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Acute Toxicity Test Of Zinc Shot On Game-Farm Mallards

Final Report

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FINAL REPORT FOR THE ACUTE TOXICITY TEST

PROJECT NAME: ACUTE TOXICITY TEST OF ZINC SHOT ON GAME-FARM MALLARDS

DATE: 13 June 1997

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SUMMARY

We conducted a 30-day Acute Toxicity Test of Zinc (Zn) shot using 40 female and 40 male, 6 to 8 month-old, wild-type game-farm mallards (*Anas platyrhynchos*). Following Canadian Wildlife Service guidelines, 40 ducks (20 males and 20 females) were dosed with 6 No. 4 candidate shot pellets containing 98% Zn and 2% tin (Sn), and the remaining 40 ducks were dosed with 6 No. 4 steel (Fe) shot and served as controls.

The Zn shot dosage resulted in high mortality (62.5%), with a greater proportion of females (80%) dying than males (45%). Survival averaged 18 and 23 days for female and male Zn-dosed ducks, respectively. All Fe-dosed ducks survived to Day 30. Ataxia and/or paresis were noted in 87.5% of Zn-dosed ducks, with 74% of these categorized as displaying moderate or severe signs. 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 30 days, percent loss of original shot weight and the dissolution rate were higher in Zn-dosed ducks.

Male Zn-dosed ducks lost an average of 14.8%, and male Fe-dosed ducks lost an average of 1.9%, of their body weight between Day 0 and Day 15 or death (if < 15 days), and 16.5% and 2.4%, respectively, of their body weight between Day 0 and Day 30 or death (if < 30 days). Loss of body weight was much greater in male Zn-dosed ducks that died prior to Days 15 or 30 than in those that survived to either day.

Female Zn-dosed ducks lost an average of 30.9%, and female Fe-dosed ducks lost an average of 4.6%, of their body weight between Day 0 and Day 15 or death (if < 15 days), and

31.9% and 5.9%, respectively, of their body weight between Day 0 and Day 30 or death (if < 30 days). Loss of body weight was much greater in female Zn-dosed ducks that died prior to Day 30 than in those that survived.

The livers (males) and kidneys of Zn-dosed ducks that died as a result of Zn intoxication were heavier and the livers, kidneys, and pancreases represented a greater proportion of total body weight, as compared to those that survived. The kidneys of Zn-dosed ducks as a group were heavier, and the pancreases and gizzards lighter, as compared with Fe-dosed ducks. Although we detected no significant differences in gonadal weight between Zn- and Fe-dosed ducks, the gonads of Zn-dosed animals dying prior to Day 30 were smaller (especially in males) than in those that survived 30 days. Similarly, the gizzards of ducks that succumbed to Zn toxicosis were smaller than Zn-dosed ducks that survived.

Mean hematocrit values decreased between Days 0 and 15, and increased between Days 15 and 30, in Zn-dosed ducks; variability in hematocrits was highest at Day 15. Some individual Zn-dosed ducks that survived to Day 30 experienced increases in hematocrit values between Days 15 and 30. Mean hematocrits decreased 9.0% between Days 0 and 15 in male Zn-dosed ducks, and increased slightly in Fe-dosed males during the same period; both groups experienced similar changes between Days 0 and 30. Hematocrits decreased 29.6%, on average, between Days 0 and 15 in female Zn-dosed ducks, and 0.2% in Fe-dosed females during the same period; both groups experienced similar changes between Days 0 and 30.

We detected high concentrations of Zn in tissues and alterations in levels of other minerals examined in Zn-dosed ducks, relative to Fe-dosed controls. Zn levels in the kidneys, livers, and pancreases of Zn-intoxicated mallards were similar to levels associated with toxic

livers, and pancreases of Zn-intoxicated mallards were similar to levels associated with toxic effects reported in other studies. Changes in mineral concentrations in tissues tended to be more dramatic in ducks that died as a result of Zn intoxication than in Zn-dosed ducks that survived the experiment. Female mallards were more vulnerable to Zn intoxication than were males, and individual variation in susceptibility was apparent.

Gross lesions observed in Zn-dosed ducks included pectoral muscle atrophy (n=15), pericardial abnormalities (n=15), air sacculitis (n=3), hepatic granulomas (n=10), pancreatic pallor or adhesions (n=4), proventricular erosions (n=8), renal pallor (n=5), cecal impaction with necrotizing enteritis (n=23), and enteritis involving the small and large intestines (n=12). No macroscopic lesions were observed in the Fe-dosed ducks.

Histologic lesions in Fe-dosed ducks included mild to moderate lymphocytic inflammation in the small and large intestines, proventriculus, and ceca, lymphocytic periportal inflammatory lesions and/or hepatic lipidosis, hepatic parenchymal granulomas (n=2), peribronchiolar lymphoid hyperplasia (n=9), and lymphocytes surrounding the ureters. These lesions were considered within normal limits for game-farm raised ducks.

Histologic lesions in Zn-dosed mallards included typhlitis (n=25), necrotizing to necrohemorrhagic inflammation of the small (n=17) and large intestine (n=14), pancreatic apoptosis (n=34), hepatic granulomas (n=11), hemosiderosis (n=23), and cellular atrophy or apoptosis (n=15), splenic hemosiderosis (n=19) and/or lymphoid depletion and/or lympholysis (n=27), superficial necrosis of the proventriculus (n=13) with associated glandular atrophy or apoptosis, glandular atrophy and/or inflammation of the ventriculus (n=13 of 15 examined), increased bone marrow heterophil populations (n=24 of 29 examined), mild to moderate necrosis

of the epithelial cells of the renal tubules (n=24), adrenal medullary apoptosis/atrophy (n=16 of 27 examined), vacuolization of the adrenal cortex (n=6), mild myofiber vacuolization to multifocal degeneration or necrosis (n=21), and pericardial/epicardial mineralization (n=3).

INTRODUCTION

As a result of increased recognition of the problems associated with the ingestion of spent lead (Pb) shot by waterfowl, the United States (US) began the phase-in of nontoxic shot regulations in the early 1970s, culminating in a nationwide ban on the use of Pb shot for all waterfowl hunting in 1991. Canada first established nontoxic shot zones in 1991. In 1995, the Canadian government announced intentions to ban the use of Pb shot for the hunting of migratory birds on federal lands in 1996, with implementation of a nationwide ban in 1997 (Canadian Wildlife Service, 1995). Currently, only iron or steel (Fe) and bismuth/tin (Bi/Sn) shot are approved as nontoxic shot in both the US and Canada. Tungsten/iron (W/Fe) shot was recently granted conditional approval for the 1997-98 hunting season in the US.

Because Fe shot is harder and lighter in weight and Bi shot is more expensive, as compared with Pb, industry has continued to search for non-toxic alternatives to Pb shot. Zinc (Zn) shot is currently in use as a Pb shot substitute for waterfowl hunting in Great Britain and parts of Europe. Zinc coatings of less than 0.0002" thickness or less than 1.0% of the weight of the shot pellet are currently approved in the United States (Cyndi Perry, USFWS, pers. commun.). 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 cost about the same as Fe and less than Bi. Anticipation of new and expanding markets for nontoxic shot has led manufacturers to focus on the development and testing of candidate shot for subsequent approval as non-toxic shot.

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; Eisler 1993). Zinc 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 is affected by intake level, form ingested, and presence of other elements and compounds in the diet (Underwood 1971). Copper (Cu), calcium (Ca), cadmium (Cd), and phytates decrease the absorption of dietary Zn (Underwood 1971; Eisler 1993; Walsh et al. 1994). 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 2330 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 > 2100 mg/kg DW and in mammals when kidney, liver, or pancreas levels exceed 274, 465, or 752 mg/kg DW, respectively. Puls (1988) indicated that, in poultry, concentrations in the liver, kidney, and pancreas of 200-700, 300-800, or 1000-35000 mg/Kg fresh weight (FW), respectively, were indicative of Zn intake at levels likely to cause subclinical, clinical, or pathological signs of toxicity.

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; Underwood 1971; 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). Metal fume fever resulting from inhalation of Zn oxides produced by industrial processes such as smelting produces fever, tachycardia, dyspnea, and chest pains in humans (Eisler 1993).

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 other elements (Eisler 1993). In the right form and under the proper 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 (Eisler 1993). The effects of Zn on immunological systems are unclear (Walsh et al. 1994).

Poultry are routinely fed excessive levels of Zn to induce molting in order to promote long-term egg production. Although hens can tolerate levels exceeding 8,000 mg Zn/kg in their diet, this quantity is fatal to chicks. Much lower levels can inhibit growth, suppress immune system function, cause Fe and Cu deficiencies, and impair pancreatic function in chicks (see Eisler 1993).

Irby et al. (1967) tested the toxicity of 10 shot types on 1-year-old male game-farm mallards, 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 4 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 (12 of 15 ducks), kidney histopathology (1 duck), high Fe concentrations in livers of affected ducks, and increased mortality (Grandy et al. 1968). The authors suggested that consideration of Zn as a non-toxic 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 (*Anas platyrhyncos*). Reductions in food consumption and body weight were associated with dietary Zn levels. Compared with controls, Zn-fed ducks exhibited reductions in pancreas, liver, and gonadal weights, increased adrenal and kidney weights, increased tissue Zn concentrations, lowered hematocrit 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 yr-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.

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. Diet, including ingestion of soil and grit, can have a dramatic effect on Pb shot erosion and Pb absorption, retention, and excretion rates, and is therefore important in mitigating or exacerbating the 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 birds and mammals to high Zn concentrations, known antagonisms between Zn and elements such as Ca, Cu, and Fe, and the effects of other dietary inhibitors of Zn absorption such as phytate, lignin, and hemicellulose (Underwood 1971; Eisler 1993; Walsh et al. 1994).

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

The Acute Toxicity Study was conducted using 40 female and 40 male wild-type game-farm mallards, 6 to 8 months of age, purchased from Whistling Wings, Hanover, Illinois. The ducks, reared on a 60-acre lake, were transported from Hanover to Champaign, Illinois, by truck in crates on 7 August 1996.

Upon arrival at the Illinois Natural History Survey's (INHS) facility in Champaign, the ducks were weighed and randomly assigned to pens, with one duck per pen. Forty ducks (20 females and 20 males) were randomly assigned to one of the 2 treatments (dosed with 6 No. 4 Zn or 6 No. 4 Fe shot).

The pens are consecutively-numbered, elevated, outdoor, 1 m^3 pens, constructed of vinyl-coated, 1-inch (25.4-mm) mesh, 14-gauge wire (see Sanderson et al. 1992 for more details). A 10- x 40-yard pole barn without sides 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 Committee, University of Illinois, prior to the study. Committee members also visited the facilities several times during the study. Commercial duck pellets (Heinhold 17% Duck Finisher Pellet, Heinhold Feeds, Inc., Kouts, IN) were provided *ad libitum* during the 20-day acclimatization period. The duck pellets contained a minimum of 17.0% protein. On the date of dosing, the pellets were removed and whole-kernal corn was provided *ad libitum* for the duration of the study.

The study was begun 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" dia.}) of Zn or Fe shot were counted, weighed, and placed in individual vials in the laboratory. The type, number, and weight of shot were recorded on the top of each vial and on a computer printout for each duck. At dosing, the shot dose was matched with the corresponding duck.

Blood was collected from a brachial vein in heparinized microhematocrit capillary tubes for hematocrit determination and in 2.5-mL syringes fitted with twenty-gauge, 1-inch (25.4-mm)

needles to obtain samples to ascertain concentrations of selected elements. The whole blood was injected into 10-mL lithium heparinized Vacutainer® tubes and centrifuged to separate cells and plasma. Body weights were recorded and blood samples collected on Days 0, 15, and 30.

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

Whole blood samples were also centrifuged at the site when 12 samples (capacity of the centrifuge) were collected. The tubes were spun for 10 minutes at 3,000 RPM. Plasma was removed with micropipettes and placed in 5-ml non-heparinized Vacutainer® tubes; cells remained in the 10-mL lithium heparinized tubes. As the plasma and cells were separated, the tubes were placed in metal racks and kept in a refrigerator until transported to freezers at the nearby State Water Survey Laboratory. Samples were stored at -10^0 C until thawed in preparation for chemical analysis.

Detailed behavioral observations were collected 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 it's 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 ½ gallon paper milk carton with a hole cut in the bottom, which allowed the head and neck to protrude. The 4-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.

Preliminary analyses of chemical composition of the shot were conducted by the Illinois State Water Survey's Analytical Laboratory, Champaign, Illinois, using Inductively Coupled Argon Emission Plasma Spectroscopy (ICP). Ten randomly-selected pellets were composed of an average of 98% Zn and 2% Sn; other elements were essentially undetectable (< 0.1% each). The steel shot were commercially-available pellets sold for reloading shotshells.

All surviving ducks used in the study were weighed and blood was collected from the brachial veins as scheduled on 11 September (Day 15) and 26 September 1996 (Day 30). Subsequently, 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 veterinary pathologist (GLF) necropsied all ducks dying before Day 30 at the University of Illinois' School of Veterinary Medicine's Diagnostic Laboratory. The condition of all major tissues and organs was noted, organs weighed, and specimens preserved for histopathological examination (see Pathology Report).

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 (Days 30 and 31) to insure that fresh specimens would be examined. The organs from the remaining ducks were examined,

removed, and weighed by project personnel on Day 30. 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 State Water Survey and stored with the blood samples until thawed for analysis. Methods, data conversions, and quality assurance for the ICP analyses are provided in Appendix A.

Statistical Methods

We examined variation in whole body and organ weights, hematocrits, and concentrations of selected elements in tissues by a randomized, 2 x 2 factorial, Model 1 ANOVA, using sex and dose as grouping factors. Levene's test was used to assess homogeneity of variances between groups; when variances were not assumed equal ($P \leq 0.01$) variables were log-transformed. Log-transformations are not included in this report as they did not result in substantial improvements in distribution (significant heterogeneity still detected) and/or tests using transformed variables yielded the same statistical conclusions as the raw data. ANOVA and *t* test are robust to all but the most severe departures from normality or heterogeneity of variance (Zar 1984).

Student's *t* test was used to compare differences between treatment groups (Fe- vs. Zn-dosed) within a sex 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. 1996b). Pooled variances and adjusted degrees of freedom were used for *t* tests when significant heteroscedasticity was detected using Levene's test (SPSS, Inc. 1996c). For one-tailed non-parametric testing, $U^1 = n_1 n_2 - U$. (Zar 1984).

