	F	Zn30[c]	26.8 ± 1.8 (0.1)
	F	Zn < 30[d]	14.8 ± 0.7 (0.2)

[a] n = 20 for each group unless otherwise specified below

[b] includes Zn-dosed ducks surviving ≤ 30 days

[c] Zn-dosed ducks surviving 30 days; for males n = 11; for females n = 4

[d] Zn-dosed ducks surviving < 30 days; for males n = 9; for females n = 16

Results of ANOVA testing:

Liver weight
Dose $F_{1,76} = 5.3$; $P < 0.05$

Pancreas weight
Dose $F_{1,76} = 79.1$; $P < 0.001$
Sex $F_{1,76} = 13.7$; $P = 0.001$

Kidney weight
Dose $F_{1,76} = 23.4$; $P < 0.001$

Gonad weight
Dose $F_{1,76} = 6.8$; $P < 0.05$
Sex $F_{1,76} = 78.2$; $P < 0.001$

Gizzard weight
Dose $F_{1,76} = 54.1$; $P < 0.001$
Sex $F_{1,76} = 2.8$; $P < 0.001$
Inter. $F_{1,76} = 5.9$; $P < 0.05$

The liver represented a slightly greater proportion of total body weight in Zn-dosed than in Fe-dosed Mallards (Fig. 2). The livers of Zn-dosed ducks surviving < 30 days represented nearly twice the proportion of total body weight as compared with livers of ducks surviving to Day 30.

Pancreas

Mean pancreas weight was significantly greater in males and in Fe-dosed ducks, compared to females and Zn-dosed ducks, respectively (Table 2). Pancreas weight did not differ significantly between Zn-dosed males surviving to Day 30 and those that did not ($t_{0.05\,(1),18}$ = 0.72, P = 0.48). Similarly, pancreas weight did not differ between Zn-dosed females surviving to Day 30 and those that did not ($U^{1}_{0.05(1)4,16}$ = 34, P = 0.45).

The pancreas represented a greater proportion of total body weight in Fe- than in Zn-dosed ducks as well as in male and female Zn-dosed ducks that died prior to Day 30, compared with those that survived (Fig. 3).

Kidneys

Average kidney weights were significantly greater in Zn- than in Fe-dosed Mallards (Table 2). We detected no differences in mean kidney weight between the sexes. Kidney weights in male and female Zn-dosed ducks surviving 30 days averaged less than in ducks that died prior to Day 30 (for males, $t_{0.05(1)18}$ = –5.37, P < 0.001; for females, $U^{1}_{0.05(1)4,16}$ = 63, P = 0.0005).

The kidneys represented a greater proportion of total body weight in Zn-dosed ducks than in Fe-dosed ducks (Fig. 4). The kidneys of Zn-dosed males surviving < 30 days represented twice the proportion of total body weight as compared with male Zn-dosed ducks surviving to Day 30. Similarly, kidney weight in Zn-dosed females surviving < 30 days accounted for more than twice the proportion of total body weight as compared with ducks surviving to Day 30.

Gonads

Mean gonadal weights were significantly greater in males and in Fe-dosed ducks as compared with females and Zn-dosed ducks, respectively (Table 2). The gonads were heavier in male ($t_{0.05(1)18}$ = 4.73, P < 0.001) and female ($U^{1}_{0.05(1),4,16}$ = 52, P < 0.05) Zn-dosed ducks that survived to Day 30 than in those that did not.

The gonads accounted for a greater proportion of body weight in Fe-dosed ducks than in Zn-dosed ducks (Fig. 5). The contribution of the gonad to body weight was much greater in Zn-dosed males that survived 30 days, as compared with those that did not, whereas in females these percentages were similar.

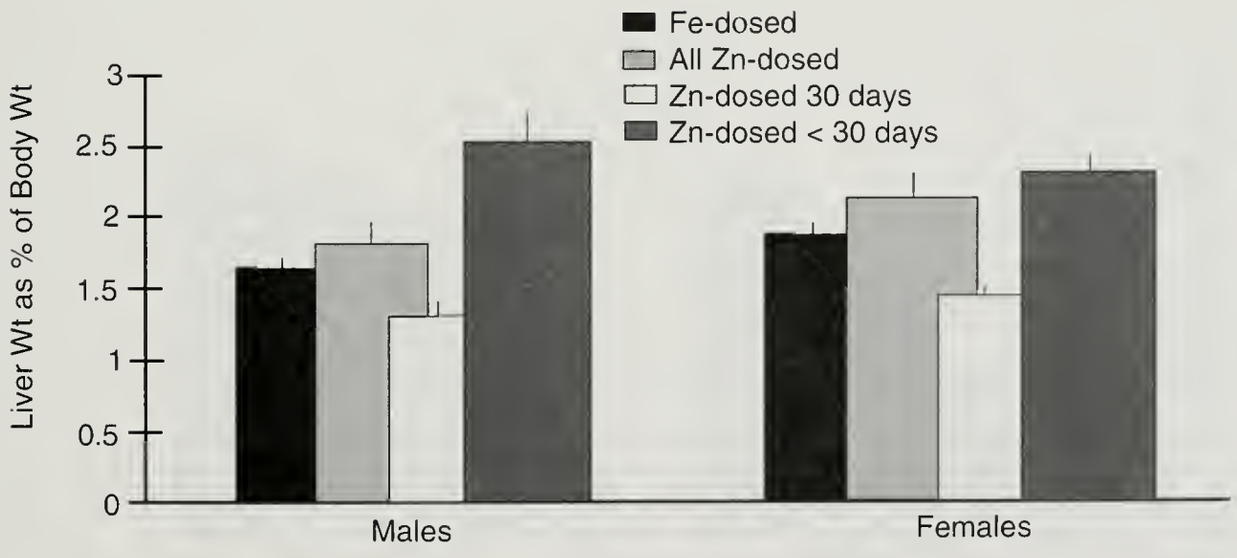

Figure 2. Weight of liver expressed as a proportion of body weight in game-farm Mallards dosed with 6 No. 4 Zn or Fe shot. Sample sizes equal 20 except for Zn-dosed males surviving 30 days, n = 11; males surviving < 30 days, n = 9; females surviving 30 days, n = 4; females surviving < 30 days, n = 16. Vertical lines represent +S.E.

Gizzards

Gizzards averaged significantly heavier in males and in Fe-dosed ducks, respectively, than in females and Zn-dosed ducks (Table 2). We also detected a significant interaction between sex and dose. Male Zn-dosed Mallards had smaller gizzards than female Fe-dosed, and female Zn-dosed had smaller gizzards than male Zn-dosed ducks. Mean gizzard weight was greater in male ($t_{0.05(1)18} = 9.0$, $P < 0.001$) and female ($U^{1}_{0.05(1)4,16} = 64$, $P < 0.001$) Zn-dosed ducks that survived to 30 days than in those that did not.

The gizzard accounted for slightly greater proportions of body weight in Fe- than in Zn-dosed ducks (Fig. 6). The contribution of the gizzard to body weight was higher in male and female Zn-dosed ducks that survived to Day 30 as compared with those that did not.

Figure 3. Weight of pancreas expressed as a proportion of body weight in game-farm Mallards dosed with 6 No. 4 Zn or Fe shot. Sample sizes equal 20 except for Zn males surviving 30 days, n = 11; Zn males surviving < 30 days, n = 9; Zn females surviving 30 days, n = 4; Zn females surviving < 30 days, n = 16. Vertical lines represent +S.E.

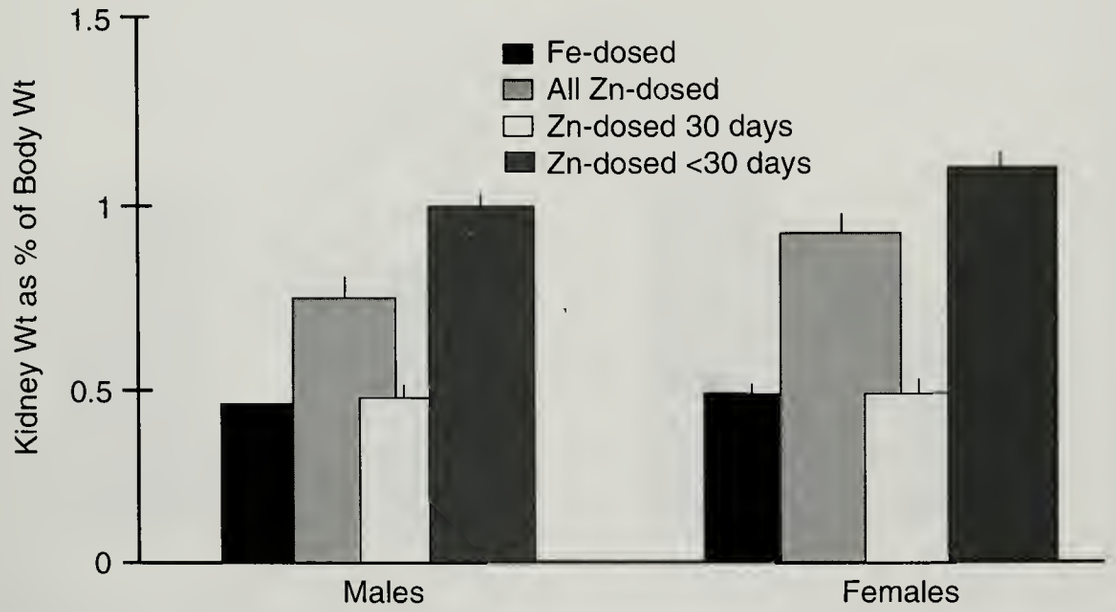

Figure 4. Weighty of kidneys expressed as a proportion of body weight in game-farm Mallards dosed with 6 No. 4 Zn or Fe shot. Sample sizes equal 20 except for Zn-dosed males surviving 30 days, n = 11; males surviving < 30 days, n = 9; females surviving 30 days, n = 4; females surviving < 30 days, n = 16. Vertical lines represent +S.E.

Packed Cell Volume

Packed cell volume (PCV) increased, on average, in Fe-dosed males over the course of the study and decreased slightly in Fe-dosed females from Days 0 to 15 before increasing between Days 15 and 30 (Table 3). Mean PCV decreased in surviving male and female Zn-dosed ducks between Days 0 and 15, then increased between Days 15 and 30. For male Zn-dosed ducks, mean PCV at Day 30 was greater than at Day 0.

Variability in PCVs was highest at Day 15 due to severe anemia in ducks experiencing moderate to severe Zn toxicosis. PVCs increased in Zn-dosed ducks as follows: 7 males and 1 female between Days 0 and 15, 7 males and 1 female between Days 15 and 30, and 7 males between Days 0 and 30.

We detected no significant differences in mean PCV between doses at Days 0 or 30, or between sexes at Day 0 (Table 3). Mean PCV values were higher in Fe-dosed ducks than in Zn-dosed ducks at Day 15, and higher in males than in females at Days 15 and 30. Although some Zn-dosed ducks experienced dramatic declines in PCV values between data collection dates, the effects on mean values were apparently masked by those ducks that were not experiencing Zn-mediated anemia and the deaths of severely Zn-intoxicated ducks prior to sampling.

The mean change in PCV indicated a dramatic decline in male and female Zn-dosed Mallards between Days 0 and 15, compared with a slight decrease in female and a small increase in male Fe-dosed Mallards (Fig. 7). Individual values ranged from –63.0% to 12.5% and –4.4% to 10.4% in Zn- and Fe-dosed male Mallards, respectively. Individual values in Zn- and Fe-dosed female Mallards, respectively, ranged from –56.5% to 6.7% and –10.6% to 14.0%.

The average change (decline) in PCV in Zn-dosed ducks between Days 0 and 30 was less, particularly in females, than from Days 0 to 15 (Fig. 7). Both male and female Fe-dosed ducks exhibited mean increases in PCV. Individual values ranged from –2.2% to 17.5% and –4.3% to 17.5% in Zn- and Fe-dosed males, respectively. Individual values in female Zn- and Fe-dosed Mallards, respectively, ranged from –12.2% to 7.3% and –6.5% to 20.9%.

Gross Pathology

None of the control (Fe-dosed) ducks had macroscopic lesions at necropsy. Zn-dosed

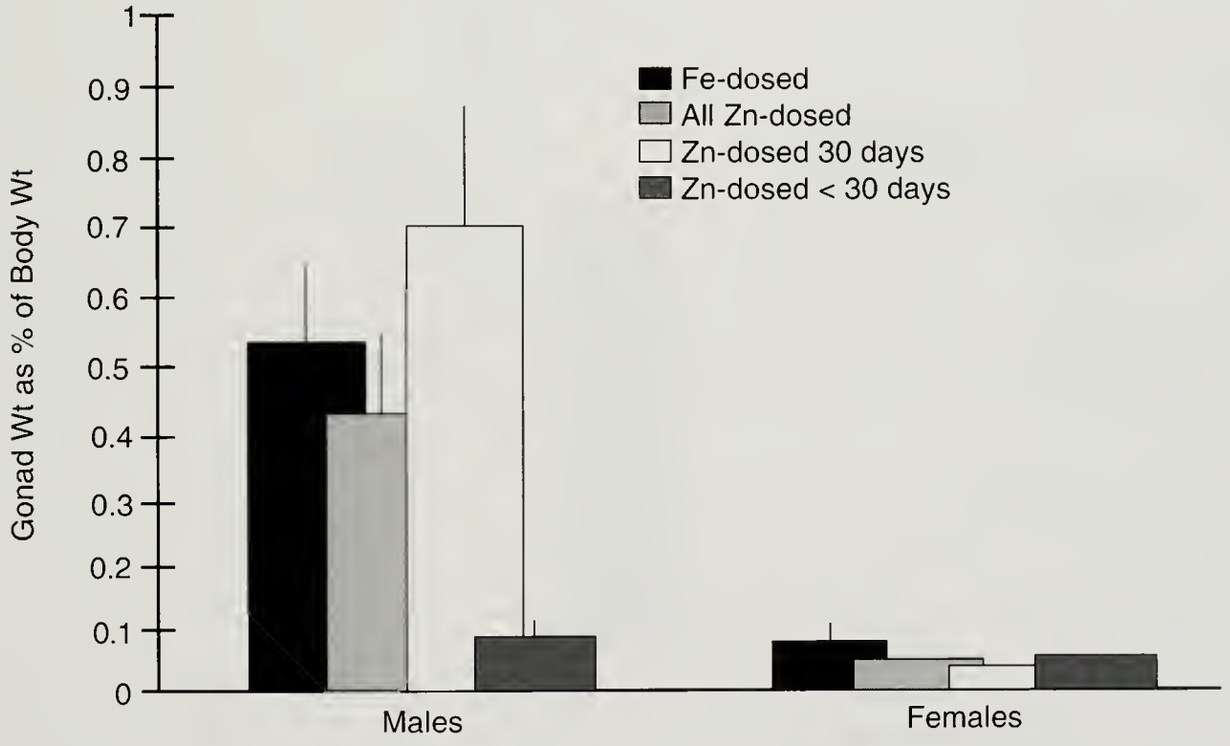

Figure 5. Weight of gonads expressed as a proportion of body weight in game-farm Mallards dosed with 6 No. 4 Zn or Fe shot. Sample sizes equal 20 except for Zn-dosed males surviving 30 days, n = 11; males surviving < 30 days, n = 9; females surviving 30 days, n = 4; females surviving < 30 days, n = 16. Vertical lines represent +S.E.

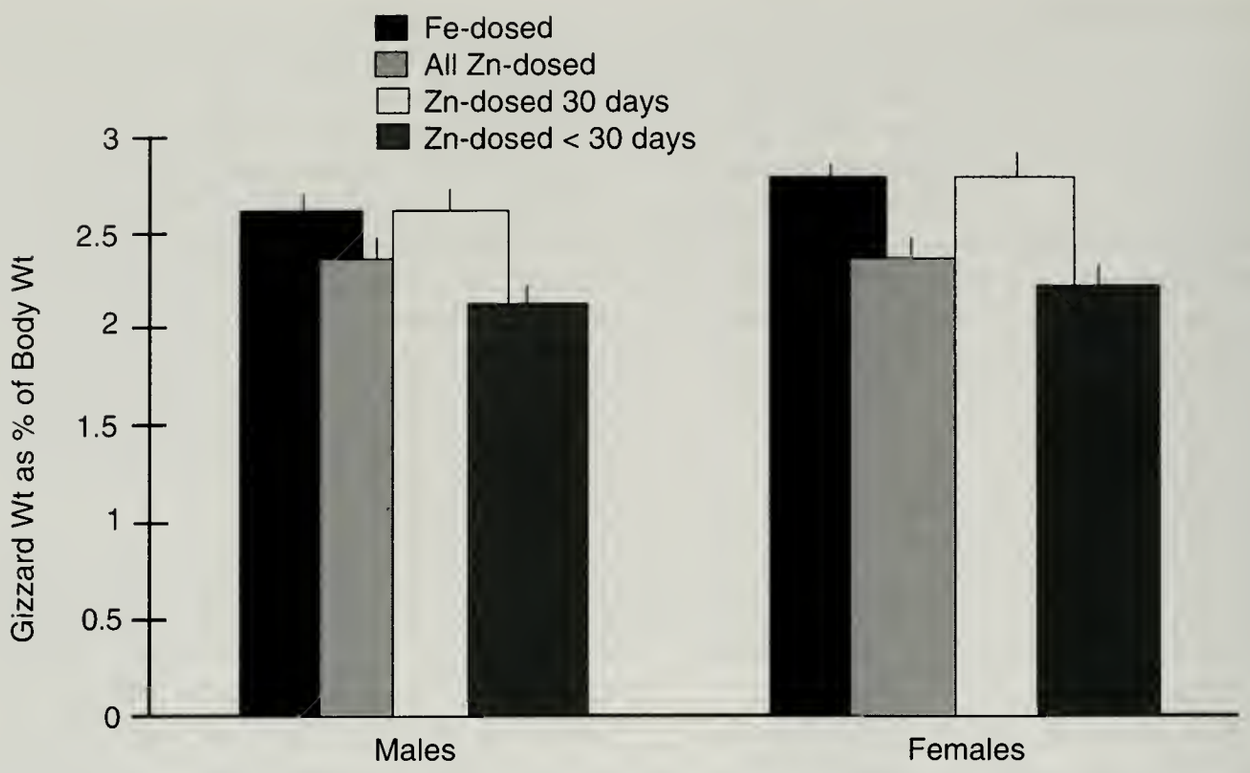

Figure 6. Weight of gizzard expressed as a proportion of body weight in game-farm Mallards dosed with 6 No. 4 Zn or Fe shot. Sample sizes equal 20 except for Zn-dosed males surviving 30 days, n = 11; males surviving < 30 days, n = 9; females surviving 30 days, n = 4; females surviving < 30 days, n = 16. Vertical lines represent +S.E.

Table 3. Mean PCV ± SE (CV) in 6- to 8-month-old male and female game-farm Mallards dosed with 6 No. 4 Zn or Fe shot.[a]

		PCV		
Dose	Sex	Day 0	Day 15	Day 30
Zn	M	44.65 ± 0.58 (0.06)	40.87 ± 2.81 (0.22)	47.64 ± 0.79 (0.06)
	F	46.15 ± 0.02 (0.06)	32.46 ± 3.19 (0.35)	44.25 ± 0.63 (0.03)
Fe	M	45.15 ± 0.60 (0.06)	46.60 ± 0.79 (0.08)	46.80 ± 0.46 (0.04)
	F	44.80 ± 0.58 (0.06)	44.60 ± 0.49 (0.05)	45.95 ± 0.61 (0.06)

[a] n = 20 except male Zn-dosed Day 15, n = 15, Day 30, n = 11; female Zn-dosed Day 15, n = 13, Day 30, n = 4

Results of ANOVA testing:

PCV

Day 15

Dose $F_{1,64} = 23.3$; $P < 0.001$

Sex $F_{1,64} = 8.1$; $P < 0.01$

Day 30

Sex $F_{1,51} = 7.0$; $P < 0.05$

ducks had gross lesions in multiple organs with varying degrees of severity.

Pectoral Muscle Atrophy
Of the 39 Zn-dosed ducks examined, 15 had pectoral muscle atrophy. The atrophy was subjectively graded as mild, moderate, or severe. Of the 15 ducks with atrophy, 4, 8, and 3 ducks were ranked as having mild, moderate, and severe lesions, respectively.

Pericardial Lesion
Fifteen of the 39 Zn-dosed ducks examined had increased pericardial fluid and/or white cloudiness to white plaques on the pericardial membrane and to a lesser extent on the epicardial surface. Severity was subjectively evaluated as mild (9 ducks), moderate (5 ducks), or severe (1 duck). In some ducks the white plaque material extended onto the adjacent air sacs or parenchymal organs.

Air Sacculitis
Cloudiness of the air sacs was noted in 3 of the Zn-dosed ducks examined. One duck had mild cloudiness whereas 2 ducks had severe lesions consisting of opaque plaques on the surface.

Hepatic Granulomas
Ten of the Zn-dosed ducks had liver lesions, which ranged from single small white foci in the liver parenchyma to hundreds of nodules. Of the 10 ducks with macroscopic lesions, 4, 2, and 4 ducks had mild, moderate, and severe to marked lesions, respectively.

Pancreatic Lesions
Pancreatic lesions were detected in only 4 of the Zn-dosed ducks examined and typically were manifested as pallor to the parenchyma or white plaques on the surface. Two ducks had nodular lesions, and in another the pancreas had an adhesion resulting from intestinal rupture.

Proventricular Erosions
Eight of the 39 Zn-dosed ducks examined had proventricular lesions. The lesions ranged from roughening of the mucosa to raised discolored plaques.

Ventricular Lesions
The gizzard linings in Zn-dosed ducks that died or were euthanized during the experiment were walnut brown and brittle. Gizzard linings in

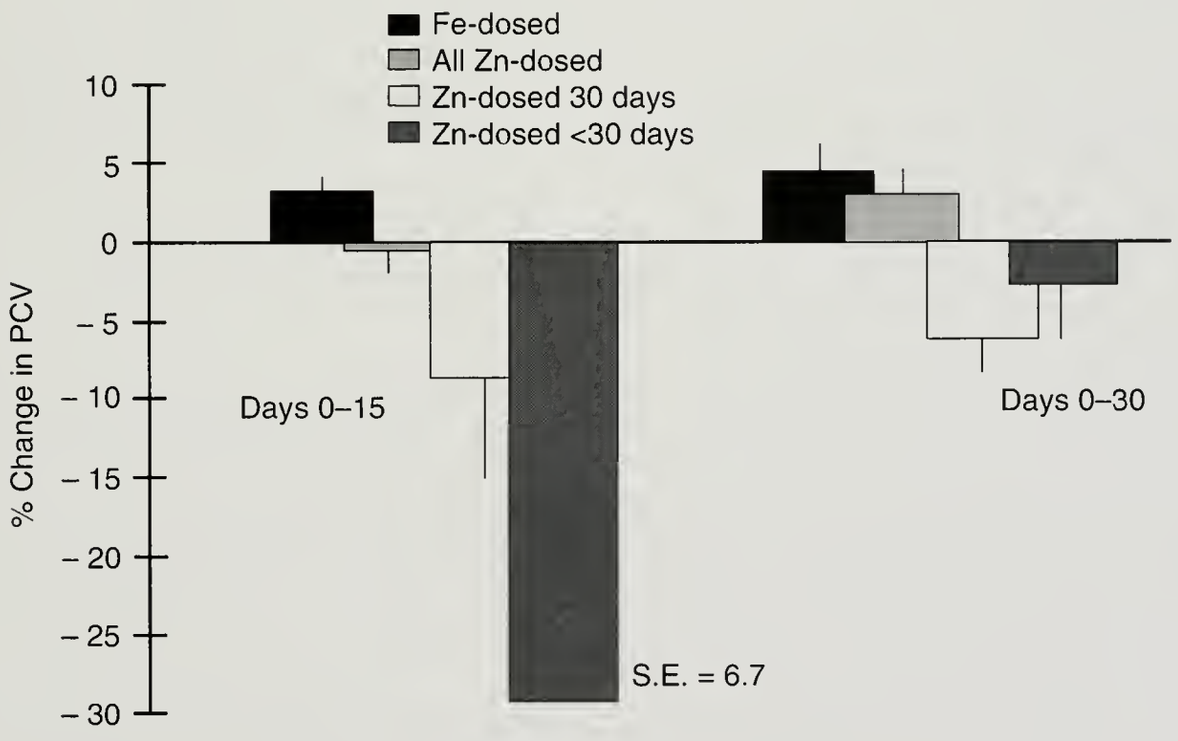

Figure 7. Change in packed cell volume between Days 0 and 15 and Days 15 and 30 in game-farm Mallards dosed with 6 No. 4 Zn or Fe shot. Sample sizes equal 20 except for Zn-dosed males (n = 15) and females (n = 13) Days 0–15; Zn-dosed males (n = 11) and females (n = 4) Days 15–30. Vertical lines represent +S.E.

Fe-dosed and Zn-dosed ducks surviving to Day 30 were stained yellow (with the exception of one Zn-dosed, which was green) and normal in texture. Gizzard linings were examined by the first author.

Renal Pallor
The kidneys of 5 of the 39 Zn-dosed ducks examined were paler than normal on gross examination. These lesions were all judged to be mild.

Cecal Lesions
Cecal lesions, which were noted in 23 Zn-dosed ducks, ranged from mildly dilated ceca (unilateral or bilateral) to massively enlarged ceca with transmural necrosis, rupture, and extensive adhesions. Of the 23 ducks, 3 had mild lesions, 14 moderate, 5 severe, and 1 marked.

Intestinal Lesions
Intestinal lesions (small and/or large) were similar, although typically less severe, than the cecal lesions described. Twelve Zn-dosed ducks had lesions of the intestine. Typically these lesions were associated with cecal lesions.

Histopathology

None of the control (Fe-dosed) ducks had significant histologic lesions in the sections examined. Many control ducks had mild to moderate lymphocytic inflammation in the small and large intestines, proventriculus, and ceca. Control ducks also had liver lesions consisting of a lymphocytic periportal inflammatory lesion and/or hepatic lipidosis. Two control ducks also had small numbers of granulomas within the hepatic parenchyma. Lung lesions noted in controls included 9 ducks with peribronchiolar lymphoid hyperplasia. Most ducks had a small population of lymphocytes around the ureters. These lesions were considered within normal limits for a population of game-farm-raised ducks.