Sexes were treated separately where deemed appropriate (e.g. where sex effects were

detected, or in comparisons of surviving ducks and those that died during the study). Kaplan-Meier survival functions were calculated using the KM procedure of SPSS (SPSS, Inc. 1996a); 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). 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. 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.

RESULTS AND DISCUSSION

Survival

All Fe-dosed ducks survived to Day 30/31, when the ducks were euthanized. Sixteen of 20 (80%) female, 9 of 20 (45%) male, and 25 of all 40 (62.5%) Zn-dosed ducks died prior to Day 30 (3 of these individuals were euthanized after being found on their backs and unable to right themselves). Mortality in Pb-dosed mallards generally approaches or reaches 100% (Sanderson and Irwin 1976; Sanderson et al. 1992; Sanderson et al. 1996). Grandy et al. (1968) reported 20% mortality to 30 days in 15 male mallards dosed with 8 No. 6 Zn shot, and 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 their 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.

In the present study, survival in male Zn-dosed ducks averaged 23 days, and ranged from 9 to 30 days. Survival rates differed between Zn- and Fe-dosed male ducks (X_2= 11.24; $P < 0.001$). Survival in female Zn-dosed ducks averaged 18 days, and ranged from 5 to 30 days. Survival rates differed between Zn- and Fe-dosed female ducks (X_2= 25.24; $P < 0.0001$).

Survival rates did not differ between male and female Zn-dosed ducks (X_2= 3.21; $P = 0.07$). Similarly, Sanderson et al. (1996) found no difference in survival times between sexes in Pb-dosed mallards. Mean survival times in our study were similar to the 20 days 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).

Behavioral Abnormalities

Signs of zinc toxicosis observed were consistent with the obervations of Grandy et al. (1968) and/or Gasaway and Buss (1972). These signs included ataxia, paresis, reduction or cessation of food intake, and severe anemia. Behavioral 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. Mortality first occurred on Day 5 (1 female) before any signs were noted in that animal. Grandy et al. (1968) reported that one of the Zn-dosed ducks in their study died without exhibiting any 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. Grandy et al. (1968) 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.

Zinc-dosed ducks typically exhibited a reduction and ultimately cessation of feeding several days before showing moderate signs of toxicosis. Five 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. No animals showing behavioral abnormalities completely recovered from the effects of Zn-intoxication over the course of the experiment. Grandy et al. (1968) reported that 1 of 3 ducks retaining shot to the end of their study showed no signs of intoxication, whereas another exhibited signs for 5 days but had "fully recovered" by Day 30 .

The severely-anemic condition of Zn-dosed animals became apparent through pallor of the oral cavity (and documented by hematocrits). Dark or bright green feces were noted within 1 or 2 days of dosing. Five ducks passed blood with their feces, however, this condition persisted to death only in 1 female. Another duck, also a female, was observed to have passed blood on 1 occasion, and exhibited no other signs throughout the experiment.

Other signs of Zn toxicosis noted included diarrhea, excreta having 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 cage).

Body Weight

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, whereas surviving males gained weight between Days 15 and 30 (Table 1, Fig. 1). These data included body weights for ducks that died between Day 0 and 15 and Day 15 and 30, respectively. Variability increased over time, presumably 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 (Fig. 1). This pattern was similar to that reported by Sanderson et al. (1995) for Bi-, Fe-, and 0-dosed mallards. Variability in weight in Fe-dosed ducks did not change remarkably throughout our study (Table 1).

Males weighed more than females at Days 0, 15, and 30, and Fe-dosed ducks weighed more than Zn-dosed ducks at Days 15 and 30 (Table 1, Fig. 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 pronounced in females, presumably due to a greater dose/unit body weight. We detected a relationship between dose/unit body weight and the number of days a duck survived in males that died prior to Day 30 (r= -0.62, P=0.04), but not in females (r= -0.20, P=0.23), suggesting that the lesser weight of females may have made them so highly susceptible to Zn toxicosis as to preclude a dose/unit body weight relationship.

Male Zn-dosed ducks as a group experienced greater weight loss (expressed as percentage body weight change) between Days 0 and 15, compared with Fe-dosed males (Table 2). These differences in mean percent weight change were significant ($t_{0.05\,(1)20}$= -4.0, $P < 0.001$).

Mean percent weight loss in male Zn-dosed ducks surviving less than 15 days was considerably greater than in those that survived at least 15 days (Fig. 2). These differences in mean percent weight change were significant ($U'_{0.05(1),5,15}$= 57, $P = 0.05$).

Females in both dosing groups lost weight between Days 0 and 15 (Table 2). The mean percent weight lost in Zn-dosed females between Days 0 and 15 or death (if <15 days) was marked and greater than in Fe-dosed females ($t_{0.05(2)21}$= - 6.0, $P< 0.001$). Weight loss in Zn-dosed females surviving at least 15 days and those surviving <15 days was similar ($U'_{0.05(1),4,14}$= 27, $P > 0.10$) (Fig. 2).

Male Zn-dosed ducks lost 16.5% of their body weight, on average, to Day 30 or death (if <30 days), whereas Fe-dosed males lost only 2.4% percent (Table 3). This difference in mean percent weight change was significant ($t_{0.05(1)20}$= - 3.9, $P <0.001$). Male Zn-dosed ducks surviving to Day 30 lost 3.2% of their body weight, as a group, whereas those surviving < 30 days lost 32.8% of their body weight between Day 0 and death (Fig. 2). The difference in mean percent weight change between these 2 groups was significant ($t_{0.05(1),11}$= 11.2, $P <0.001$).

Zn-dosed females lost 31.0% of their body weight, on average, between Day 0 and Day 30 or death (Table 3, Fig.2). In comparison, Fe-dosed females as a group lost only 5.9% of their body weight from the start to the end of the experiment. The difference in mean percent weight change between these dosing groups was significant ($t_{0.05(1),19}$= -6.7; $P <0.001$).

Female Zn-dosed ducks that died prior to Day 30 lost 39.7% of their body weight

between Day 0 and death (Table 3, Fig. 2), which was considerably greater than the 4.8% of body weight lost by Zn-dosed females surviving to Day 30 ($U'_{0.05(1),4,14} = 64$; $P < 0.001$).

Mean percent loss of 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. Weight loss in Zn-dosed ducks that survived to Day 30 was similar to weight loss in Fe-dosed ducks, all of which survived to Day 30. 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. Mean 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 gained weight. Weight loss in Pb and Zn dosing experiments involving ducks has been attributed to a reduction or cessation of feeding.

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, one male and one female each had 2, one female had 3, and one 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 linings of the gizzard in Zn-dosed ducks that died or were euthanized during the experiment were stained a walnut-brown color and were brittle. Gizzard linings in 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. According to Sanderson and Bellrose (1986:8), the gizzard lining of Pb-poisoned waterfowl is often "dark, soft, decayed, easily eroded, inflamed, corroded, and incomplete". Droual et al. (1991) documented severe gizzard erosion in a gray-headed chachalaca (*Ortalis cinereiceps*) that had ingested a copper-plated Zn coin.

The Fe and Zn shot recovered from the gizzards differed in appearance. The Fe shot were generally round, with pits or empty spaces on the surface. The Zn shot recovered from ducks that died prior to Day 30 were round with a dimpled surface, reminiscent of a tiny golf ball. Most of the Zn shot recovered from ducks surviving to Day 30 were greatly reduced in size and worn smooth, although some were fragmented.

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 a relatively high proportion of the dosed shot (Table 4). Although the mean number of shot retained was higher for Fe-dosed than for Zn-dosed ducks, this difference was not significant ($t_{0.05(2),78} = -0.71$, $P = 0.48$). Retention rates were slightly lower than those reported by Sanderson et al. (1995) for Bi- (100%) and Fe-dosed (98.8%) mallards. 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.

For ducks that retained 6 shot, the percentage of the original shot weight that had dissolved was slightly higher in Fe-dosed than in Zn-dosed ducks (Table 4); 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 (g/day) were identical in these 2 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 than in Fe-dosed ducks (Table 4) ($t_{0.05(2),49}$ = 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 compared with Fe-dosed 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. Mean absorption rates were 0.009 and 0.01 g/day, respectively, in mallards dosed with 5 or 10 No. 6 pure Zn pellets (French et al. 1987).

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. Shot dissolution ranged from 38.2% to 96.5% 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. 1995). The authors attributed the higher dissolution to increased food consumption by females in preparation for the breeding season.

Organ Weights

Liver

Mean liver weight did not differ between doses or sexes, and the liver represented a similar proportion of total body weight in male Fe- and Zn-dosed mallards ($t_{0.05(1)22} = 1.31$, $P > 0.10$) (Table 5). Liver weight in Zn-dosed males that died prior to Day 30 was greater than in those surviving to Day 30 ($t_{0.05(1)18} = -2.44$, $P < 0.05$). The livers of Zn-dosed males surviving <30 days represented nearly twice the proportion of total body weight as compared with ducks surviving to Day 30 ($t_{0.05(1)18} = -5.61$, $P < 0.0001$).

Females exhibited a pattern similar to males for liver weights, however, liver weight as a percentage of body weight was greater in Zn-dosed than in Fe-dosed females ($t_{0.05(1)22} = 2.29$, $P < 0.05$) (Table 5). Mean liver weight did not differ between female Zn-dosed ducks that survived to Day 30 and those that did not ($U^{1}_{0.05(1)4,16} = 46$, $P > 0.10$), however, liver weight represented a greater proportion of body weight in those that died prior to Day 30 ($U^{1}_{(0.05)1,4,14} = 64$, $P < 0.0005$).

Pancreas

Pancreas weight averaged greater in males and in Fe-dosed ducks (Table 6, Fig. 3), respectively, as compared to females and Zn-dosed ducks. Mean pancreas weight represented a greater proportion of total body weight ($t_{0.05\,(1),38} = -3.2$, $P < 0.01$) in Fe- than in Zn-dosed males. Pancreas weight did not differ between Zn-dosed males surviving to Day 30 and those that did not ($t_{0.05\,(1),18} = 0.70$, $P > 0.10$); however, the pancreas represented a greater proportion of body weight in those that died prior to Day 30 ($t_{0.05\,(1),18} = -2.10$, $P < 0.05$).

Pancreases represented a larger proportion of body weight ($t_{0.05\,(1),36} = -3.9$, $P < 0.001$) in

Fe- than in Zn-dosed females (Table 6). 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), however, the pancreas represented a greater proportion of body weight in those that died prior to Day 30 ($U^1_{0.05(1)4,16}$= 53, $P < 0.05$).

Kidneys

Average kidney weights were greater in Zn- than in Fe-dosed mallards (Table 7, Fig. 4). We detected no differences in mean kidney weight between the sexes. The kidneys represented a higher proportion of total body weight ($t_{0.05(1)20}$ = 4.12, $P< 0.001$) in Zn- than in Fe-dosed males (Table 7). Kidney weights in Zn-dosed males surviving 30 days averaged less than in ducks that died prior to Day 30 ($t_{0.05(1)18}$ = -5.10, $P< 0.001$). 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 ($t_{0.05(1)18}$ = -12.84, $P< 0.0001$).

Mean kidney weight accounted for a greater proportion of body weight in Zn-dosed females than in Fe-dosed females ($t_{0.05(1)20}$ = 5.92, $P< 0.001$) (Table 7). Kidney weights in Zn-dosed females surviving to Day 30 averaged less than in those that died prior to 30 days ($U^1_{0.05(1)4,16}$ = 63, P= 0.0005) . The kidneys of Zn-dosed females surviving <30 days represented more than twice the proportion of total body weight as compared with ducks surviving to day 30 ($U^1_{(0.05)1,4,14}$= 56, P= 0.0005).

Gonads

Mean gonadal weights were greater in males than in females (Table 8). The proportion of body weight accounted for by the gonads ($t_{0.05(1)38}$ = -0.62, $P> 0.25$) did not differ between male Zn- and Fe-dosed ducks. The gonads were heavier ($t_{0.05(1)10}$ = 3.17, $P< 0.01$) and represented a

much greater proportion of body weight ($t_{0.05(1)10} = 3.40$, $P< 0.01$) in Zn-dosed males surviving 30 days, as compared with those that died prior to Day 30. Mean gonadal weight did not differ between Fe-dosed and Zn-dosed males surviving to Day 30 ($t_{0.05(1)29} = 0.98$, $P> 0.10$).

The proportion of body weight accounted for by the gonads ($t_{0.05(1)36} = -0.97$, $P> 0.10$) did not differ between female Zn- and Fe-dosed ducks (Table 8). Although the gonads were heavier ($U^1_{0.05(1),4,16}= 52$, $P< 0.05$) in Zn-dosed females surviving 30 days, the contribution of the gonad to body weight did not differ ($U^1_{0.05(1),4,16}= 41$, $P> 0.10$), as compared with those that died prior to Day 30.

Gizzards

Gizzards averaged heavier in males and in Fe-dosed ducks (Table 9, Fig. 5), respectively, than in females and Zn-dosed ducks. We also detected an interaction between sex and dose, with male Zn-dosed mallards having smaller gizzards than female Fe-dosed, and female Zn-dosed having smaller gizzards than male Zn-dosed ducks. Gizzard weights accounted for a greater proportion of body weight ($t_{0.05(1)37} = -2.10$, $P= 0.05$) in Fe-dosed as compared with Zn-dosed males (Table 9). Mean gizzard weight ($t_{0.05(1)13} = 8.4$, $P< 0.001$) and gizzard weight as a percentage of body weight ($t_{0.05(1)18} = 4.10$, $P< 0.001$) were greater in Zn-dosed males surviving 30 days than in those that did not.

The same pattern was apparent in females, with gizzard weights accounting for a larger proportion of body weight ($t_{0.05(1)36} = -4.1$, $P< 0.001$) in Fe-dosed as compared with Zn-dosed ducks (Table 9). Similarly, mean gizzard weight ($U^1_{0.05(1)4,16} = 64$, $P< 0.001$) and gizzard weight as a percentage of body weight ($U^1_{0.05(1)4,14} = 49$, $P< 0.05$) were greater in Zn-dosed females surviving to Day 30 than in those that did not.

Discussion of Organ Weights

The organs of Pb-poisoned waterfowl may be reduced or enlarged at death, depending on the nature of the toxicosis (see Sanderson and Bellrose 1986:10-11). In the present study the livers (males) and kidneys of Zn-dosed ducks that died as a result of Zn intoxication were heavier and the livers, kidneys, and pancreases represented a greater proportion of total body weight, as compared to those that survived. The kidneys of Zn-dosed ducks as a group were heavier, and the pancreases and gizzards lighter, as compared with Fe-dosed ducks. Gasaway and Buss (1972) found that ducks fed dietary Zn experienced significant reductions in the weight of the pancreas, liver, and gonad, compared with controls, whereas the kidneys represented a larger proportion of body weight. Zee et al. (1985) noted liver and kidney hypertrophy in a captive Nicobar pigeon (*Caloenas nicobarica*) that had ingested Zn fragments.

Although we detected no significant differences in gonadal weight between Zn- and Fe-dosed ducks, the gonads of Zn-dosed animals dying prior to Day 30 were smaller (especially in males) than in those that survived 30 days. Similarly, the gizzards of ducks that succumbed to Zn toxicosis were smaller than the gizzards of Zn-dosed ducks that survived. Gasaway and Buss (1972) suggested that Zn-mediated retardation of gonadal development could impact breeding success, however, Locke et al. (1969) reported that 1-year old mallards dosed with 8 No. 6 Zn-coated Fe shot for 60 days exhibited active spermatogenesis. Some of the "reduction" (or lack of increase) in organ weights in our study is likely attributable to the younger age of those ducks that died before the end of the experiment.

Hematocrit

Hematocrits 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 10). Mean hematocrit values 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 hematocrit at Day 30 was greater than at Day 0.

Variability in hematocrits was highest at Day 15, presumably due to severe anemia in ducks experiencing moderate to severe Zn toxicosis. Of those Zn-dosed ducks surviving to Day 30, 8 (1 female), 8 (1 female), and 7 (all males) individuals exhibited increased hematocrits between Days 0 and 15, 15 and 30, and 0 and 30, respectively.

We detected no differences in mean hematocrit values between doses at Days 0, 15, or 30, or between sexes at Days 0 or 15 (Table 10). Mean hematocrit values were greater in males than in females at Day 30. Although some Zn-dosed ducks experienced dramatic declines in hematocrit values between data collection dates, the effects on mean values were apparently masked by those ducks that were not experiencing Zn-mediated anemia (see below) and the deaths of severely Zn-intoxicated ducks prior to sampling.

Hematocrits decreased by an average of nearly 9% in Zn-dosed male mallards, and increased slightly in Fe-dosed males, between Days 0 and 15 (Table 11). These differences in mean percent change in hematocrits were significant ($t_{(0.01),\,15} = -1.9$, $P < 0.05$). Individual values ranged from -63.0% to 12.5% and -4.4% to 10.4% in Zn- and Fe-dosed males, respectively.

Both Zn- and Fe-dosed male mallards experienced similar ($t_{(0.01),\,29} = 1.1$, $P > 0.10$) changes in hematocrits (expressed as mean percent change in hematocrit) between Days 0 and 30 (Table 11). Individual values ranged from -2.2% to 17.5% and -4.3% to 17.5% in Zn- and Fe-dosed males, respectively.

Hematocrit values decreased between Days 0 and 15 in both Zn- and Fe-dosed females though this decrease in hematocrits (expressed as percent change in hematocrit) was much greater in Zn-dosed female mallards surviving to 15 days than in female Fe-dosed mallards, all of which survived to Day 15 (Table 11) ($t_{0.01,13} = -4.3$, $P < 0.001$). Individual values ranged from -56.5% to 6.7% and -10.6% to 14.0% in Zn- and Fe-dosed females, respectively.

Zn-dosed female mallards surviving to Day 30 experienced a slight decline, on average, in hematocrits between Days 0 and 30, whereas mean percent change in hematocrits was positive in Fe-dosed females (Table 11). This difference between dosing groups was not significant ($U^{1}_{(0.01),4,20} = 58$, $P = 0.10$). Individual values ranged from -12.2% to 7.3% and -6.5% to 20.9% in Zn- and Fe-dosed females, respectively

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. Although Gasaway and Buss (1972) noted no remarkable reduction in hematocrits until day 30 in mallards fed Zn carbonate, we documented considerable reductions in hematocrit values in Zn-intoxicated ducks between Days 0 and 15. This finding can be attributed to the large number of ducks experiencing Zn toxicosis in our study.

Element Concentrations in Organs and Tissues

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^{0} 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%.

Plasma

Mean plasma Ca levels generally decreased over the course of the study (Table 12). Ca concentrations did not differ between doses or sexes at Days 0, 15, or 30. Ca levels in Fe-dosed mallards ranged from 90.9 μg/g to 266.8 μg/g (Day 15). Ca concentrations in plasma of Zn-dosed mallards ranged from 49.3 μg/g to 153.6 μg/g (Day 0).

The MDLs for Cu in plasma were 0.21 μg/g for Day 0, 0.20 μg/g for Day 15, and 0.18 μg/g for Day 30. 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 12). Mean Cu concentrations were < MDLs in Zn-dosed males at Day 15 and in Fe-dosed females at Day 0.

Copper concentrations in plasma did not differ between doses or sexes at Days 0, 15, or 30 (Table 12). Copper concentrations in Fe-dosed mallards ranged from < MDL to 0.7 μg/g (Day 0). Plasma Cu levels in Zn-dosed mallards ranged from < MDL (Day 30) to 1.0 μg/g (Day 0).

The MDLs for Fe in plasma were 0.96 μg/g at Day 0, 0.92 μg/g at Day 15, and 0.81 μg/g at Day 30. Mean Fe levels decreased between Days 0 and 15 in Fe-dosed ducks, before increasing between Days 15 and 30 (Table 12). Iron concentrations increased dramatically between Days 0 and 15, and decreased between Days 15 and 30, in Zn-dosed ducks.

There were no differences between sexes or doses in plasma Fe levels at Days 0, 15, or 30

(Table 12). Plasma Fe levels in Fe-dosed ducks ranged from < MDL to 28.4 μg/g (Day 0). Plasma Fe levels in Zn-dosed ducks ranged from < MDL (Day 0) to 48.3 μg/g (Day 15).

Plasma Fe levels at Day 15 were higher in Zn-dosed males that died after Day 15 ($\bar{x}$= 29.4 μg/g, n= 5) than in those that survived to Day 30 ($\bar{x}$= 3.6 μg/g, n= 11, Fig. 6) ($U'_{0.05(1),5,11}$ = 50.0, P<0.01). Plasma Fe concentrations at Day 15 were higher in Zn-dosed females that died after Day 15 ($\bar{x}$= 15.7 μg/g, n= 8) than in those that survived to Day 30 ($\bar{x}$= 2.5μg/g, n= 4, Fig. 6) ($U'_{0.05(1),4,8}$ = 28.0, P<0.01).

Magnesium concentrations changed little over the course of the study (Table 12). Mean Mg levels did not differ between dosing groups or sexes at Days 0, 15, or 30. Magnesium concentrations in Fe-dosed ducks ranged from 16.6 μg/g (Day 0) to 33.9 μg/g (Day 15). Plasma Mg levels in Zn-dosed ducks ranged from 8.3 μg/g (Day 0) to 26.6 μg/g (Day 15).

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 12). Phosphorus concentrations were higher in Fe-dosed than in Zn-dosed ducks at Days 15 and 30 (Table 12). We detected no sex effects in mean plasma P levels. Phosphorus levels in blood plasma in Fe-dosed mallards ranged from 51.6 μg/g (Day 0) to 444.8 μg/g (Day 15). Plasma P levels in Zn-dosed mallards ranged from 44.8 μg/g (Day 0) to 563.1 μg/g (Day 15).

The MDLs for Sn in blood plasma were 2.5 μg/g for Day 0, 2.35 μg/g for Day 15, and 2.1 μg/g for Day 30. 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 μg/g 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, i.e. there was an equal number of Zn- and Fe-dosed ducks with Sn levels > MDLs.

Mean Zn levels changed little in Fe-dosed ducks over the course of the study (Table 12). 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 12, Fig. 7). There were no significant differences in Zn levels between the sexes. Zinc levels in blood plasma in Fe-dosed mallards ranged from 1.7 μg/g (Day 30) to 6.7 μg/g (Day 15). Plasma Zn concentrations in Zn-dosed mallards ranged from 1.2 μg/g (Day 0) to 25.5 μg/g (Day 15). Plasma Zn levels at Day 15 were higher in Zn-dosed males that died after Day 15 ($\bar{x}$= 18.9 μg/g, n= 5) than in those that survived to Day 30 ($\bar{x}$= 11.8 μg/g, n= 11, Fig. 8) ($U'_{0.05(1),5,11} = 51.0$, $P<0.01$).

Red Blood Cells

Mean Ca levels in red blood cells decreased between Days 0 and 15 in all groups, before increasing between Days 15 and 30 in all but Zn-dosed females (Table 13). Mean Ca concentrations were greater in female than in male ducks at Day 0. At Day 0, Fe-dosed males exhibited similar concentrations of Ca as Zn-dosed females, and Zn-dosed males had lower Ca levels in red blood cells than Fe-dosed females. Calcium levels in Fe-dosed mallards ranged from 14.6 μg/g in a male at Day 15 to 123.9 μg/g in a female at Day 0. Erythrocyte Ca concentrations in Zn-dosed mallards ranged from 14.4 μg/g to 172.0 μg/g, with both values observed in Zn-dosed female ducks at Day 15. Calcium levels at Day 15 were higher in male Zn-dosed ducks that died between Days 15 and 30 ($\bar{x}$= 63.9 μg/g, n= 5), as compared with those

that survived to Day 30 ($\bar{x}$= 26.3 μg/g, n= 11) ($U1_{0.05(1),5,11}$ = 43, P= 0.05).

The MDLs for Cu in red blood cells were 0.18 μg/g for Day 0, 0.37 μg/g for Day 15, and 0.27 μg/g for Day 30. Copper levels in red blood cells increased between Days 0 and 15 and decreased between Days 15 and 30 in all but male Fe-dosed mallards (Table 16). Mean Cu concentrations did not differ significantly between dosing groups or sexes (Table 16). Copper concentrations in Fe-dosed mallards ranged from < MDL (Day 30) to 10.6 μg/g (Day 30). Copper levels in red blood cells in Zn-dosed mallards ranged from < MDL (Day 30) to 1.6 μg/g (Day 15).

Mean Fe levels in red blood cells increased in Fe-dosed, and decreased in Zn-dosed, mallards between Days 0 and 15 (Table 13). Iron concentrations increased between Days 15 and 30 in all groups with the exception of Fe-dosed females. There were no differences between sexes or dosing groups in mean Fe levels (Table 13). Iron concentrations in red blood cells in Fe-dosed mallards ranged from 292.3 μg/g to 1030 μg/g (Day 30). Iron levels in Zn-dosed mallards ranged from 247.0 μg/g (Day 15) to 1013 μg/g (Day 0).

Iron levels at Day 15 were higher in Zn-dosed males that survived to Day 30 ($\bar{x}$= 917.5 , n= 11), than in those that died after Day 15 ($\bar{x}$= 694.2, n= 5, Fig. 9) ($U^1_{0.05(1),5,11=}$ 54, P< 0.05). Similarly, Fe levels at Day 15 were higher in Zn-dosed females that survived to Day 30 ($\bar{x}$= 866.1, n= 4), than in those that died after Day 15 ($\bar{x}$= 636.3, n= 8, Fig. 9) ($U^1_{0.05(1),4,8=}$ 28, P= 0.025).

Mean Mg levels in red blood cells decreased between Days 0 and 15 in all but Zn-dosed males (Table 13). Mean Mg levels were higher in Zn-dosed mallards at Day 15 (Table 13). No sex effect on Mg levels was detected. Concentrations in red blood cells of Fe-dosed mallards

ranged from 51.0 μg/g (Day 30) to 150.1 μg/g (Day 0). Magnesium levels in Zn-dosed mallards ranged from 52.9 μg/g to 171.5 μg/g (Day 15).

Phosphorus levels in red blood cells increased between Days 0 and 15 in all groups (Table 13). The difference in mean P levels between Zn- and Fe-dosed ducks at Day 15 was nearly significant. Red blood cell P levels did not differ between the sexes. Concentrations in red blood cells in Fe-dosed mallards ranged from 964.1 μg/g to 2958 μg/g (Day 30). Phosphorus levels in Zn-dosed mallards ranged from 1029 μg/g to 3074 μg/g (Day 15).

The MDLs for Sn in red blood cells were 2.1 μg/g for Day 0, 4.3 μg/g for Day 15, and 3.2 for Day 30. Mean Sn concentrations in red blood cells 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 μg/g 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 μg/g to 6.8 μg/g.

Mean Zn concentrations in red blood cells changed little during the study in Fe-dosed ducks (Table 13, Fig. 10). Zinc levels increased markedly in Zn-dosed ducks between Days 0 and 15, before decreasing between Days 15 and 30. Mean Zn levels were higher in Zn-dosed ducks, as compared with Fe-dosed ducks, at Days 15 and 30 (Table 13, Fig. 10). There was no difference in Zn levels between the sexes. Zinc concentrations in red blood cells of Fe-dosed mallards ranged from 4.6 μg/g to 11.0 μg/g (Day 30). Zn levels in Zn-dosed mallards ranged from 6.5 μg/g (Day 0) to 387.9 μg/g (Day 15).

Zinc levels at Day 15 were considerably higher in Zn-dosed males that died after Day 15 ($\bar{x}$= 179.9 , n= 5) than in those that survived to Day 30 ($\bar{x}$= 12.0, n= 11, Fig. 11) ($U^{1}_{0.05(1),5,11=}$ 55,

$P< 0.001$). Similarly, Zn levels at Day 15 were higher in Zn-dosed females that died after Day 15 ($\bar{x}= 164.3$, n= 8) than in those that survived to Day 30 ($\bar{x}= 13.7$, n= 4, Fig. 11) ($U^1_{0.05(1),4,8=} 32$, $P= 0.025$).