The Zn-dosed ducks had remarkable histologic lesions in addition to those noted in the control cohort. No important lesions were noted in the gonads of treated or control ducks nor were significant lesions noted in the sciatic nerves from treated or control ducks.

Ceca
Histologic lesions of necrohemorrhagic typhlitis (inflammation of the ceca) were present in 25 of 38 Zn-dosed ducks examined. Thirteen Zn-dosed ducks had only a mild typhlitis (as seen in Fe-dosed controls) or no significant lesion in the ceca.
Necrohemorrhagic lesions ranged from moderate superficial necrosis (n = 5) to severe necrohemorrhagic (n = 3) or severe necrohemorrhagic transmural (n = 17) typhlitis. In the latter the inflammatory process extended into the body cavity.

Small and Large Intestines
Many of the Zn-dosed ducks had mild lymphocytic inflammatory lesions (small intestine n = 17, large intestine n = 14) similar to control animals. Two large intestinal segments and 1 small intestinal segment had extensive autolysis (postmortem change). The remaining ducks had varying degrees of necrotizing to necrohemorrhagic inflammatory lesions in the small and large intestines. In some animals the lesions were segmental, appearing in some sections of the gastrointestinal tract and not in others.

Pancreas
Of the 38 Zn-dosed ducks, all but 4 had apoptosis (individual cell death) in the pancreas, the most consistent lesion of Zn exposure. Only 1 control duck had a mild amount of apoptosis whereas all but 4 of the Zn-dosed ducks had moderate, severe, or marked apoptosis. In some ducks the degree of apoptosis had progressed to necrosis.

Liver
Control (Fe-dosed) ducks had lymphocytic periportal hepatitis and occasional hepatic lipidosis, which were considered normal for these animals. Hepatic lesions in Zn-dosed ducks ranged from granuloma(s) (n = 11), hemosiderosis (n = 23), and hepatocellular atrophy to apoptosis (n = 15). The hemosiderosis was most likely related to the observed anemia. The atrophy/cell death could be related to the cachectic state of the animals or could be a direct effect of the Zn on hepatic metabolism.

Spleen
Of the Zn-dosed ducks, 9 had no significant splenic lesions and 2 spleens were not available for examination. Of the remaining ducks, 19 had hemosiderosis and this lesion was often concurrent with lymphoid depletion and/or lympholysis. The hemosiderosis was most likely similar to the hepatic lesion of hemosiderosis whereas the lymphoid depletion/ lympholysis probably was related to the intestinal inflammatory lesion(s).

Proventriculus
Zn-induced lesions of the proventriculus were characterized by a superficial necrosis (n=13), often associated with glandular atrophy or apoptosis. The proventriculus was not examined in 4 Zn-dosed ducks.

Ventriculus
Only 15 gizzards (ventriculi) were examined histologically. One Zn-dosed duck had no remarkable lesions, whereas 13 had evidence of glandular atrophy and/or inflammation with variable amounts of intraluminal hemorrhage.

Bone Marrow
Bone marrow samples from 29 of the 39 Zn-dosed ducks were examined. Thirteen ducks had no important lesions whereas 5 had an increased number of heterophils in the marrow. An additional 11 animals had increased heterophil populations concurrent with a mucinous appearance to the marrow stroma.

Kidney
Twelve of the 39 Zn-dosed ducks examined had no significant lesion in the renal parenchyma and renal tissue from 1 duck was not available for examination. Of the remaining Zn-dosed ducks (n = 24), all had a mild to moderate necrosis of the epithelial cells of the renal tubules. This lesion was often concurrent with hyaline cast formation and a few animals had granular casts (cellular casts) due to cells sloughing into the lumen.

Adrenal
Zn-induced lesions in the adrenal consisted of adrenal medullary apoptosis/atrophy (n = 16). This condition caused a moderate to marked reduction in the amount of renal medullary tissue present. Six of the affected ducks also had vacuolization of the adrenal cortex, most likely related to systemic illness. Cortical hypertrophy/hyperplasia was not measured due to a lack of standard sectioning in the small gland. Nine adrenals of the Zn-dosed group were not examined.

Heart
Seventeen Zn-dosed ducks had no significant lesion in the myocardial sections examined. Tissues from the other ducks (n = 21) ranged from mild myofiber vacuolization to multifocal degeneration or necrosis. Histologic lesion of pericardial/epicardial mineralization was noted in 3 ducks. Grossly the pericardial lesion was detected more frequently, which was probably related to loss of the pericardial membrane during processing and embedding.

Tissue Element Concentrations

Plasma
Mean plasma Ca levels generally decreased over the course of the study (Table 4). Ca concentrations did not differ significantly between doses or sexes at Days 0, 15, or 30. Plasma Ca levels ranged from 49.3 to 266.8 µg/g.

Mean plasma Cu levels decreased slightly between Days 0 and 15 in all groups, with the exception of Fe-dosed females, and increased between Days 15 and 30 in all but Zn-dosed females (Table 4). Mean Cu concentrations were < MDL in Zn-dosed males at Day 15 and in Fe-dosed females at Day 0. Copper concentrations in plasma did not differ significantly between doses or sexes at Days 0, 15, or 30 (Table 4). Plasma Cu concentrations ranged from < MDL to 1.0 µg/g.

Mean Fe levels decreased between Days 0 and 15 in Fe-dosed ducks, before increasing between Days 15 and 30 (Table 4). Iron concentrations increased dramatically between Days 0 and 15, and decreased between Days 15 and 30, in Zn-dosed ducks. Plasma Fe concentrations were significantly greater in Zn-dosed, as compared with Fe-dosed, ducks at day 15. We did not detect any significant differences in plasma Fe concentrations between doses at Days 0 or 30, or between sexes at Days 0, 15, or 30. Plasma Fe levels ranged from < MDL to 48.3 µg/g.

Mg concentrations changed little over the course of the study (Table 4). Mean Mg levels did not differ between dosing groups or sexes

Table 4. Mean levels (µg/g wet wt) ± SE(CV) of Ca, Cu, Fe, Mg, P, and Zn in plasma of game-farm Mallards dosed with 6 No. 4 Fe or Zn shot.[a]

Element	Dose	Sex	Day After Dosing 0	15	30
Ca	Fe	M	120.3 ± 2.5 (0.1)	110.7 ± 1.6 (0.1)	105.3 ± 1.2 (0.1)
	Zn		117.5 ± 3.1 (0.1)	109.3 ± 2.7 (0.1)[b]	106.7 ± 1.5 (0.1)[c]
	Fe	F	114.3 ± 2.6 (0.1)	116.0 ± 8.1 (0.3)	108.8 ± 5.0 (0.2)
	Zn		112.5 ± 4.9 (0.2)	108.0 ± 3.3 (0.1)[d]	103.0 ± 1.7 (0.0)[e]
Cu	Fe	M	0.23 ± 0.0 (0.6)	0.20 ± 0.0 (0.6)	0.25 ± 0.0 (0.5)
	Zn		0.22 ± 0.0 (0.6)	f	0.25 ± 0.0 (0.5)
	Fe	F	f	0.23 ± 0.0 (0.7)	0.32 ± 0.0 (0.5)
	Zn		0.27 ± 0.0 (0.8)	0.26 ± 0.0 (0.5)	0.25 ± 0.0 (0.3)
Fe	Fe	M	4.6 ± 0.9 (0.9)	3.8 ± 0.4 (0.5)	4.7 ± 0.7 (0.7)
	Zn		4.3 ± 0.6 (0.6)	11.7 ± 3.8 (1.3)	5.7 ± 2.0 (1.2)
	Fe	F	6.3 ± 1.7 (1.2)	5.1 ± 1.0 (0.9)	5.2 ± 0.8 (0.7)
	Zn		5.5 ± 1.2 (1.0)	11.3 ± 3.5 (1.1)	2.4 ± 0.2 (0.1)
Mg	Fe	M	22.1 ± 0.6 (0.1)	22.3 ± 0.4 (0.1)	21.6 ± 0.4 (0.1)
	Zn		22.2 ± 0.7 (0.2)	21.0 ± 0.9 (0.2)	22.0 ± 0.5 (0.1)
	Fe	F	21.5 ± 0.7 (0.2)	22.2 ± 0.6 (0.1)	21.3 ± 0.4 (0.1)
	Zn		20.9 ± 1.2 (0.3)	21.8 ± 0.8 (0.1)	22.1 ± 0.5 (0.1)
P	Fe	M	209.2 ± 8.6 (0.2)	241.9 ± 7.3 (0.1)	239.5 ± 8.7 (0.2)
	Zn		188.0 ± 13.2 (0.3)	246.9 ± 23.5 (0.4)	215.5 ± 14.1 (0.2)
	Fe	F	182.1 ± 9.9 (0.2)	241.9 ± 12.9 (0.2)	255.5 ± 10.7 (0.2)
	Zn		163.8 ± 13.8 (0.4)	202.2 ± 21.0 (0.4)	192.7 ± 27.3 (0.3)
Zn	Fe	M	3.1 ± 0.1 (0.2)	3.2 ± 0.1 (0.2)	2.9 ± 0.1 (0.2)
	Zn		3.0 ± 0.2 (0.2)	14.0 ± 1.3 (0.4)	9.3 ± 1.3 (0.5)
	Fe	F	2.9 ± 0.1 (0.2)	3.5 ± 0.2 (0.3)	3.0 ± 0.1 (0.2)
	Zn		2.7 ± 0.2 (0.3)	15.9 ± 1.3 (0.3)	8.6 ± 0.6 (0.1)

[a] n = 20 unless otherwise specified
[b] n = 16 for Zn-dosed males at Day 15
[c] n = 11 for Zn-dosed males at Day 30
[d] n = 12 for Zn-dosed females at Day 15
[e] n = 4 for Zn-dosed females at Day 30
[f] mean < MDL

MDLs (in µg/g) for Days 0, 15, and 30, respectively, were as follows: Ca, 0.77, 0.73, 0.64; Cu, 0.21, 0.20, 0.18; Fe, 0.96, 0.92, 0.81; Mg, 0.15, 0.14, 0.12; P, 10.1, 9.7, 8.5; Sn, 2.5, 2.4, 2.1; Zn, 0.25, 0.23, 0.21.

Results of ANOVA testing:

Fe Day 15
Dose $F_{1,51} = 4.1$; $P = 0.05$
P Day 15
Dose $F_{1,51} = 6.8$; $P = 0.01$
P Day 30
Dose $F_{1,51} = 10.5$; $P = 0.002$
Zn Day 15
Dose $F_{1,51} = 222.8$; $P < 0.001$
Zn Day 30
Dose $F_{1,51} = 145.0$; $P < 0.001$

at Days 0, 15, or 30. Plasma Mg concentrations ranged from 8.3 to 33.9 µg/g.

Mean P levels increased between Days 0 and 15 in all groups, and decreased between days 15 and 30 in all but Fe-dosed females (Table 4). Phosphorus concentrations were higher in Fe-dosed than in Zn-dosed females at Day 15 and in both sexes at Day 30 (Table 4). We detected no gender effects in mean plasma P levels. Phosphorus levels in blood plasma in Fe-dosed Mallards ranged from 51.6 to 444.8 µg/g; plasma P levels in Zn-dosed Mallards ranged from 44.8 to 563.1 µg/g .

Mean Sn concentrations were < MDL at Days 0, 15, and 30. For Fe-dosed ducks, Sn concentrations were > MDLs in 2, 6, and 2 individuals at Days 0, 15, and 30, respectively; values ranged from 2.4 to 4.7 µg/g. For Zn-dosed Mallards, Sn concentrations were > MDLs in 5, 5, and 0 individuals at Days 0, 15, and 30, respectively; values ranged from 2.4 µg/g to 3.3 µg/g. No effect of dose was apparent; that is, there was an equal number of Zn- and Fe-dosed ducks with Sn levels > MDLs.

Mean plasma Zn levels changed little in Fe-dosed ducks over the course of the study (Table 4). Zinc levels in plasma increased markedly between Days 0 and 15, and decreased between Days 15 and 30, in Zn-dosed ducks. Plasma Zn concentrations were higher in Zn-dosed ducks at Days 15 and 30, as compared with Fe-dosed ducks (Table 4). There were no significant differences in Zn levels between the sexes. Zn levels in blood plasma in Fe-dosed Mallards ranged from 1.7 to 6.7 µg/g; plasma Zn concentrations in Zn-dosed Mallards ranged from 1.2 to 25.5 µg/g.

Erythrocytes

Mean Ca levels in erythrocytes decreased between Days 0 and 15 in all groups, before increasing between Days 15 and 30 in all but Zn-dosed females (Table 5). Calcium concentrations did not differ significantly by dose, or by gender at Days 15 and 30, and ranged from 14.4 to 172.0 µg/g.

Copper levels in erythrocytes increased between Days 0 and 15 and decreased between Days 15 and 30 in all but male Fe-dosed Mallards (Table 5). Mean Cu concentrations did not differ significantly between dosing groups or sexes (Table 5). Erythrocyte Cu concentrations ranged from < MDL to 10.6 µg/g.

Mean Fe levels in erythrocytes increased in Fe-dosed, and decreased in Zn-dosed, Mallards between Days 0 and 15 (Table 5). Mean Fe concentrations increased between Days 15 and 30 in all groups with the exception of Fe-dosed females. There were no significant differences between sexes or dosing groups in mean Fe levels. Fe concentrations in erythrocytes ranged from 247.0 to 1,030 µg/g.

Fe levels at Day 15 were higher in Zn-dosed males that survived to Day 30 ($\bar{x}$ = 917.5 µg/g, n = 11) than in those that died after Day 15 ($\bar{x}$ = 694.2 µg/g, n = 5) ($U^{1}_{0.05(1),5,11} = 54$, $P < 0.05$). Similarly, Fe levels at Day 15 were higher in Zn-dosed females that survived to Day 30 ($\bar{x}$ = 866.1µg/g, n = 4) than in those that died after Day 15 ($\bar{x}$ = 636.3 µg/g, n = 8) ($U^{1}_{0.05(1),4,8} = 28$, $P = 0.025$).

Mean Mg concentrations in erythrocytes decreased between Days 0 and 15 in all but Zn-dosed males (Table 5). Mg concentrations were significantly higher in Zn-dosed ducks at Day 15, otherwise we did not detect any significant differences attributable to dose or gender. Mg concentrations in erythrocytes ranged from 51.0 to 171.5 µg/g.

P levels in erythrocytes increased between Days 0 and 15 in all groups (Table 5). Mean P concentrations were marginally higher in Zn-dosed ducks at Day 15. Erythrocyte P levels did not differ significantly between the sexes. Phosphorus concentrations in erythrocytes in Fe-dosed Mallards ranged from 964.1 to 2958 µg/g. P levels in Zn-dosed Mallards ranged from 1029 to 3074 µg/g.

Mean Sn concentrations in erythrocytes were < MDLs at Days 0, 15, and 30. For Fe-dosed ducks, Sn concentrations were > MDLs in 12, 2, and 8 individuals at Days 0, 15, and 30, respectively; values ranged from 2.1 to 6.8 µg/g. For Zn-dosed ducks, Sn concentrations were > MDLs for 14, 1, and 2 individuals at Days 0, 15, and 30, respectively; values ranged from 2.1 to 6.8 µg/g.

Mean Zn concentrations in erythrocytes changed little during the study in Fe-dosed ducks (Table 5). Zn levels increased markedly in Zn-dosed ducks between Days 0 and 15, before decreasing between Days 15 and 30. Mean Zn levels were significantly higher in Zn-dosed ducks, as compared with Fe-dosed ducks, at Days 15 and 30 (Table 5). Erythrocyte Zn concentrations were marginally higher in female than in male Zn-dosed ducks at Day

Table 5. Mean levels (µg/g wet wt) ± SE(CV) of Ca, Cu, Fe, Mg, P, and Zn in erythrocytes of game-farm Mallards dosed with 6 No. 4 Fe or Zn shot.[a]

Element	Dose	Sex	Day After Dosing 0	15	30
Ca	Fe	M	58.3 ± 7.0 (0.5)	32.5 ± 4.5 (0.6)	35.9 ± 3.3 (0.4)
	Zn		40.0 ± 3.4 (0.4)	38.0 ± 7.7 (0.8)[b]	40.5 ± 7.0 (0.6)[c]
	Fe	F	46.5 ± 4.9 (0.5)	31.2 ± 2.6 (0.4)	40.0 ± 4.0 (0.4)
	Zn		56.2 ± 6.5 (0.5)	50.2 ± 12.8 (0.9)[d]	33.2 ± 8.1 (0.5)[e]
Cu	Fe	M	0.64 ± 0.1 (0.4)	0.56 ± 0.1 (0.4)	1.00 ± 0.5 (2.3)
	Zn		0.59 ± 0.1 (0.4)	0.68 ± 0.1 (0.3)	0.62 ± 0.1 (0.3)
	Fe	F	0.60 ± 0.0 (0.3)	0.66 ± 0.1 (0.3)	0.57 ± 0.1 (0.5)
	Zn		0.65 ± 0.1 (0.5)	0.86 ± 0.1 (0.5)	0.70 ± 0.1 (0.3)
Fe	Fe	M	861.3 ± 13.7 (0.1)	902.1 ± 17.5 (0.0)	907.1 ± 16.9 (0.1)
	Zn		860.9 ± 14.4 (0.1)	847.7 ± 40.4 (0.2)	880.7 ± 54.5 (0.2)
	Fe	F	851.2 ± 13.2 (0.1)	898.7 ± 13.4 (0.1)	848.6 ± 41.2 (0.2)
	Zn		880.5 ± 14.1 (0.1)	712.9 ± 64.4 (0.3)	897.8 ± 14.2 (0.1)
Mg	Fe	M	131.9 ± 2.6 (0.1)	122.7 ± 2.5 (0.1)	125.3 ± 2.6 (0.1)
	Zn		129.2 ± 2.5 (0.1)	131.9 ± 3.9 (0.1)	125.0 ± 6.7 (0.2)
	Fe	F	130.8 ± 2.3 (0.1)	122.3 ± 2.0 (0.1)	117.9 ± 5.0 (0.2)
	Zn		135.4 ± 8.0 (0.1)	130.4 ± 8.0 (0.2)	131.7 ± 2.5 (0.0)
P	Fe	M	2467.0 ± 40.5 (0.1)	2495.3 ± 56.3 (0.1)	2525.9 ± 52.8 (0.1)
	Zn		2488.4 ± 40.7 (0.1)	2677.6 ± 46.5 (0.0)	2568.1 ± 143.8 (0.2)
	Fe	F	2430.0 ± 29.5 (0.1)	2518.2 ± 43.5 (0.1)	2400.7 ± 114.6 (0.2)
	Zn		2490.7 ± 49.6 (0.1)	2585.0 ± 154.0 (0.2)	2773.8 ± 47.2 (0.0)
Zn	Fe	M	7.7 ± 0.2 (0.1)	7.8 ± 0.2 (0.1)	7.6 ± 0.2 (0.1)
	Zn		7.4 ± 0.1 (0.1)	64.5 ± 26.2 (1.6)	11.2 ± 1.3 (0.4)
	Fe	F	7.6 ± 0.1 (0.1)	8.0 ± 0.2 (0.1)	8.0 ± 0.3 (0.2)
	Zn		7.8 ± 0.1 (0.3)	114.1 ± 31.2 (1.0)	10.9 ± 0.6 (0.1)

[a] n = 20 unless otherwise specified
[b] n = 16 for Zn-dosed males at Day 15
[c] n = 11 for Zn-dosed males at Day 30
[d] n = 12 for Zn-dosed females at Day 15
[e] n = 4 for Zn-dosed females at Day 30

MDLs (in µg/g) at Days 0, 15, and 30, respectively, were as follows: Ca, 0.64, 1.4, 1.0; Cu, 0.18, 0.37, 0.27; Fe, 0.81, 1.7, 1.3; Mg, 0.13, 0.26, 0.19; P, 8.5, 17.8, 13.2; Sn, 2.1, 4.3, 3.2; Zn, 0.21, 0.43, 0.32.

Results of ANOVA testing:

Mg Day 15
Dose $F_{1,51} = 4.7$; $P = 0.035$
P Day 15
Dose $F_{1,51} = 3.8$; $P = 0.056$
Zn Day 15
Dose $F_{1,51} = 75.2$; $P < 0.001$
Sex $F_{1,51} = 3.6$; $P = 0.06$
Zn Day 30
Dose $F_{1,51} = 25.5$; $P < 0.001$

Table 6. Mean levels (μg/g wet wt) ± SE(CV) of Ca, Cu, Fe, Mg, P, and Zn in kidneys, livers, and pancreases of game-farm Mallards dosed with 6 No. 4 Fe or Zn shot.[a]

Element	Dose	Sex	Tissue		
			Kidney	Liver	Pancreas
Ca	Fe	M	89.9 ± 3.2 (0.2)	61.8 ± 3.6 (0.3)	169.5 ± 6.7 (0.2)
	Zn		151.3 ± 19.7 (0.6)	93.6 ± 17.2 (0.8)	91.4 ± 5.6 (0.3)
	Fe	F	93.2 ± 3.6 (0.2)	64.8 ± 4.3 (0.3)	172.6 ± 8.4 (0.2)
	Zn		249.7 ± 33.1 (0.6)	92.3 ± 5.7 (0.3)	133.3 ±22.4 (0.8)
Cu	Fe	M	6.4 ± 0.3 (0.2)	380.9 ± 74.3 (0.9)	2.0 ± 0.1 (0.3)
	Zn		26.9 ± 3.9 (0.7)	167.5 ± 44.7 (1.2)	4.1 ± 0.2 (0.2)
	Fe	F	5.5 ± 0.2 (0.2)	160.0 ± 38.2 (1.1)	2.0 ± 0.1 (0.3)
	Zn		22.0 ± 4.5 (0.9)	123.3 ± 35.6 (1.3)	5.1 ± 0.4 (0.4)
Fe	Fe	M	175.4 ± 7.9 (0.2)	1509.3 ± 81.8 (0.2)	69.0 ± 6.6 (0.4)
	Zn		161.0 ± 13.5 (0.4)	1622.1 ± 205.7 (0.6)	78.7 ± 6.4 (0.4)
	Fe	F	166.1 ± 7.1 (0.2)	1854.2 ± 106.9 (0.3)	59.0 ± 2.8 (0.2)
	Zn		157.6 ± 12.5 (0.4)	2647.7 ± 299.4 (0.5)	81.9 ± 6.7 (0.4)
Mg	Fe	M	219.9 ± 3.0 (0.1)	253.3 ± 9.8 (0.2)	375.5 ± 7.0 (0.1)
	Zn		220.1 ± 4.5 (0.1)	257.9 ± 8.1 (0.1)	269.8 ±10.2 (0.2)
	Fe	F	218.8 ± 2.4 (0.1)	250.4 ± 10.3 (0.2)	385.7 ± 6.1 (0.1)
	Zn		227.1 ± 4.4 (0.1)	245.8 ± 9.0 (0.2)	257.1 ± 4.0 (0.1)
P	Fe	M	3396.7 ± 30.0 (0.0)	3517.8 ± 69.2 (0.1)	6046.0 ±114.6 (0.1)
	Zn		3207.1 ± 51.7 (0.1)	3490.6 ± 87.3 (0.1)	4173.7 ±158.8 (0.2)
	Fe	F	3361.9 ± 24.6 (0.0)	3532.4 ± 89.3 (0.1)	6158.1 ± 86.0 (0.1)
	Zn		3144.4 ± 44.0 (0.1)	3397.2 ± 82.8 (0.1)	4143.1 ± 78.4 (0.1)
Zn	Fe	M	27.2 ± 0.4 (0.1)	63.0 ± 2.3 (0.2)	99.8 ± 9.7 (0.4)
	Zn		268.0 ± 25.4 (0.4)	360.3 ± 28.4 (0.4)	2070.9 ±171.5 (0.4)
	Fe	F	26.3 ± 0.4 (0.1)	61.5 ± 3.1 (0.2)	76.9 ± 5.5 (0.3)
	Zn		359.8 ± 24.3 (0.3)	401.8 ± 20.9 (0.2)	2254.7 ±184.5 (0.4)

[a] n = 20 for all groups

MDLS (in μg/g) for kidneys, liver, and pancreas, respectively, were as follows: Ca, 1.0, 1.1, 1.1; Cu, 9.1, 0.30, 0.30; Fe, 1.3, 1.4, 1.4; Mg, 0.20, 0.21, 0.21; P, 13.7, 14.4, 14.3; Sn, 3.3, 3.5, 3.5; Zn, 0.33, 0.35, 0.35.

Results of ANOVA testing:

Kidney Ca
- Dose $F_{1,76} = 45.8$; $P < 0.001$
- Sex $F_{1,76} = 8.5$; $P = 0.005$

Kidney Cu
- Dose $F_{1,76} = 115.5$; $P < 0.001$

Kidney P
- Dose $F_{1,76} = 27.3$; $P < 0.001$

Kidney Zn
- Dose $F_{1,76} = 1021.4$; $P < 0.001$
- Sex $F_{1,76} = 5.4$; $P = 0.023$
- Inter. $F_{1,76} = 7.7$; $P = 0.007$

Liver Ca
- Dose $F_{1,76} = 18.0$; $P < 0.001$

Liver Cu
- Dose $F_{1,76} = 11.1$; $P = 0.001$
- Sex $F_{1,76} = 5.2$; $P = 0.026$

Liver Fe
- Sex $F_{1,76} = 7.4$; $P = 0.005$

Liver Zn
- Dose $F_{1,76} = 858.3$; $P < 0.001$

Pancreas Ca
- Dose $F_{1,76} = 62.7$; $P < 0.001$
- Sex $F_{1,76} = 5.8$; $P = 0.018$
- Inter. $F_{1,76} = 5.0$; $P = 0.028$

Pancreas Cu
- Dose $F_{1,76} = 191.4$; $P < 0.001$

Pancreas Fe
- Dose $F_{1,76} = 8.8$; $P = 0.004$

Pancreas Mg
- Dose $F_{1,76} = 272.8$; $P < 0.001$

Pancreas P
- Dose $F_{1,76} = 287.0$; $P = 0.006$

Pancreas Zn
- Dose $F_{1,76} = 1395.8$; $P < 0.001$

15 (Table 5). Zn concentrations in erythrocytes of Fe-dosed Mallards ranged from 4.6 to 11.0 µg/g; Zn levels in Zn-dosed Mallards ranged from 6.5 to 387.9 µg/g.