Kidneys

Mean kidney Ca concentrations were higher in Zn-dosed and female mallards, as compared with Fe-dosed and male ducks, respectively (Table 14, Fig. 12). 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 μg/g in a male to 143.6 μg/g in a female. Calcium concentrations in kidneys of Zn-dosed mallards ranged from 71.7 μg/g in a male to 578.1 μg/g in a female.

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

Mean kidney Cu levels were higher in Zn- than in Fe-dosed ducks (Table 14); no sex effect was detected (Fig. 13). Copper concentrations in Fe-dosed mallards ranged from 3.7 μg/g to 9.9 μg/g. Copper levels in Zn-dosed mallards ranged from 6.3 μg/g to 82.3 μg/g.

Kidney Cu levels were lower in Zn-dosed males that died prior to Day 30 ($\bar{x}= 16.2$, n= 9) than in those that survived to Day 30 ($\bar{x}= 35.7$, 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 ($\bar{x}= 15.8$, n= 16) than in those

that survived to Day 30 ($\bar{x}$= 46.8, n= 4) ($U^{1}_{0.05(1),4,16=}$ 54, $P< 0.05$).

Mean kidney Fe levels did not differ between sexes or doses (Table 14). Kidney Fe levels in Fe-dosed ducks ranged from 115.4 μg/g to 270.4 μg/g. Iron levels in Zn-dosed ducks ranged from 85.6 μg/g to 332.6 μg/g.

Kidney Fe levels were higher in Zn-dosed males that died prior to Day 30 ($\bar{x}$= 191.4, n= 9) than in those that survived to Day 30 ($\bar{x}$= 136.2, 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, n= 16) than in those that survived to Day 30 ($\bar{x}$= 120.6, n= 4) ($U^{1}_{0.05(1),4,16=}$ 52, $P< 0.05$).

Magnesium concentrations did not differ between sexes or doses (Table 14). Kidney Mg levels in Fe-dosed ducks ranged from 190.3 μg/g to 248.0 μg/g. Magnesium levels in Zn-dosed ducks ranged from 181.2 μg/g to 263.2 μg/g.

Mean kidney P levels were higher in Fe- than in Zn-dosed ducks (Table 14). Kidney P levels in Fe-dosed mallards ranged from 3102 μg/g in a male to 3585 μg/g in a female. Phosphorus levels in kidneys of Zn-dosed mallards ranged from 2691 μg/g in a male to 3550 μg/g in a female.

The MDL for Sn in kidneys was 3.3 μg/g. Mean Sn concentrations were < MDL for all groups. For Fe-dosed ducks, kidney Sn concentrations were > MDLs in 5 individuals; values ranged from 3.5 μg/g to 4.0 μg/g. For Zn-dosed mallards, kidney Sn concentrations were > MDLs in only 1 individual (4.5 μg/g).

Mean kidney Zn levels were higher in Zn- than in Fe-dosed mallards (Table 14, Fig. 14). No difference in kidney Zn levels between the sexes was detected. Kidney Zn levels in Fe-dosed mallards ranged from 23.6 μg/g to 30.1 μg/g. Kidney Zn concentrations in Zn-dosed mallards

ranged from 62.3 μg/g in a male to 608.7 μg/g in a female.

Kidney Zn levels were higher in Zn-dosed males that died prior to Day 30 ($\bar{x}$= 347.7 , n= 9) than in those that survived to Day 30 ($\bar{x}$= 202.7, n= 11, Fig. 15) ($t_{0.05(1),18=}$ -3.7, P< 0.001). Similarly, Zn levels were higher in Zn-dosed females that died prior to Day 30 ($\bar{x}$= 388.5, n= 16) than in those that survived to Day 30 ($\bar{x}$= 245.2, n= 4, Fig. 15) ($U^1_{0.05(1),4,16=}$ 58, P< 0.01).

Liver

Mean liver Ca concentration was higher in Zn-dosed, as compared with Fe-dosed, ducks (Table 14, Fig. 12). Calcium concentrations in livers of Fe-dosed ducks ranged from 36.2 μg/g to 122.0 μg/g. Liver Ca concentrations in Zn-dosed mallards ranged from 46.0 μg/g to 395.4 μg/g. Liver Ca levels were higher in Zn-dosed females that died prior to Day 30 ($\bar{x}$= 97.8, n= 16) than in those that survived to Day 30 ($\bar{x}$= 70.7, n= 4, Fig. 12) ($U^1_{0.05(1),4,16=}$ 51, P< 0.05).

Mean liver Cu levels were higher in Fe-dosed and male ducks, as compared with Zn-dosed and females ducks, respectively (Table 14, Fig. 16). Copper concentrations in Fe-dosed mallards ranged from 20.3 μg/g in a female to 1158 μg/g in a male. Liver Cu levels in Zn-dosed mallards ranged from 5.0 μg/g in a female to 741.3 μg/g in a male.

Mean liver Fe levels did not differ between doses, however, liver Fe concentrations were greater in female than in male mallards (Table 14). Liver Fe levels in Fe-dosed ducks ranged from 960.4 μg/g in a male to 2646 μg/g in a female. Iron levels in livers of Zn-dosed ducks ranged from 285.5 μg/g to 4788 μg/g, with both values recorded in females.

Liver Fe levels were higher in Zn-dosed males that died prior to Day 30 ($\bar{x}$= 2459, n= 9) than in those that survived to Day 30 ($\bar{x}$= 937.5, n= 11, Fig. 17) ($t_{0.05(1),18=}$ -6.7, P< 0.001). Similarly, mean Fe levels were higher in Zn-dosed females that died prior to Day 30

($\bar{x}$= 3118, n= 16) than in those that survived to Day 30 ($\bar{x}$= 765.3, n= 4, Fig. 17) ($U^1_{0.05(1),4,16=}$ 64, $P< 0.001$).

Mean liver Mg concentrations did not differ between sexes or doses (Table 14). Magnesium levels in Fe-dosed ducks ranged from 181.0 μg/g to 343.0 μg/g. Magnesium levels in Zn-dosed ducks ranged from 179.7 μg/g to 343.1 μg/g.

Liver Mg levels were lower in Zn-dosed males that died prior to Day 30 ($\bar{x}$= 237.9, n= 9) than in those that survived to Day 30 ($\bar{x}$= 274.2 , 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, n= 16) than in those that survived to Day 30 ($\bar{x}$= 300.8, n= 4) ($U^1_{0.05(1),4,16=}$ 62, $P< 0.001$).

Mean liver P concentrations did not differ between doses or sexes (Table 14). Liver P levels in Fe-dosed mallards ranged from 2836 to 4236 μg/g. Phosphorus levels in livers of Zn-dosed mallards ranged from 2633 μg/g to 4210 μg/g.

Liver P levels were lower in male Zn-dosed ducks that died prior to Day 30 ($\bar{x}$= 3231, n= 9) than in those that survived to Day 30 ($\bar{x}$= 3703, 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 ($\bar{x}$= 3102, n= 16) than in those that survived to Day 30 ($\bar{x}$= 3314, n= 4) ($U^1_{0.05(1),4,16=}$ 64, $P< 0.001$).

The MDL for Sn in liver was 3.5 μg/g. Mean Sn concentrations were < MDL for all groups. For Fe-dosed ducks, liver Sn concentrations were > MDL in one individual (3.9 μg/g). For Zn-dosed mallards, liver Sn concentrations were > MDL in 3 individuals; values ranged from 3.6 μg/g to 4.4 μg/g.

Mean liver Zn levels were higher in Zn- than in Fe-dosed mallards (Table 14, Fig. 14). No difference in mean liver Zn levels between the sexes was detected. Liver Zn levels in Fe-dosed mallards ranged from 41.4 μg/g to 88.5 μg/g. Concentrations in Zn-dosed mallards ranged from 142.4 μg/g to 597.2 μg/g. Hepatic Zn levels were higher in Zn-dosed males that died prior to Day 30 ($\bar{x}$= 425.5, n= 9) than in those that survived to Day 30 ($\bar{x}$= 307.0, n= 11, Fig. 15) ($t_{0.05(1),18=}$ -2.3, $P< 0.05$).

Pancreas

Mean pancreas Ca concentrations were higher in Zn than in Fe-dosed ducks (Table 14, Fig. 12). Calcium concentrations in Fe-dosed ducks ranged from 106.7 μg/g to 268.2 μg/g. Calcium concentrations in Zn-dosed mallards ranged from 61.5 μg/g to 548.8 μg/g.

Mean pancreatic Cu levels were higher in Zn- than in Fe-dosed ducks, and were higher in females than in males (Table 14). Copper concentrations in Fe-dosed mallards ranged from 1.3 μg/g to 3.6 μg/g. Copper levels in Zn-dosed mallards ranged from 2.4 μg/g to 11.1 μg/g.

Mean pancreas Fe levels were higher in Zn- than in Fe-dosed ducks (Table 14, Fig. 18). Pancreatic Fe levels in Fe-dosed ducks ranged from 37.2 μg/g to 176.6 μg/g. Iron levels in Zn-dosed ducks ranged from 38.5 μg/g to 156.4 μg/g.

Magnesium concentrations were higher in Fe- than in Zn-dosed ducks (Table 14). Pancreas Mg levels in Fe-dosed ducks ranged from 283.6 μg/g to 449.3 μg/g. Magnesium levels in Zn-dosed ducks ranged from 220.2 μg/g to 406.3 μg/g.

Mean pancreas P levels were higher in Fe- than in Zn-dosed ducks (Table 14). Pancreas P levels in Fe-dosed mallards ranged from 4435 μg/g to 6793 μg/g. Phosphorus levels in Zn-dosed mallards ranged from 3438 μg/g to 6502 μg/g.

The MDL for Sn in pancreas was 3.5 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 3.6μg/g - 5.5 μg/g).

Mean pancreatic Zn levels were higher in Zn- than in Fe-dosed mallards (Table 14, Fig. 14). No difference in pancreas Zn levels between the sexes was detected. Pancreas Zn levels in Fe-dosed mallards ranged from 39.8 μg/g to 221.8 μg/g. Zn concentrations in Zn-dosed mallards ranged from 751.6 μg/g to 3844 μg/g.

Pancreas Zn levels were higher in Zn-dosed males that died prior to Day 30 ($\bar{x}$= 2516, n= 9) than in those that survived to Day 30 ($\bar{x}$= 1707, n= 11, Fig. 19) ($t_{0.05(1),18=}$ -2.7, P< 0.01). Similarly, Zn levels were higher in Zn-dosed females that died prior to Day 30 ($\bar{x}$= 2427, n= 16) than in those that survived to Day 30 ($\bar{x}$= 1567, n= 4, Fig. 19) ($U^{1}_{0.05(1),4,16=}$ 50, P= 0.05).

Discussion of Element Levels in Tissues

Calcium- Calcium levels in our study were higher in the kidneys and liver, and lower in the pancreas, of Zn-dosed duc

ks, as compared with Fe-dosed mallards. Calcium concentrations tended to be higher in Zn-dosed ducks that died prior to the end of the experiment, than in those that did not, although this effect varied by sex and the tissue examined. Mean kidney Ca levels in Fe-dosed ducks were below the range of values (200-600 μg/g) provided by Puls (1988) for poultry on a Ca adequate diet, whereas mean concentrations in Zn-dosed ducks fell just below and within this range for males and females, respectively. Mean liver Ca levels in Fe-dosed mallards where also below the range of values (75-80 pm) for poultry on a Ca adequate diet (Puls 1988), whereas mean

concentrations in Zn-dosed ducks were above this range.

Sanderson et al. (1995) reported that kidney and liver Ca levels were lower in Fe- and Bi/Sn-dosed mallards, as compared with 0-dosed controls. Mean kidney and liver Ca concentrations in Zn-dosed ducks in our study were considerably higher than reported in their study.

In contrast to Sanderson et al. (1995), who noted that Ca levels in the plasma and red blood cell fractions tended to increase over time, we found that blood Ca levels generally decreased over the course of our experiment. Differences in Ca levels between Sanderson et al. (1995) and the present study are likely attributable to seasonality; their study was conducted in the spring of the year whereas ours was conducted in the autumn.

The antagonistic relationship between Zn and Ca has been well-documented; the site of interaction is apparently at the intestinal level, where one inhibits absorption of the other (Underwood 1971; Prasad 1979; Spencer et al. 1980). Corn is low in Ca, and the ducks in our study were fed only corn during the 30-day experiment, thus the source of high levels of Ca in some tissues may have been other than dietary. The higher Ca concentrations in organs of Zn-intoxicated ducks may have resulted from organs sequestering excess Ca competitively excluded from other sites (i.e. other than in the intestinal mucosa) by high levels of Zn.

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. (1995) 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 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 hematocrit values between doses, we did document differences between doses in percent change in hematocrit from Days 0 to 15. Mean values may have been biased towards those animals that experienced only mild Zn toxicosis, as 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; Walsh et al. 1994). French et al. (1987) indicated that liver Cu levels did not differ between Zn-dosed mallards and sham-dosed controls, and Sanderson et al. (1995) 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 reported by Puls (1988) for ducks on a high-Cu diet (25 - 300 μg/g). Levels in Fe-dosed ducks fell within this range for females, however, the mean level for males was somewhat

higher. Individual levels in some Zn- and Fe-dosed mallards were well above the level (540 μg/g) associated with toxic effects in ducks (Puls 1988). Mean hepatic Cu concentrations in our Zn-dosed ducks were higher in females and lower in males, respectively, as compared with those reported for female and male sham-dosed mallards (Sanderson et al. 1995). We observed higher liver Cu levels in male as opposed to female ducks, as was previously reported by Sanderson et al. (1995).

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. 1995), 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. High levels of Cu in the kidneys may have reflected an attempt at excreting excess Cu (e.g. released from liver); increased urinary Cu output has been noted in Wilson's disease, a human condition characterized by excessive tissue Cu loads (Underwood 1971).

We cannot explain the higher pancreatic Cu concentrations in Zn-dosed mallards, given a Zn-Cu antagonism and a pancreatic affinity for Zn. Stahl et al. (1989) found that excessive Zn intake resulted in reduced pancreatic and hepatic Cu concentrations in young chickens (*Gallus* sp.). Tissue Cu concentrations of older chickens, however, were less sensitive to increased Zn intake (Stahl et al. 1990).

Iron- With the exception of the pancreas, we found no differences in tissue Fe levels between doses, however, mean Fe concentrations were higher in the plasma, kidneys, and liver of

Zn-dosed ducks that died during the experiment, than in those that survived. Iron concentrations in pancreases 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. (1995) 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. (1995) 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; Puhlator 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. (1995) found higher Fe levels in the livers and kidneys of Fe-dosed, 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. (1995) reported for their 3 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 Fe observed in our Zn-dosed ducks (4788 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 noted in some animals, including birds (Underwood 1971). Sanderson et al. (1995) 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- Magnesium concentrations in plasma and kidneys did not differ between dosing groups. Erythrocyte Mg levels were higher, and renal Mg concentrations were lower at Day 15 in Zn-dosed than in Fe-dosed mallards. Liver Mg concentrations were lower in Zn-dosed mallards that died during the study than in those that survived. Hepatic Mg levels (mean and maximum) were lower than reported by Puls (1988) for poultry on a Mg-adequate diet (400-500 μg/g). Mean erythrocytic, renal, and hepatic Mg levels in our study were generally higher than those reported by Sanderson et al. (1995) for 0-, Fe-, and Bi/Sn-dosed mallards. Although plasma Mg concentrations did not vary dramatically between our study and Sanderson et al.

(1995), we did not note a progressive increase over our 30-day study, as documented by Sanderson et al. (1995).

Borovansky and Riley (1989) found that Mg was not effective in reducing Zn cytotoxicity, suggesting lack of a direct antagonism between these 2 elements. 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. Plasma Mg levels increased slightly between Days 0 and 15 in male Zn-dosed ducks, whereas Ca levels decreased and P levels increased. In female Zn-dosed mallards, plasma Mg and P levels increased and Ca concentrations decreased from Day 0 to Day 30. Pancreatic Mg, Ca, and P levels were all lower in Zn- than in Fe-dosed mallards.

Phosphorus- Plasma, kidney, and pancreas P concentrations were lower in Zn-dosed as compared with Fe-dosed ducks. Mean plasma and renal P levels were higher in our Zn-dosed ducks than was reported by Sanderson et al. (1995) in 0-dosed ducks in the spring of the year. Relatively little is known with regard to the interaction between P and Zn in animals, although a Zn-P antagonsim in plants in 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). Interactions between P and Ca and Mg have been reported (Puls 1988).

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. (1995) 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. 1995).

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 did not. 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). The mean value for all Zn-dosed males was lower than those reported above, due to a higher proportion of males that survived the study with only mild toxicosis. Our highest value (original datum converted to dry weight for comparison; 2424μg/g) was higher than that (2102 μg/g) reported for an acutely-intoxicated Nicobar pigeon (van der Zee et at. 1985). Our mean values (original data converted to dry weight) for male (1043 μg/g) and female (1400 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.

In their review, Walsh et al. (1994) concluded that increased Zn intake, even at excessive levels, was not likely to be hepatotoxic. 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; 1851 μg/g) was lower than that (3579 μg/g) reported for a Nicobar pigeon that became acutely-intoxicated after ingesting Zn-plated wire (van der Zee et at. 1985) but was similar to that reported for a gray-headed chachalaca (presumed dry weight, 1910 μg/g) that had ingested a copper-coated Zn coin (Droual et al. 1991). A mean liver Zn concentration of 1144 μg/g (dry weight) was reported for poultry chicks *(Gallus* sp.) fed 4000 mg/kg dietary Zn, a level that produced Zn toxicosis. Mean values (converted to dry weight) for male (1116 μg/g) and female (1246 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.

Zinc concentrations in pancreases of mallards dosed with Zn shot were much higher than in the other tissues examined. Increased pancreatic Zn levels are not suprising, 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 (1000-3500 μg/g) associated with subclinical, clinical, and pathological effects in poultry (Puls 1988). The highest value in our study (3844 μg/g wet weight; 11455 μg/g dry weight) was higher than the range of values provided by Puls (1988), and mean values reported (1252 to 2672 μg/g wet) for mallards (Gasaway and Buss 1972) or poultry (Southern and Baker 1983; 8201 μg/g dry) fed excess dietary Zn.

PATHOLOGY REPORT

George L. Foley DVM PhD, Diplomate ACVP

A total of 59 (20 Fe-dosed controls, 39 Zn-dosed) ducks were necropsied 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 organ 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 post

mortem interval (died 9/10 and necropsied on 9/20). 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.

Gross Necropsy Results

None of the control (Fe-dosed) ducks had macroscopic lesions at necropsy. Zn-dosed ducks had gross lesions in multiple organs with varying degrees of severity. Lesions observed grossly included: pectoral muscle atrophy, pericardial abnormalities, air sacculitis, hepatic granulomas, pancreatic pallor or adhesions, proventricular erosions, renal pallor, cecal impaction with necrotizing enteritis, and similar enteritis involving the small and large intestines.

Pectoral Muscle Atrophy

Of the 39 Zn-dosed ducks examined, 15 had pectoral muscle atrophy (significant loss of breast muscle mass). The atrophy was subjectively graded as mild, moderate or severe. Of the 15 ducks with atrophy, 4 ducks had mild, 8 ducks had moderate and 3 ducks had severe lesions.

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 only 3 of the Zn-dosed ducks examined. One

duck had mild cloudiness while 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 were mild, 2 were moderate and 4 were severe to marked.

Pancreatic Lesions

Pancreatic lesions were detected in only 4 of the Zn-dosed ducks examined and typically manifested as pallor to the parenchyma or white plaques on the surface. In 2 ducks there were nodular lesions (#12 and 87). In one duck (#97) the pancreas had adhesion resulting from intestinal rupture.

Proventricular Erosions

Eight of the 39 Zn-dosed ducks examined had proventricular lesions. The lesions manifested as roughening of the mucosa to raised discolored plaques.

Renal Pallor

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

Cecal Lesions

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

Intestinal Lesions

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

Histopathology Results

None of the control (Fe-dosed) ducks had significant histologic lesions in the sections examined. There were many control ducks with a 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 in the intestines, liver, lungs and kidney were considered within normal limits for the population of game-farmed raised ducks.

The Zn-dosed ducks had significant histologic lesions in addition to those noted in the control cohort. Lesions were noted in the ceca, intestines, pancreas, liver, spleen, proventriculus, ventriculus, bone marrow, kidney, adrenal, and heart. No significant lesions were noted in the gonads of treated or control ducts 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) to severe necrohemorrhagic transmural typhlitis (n=17). Cecal lesion with transmural inflammation had extension of the inflammatory process 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. There were 2 large intestinal segments and 1 small intestinal segment that had extensive autolysis (post mortem change). The remaining of the ducks had varying degrees of necrotizing to necrohemorrhagic inflammatory lesions in the small and large intestine. In some animals the lesion was 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 in the pancreas. Pancreatic apoptosis was the most consistent lesion of Zn exposure. Only one 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

In control (Fe-dosed) ducks there was a 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 individual cell death (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 (n=27). The hemosiderosis was most likely similar to the hepatic lesion of hemosiderosis while the lymphoid depletion/lympholysis probably is related to the intestinal inflammatory lesion(s).

Proventriculus

Zn 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 (ventriculus) were examined histologically. One Zn-dosed duck had no significant lesions and 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 significant lesion in the marrow 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. Due to the syncytial function of the cardiac myofibers, even mild lesions are considered significant. Histologic lesion of pericardial/epicardial mineralization was noted in 3 ducks. Grossly the pericardial lesion was detected more frequently, and this was probably related to loss of the pericardial membrane during processing and embedding.

CONCLUSIONS

The results of this 30-day Acute Toxicity Test, conducted according to CWS guidelines for candidate nontoxic shot (i.e. fed a corn diet and individually housed in 1 m^3 pens), indicated that dosing of 6-8 month-old, wild type, game-farm mallards with 6 No. 4 Zn shot pellets (98% Zn/2% Sn) produced toxic effects. The Zn-dosed group experienced high mortality, and a large

proportion developed behavioral signs of Zn toxicosis. Zn-intoxicated ducks experienced reduced hematocrits and body weight, changes in kidney, liver, pancreas, gonad, and gizzard weights, greatly elevated tissue Zn concentrations, changes in tissue concentrations of other elements examined, and gross and histological 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 histological 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 2 studies varied dramatically. Differences in diet between these 2 studies were readily apparent in that, according to CWS protocol, mallards in our study 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, Fe, as well as other dietary inhibitors of Zn absorption such as phytate, lignin, and hemicellulose (Underwood 1971; Eisler 1993; Walsh et al. 1994). Therefore,

additional research is necessary to better understand the influence of diet and soil/grit ingestion on the toxicity of Zn shot to game-farm mallards.

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

Dose	Sex	Mean Body Weight (Kg) 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) (n= 15)
	F	1.04 ± 0.02 (0.09)	0.71 ± 0.04 (0.25) (n= 18)	0.71 ± 0.05 (0.25) (n= 13)
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 unless otherwise specified
[b] includes ducks surviving < 15 days
[c] includes ducks that survived > 15 and < 30 days

Results of ANOVA testing:

Day 0
Sex $F_{1,76}= 37.2$; $P<0.001$

Day 15
Dose $F_{1,74}= 44.5$; $P<0.001$
Sex $F_{1,74}= 46.5$; $P<0.001$
Inter. $F_{1,74}= 3.9$; $P=0.05$

Day 30
Dose $F_{1,64}= 32.8$; $P<0.001$
Sex $F_{1,64}= 51.6$; $P<0.001$
Inter. $F_{1,64}= 5.4$; $P=0.02$

Table 2. Mean percent body weight change ±SE (CV) between Days 0 and 15 in 6-8 month old game-farm mallards dosed with 6 No. 4 Zn or Fe shot.[a]

Dose	Percent Change Day 0 - 15 Males	Females
Zn	- 14.8 ± 3.2 (1.0)	- 30.9 ± 4.2 (0.6) (n= 18)
Fe	- 1.9 ± 0.6 (1.4)	- 4.6 ± 1.4 (1.3)
Zn[b]	- 10.6 ± 3.7 (1.4) (n= 15)	- 30.1 ± 5.3 (0.7) (n= 14)
Zn[c]	- 27.4 ± 1.3 (0.1) (n= 5)	- 33.5 ± 3.2 (0.2) (n= 4)

[a] n= 20 unless otherwise specified
[b] Zn-dosed ducks surviving at least 15 days
[c] Zn-dosed ducks surviving < 15 days

Table 3. Mean percent body weight change ±SE (CV) between Days 0 and 30 in 6-8 month old game-farm mallards dosed with 6 No. 4 Zn or Fe shot.[a]

Dose	Percent Change Day 0 - 30 Males	Females
Zn	-16.5 ± 3.6 (1.0)	- 31.9 ± 3.8 (0.5) (n= 18)
Fe	- 2.4 ± 0.6 (1.1)	- 5.9 ± 1.0 (0.7)
Zn[b]	- 3.2 ± 1.0 (1.1) (n= 11)	- 4.8 ± 1.8 (0.8) (n= 4)
Zn[c]	- 32.8 ± 2.5 (0.2) (n= 9)	- 39.7 ± 1.6 (0.2) (n= 14)

[a] n= 20 unless otherwise specified
[b] Zn-dosed ducks surviving to 30 days
[c] Zn-dosed ducks surviving < 30 days

Table 4. Shot retention and dissolution rate in game-farm mallards (sexes combined) dosed with 6 No. 4 Zn or Fe shot.

Dose	Total Shot Retained	Mean Number Shot Retained	Mean % Loss Shot Weight	Dissolution Rate (g/day)
All Ducks				
Fe	235/240 (97.9%)	5.88	--	--
Zn	231/240 (96.3%)	5.78	--	--
All Ducks retaining 6 shot				
Fe (n=36)	--	--	56.3	0.017
Zn (n=36)	--	--	53.3	0.017
All Ducks retaining 6 shot and surviving to Day30				
Fe (n=36)	--	--	53.3	0.017
Zn (n=15)	--	--	88.1	0.022

Table 5. Mean ± SE (CV) weight of liver and the mean percentage it contributed to total body weight in game-farm mallards dosed with 6 No. 4 Zn or Fe shot[a]

Sex	Dose	Mean Weight (g)	Mean % of Body Weight
M	Zn[b]	16.8 ± 1.2 (0.3)	1.8
M	Fe	18.0 ± 0.5 (0.1)	1.6
F	Zn[b]	15.3 ± 1.2 (0.3)	2.1 (n= 18)
F	Fe	16.9 ± 0.6 (0.2)	1.8
M	Zn_{30}[c]	14.5 ± 0.9 (0.2) (n=11)	1.3 (n=11)
M	$Zn_{<30}$[d]	19.7 ± 2.0 (0.3) (n= 9)	2.5 (n= 9)
F	Zn_{30}[c]	12.6 ± 0.2 (0.0) (n= 4)	1.3 (n= 4)
F	$Zn_{<30}$[d]	16.0 ± 1.4 (0.4) (n= 16)	2.3 (n= 16)

[a]n= 20 for each group unless otherwise specified
[b]includes Zn-dosed ducks surviving < 30 days
[c]Zn-dosed ducks surviving 30 days
[d]Zn-dosed ducks surviving < 30 days

Results of ANOVA testing:

Liver weight
Dose $F_{1,76}$= 2.3; P=0.13

Table 6. Mean ± SE (CV) weight of pancreas and the mean percentage it contributed to total body weight in game-farm mallards dosed with 6 No. 4 Zn or Fe shot[a]

Sex	Dose	Mean Weight (g)	Mean % of Body Weight
M	Zn[b]	1.8 ± 0.1 (0.3)	0.19
M	Fe	2.7 ± 0.1 (0.1)	0.24
F	Zn[b]	1.3 ± 0.1 (0.3)	0.1 (n= 18)
F	Fe	2.4 ± 0.1 (0.2)	0.3
M	Zn_{30}[c]	1.9 ± 0.2 (0.3) (n=11)	0.17 (n=11)
M	$Zn_{<30}$[d]	1.7 ± 0.2 (0.3) (n= 9)	0.22 (n= 9)
F	Zn_{30}[c]	1.3 ± 0.0 (0.1) (n= 4)	0.1 (n= 4)
F	$Zn_{<30}$[d]	1.4 ± 0.1 (0.4) (n= 16)	0.2 (n= 16)

[a]n= 20 for each group unless otherwise specified
[b]includes Zn-dosed ducks surviving < 30 days
[c]Zn-dosed ducks surviving 30 days
[d]Zn-dosed ducks surviving < 30 days