Kidneys

Mean kidney Ca concentrations were significantly higher in Zn-dosed and female Mallards, as compared with Fe-dosed and male ducks, respectively (Table 6). We also detected an interaction between sex and dose, with Zn-dosed females exhibiting higher Ca levels than all other groups, and male Fe-dosed Mallards exhibiting the lowest levels. Kidney Ca concentrations in Fe-dosed ducks ranged from 73.6 to 143.6 µg/g; Ca concentrations in kidneys of Zn-dosed Mallards ranged from 71.7 to 578.1 µg/g.

Kidney Ca concentrations were considerably higher in Zn-dosed males that died prior to Day 30 ($\bar{x}$ = 222.2 µg/g, n = 9) than in those that survived to Day 30 ($\bar{x}$ = 93.3 µg/g, n = 11) ($t_{0.05(1),9} = -4.3$, $P < 0.001$). Similarly, Ca levels were higher in Zn-dosed females that died prior to Day 30 ($\bar{x}$ = 289.4 µg/g, n = 16) than in those that survived to Day 30 ($\bar{x}$ = 90.9 µg/g, n = 4) ($U^1_{0.05(1),4,16} = 64$, $P < 0.001$).

Mean kidney Cu levels were significantly higher in Zn- than in Fe-dosed ducks (Table 6); no gender effect was detected. Cu concentrations in Fe-dosed Mallards ranged from 3.7 to 9.9 µg/g; Cu levels in Zn-dosed Mallards ranged from 6.3 to 82.3 µg/g.

Kidney Cu levels were lower in Zn-dosed males that died prior to Day 30 ($\bar{x}$ = 16.2 µg/g, n = 9) than in those that survived to Day 30 ($\bar{x}$ = 35.7 µg/g, n = 11) ($t_{0.05(1),18} = 2.9$, $P < 0.01$). Similarly, Cu levels were lower in Zn-dosed females that died prior to Day 30 ($\bar{x}$ = 15.8 µg/g, n = 16) than in those that survived to Day 30 ($\bar{x}$ = 46.8 µg/g, n = 4) ($U^1_{0.05(1),4,16} = 54$, $P < 0.05$).

Mean kidney Fe levels did not differ significantly between sexes or doses (Table 6). Kidney Fe levels ranged from 85.6 to 332.6 µg/g. Kidney Fe levels were higher in Zn-dosed males that died prior to Day 30 ($\bar{x}$ = 191.4 µg/g, n = 9) than in those that survived to Day 30 ($\bar{x}$ = 136.2 µg/g, n = 11) ($t_{0.05(1),18} = -2.2$, $P < 0.05$). Similarly, Fe levels were higher in Zn-dosed females that died prior to Day 30 ($\bar{x}$ = 166.9 µg/g, n = 16) than in those that survived to Day 30 ($\bar{x}$ = 120.6 µg/g, n = 4) ($U^1_{0.05(1),4,16} = 52$, $P < 0.05$).

Magnesium concentrations did not differ significantly between sexes or doses (Table 6). Kidney Mg levels ranged from 181.2 µg/g to 263.2 µg/g.

Mean kidney P concentrations were significantly higher in Fe- than in Zn-dosed ducks (Table 6). Kidney P levels in Fe-dosed Mallards ranged from 3102 to 3585 µg/g; P levels in kidneys of Zn-dosed Mallards ranged from 2691 to 3550 µg/g.

Mean Sn concentrations were < MDL for all groups. For Fe-dosed ducks, Sn concentrations were > MDLs for kidney in 5 individuals; values ranged from 3.5 to 4.0 µg/g. For Zn-dosed Mallards, Sn concentrations were > MDLs for kidney in only 1 individual (4.5 µg/g).

Kidney Zn concentrations were higher in Zn-dosed and female Mallards, as compared with Fe-dosed and male ducks, respectively (Table 6; Fig. 8). We also detected an interaction between dose and sex. Kidney Zn levels in Fe-dosed Mallards ranged from 23.6 to 30.1 µg/g; kidney Zn concentrations in Zn-dosed Mallards ranged from 62.3 to 608.7 µg/g. Kidney Zn levels were higher in male ($t_{0.05(1),18} = -3.7$, $P < 0.001$) and female ($U^1_{0.05(1),4,16} = 58$, $P < 0.01$) Zn-dosed ducks that died prior to Day 30 than in those that survived to Day 30 (Fig. 8).

Liver

Mean liver Ca concentrations were significantly higher in Zn-dosed than in Fe-dosed ducks (Table 6). Calcium concentrations in livers of Fe-dosed ducks ranged from 36.2 to 122.0 µg/g; liver Ca concentrations in Zn-dosed Mallards ranged from 46.0 to 395.4 µg/g. Liver Ca levels were higher in Zn-dosed females that died prior to Day 30 ($\bar{x}$ = 97.8 µg/g, n = 16) than in those that survived to Day 30 ($\bar{x}$ = 70.7 µg/g, n = 4, Fig. 12) ($U^1_{0.05(1),4,16} = 51$, $P < 0.05$).

Mean liver Cu concentrations were significantly higher in Fe-dosed and male ducks, as compared with Zn-dosed and females ducks, respectively (Table 6). Copper concentrations in Fe-dosed Mallards ranged from 20.3 to 1158 µg/g; liver Cu levels in Zn-dosed Mallards ranged from 5.0 to 741.3 µg/g.

Mean liver Fe levels did not differ significantly between doses; however, liver Fe concentrations were greater in female than in male Mallards (Table 6). Liver Fe levels

ranged from 285.5 to 4788 µg/g. Liver Fe levels were higher in Zn-dosed males that died prior to Day 30 ($\bar{x}$ = 2459 µg/g, n = 9) than in those that survived to Day 30 ($\bar{x}$ = 937.5 µg/g, n = 11) ($t_{0.05(1),18} = -6.7$, $P < 0.001$). Similarly, mean Fe levels were higher in Zn-dosed females that died prior to Day 30 ($\bar{x}$ = 3118 µg/g, n = 16) than in those that survived to Day 30 ($\bar{x}$ = 765.3 µg/g, n = 4) ($U^1_{0.05(1),4,16} = 64$, $P < 0.001$).

Mean liver Mg concentrations did not differ significantly between sexes or doses (Table 6). Mg levels ranged from 179.7 to 343.1 µg/g. Liver Mg levels were lower in Zn-dosed males that died prior to Day 30 ($\bar{x}$ = 237.9 µg/g, n = 9) than in those that survived to Day 30 ($\bar{x}$ = 274.2 µg/g, n = 11) ($t_{0.05(1),18} = -2.5$, $P < 0.05$). Similarly, Mg levels were lower in Zn-dosed females that died prior to Day 30 ($\bar{x}$ = 232.1 µg/g, n = 16) than in those that survived to Day 30 ($\bar{x}$ = 300.8 µg/g, n = 4) ($U^1_{0.05(1),4,16} = 62$, $P < 0.001$).

Mean liver P concentrations did not differ significantly between doses or sexes (Table 6). Liver P levels ranged from 2633 to 4236 µg/g. Liver P levels were lower in male Zn-dosed ducks that died prior to Day 30 ($\bar{x}$ = 3231 µg/g, n = 9) than in those that survived to Day 30 ($\bar{x}$ = 3703 µg/g, n = 11) ($t_{0.05(1),18} = 3.3$, $P < 0.01$). Similarly, P levels were lower in Zn-dosed females that died prior to Day 30 ($\bar{x}$ = 3,102 µg/g, n = 16) than in those that survived to Day 30 ($\bar{x}$ = 3,314 µg/g, n = 4) ($U^1_{0.05(1),4,16} = 64$, $P < 0.001$).

Mean Sn concentrations were < MDL for all groups. For Fe-dosed ducks, Sn concentrations were > MDL for liver in 1 individual (3.9 µg/g). For Zn-dosed Mallards, Sn concentrations were > MDL for liver in 3 individuals; values ranged from 3.6 µg/g to 4.4 µg/g.

Mean liver Zn levels were significantly higher in Zn- than in Fe-dosed Mallards (Table 6, Fig. 9). No difference in mean liver Zn levels between the sexes was detected. Liver Zn levels in Fe-dosed Mallards ranged from 41.4 to 88.5 µg/g; liver Zn concentrations in Zn-dosed Mallards ranged from 142.4 to 597.2 µg/g. Liver Zn levels were higher in Zn-dosed males that died prior to Day 30 than in those that survived to Day 30 ($t_{0.05(1),18} = -2.3$, $P < 0.05$) (Fig. 9).

Pancreas

Mean pancreas Ca concentrations were

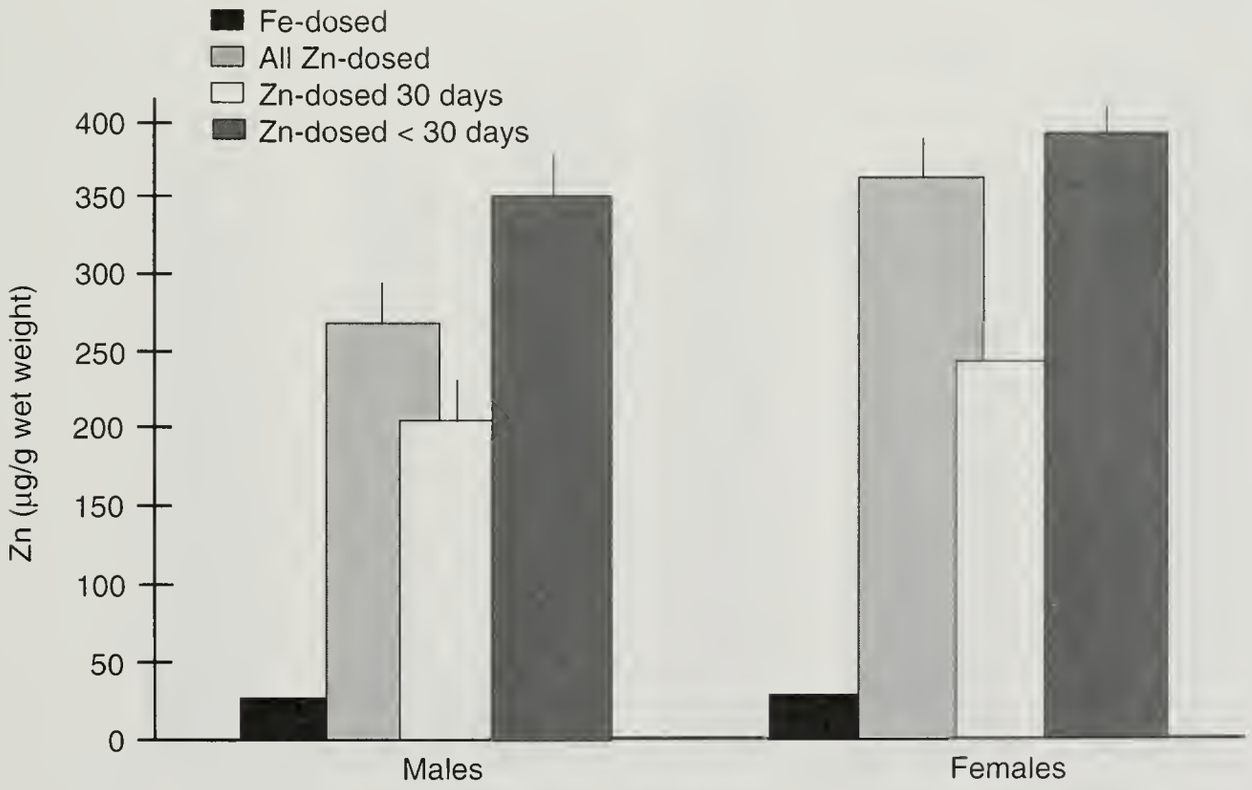

Figure 8. Zn concentrations in the kidneys of game-farm Mallards dosed with 6 No. 4 Zn or 6 No. 4 Fe shot. Sample sizes equal 20 except for Zn-dosed males surviving 30 days, n = 11; males surviving < 30 days, n = 9; females surviving 30 days, n = 11; females surviving < 30 days, n = 4. Vertical lines represent +S.E.

significantly higher in Fe-dosed and female ducks, as compared with Zn-dosed and male ducks (Table 6). We also detected an interaction effect; male Zn-dosed ducks had the lowest concentrations, and female Fe-dosed ducks had the highest concentrations. Calcium concentrations in Fe-dosed ducks ranged from 106.7 to 268.2 µg/g; Ca concentrations in Zn-dosed Mallards ranged from 61.5 to 548.8 µg/g.

Mean pancreatic Cu levels were significantly higher in Zn- than in Fe-dosed ducks; no gender effect was detected (Table 6). Cu concentrations in Fe-dosed Mallards ranged from 1.3 to 3.6 µg/g; Cu levels in Zn-dosed Mallards ranged from 2.4 to 11.1 µg/g.

Mean pancreas Fe levels were significantly higher in Zn- than in Fe-dosed ducks (Table 6). Pancreas Fe levels in Fe-dosed ducks ranged from 37.2 to 176.6 µg/g; Fe levels in Zn-dosed ducks ranged from 38.5 to 156.4 µg/g.

Magnesium concentrations were significantly higher in Fe- than in Zn-dosed ducks (Table 6). Pancreas Mg levels in Fe-dosed ducks ranged from 283.6 to 449.3 µg/g; Mg levels in Zn-dosed ducks ranged from 220.2 to 406.3 µg/g.

Mean pancreas P levels were significantly higher in Fe- than in Zn-dosed ducks (Table 6). Pancreas P levels in Fe-dosed Mallards ranged from 4,435 to 6,793 µg/g; P levels in Zn-dosed Mallards ranged from 3,438 to 6,502 µg/g.