Results of ANOVA testing:

Pancreas weight
Dose $F_{1,76}$= 90.6; P<0.001
Sex $F_{1,76}$= 12.7; P=0.001

Table 7. Mean ± SE (CV) weight of kidneys and the mean percentage it contributed to total body weight in game-farm mallards dosed with 6 No. 4 Zn or Fe shot[a]

Sex	Dose	Mean Weight (g)	Mean % of Body Weight
M	Zn[b]	6.7 ± 0.4 (0.3)	0.7
M	Fe	5.4 ± 0.1 (0.1)	0.5
F	Zn[b]	6.5 ± 0.4 (0.3)	0.9
F	Fe	5.1 ± 0.2 (0.2)	0.5
M	Zn_{30}[c]	5.6 ± 0.3 (0.2) (n=11)	0.5 (n=11)
M	$Zn_{<30}$[d]	8.1 ± 0.4 (0.4) (n= 9)	1.0 (n= 9)
F	Zn_{30}[c]	4.8 ± 0.2 (0.1) (n= 4)	0.5 (n= 4)
F	$Zn_{<30}$[d]	6.9 ± 0.4 (0.2) (n= 16)	1.1 (n= 16)

[a]n= 20 for each group unless otherwise specified
[b]includes Zn-dosed ducks surviving < 30 days
[c]Zn-dosed ducks surviving 30 days
[d]Zn-dosed ducks surviving < 30 days

Results of ANOVA testing:

Kidney weight
Dose $F_{1,76}$= 22.3; P<0.001

Table 8. Mean ± SE (CV) weight of gonads and the mean percentage they contributed to total body weight in game-farm mallards dosed with 6 No. 4 Zn or Fe shot[a]

Sex	Dose	Mean Weight (g)	Mean % of Body Weight
M	Zn[b]	4.8 ± 1.5 (1.4)	0.4
M	Fe	5.8 ± 1.2 (0.9)	0.5
F	Zn[b]	0.3 ± 0.0 (0.4)	0.1
F	Fe	0.7 ± 0.2 (0.2)	0.1
M	Zn_{30}[c]	8.1 ± 2.3 (0.9) (n=11)	0.7 (n=11)
M	$Zn_{<30}$[d]	0.7 ± 0.2 (0.7) (n= 9)	0.1 (n= 9)
F	Zn_{30}[c]	0.4 ± 0.1 (0.2) (n= 4)	0.0 (n= 4)
F	$Zn_{<30}$[d]	0.3 ± 0.0 (0.4) (n= 16)	0.1 (n= 16)

[a]n= 20 for each group unless otherwise specified
[b]includes Zn-dosed ducks surviving < 30 days
[c]Zn-dosed ducks surviving 30 days
[d]Zn-dosed ducks surviving < 30 days

Results of ANOVA testing:

Gonad weight
Dose $F_{1,76}$= 0.5; P=0.47
Sex $F_{1,76}$= 23.1; P<0.001

Table 9. Mean ± SE (CV) weight of gizzard and the mean percentage it contributed to total body weight in game-farm mallards dosed with 6 No. 4 Zn or Fe shot[a]

Sex	Dose	Mean Weight (g)	Mean % of Body Weight
M	Zn^b	23.5 ± 1.6 (0.3)	2.4
M	Fe	29.2 ± 0.5 (0.1) (n=19)	2.6
F	Zn^b	17.2 ± 1.3 (0.3)	2.4 (n= 18)
F	Fe	27.2 ± 0.8 (0.1)	2.8
M	Zn_{30}^c	29.0 ± 1.4 (0.2) (n=11)	2.6 (n=11)
M	$Zn_{<30}^d$	16.9 ± 0.5 (0.1) (n= 9)	2.1 (n= 9)
F	Zn_{30}^c	26.8 ± 1.8 (0.1) (n= 4)	2.8 (n= 4)
F	$Zn_{<30}^d$	14.8 ± 0.7 (0.2) (n= 16)	2.3 (n= 16)

[a]n= 20 for each group unless otherwise specified
[b]includes Zn-dosed ducks surviving < 30 days
[c]Zn-dosed ducks surviving 30 days
[d]Zn-dosed ducks surviving < 30 days

Results of ANOVA testing:

Gizzard weight
Dose $F_{1,76}$= 48.2; P<0.001
Sex $F_{1,76}$= 23.1; P<0.001
Inter. $F_{1,76}$= 4.1; P=0.05

Table 10 . Mean hematocrit ± SE (CV) of 6-8 month-old male and female game-farm mallards dosed with 6 No. 4 Zn or Fe shot.[a]

Dose	Sex	Hematocrit (% PCV) Day 0	Day 15[b]	Day 30[c]
Zn	M	44.65 ± 0.58 (0.06)	40.87 ± 2.81 (0.22) (n= 15)	47.64 ± 0.79 (0.06) (n= 11)
	F	46.15 ± 0.02 (0.06)	32.46 ± 3.19 (0.35) (n= 13)	44.25 ± 0.63 (0.03) (n= 4)
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 unless otherwise specified

Results of ANOVA testing:

Hematocrit

Day 15
Dose $F_{1,51}$= 0.2; P=0.67

Day 30
Dose $F_{1,51}$= 0.3; P=0.59
Sex $F_{1,51}$= 7.1; P=0.01

Table 11. Mean percent change in hematocrit ± SE (CV) between Days 0 and 15 and between Days 0 and 30 in 6-8 month old game-farm mallards dosed with 6 No. 4 Zn or Fe shot.[a]

Sex	Dose	Percent Change Days 0 - 15[b]	Days 0 - 30[c]
M	Zn	- 8.9 ± 6.3 (2.8) (n=15)	- 6.3 ± 1.8 (1.0) (n= 11)
	Fe	3.2 ± 1.1 (1.6)	3.9 ± 1.3 (1.5)
F	Zn	-29.6 ± 6.7 (0.8) (n=13)	- 2.8 ± 4.0 (2.9) (n= 4)
	Fe	- 0.2 ± 1.5 (33.0)	2.7 ± 1.4 (2.2)

[a] n= 20 unless otherwise specified
[b] Zn-dosed ducks surviving at least 15 days
[c] Zn-dosed ducks surviving to Day 30

Table 12. Mean concentrations (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

Results of ANOVA testing:

Ca Day 15
Dose $F_{1,50}$= 0.4; P=0.85

Ca Day 30
Dose $F_{1,50}$= 0.2; P=0.67

Cu Day 15
Dose $F_{1,50}$= 0.4; P=0.54

Cu Day 30
Dose $F_{1,50}$= 0.9; P=0.34

Fe Day 15
Dose $F_{1,50}$= 2.1; P=0.15

Fe Day 30
Dose $F_{1,50}$= 0.6; P=0.46

Mg Day 15
Dose $F_{1,50}$= 0.6; P=0.43

Mg Day 30
Dose $F_{1,50}$= 1.5; P=0.23

P Day 15
Dose $F_{1,50}$= 5.4; P=0.03

P Day 30
Dose $F_{1,50}$= 8.6; P=0.005

Zn Day 15
Dose $F_{1,50}$= 152.8; P<0.001

Zn Day 30
Dose $F_{1,50}$= 82.8; P<0.001

Table 13. Mean concentrations (μg/g wet wt) ± SE (CV) of Ca, Cu, Fe, Mg, P, and Zn in red blood cells 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

Results of ANOVA testing:

Ca Day 0	
Dose	$F_{1,51}$= 4.0; P=0.05
Sex	$F_{1,51}$= 7.0; P=0.01
Inter.	$F_{1,51}$= 16.4; P<0.001
Ca Day 15	
Dose	$F_{1,51}$= 0.2; P=0.67
Ca Day 30	
Dose	$F_{1,51}$= 0.0; P=0.85
Cu Day 15	
Dose	$F_{1,51}$= 2.2; P=0.15
Cu Day 30	
Dose	$F_{1,51}$= 0.1; P=0.79
Fe Day 15	
Dose	$F_{1,51}$= 0.2; P=0.68
Fe Day 30	
Dose	$F_{1,51}$= 0.1; P=0.82
Mg Day 15	
Dose	$F_{1,51}$= 4.8; P=0.03
Mg Day 30	
Dose	$F_{1,51}$= 1.2; P=0.27
P Day 15	
Dose	$F_{1,51}$= 3.9; P=0.05
P Day 30	
Dose	$F_{1,51}$= 2.4; P=0.13
Zn Day 15	
Dose	$F_{1,51}$= 43.7; P<0.001
Zn Day 30	
Dose	$F_{1,51}$= 19.7; P<0.001

Table 14. Mean concentrations (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	Organ		
			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

Results of ANOVA testing:

Kidney Ca	
Dose	$F_{1,76}$= 31.6; P<0.001
Sex	$F_{1,76}$= 6.9; P=0.01
Inter.	$F_{1,76}$= 6.0; P<0.02
Kidney Cu	
Dose	$F_{1,76}$= 38.1; P<0.001
Kidney Fe	
Dose	$F_{1,76}$= 1.2; P=0.29
Kidney Mg	
Dose	$F_{1,76}$= 1.3; P=0.26
Kidney P	
Dose	$F_{1,76}$= 27.2; P<0.001
Kidney Zn	
Dose	$F_{1,76}$= 267.2; P<0.001
Sex	$F_{1,76}$= 6.7; P=0.01
Inter.	$F_{1,76}$= 7.0; P=0.01
Liver Ca	
Dose	$F_{1,76}$= 9.8; P=0.002
Liver Cu	
Dose	$F_{1,76}$= 6.1; P=0.02
Sex	$F_{1,76}$= 6.9; P=0.01
Liver Fe	
Dose	$F_{1,76}$= 5.5; P=0.02
Sex	$F_{1,76}$= 12.5; P=0.001
Liver Mg	
Dose	$F_{1,76}$= 0.0; P=1.0
Liver P	
Dose	$F_{1,76}$= 1.0; P=0.33
Liver Zn	
Dose	$F_{1,76}$= 323.2; P<0.001
Pancreas Ca	
Dose	$F_{1,76}$= 21.2; P<0.001
Pancreas Cu	
Dose	$F_{1,76}$= 107.1; P<0.001
Sex	$F_{1,76}$= 4.0; P=0.05
Pancreas Fe	
Dose	$F_{1,76}$= 7.7; P=0.007
Pancreas Mg	
Dose	$F_{1,76}$= 266.6; P<0.001
Pancreas P	
Dose	$F_{1,76}$= 291.3; P<0.001
Pancreas Zn	
Dose	$F_{1,76}$= 270.7; P<0.001

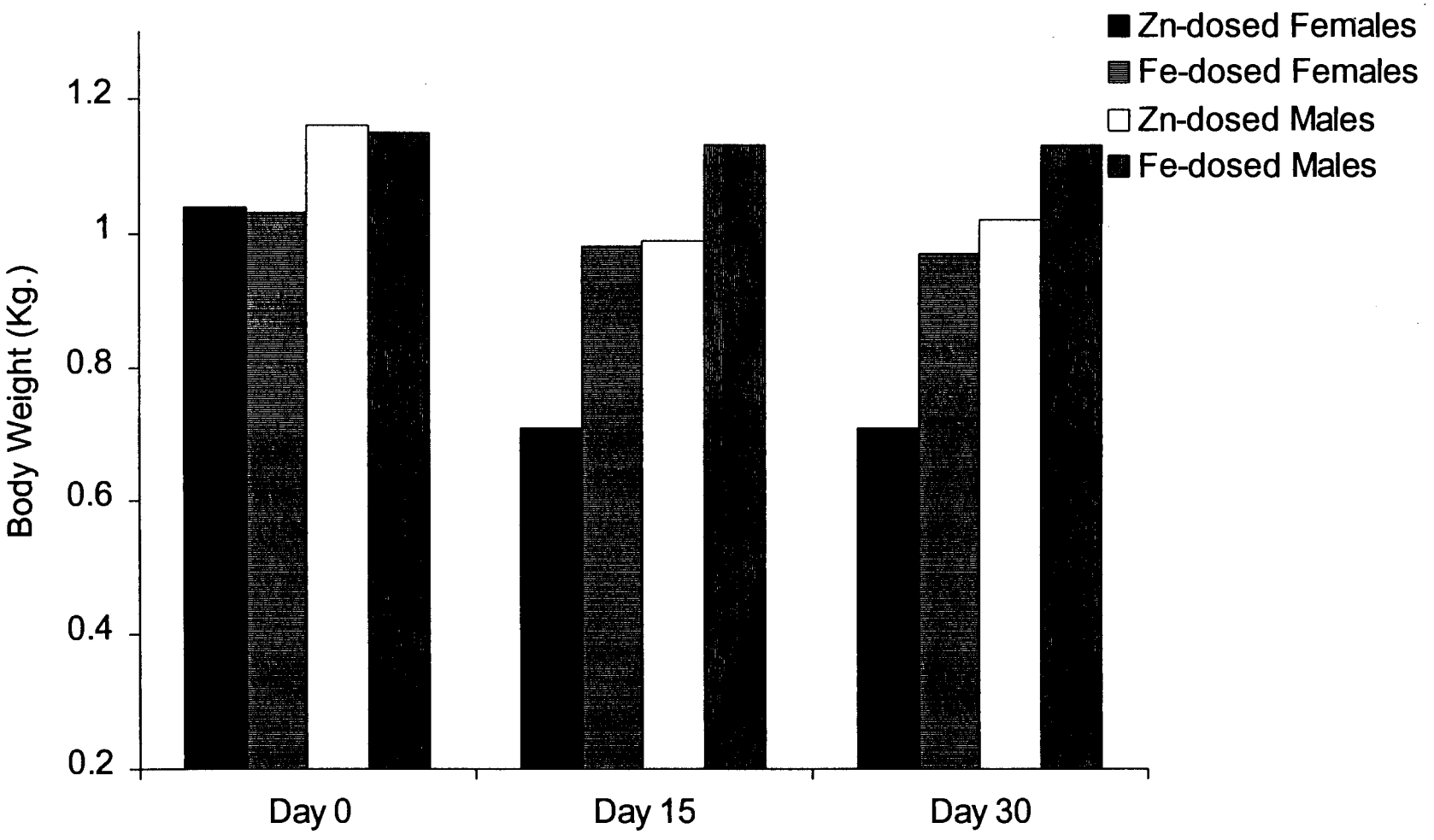

Figure 1. Whole body weight of game-farm mallards dosed with 6 No. 4 Zn or 6 No. 4 Fe shot. Sample size for each group equals 20 except as follows: Zn-dosed males at Day 30, n= 15; Zn-dosed females at Day 15, n= 18, and at Day 30, n= 13. Samples include weights of ducks dying before that data collection period.

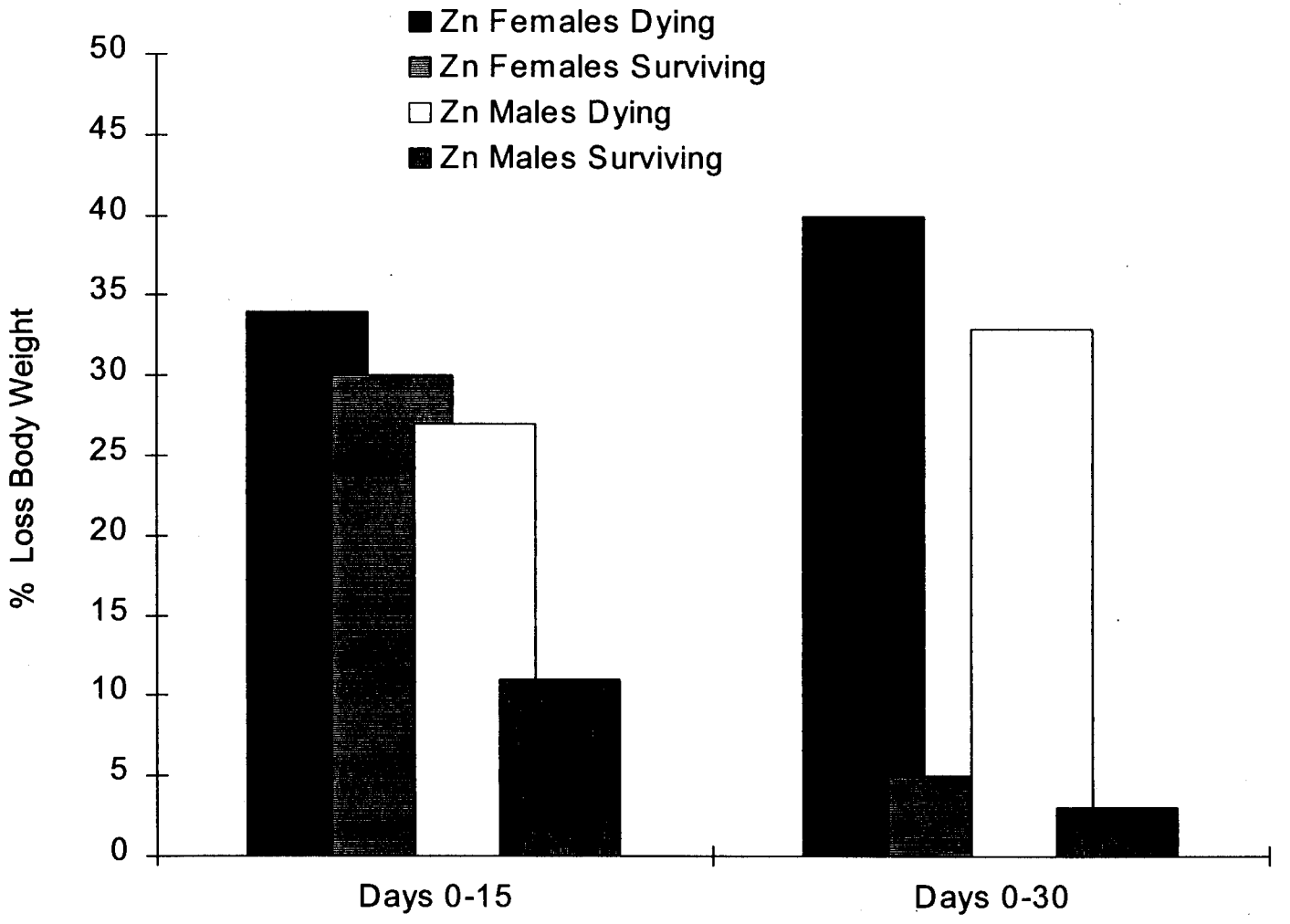

Figure 2. Change in body weight between Days 0 and 15 (or death) and between Days 0 and 30 (or death) in game-farm mallards dosed with 6 No. 4 Zn shot. Sample sizes are as follows: for Days 0-15, males surviving, n= 15; males dying, n= 5; females surviving, n= 14; females dying, n= 4; for Days 0-30, males surviving , n= 11, males dying, n= 9; females surviving, n= 4; females dying, n= 14.

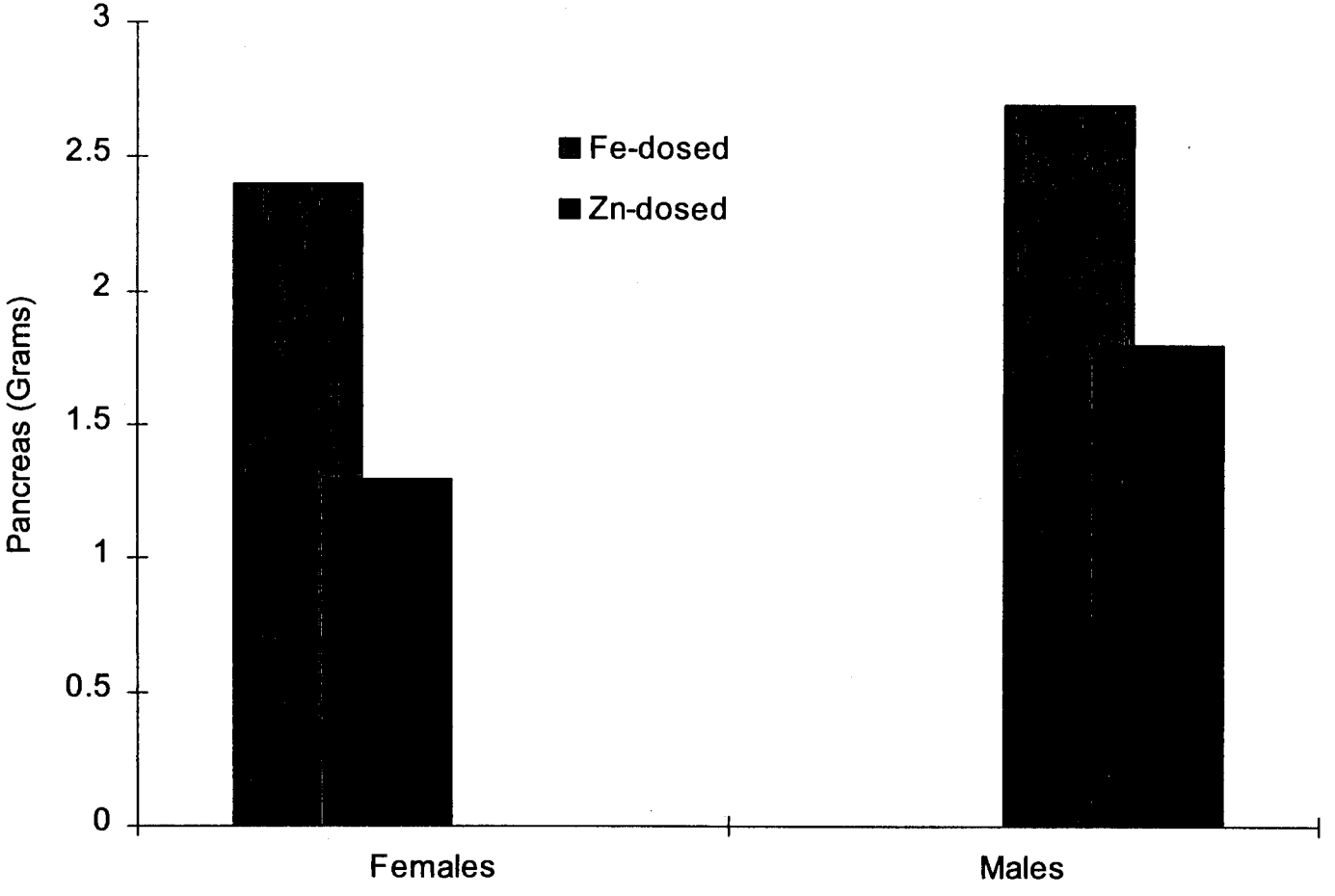

Figure 3. Pancreas weight in game-farm mallards dosed with 6 No. 4 Zn or 6 No. 4 Fe shot. Sample size equals 20 for each group.

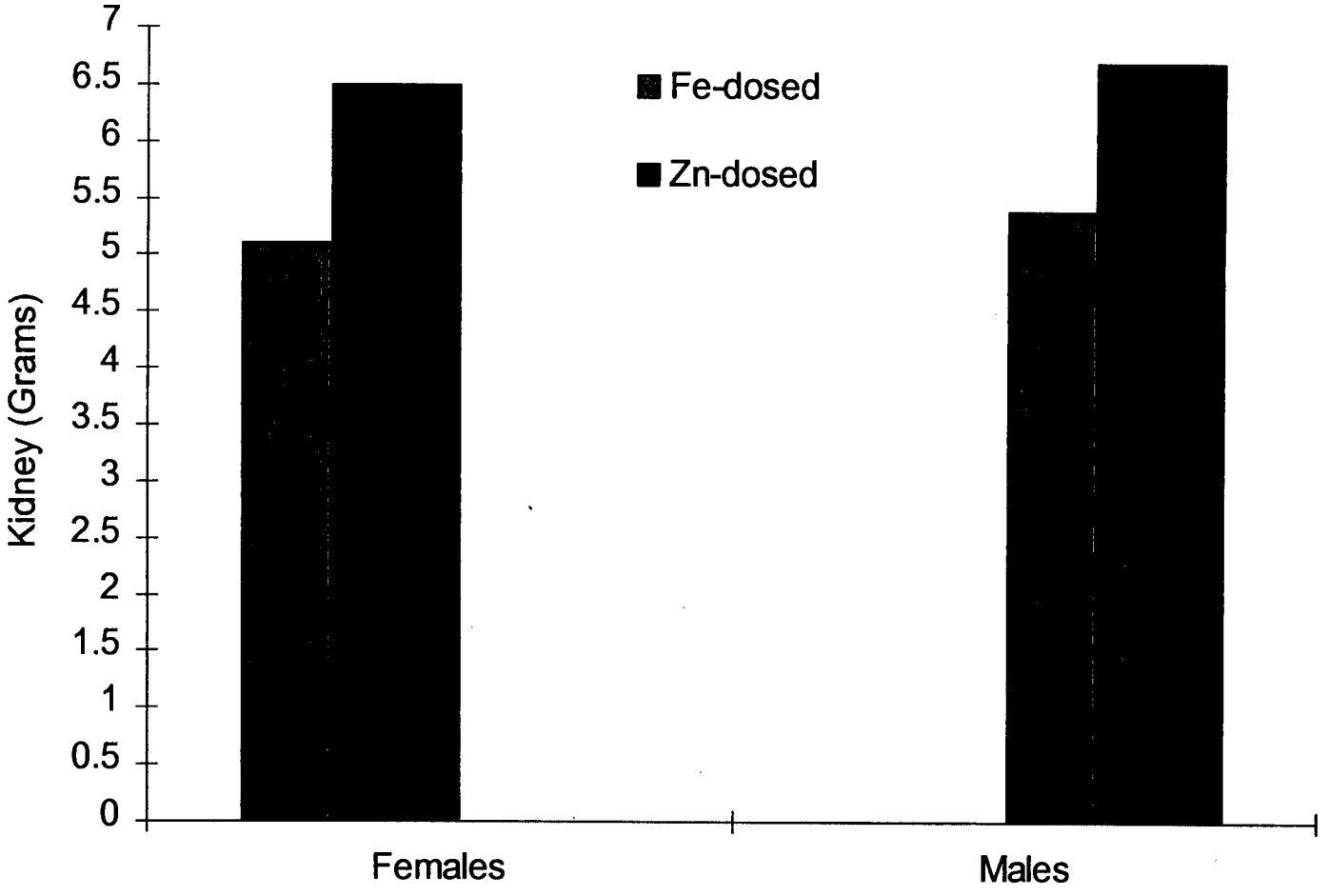

Figure 4. Kidney weight in game-farm mallards dosed with 6 No. 4 Zn or 6 No. 4 Fe shot. Sample size equals 20 for each group.