Mean Sn concentrations were below the MDL for all groups. Pancreas Sn concentrations were < MDL in all Fe-dosed ducks. For Zn-dosed Mallards, pancreatic Sn concentrations were > MDL in 7 individuals; values ranged from 3.6 to 5.5 µg/g.

Mean pancreas Zn concentrations were significantly higher in Zn- than in Fe-dosed Mallards (Table 6, Fig. 10). No difference in pancreas Zn concentrations between the sexes was detected. Pancreas Zn levels in Fe-dosed Mallards ranged from 39.8 to 221.8 µg/g; Zn concentrations in Zn-dosed Mallards ranged from 751.6 to 3,844 µg/g. Pancreas Zn levels were higher in male ($t_{0.05(1),18} = -2.7$, $P < 0.01$) and female ($U^{1}_{0.05(1),4,16} = 50$, $P = 0.05$) Zn-dosed ducks that died prior to Day 30 than in those that survived to Day 30 (Fig. 10).

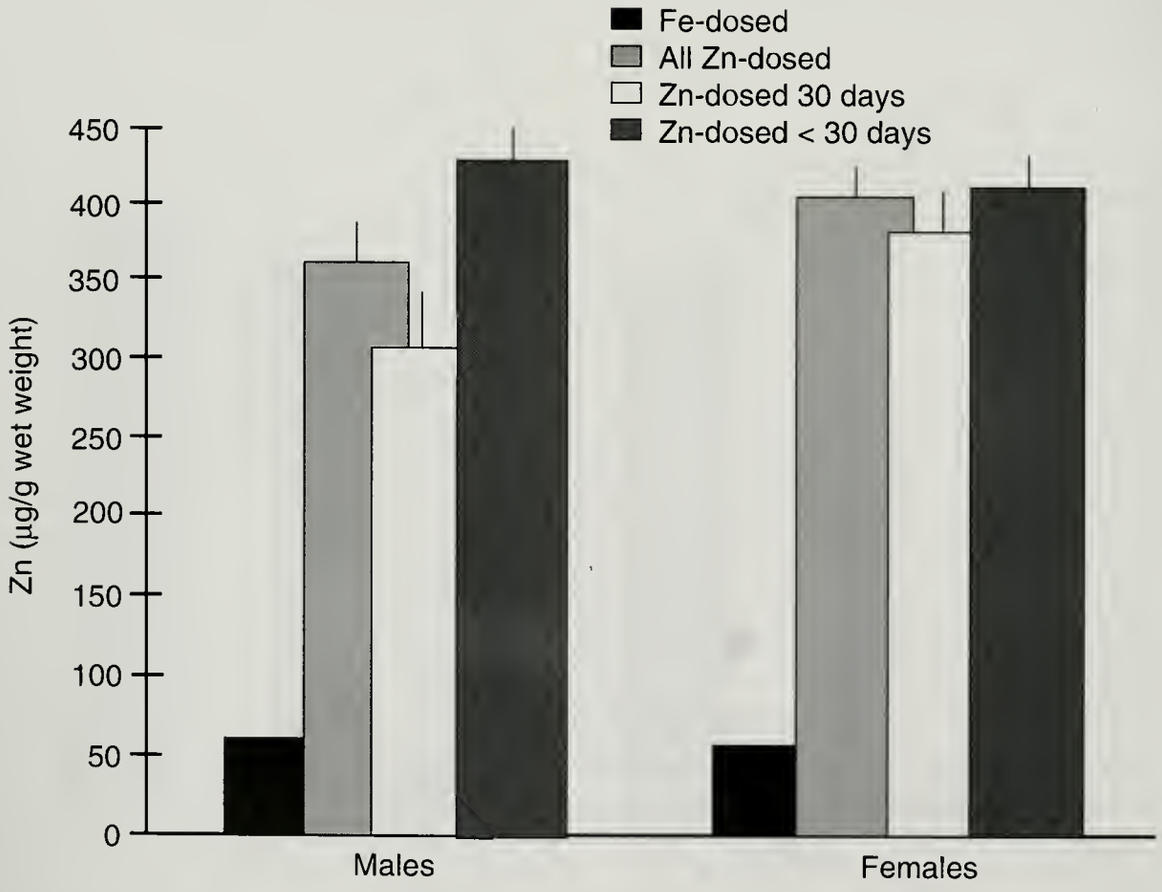

Figure 9. Zn concentrations in the liver of game-farm Mallards dosed with 6 No. 4 Zn or Fe shot. Sample sizes equal 20 except for Zn-dosed males surviving 30 days, n = 11; males surviving < 30 days, n = 4; females surviving 30 days, n = 4; females surviving < 30 days, n = 14. Vertical lines represent +S.E.

Discussion

Mortality and Behavioral Abnormalities

We observed a high rate of mortality to 30 days in male (45%) and female (80%) Zn-dosed Mallards. Grandy et al. (1968) reported 20% mortality to 30 days in 15 male Mallards dosed with 8 No. 6 Zn shot, and maintained on cracked corn, quartz, and oyster shell grit. Gasaway and Buss (1972:1114) indicated that "severe mortality occurred after 30 days," with 92% mortality to 60 days, in ducks fed 3,000 to 12,000 mg/kg Zn carbonate in a chicken developer-turkey finisher diet. In contrast, French et al. (1987) found no mortality to 28 days in Mallards dosed with 5 or 10 pure (99.9%) No. 6 Zn shot pellets; their ducks were fed a diet of wheat, barley, and "turkey crumbs." Mortality in Pb-dosed Mallards generally approaches or reaches 100% under test conditions similar to ours (Sanderson and Irwin 1976; Sanderson et al. 1992; Sanderson et al. 1997a).

Mean survival times in our study were similar to an average of 20 days, as reported by Grandy et al. (1968), for Zn-dosed ducks. The shortest survival time for an individual duck in the current study was 5 days, which is comparable to 4 days in a Mallard dosed with 8 No. 2 Pb shot (Sanderson et al. 1992).

We observed a variety of clinical signs of Zn toxicosis, which were similar to those noted in other studies. Progressive loss of muscular control has been noted in Zn-dosed Mallards (Grandy et al. 1968; Gasaway and Buss 1972) and Cockatiels (Howard 1992), and ducklings dosed with Zn sulfate had trouble standing and righting themselves (Van Vleet et al. 1981). Other signs reported for Zn-intoxicated birds that were consistent with those observed in our study have included anorexia or dysphagia, lethargy, dullness, green droppings, and diarrhea (Grandy et al. 1968; Gasaway and Buss 1972; Howard 1992). The signs were similar to those produced by Pb poisoning in Mallards (Jordan and Bellrose 1951). In contrast to these studies, French et al. (1987) noted no abnormalities in Mallards dosed with 5 or 10 No. 6 Zn shot.

Mortality first occurred on Day 5 (1 female) before any signs were noted in that animal. Similarly, Grandy et al. (1968)

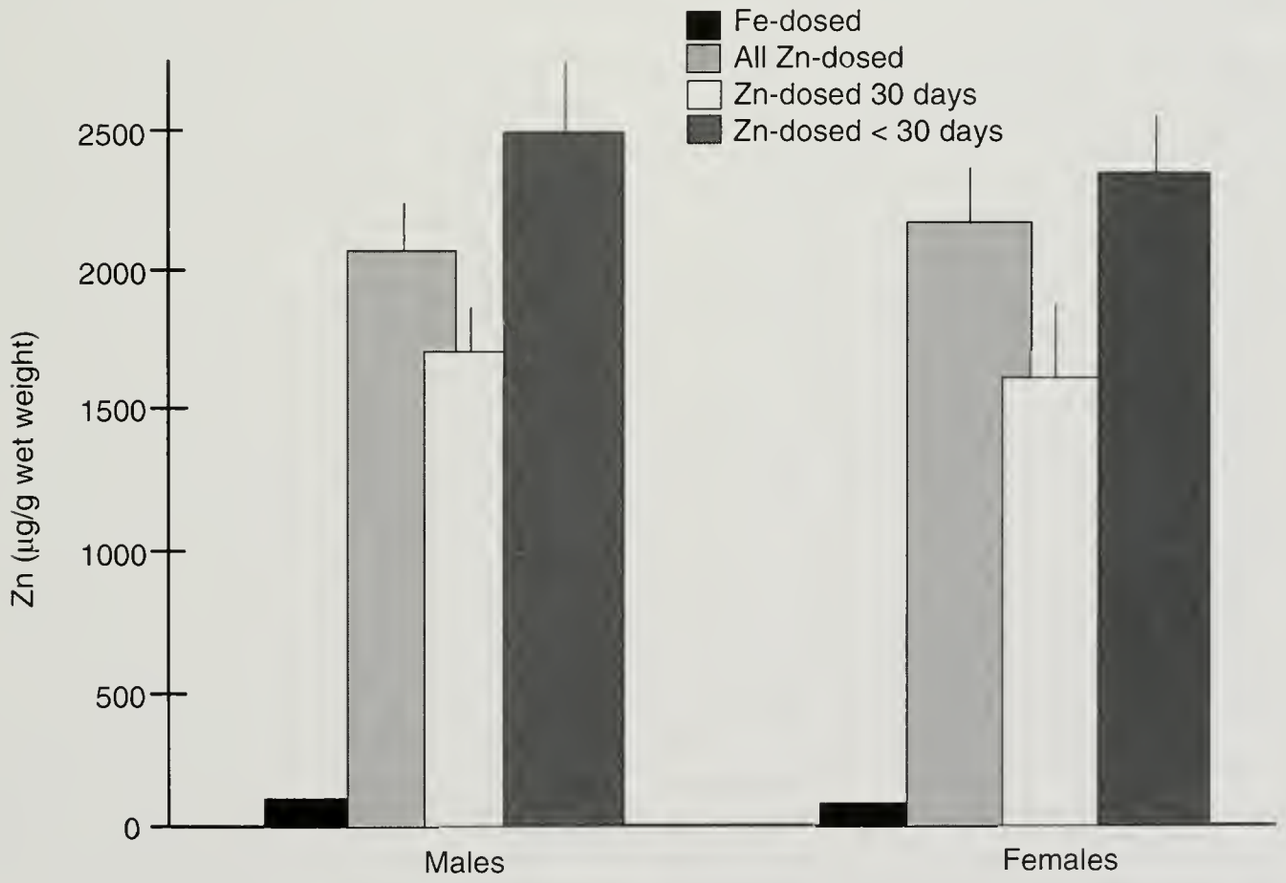

Figure 10. Zn concentrations in the pancreas of game-farm Mallards dosed with 6 No. 4 Zn or Fe shot. Sample sizes equal 20 except for Zn-dosed males surviving 30 days, n = 11; males surviving < 30 days, n = 9; females surviving 30 days, n = 4; females surviving < 30 days, n = 4. Vertical lines represent +S.E.

reported that 1 of the Zn-dosed ducks in their study died without exhibiting any signs, and van der Zee et al. (1985:68) noted that a Zn-poisoned Nicobar Pigeon (*Caloenas nicobarica*) died "unexpectedly and without any previous signs of disease." The large number of ducks exhibiting signs of intoxication agreed with Grandy et al. (1968), who detected behavioral anomalies in 12 of 15 (80%) male Mallards dosed with 8 No. 6 Zn shot. In their study of the effects of dietary Zn carbonate on Mallards, Gasaway and Buss (1972) noted severe paralysis after 20 days, with the onset being most rapid in the group receiving the lowest dosage. In the present study, none of the ducks that manifested signs completely recovered, whereas Grandy et al. (1968) reported that 1 duck in their study exhibited signs for 5 days but had "fully recovered" by Day 30. Howard (1992:1670) indicated that "many" of 80 Cockatiels dosed with Zn fragments or "white rust" (oxidized Zn) exhibited signs of intoxication but recovered spontaneously. Some of our ducks went through periods of improvement and deterioration, and Howard (1992:1670) noted that signs in chronically ill, Zn-dosed Cockatiels were "variable and intermittent."

Shot Retention and Dissolution

Retention rates in our study were higher than those reported by Sanderson et al. (1997a) for Bi- (95%) and Fe-dosed (87.5%) Mallards. Shot retention was 84.4% in Pb-dosed Mallards, which died within 14 days of dosing (Sanderson et al. 1997b). Grandy et al. (1968) found that only 3 of 15 Zn-dosed Mallards retained any shot to 30 days, with none retaining the original dose of 6 pellets. French et al. (1987) reported retention rates of 98% and 50%, respectively, 28 days after dosing in Mallards dosed with 5 or 10 No. 6 Zn shot pellets.

The average dissolution rates for Zn and Fe shot in our study were higher than the mean "absorption" rates of 0.009 and 0.01 g/day, respectively, in Mallards dosed with 5 or 10 No. 6 pure Zn pellets (French et al. 1987). Shot dissolution ranged from 38.2% to 96.4% of the original dose in Bi-dosed ducks, with no differences between the sexes, and from 38.0% to 89.6% in Fe-dosed ducks, with females dissolving a greater proportion of the original shot weight than males (Sanderson et al. 1997a). Pb-dosed ducks, all of which had died within 14 days of dosing, lost an average of 31.4% of the original shot weight (Sanderson et al. 1997b). In the present study, the mean total dissolution (% loss of original shot weight) and dissolution rates for all Zn-dosed ducks (regardless of the length of survival) that retained 6 shot was lower than in Zn-dosed ducks that survived to Day 30. Some ducks died from Zn intoxication having dissolved little of the shot pellets, while others dissolved nearly 100% of the shot weight and survived to Day 30.

Body and Organ Weights

The average percent change (loss) in body weight in our study was as high as 33% and 40% for Zn-dosed males and females, respectively, that died prior to Day 30. Although Zn-dosed ducks weighed less than Fe-dosed ducks at Days 15 and 30, the average weight change (loss) in Zn-dosed ducks that survived to Day 30 was similar to that in Fe-dosed ducks, all of which survived to Day 30. Weight loss to 30 days in Mallards fed Zn carbonate along with a chicken developer/turkey finisher diet ranged from 17% to 44%, with females losing a greater proportion of body weight than males (Gasaway and Buss 1972). Grandy et al. (1968) reported that mean weight loss in male Mallards dosed with 8 No. 6 Zn pellets and placed on a corn and grit diet was 33% for 3 birds that died, and 22% in those surviving 30 days.

In contrast, French et al. (1987) found that Mallards dosed with 5 or 10 No. 6 Zn shot pellets and kept on a varied diet of small grains, commercial feed, and grit actually gained weight. In their review of lead poisoning, Sanderson and Bellrose (1986) reported that Mallards dying of chronic Pb poisoning typically lose 40%–60% of their body weight, whereas those dying of acute Pb poisoning may lose little weight. Weight loss in Pb and Zn dosing experiments involving ducks has been attributed to a reduction or cessation of feeding.

According to Sanderson and Bellrose (1986), the organs of Pb-poisoned waterfowl may be reduced or enlarged at death, depending on the stage and nature of the toxicosis. Sanderson and Irwin (1976) suggested that changes in liver size in Pb-intoxicated ducks

were confounded by differing rates of food consumption among seasons and between sexes, diet, length of survival, and anorexia. In our study, the kidneys of Zn-dosed ducks as a group were heavier, and the pancreases, livers, and gizzards lighter, compared with Fe-dosed ducks. The livers (males) and kidneys of Zn-dosed ducks that died as a result of Zn intoxication were heavier, whereas the gonads (females) and gizzards were lighter, compared with those that survived. The organ:body weight ratios for liver, pancreas, and kidneys increased, whereas ratios for the gonads (males) and gizzards decreased, compared with those of Zn-dosed ducks that survived. Gasaway and Buss (1972) found that ducks fed dietary Zn experienced significant reductions in the weight of the pancreas, liver, and gonads, compared with controls, whereas the kidneys represented a larger proportion of body weight. van der Zee et al. (1985) noted liver and kidney hypertrophy in a captive Nicobar Pigeon (*Caloenas nicobarica*) that had ingested Zn fragments, and Howard (1992) noted that the livers were "slightly swollen" in approximately a third of a group of Cockatiels (*Nymphus hollandus*) fed pure zinc or galvanized coating.

Packed Cell Volume

Severe anemia is common in Pb-poisoned waterfowl (Sanderson and Bellrose 1986), as well as in Zn-intoxicated vertebrates (Underwood 1971; Eisler 1993; Walsh et al. 1994). Anemia resulting from Zn toxicosis has been attributed to associated Cu and Fe deficiencies; Zn intoxication can lead to faulty hematopoiesis and shortened erythrocyte life span due to Zn-mediated Cu deficiencies and Zn-Fe interactions.

PCV values in undosed, farm-raised Mallards typically average about 45%-46% (Ringelman et al. 1993; Duncan 1997; Sanderson et al. 1997a,b). Campbell (1988) considered PCVs of less than 35% in caged birds as indicative of anemia. In the present study, PCVs were below 35% in 11 of 28 (39%) Zn-dosed ducks at Day 15, and in none of the remaining 15 Zn-dosed ducks at Day 30. Although Gasaway and Buss (1972) noted no remarkable reduction in PCVs until Day 30 in Mallards fed Zn carbonate, we documented considerable reductions in PCV values in Zn-intoxicated ducks between Days 0 and 15.

Pathology

The occurrence and severity of lesions observed in Zn-dosing/feeding studies involving Mallards has varied. Grandy et al. (1968) and Gasaway and Buss (1972) observed little in the way of gross lesions in Zn-dosed ducks, despite reduced survival and a variety of clinical signs of intoxication. Grandy et al. (1968) reported that 1 of 15 Zn-dosed ducks in their study had acid-fast inclusion bodies present in its renal tubular cells, and hepatic hemosiderosis, though noted in all ducks, was more pronounced in dosed ducks (including zinc, lead, nickel, tin, and steel) than in controls. French et al. (1987) noted no detrimental effects of dosing Mallard with 5 or 10 "pure" Zn shot, including a lack of gross and microscopic lesions. In contrast, feeding 3,000 or 6,000 mg/kg Zn produced pancreatic damage in a large proportion of White Pekin ducklings (Van Vleet et al. 1981), and smaller numbers developed necrosis of skeletal and smooth (intestine and gizzard) muscle, lesions considered indicative of Se-Vitamin E deficiency. Kazacos and Van Vleet (1989) documented progressive pancreatic injury, including apoptosis and autophagocytosis, in ducklings fed 2,500 ppm Zn.

A variety of macro- and microscopic anatomical changes were observed in Zn-dosed ducks in our study. Generally, these lesions were consistent with those observed in Zn-intoxicated birds (Dewar et al. 1983; van der Zee et al. 1985; Reece et al. 1986; Wight et al. 1986; Droual et al. 1991; Howard 1992). Although enteritis is commonly associated with Zn intoxication, we did not find specific references to Zn-induced typhlitis in the literature, but this was the most consistent and dramatic lesion detected at necropsy in our study. A relatively large number of Zn-intoxicated ducks had lesions of the peri-, epi-, and/or myocardium, although lesions involving the heart were not noted in other studies we reviewed. Given the syncytial function of the cardiac myofibers, even mild lesions associated with the myocardium are considered important. Although Walsh et al. (1994) concluded that increased Zn intake, even at excessive levels, was not likely to be hepatotoxic, we documented macro- and microscopic lesions in a relatively large proportion of the Zn-intoxicated ducks. However, these changes may have been

associated with secondary effects of Zn intoxication.

Tissue Element Concentrations

Calcium

With the exception of Fe-dosed females between Days 0 and 15, plasma Ca concentrations decreased in both Zn- and Fe-dosed ducks over the course of our experiment, probably due to the nutritionally deficient diet of corn, which is low in Ca. Decreased availability of dietary Ca may have been exacerbated by anorexia in female Zn-dosed ducks, which were more susceptible to Zn intoxication. In addition, antagonistic relationships between Zn and Ca (Underwood 1971; Prasad 1979; Spencer et al. 1980) and Fe and Ca have been noted (Forth and Rummel 1971). The site of this interaction is apparently at the intestinal level, where one inhibits absorption of the other.

Ca deficiencies can result in bone disorders, reduced growth and performance, reduced egg production and hatchability, and eggshell thinning (Underwood 1981); however, values in our study were similar to those previously reported by other researchers (Sanderson et al. 1997a; Fairbrother et al. 1990). Mean kidney and liver Ca concentrations in Fe-dosed ducks in our study were similar to those Sanderson et al. (1997a) reported for 0- and Fe-dosed Mallards. Mean hepatic, and particularly renal, Ca concentrations in Zn-dosed ducks were greatly elevated by comparison. Increased Ca concentrations in these tissues may have represented pathologic calcification due to necrosis (i.e., dystrophic calcification) and kidney dysfunction (nephrocalcinosis) (Cotran et al. 1994).

Copper

Mean plasma Cu levels that were > MDL in the present study (0.20–0.32 µg/g) were similar to serum Cu levels provided by Puls (1988) for ducks on a Cu-adequate diet (0.22–0.45 µg/g). Individual values were as high as 0.7 µg/g and 1.0 µg/g in Fe- and Zn-dosed ducks, respectively.

Sanderson et al. (1997b) found no differences in plasma or erythrocyte Cu levels attributable to sex or dose (0, Fe, or Bi/Sn). Similarly, we found no differences in Cu concentrations in either blood fraction between Zn- and Fe-dosed Mallards. This finding seems surprising, given that Cu is essential for normal erythropoiesis and Zn-intoxicated ducks exhibited severe anemia prior to death. Copper deficiency and toxicity may depend on the relative levels of Fe, as well as Ca and Zn (Underwood 1971). The anemia resulting from Zn insult has been attributed to Cu, and associated Fe, deficiencies that apparently result from interference with Cu uptake in the intestine (Vallee 1959; Underwood 1971; Prasad 1979; Southern and Baker 1983; Goyer 1991; Walsh et al. 1994; Pluhator et al. 1996). Low Cu levels interfere with normal Fe metabolism, leading to an accumulation of nonhemoglobin Fe (Underwood 1971). Although we found no difference in mean PCV values between doses, we did document dramatic changes (reductions) in percent change in PCV in Zn-dosed ducks. Mean values may have been biased towards those animals that experienced only mild Zn toxicosis, because no blood samples were collected from ducks that died prior to a data collection day.

Mean Cu levels were lower in the livers of Zn- than in Fe-dosed Mallards. Excess Zn is known to prevent hepatic accumulation of Cu (Puls 1988; Stahl et al. 1989; Walsh et al. 1994). In addition, an inverse relationship exists between hepatic Fe and Cu concentrations (Sourkes et al. 1968), and we found high concentrations of Fe in livers of Fe- and Zn-dosed ducks (see section on tissue Fe concentrations). French et al. (1987) indicated that liver Cu levels did not differ between Zn-dosed Mallards and sham-dosed controls, and Sanderson et al. (1997b) reported no differences in liver Cu concentrations among 0-, Fe-, and Bi/Sn-dosed Mallards.

Mean liver Cu levels in Zn-dosed ducks in the present study were within the range of values (25–300 µg/g) reported for ducks on a high-Cu diet (Puls 1988). Levels in Fe-dosed ducks fell within this range for females; however, the mean level for males was somewhat higher. Individual levels in 1 Zn- and 8 Fe-dosed Mallards were above the level (540 µg/g) associated with toxic effects in ducks (Puls 1988). Liver Cu concentrations of 420–657 ppm were associated with reduced growth and performance in chicks fed high levels of dietary Cu (Robbins and Baker 1980). Liver pathology has been noted in sheep with

increased serum and accumulated hepatic Cu concentrations (Ishmael et al. 1971). Mean hepatic Cu concentrations in our Zn-dosed ducks were higher in females and lower in males, respectively, compared with those reported for female and male sham-dosed Mallards (Sanderson et al. 1997a). We observed higher liver Cu levels in male as opposed to female ducks, as was previously reported by Sanderson et al. (1997a).

Kidney Cu levels were higher in Zn- as compared with Fe-dosed Mallards, and were higher in Zn-dosed ducks that survived to 30 days than in those that died during the experiment. Mean renal Cu concentrations in Fe-dosed ducks in our study were similar to those in 0-dosed ducks in a previous study (Sanderson et al. 1997a); however, levels in Zn-dosed ducks were greatly elevated by comparison. French et al. (1987) found that kidney Cu levels did not differ between Zn- and sham-dosed Mallards. Scheuhammer and Templeton (1990) demonstrated that Zn and Cu coaccumulated with Cd, associated with metallothionein, in the liver and kidneys of Ringed Turtle Doves (*Streptopelia risoria*). Renal Cu concentrations increased substantially with increased dietary Cd and kidney metallothionein concentrations. It is plausible that, in a similar mechanism, Cu might also coaccumulate with Zn in some tissues (especially kidney and pancreas) following induction of metallothionein by high Zn exposure (A. Scheuhammer, Canadian Wildlife Service, pers. comm.).

Iron

With the exception of the pancreas and plasma at Day 15, we found no significant differences in tissue Fe levels between doses; however, mean Fe concentrations were higher in the kidney and liver of Zn-dosed ducks that died during the experiment than in those that survived. Fe concentrations in pancreases and plasma (Day 15) were higher in Zn- than in Fe-dosed ducks, and erythrocyte Fe levels were higher in Zn-dosed ducks that survived to Day 30 than in those that died between Days 15 and 30.

Sanderson et al. (1997b) noted no effect of sex or dose on concentrations of Fe in plasma or erythrocytes of Mallards dosed with 0, Fe, or Bi/Sn shot. Concentrations of Fe in plasma of Fe-dosed ducks in our study dropped after Day 0, which was also noted by Sanderson et al. (1997b) in 0-, Fe-, and Bi/Sn-dosed Mallards; these researchers attributed this decrease to the change in diet beginning at Day 0 when ducks were switched from commercial pellets to corn. In our study, however, plasma Fe levels increased dramatically in Zn-dosed ducks between Days 0 and 15. Increased plasma and reduced erythrocytic Fe levels at Day 15 in Zn-intoxicated ducks may have reflected increased accumulation of transferrin-bound Fe in plasma as a result of abnormal erythropoiesis due to Zn-Fe and/or Fe-Cu antagonism.

Although Zn is reported to cause a loss of Fe from liver and other tissue (Underwood 1971; Walsh et al. 1994; Pluhator et al. 1996), some workers have demonstrated increased concentrations of Fe in the liver of Zn-intoxicated birds (Grandy et al. 1968; Droual et al. 1991). Stahl et al. (1989) reported that excessive dietary Zn reduced liver Fe turnover in poultry chicks. In contrast, French et al. (1987) found that hepatic and renal Fe concentrations were similar between Zn- and sham-dosed Mallards.

Sanderson et al. (1997b) found higher Fe levels in the livers and kidneys of Fe-dosed ducks as compared with 0- and Bi/Sn-dosed ducks. Concentrations of Fe in kidneys and livers in our study were higher than Sanderson et al. (1997b) reported for their three treatment groups. These differences may be attributable to seasonal differences in tissue chemistries. The mean liver Fe concentrations in Zn-dosed females, and in male and female Zn-dosed ducks that died prior to Day 30, in the current study were above the range of values provided by Puls (1988) for poultry on a Fe-adequate diet (300–2000 μg/g), and were similar to levels in Pb-dosed Mallards reported by Sanderson et al. (1992).

The maximum value for hepatic Fe observed in our Zn-dosed ducks (4,788 μg/g in a female) fell within the range of values obtained by Grandy et al. (1968) for ducks that died of Zn intoxication. Increased hepatic Fe levels in Zn-intoxicated ducks may result from sequestering of Fe not utilized in heme synthesis; Zn intoxication can lead to faulty hematopoiesis and shortened erythrocyte life span due to Zn-mediated Cu deficiencies and Zn-Fe interactions.

In our study, liver Fe levels were higher in females than in males, which has been previ-

ously noted in other animals, including birds (Underwood 1971). Sanderson et al. (1997b) found no difference in liver Fe concentrations between male and female Mallards in the spring of the year. We found increased pancreatic Fe levels in Zn-dosed ducks, whereas other studies have detected elevated Fe concentrations in the pancreases of Zn-deficient animals (see Walsh et al. 1994).

Magnesium

Mean plasmatic, erythrocytic, renal, and hepatic Mg levels in our study, though generally higher, did not vary dramatically from those reported by Sanderson et al. (1997a,b) for 0-, Fe-, and Bi/Sn-dosed Mallards. Although Borovansky and Riley (1989) found that Mg was not effective in reducing Zn cytotoxicity, suggesting lack of a direct antagonism between these two elements, mean Mg levels were lower in livers of Zn-dosed ducks that succumbed to Zn poisoning, and mean pancreatic concentrations were lower in Zn-dosed than in Fe-dosed ducks. Puls (1988) reported that increased Ca or P intake enhanced Mg deficiency and reduced Mg toxicity; thus, changes in Mg concentrations in Zn-dosed ducks may have reflected Zn-mediated alterations in Ca and/or P levels.

Phosphorus

Mean plasma P concentrations in Zn-dosed ducks were slightly lower, whereas erythrocyte concentrations in males were similar and in females were higher, than reported by Sanderson et al. (1997a) for male and female 0-dosed Mallards in the spring. Mean plasma P concentrations in females in our study were considerably lower than serum concentrations for Mallards of differing reproductive states (Fairbrother et al. 1990); however, differences between males in their study and ours were not as dramatic. The effects of P deficiency in poultry are the same as those produced by low Ca intake; however, these effects are not as dramatic because of lower requirements and higher levels in most grain-based diets (Underwood 1981).

Although dietary P levels were considered marginal for poultry (Puls 1988), plasma and erythrocyte P concentrations generally increased during the study. Hepatic and renal (female) P concentrations were higher in our Zn-dosed ducks than in 0-dosed ducks in an earlier study (Sanderson et al. 1997a); mean renal P concentrations in males were similar between the two studies.

Relatively little is known about the interaction between P and Zn in animals, although a Zn-P antagonism in plants is well documented (Giordano and Mortvedt 1980). Increased P intake has been shown to exacerbate Zn deficiency in rats (Underwood 1971) and increase fecal Zn output in humans (Spencer et al. 1980). Thus, an antagonistic relationship between these two elements, coupled with the high Zn concentrations observed, alterations in Ca and Mg metabolism, and low dietary P levels, may have resulted in the lower plasma, kidney, and pancreas Zn concentrations in Zn- than in Fe-dosed ducks in spite of adequate dietary P levels.

Tin

Although the Zn shot utilized in our study contained an average of 2.0% Sn, mean Sn levels were < MDLs in all tissues examined, and few individuals exhibited Sn concentrations > MDLs. Sanderson et al. (1997b) also found very low tissue Sn levels in Mallards dosed with shot containing 1.9% Sn. Low tissue Sn concentrations are apparently related to poor absorption and high excretion rates for this metal (Underwood 1971; Sanderson et al. 1997b).

Zinc

Zinc levels were higher in Zn- than in Fe-dosed ducks for all tissues examined. Mean tissue Zn concentrations were also higher in Zn-dosed ducks that died during the study than in those that survived to Day 30. The exceptions were plasma and liver Zn levels in females, presumably because a greater number of females died of Zn intoxication than did males.

Plasma Zn levels at Days 15 ($r = 0.86$ to 0.93) and 30 ($r = 0.85$ to 0.95) were more highly correlated with liver, kidney, and pancreas Zn levels than were erythrocytic Zn concentrations at Days 15 ($r = 0.61$ to 0.65) and 30 ($r = 0.67$ to 0.75). Prasad (1979) indicated that changes in erythrocyte Zn were slow to appear, compared with leucocytic or plasma Zn, and that plasma Zn levels are reflective of, and commonly used to monitor changes in, Zn status.

Little is known of the relationship between Zn insult and associated renal pathology.

Increased urinary Zn output has been documented under some conditions of renal and/or hepatic disease (Underwood 1971). In the present study, mean renal Zn levels in all Zn-dosed female Mallards and in Zn-dosed males and females that died prior to Day 30 were similar to concentrations found in kidneys of Mallards fed toxic levels of Zn carbonate (Gasaway and Buss 1972), and fell within the range of values (300–800 µg/g) associated with subclinical, clinical, and pathological effects in poultry (Puls 1988). Our highest value (original datum converted to dry weight for comparison; 2,424 µg/g) was higher than that (2,102 µg/g) reported for an acutely intoxicated Nicobar Pigeon (van der Zee et al. 1985). Our mean values (original data converted to dry weight) for male (1,043 µg/g) and female (1,400 µg/g) Zn-dosed Mallards were considerably higher than those reported by French et al. (1987) for Mallards (sexes combined) dosed with 5 (79 µg/g) or 10 (72 µg/g) No. 6 "pure" (99.9%) Zn shot.

Elevated liver Zn concentrations have been associated with increased production of metallothioneins within the liver following a decrease in plasma Zn (Cousins and Failla 1980). In our study, mean liver Zn levels in all male and female Zn-dosed Mallards, as well as in Zn-dosed males that died prior to Day 30, were similar to concentrations found in kidneys of Mallards fed toxic levels of Zn carbonate (Gasaway and Buss 1972). Our mean levels fell with the range of values (200-700 µg/g) associated with subclinical, clinical, and pathological effects in poultry (Puls 1988).

Hepatic Zn concentrations did not differ between Zn-dosed females that survived to Day 30 and those that did not. Our highest value (converted to dry weight for comparison; 1,851 µg/g) was lower than that (3,579 µg/g) reported for a Nicobar Pigeon that became acutely intoxicated after ingesting Zn-plated wire (van der Zee et al. 1985) but was similar to that reported for a Gray-headed Chachalaca (*Ortalis cinereiceps*) (presumed dry weight, 1,910 µg/g) that had ingested a copper-coated Zn coin (Droual et al. 1991). A mean liver Zn concentration of 1,144 µg/g (dry weight) was reported for poultry chicks (*Gallus* sp.) fed 4,000 mg/kg dietary Zn, a level that produced Zn toxicosis. Mean values (converted to dry weight) for male (1,116 µg/g) and female (1,246 µg/g) Zn-dosed ducks in our study were considerably higher than those reported by French et al. (1987) for Mallards dosed with 5 (217 µg/g) or 10 (211 µg/g) No. 6 "pure" (99.9%) Zn shot.

Zn concentrations in pancreases of Mallards dosed with Zn shot were much higher than in the other tissues examined. Increased pancreatic Zn levels are not surprising, given the importance of the pancreas in the excretion of endogenous Zn (Underwood 1971; Walsh et al. 1994). Mean pancreas Zn levels in male and female Zn-dosed Mallards were similar to concentrations found in pancreases of Mallards fed toxic levels of Zn carbonate (Gasaway and Buss 1972), and fell with the range of values (1,000–3,500 µg/g) associated with subclinical, clinical, and pathological effects in poultry (Puls 1988). The highest value in our study (3,844 µg/g wet weight; 11,455 µg/g dry weight) was higher than the range of values provided by Puls (1988), and mean values reported (1,252–2,672 µg/g wet) for Mallards (Gasaway and Buss 1972) or poultry (Southern and Baker 1983; 8,201 µg/g dry) fed excess dietary Zn.

Conclusions

The results of this 30-day acute toxicity test indicated that dosing of 6- to 8-month-old wild type game-farm Mallards with 6 No. 4 Zn shot pellets (98% Zn/2% Sn) produced toxic effects under the conditions of the study. The Zn-dosed group experienced high mortality, and a large proportion developed behavioral signs of Zn toxicosis. Zn-intoxicated ducks experienced reduced PCVs and body mass, changes in organ mass, greatly elevated tissue Zn concentrations, changes in tissue concentrations of other elements examined, and gross and microscopic tissue alterations, relative to Fe-dosed controls.

We documented differences in the degree of ataxia and paresis, the pattern of shot erosion/dissolution, physiological parameters, gross and microscopic pathology, and tissue element concentrations between Zn-dosed Mallards that survived to Day 30 and those that did not. Females were more susceptible to Zn insult than males, presumably due to the higher dose they received relative to body weight.

Although the shot used in our study approached the purity of that utilized by French et al. (1987), the results of these two studies varied dramatically. Comparisons among

studies must be made carefully, given differences in age, sex, dosing levels, shot composition, length of studies, rate of shot voidance, and diet. Differences in diet between our study and French et al. (1987) were apparent in that our Mallards were fed a nutritionally deficient diet of shelled corn and had no access to grit beyond what was contained in their gizzards upon arrival at our facility. In contrast, French et al. (1987) fed their ducks a more balanced ration of wheat, barley, "turkey crumbs," and grit.

Diet, including ingestion of soil and grit, can have a dramatic effect on Pb shot erosion and Pb absorption, retention, and excretion rates, and can be important in mitigating the toxic effects of ingested Pb shot (Sanderson and Bellrose 1986). Diet might be expected to play an even greater role in Zn toxicosis, given the essential nature of Zn to living organisms, resistance of higher vertebrates to high Zn concentrations, and known antagonisms between Zn and elements such as Ca, Cu, and Fe, as well as other dietary inhibitors of Zn absorption, such as phytate, lignin, and hemicellulose (Underwood 1971; Eisler 1993; Walsh et al. 1994). However, Zn shot comprised of 98% Zn and 2% Sn was toxic to farm-raised Mallards under the conditions of our study, and thus presents an environmental hazard and unacceptable risk to waterfowl, and perhaps others birds, that might ingest it. Therefore, the Zn shot we tested is not an acceptable substitute for Pb shot.

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Appendix:

Results of Analyses of Spiked Samples, Blanks, and Duplicates

SPIKED SAMPLES - MEAN % RECOVERY

	Sn % recovery	Fe % recovery	Ca % recovery	P % recovery	Mg % recovery	Zn % recovery	Cu % recovery
PLASMA	106	107	98.1	99.5	109	102	104
RBC	106	108	101	105	104	106	107
KIDNEY	107	99.4	95.6	98.3	99.9	114	115
LIVER	110	119	122	101	109	122	114
PANCREAS	104	99.3	95.7	97.5	103	120	102

SPIKED BLANKS - MEAN % RECOVERY

	Sn % recovery	Fe % recovery	Ca % recovery	P % recovery	Mg % recovery	Zn % recovery	Cu % recovery
PLASMA	103	102	101	98.7	99.7	99.7	101
RBC	104	102	102	104	99.8	104	106
KIDNEY	105	101	101	101	99	97.5	98
LIVER	105	104	106	105	104	108	107
PANCREAS	100	97.0	98.7	98.9	97.1	108	98.8

LABORATORY DUPLICATES - RELATIVE % DIFFERENCE (RPD)

	Sn, µg/g mean conc.	Sn RPD	Fe µg/g mean conc.	Fe RPD	Ca µg/g mean conc.	Ca RPD	P µg/g mean conc.	P RPD	Mg µg/g mean conc.	Mg RPD	Zn µg/g mean conc.	Zn RPD	Cu µg/g mean conc.	Cu RPD
PLASMA	-0.11	411	5.81	20.5	125	5.15	204	15.8	27.8	3.26	4.72	11.4	0.39	48.9
RBC	0.46	366	870	7.18	34.7	32.2	2482	6.35	124	5.95	8.92	6.15	0.63	34.2
KIDNEY	1.80	92	166	4.82	112	17.7	3302	2.57	221	2.76	179	1.77	16.9	3.51
LIVER	-0.38	170	1783	8.73	65.5	19.7	3557	3.26	261	3.67	202	3.56	194	6.85
PANCREAS	1.49	387	66	12.7	146	12.1	5637	4.86	357	4.11	460	16.2	2.44	12.9

Illinois Natural History Survey
Natural Resources Building
607 East Peabody Drive
Champaign, Illinois 61820
217-333-6880

A Division of the Illinois Department of Natural Resources