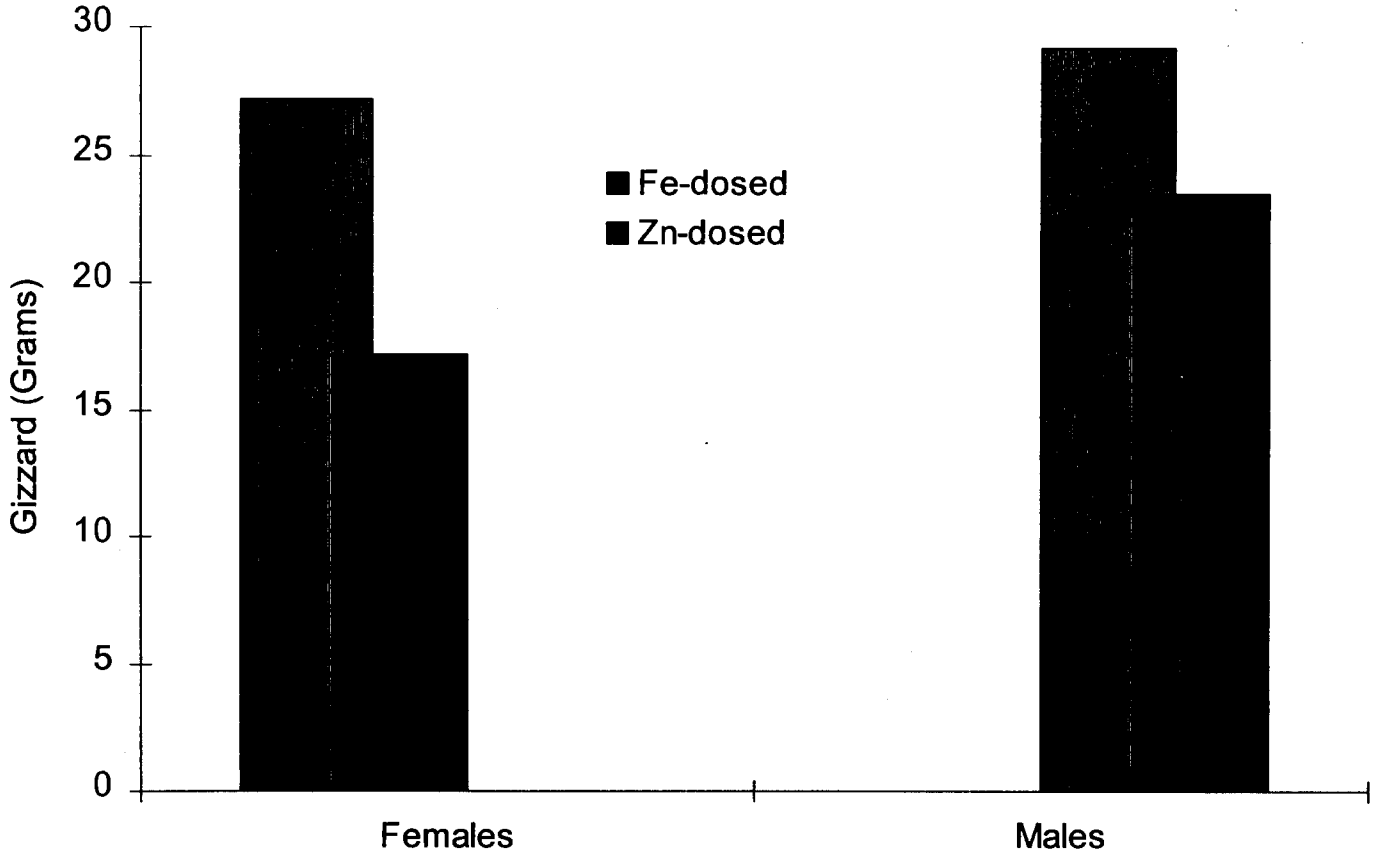

Figure 5. Gizzard weight in game-farm mallards dosed with 6 No. 4 Zn or 6 No. 4 Fe shot. Sample size equals 20 for each group.

Figure 6. Fe concentrations at Day 15 in plasma of game-farm mallards dosed with 6 No. 4 Zn shot.

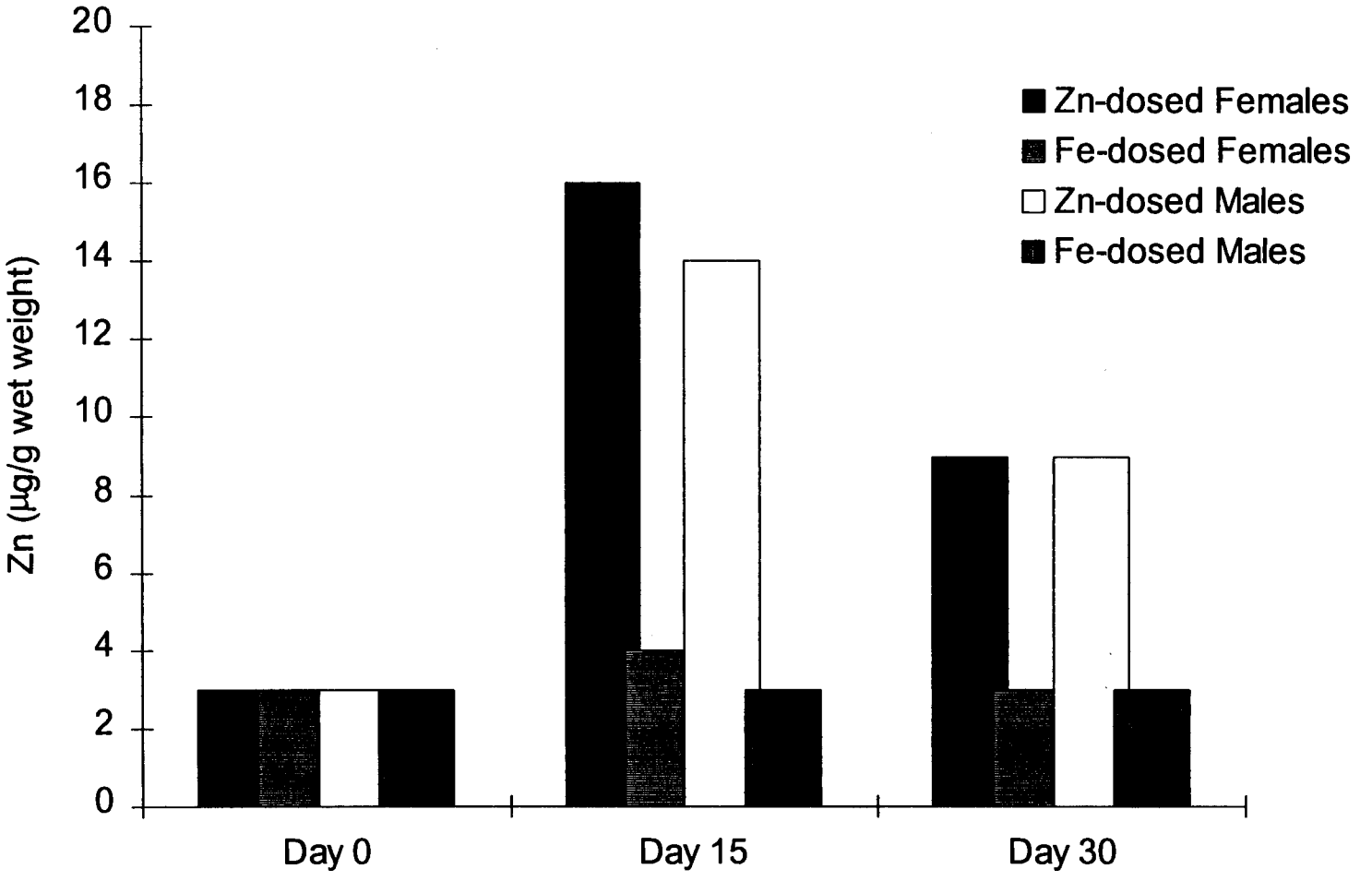

Figure 7. Zn concentrations in plasma of game-farm mallards dosed with 6 No. 4 Zn or 6 No. 4 Fe shot. Sample size for each group equals 20 except as follows: Zn-dosed males and females Day 15, n=16 and 12, respectively; Zn-dosed males and females Day 30, n= 11 and 4, respectively.

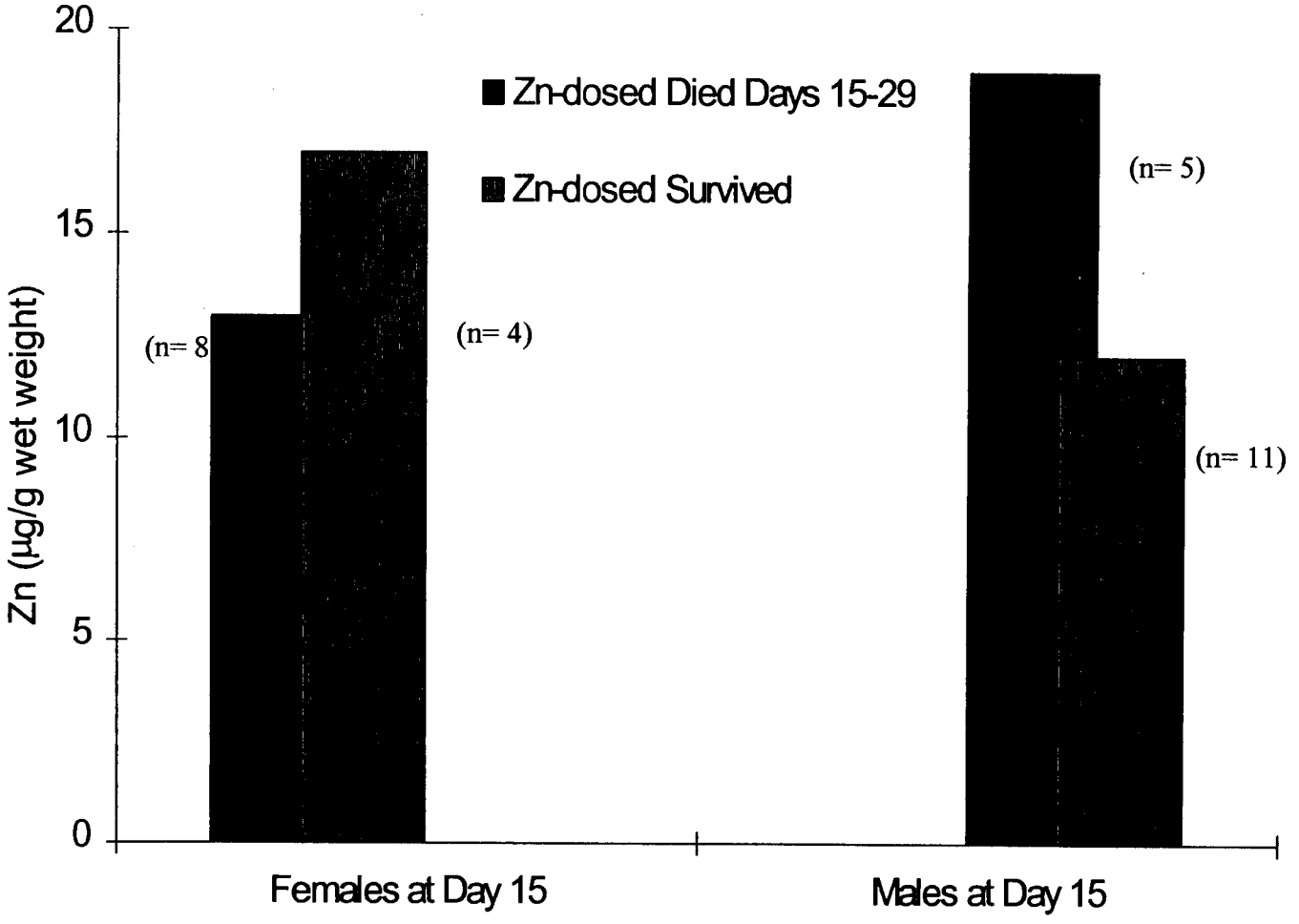

Figure 8. Zn concentrations at Day 15 in plasma of game-farm mallards dosed with 6 No. 4 Zn shot

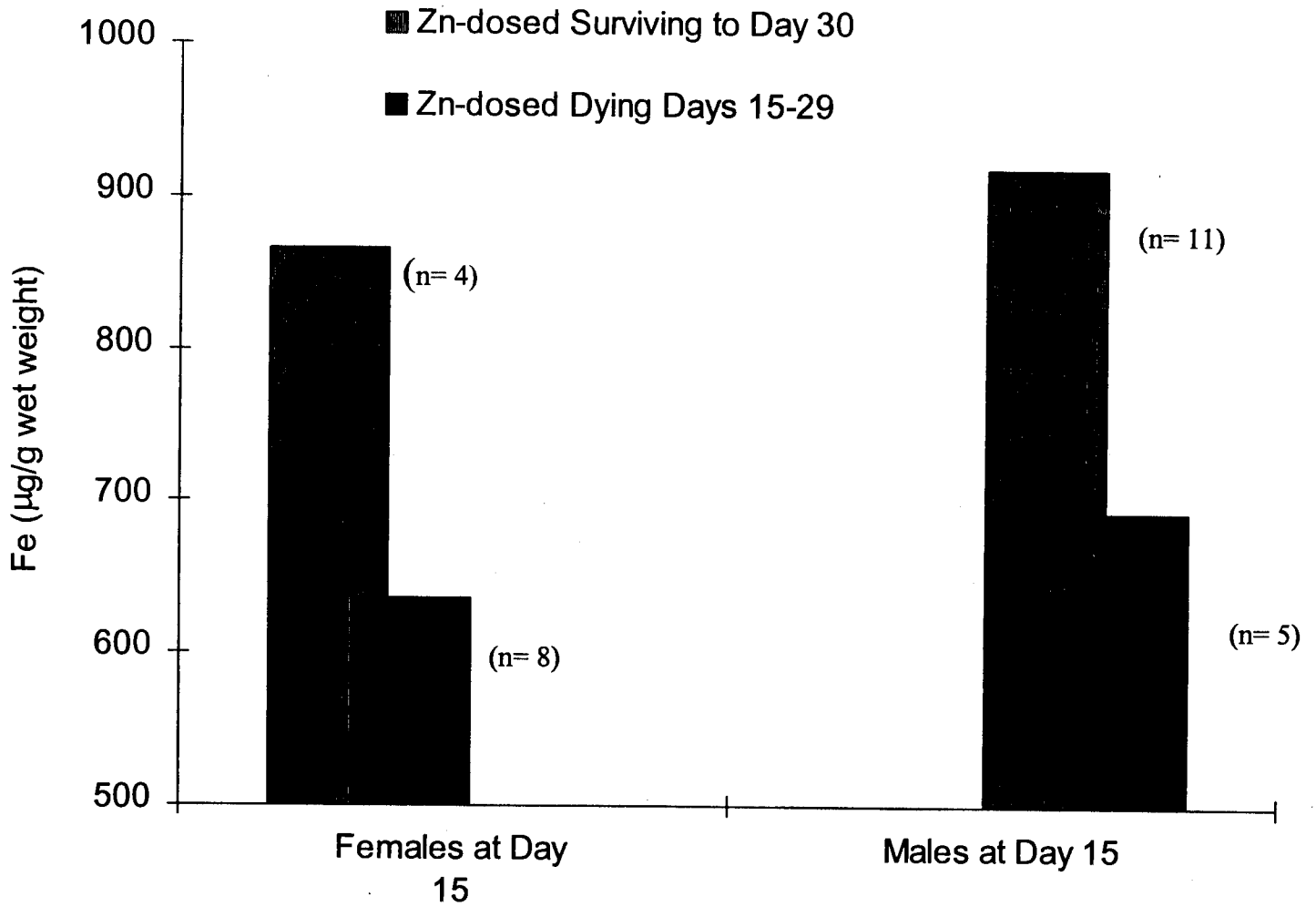

Figure 9. Fe concentrations at Day 15 in erythrocytes of game-farm mallards dosed with 6 No. 4 Zn shot.

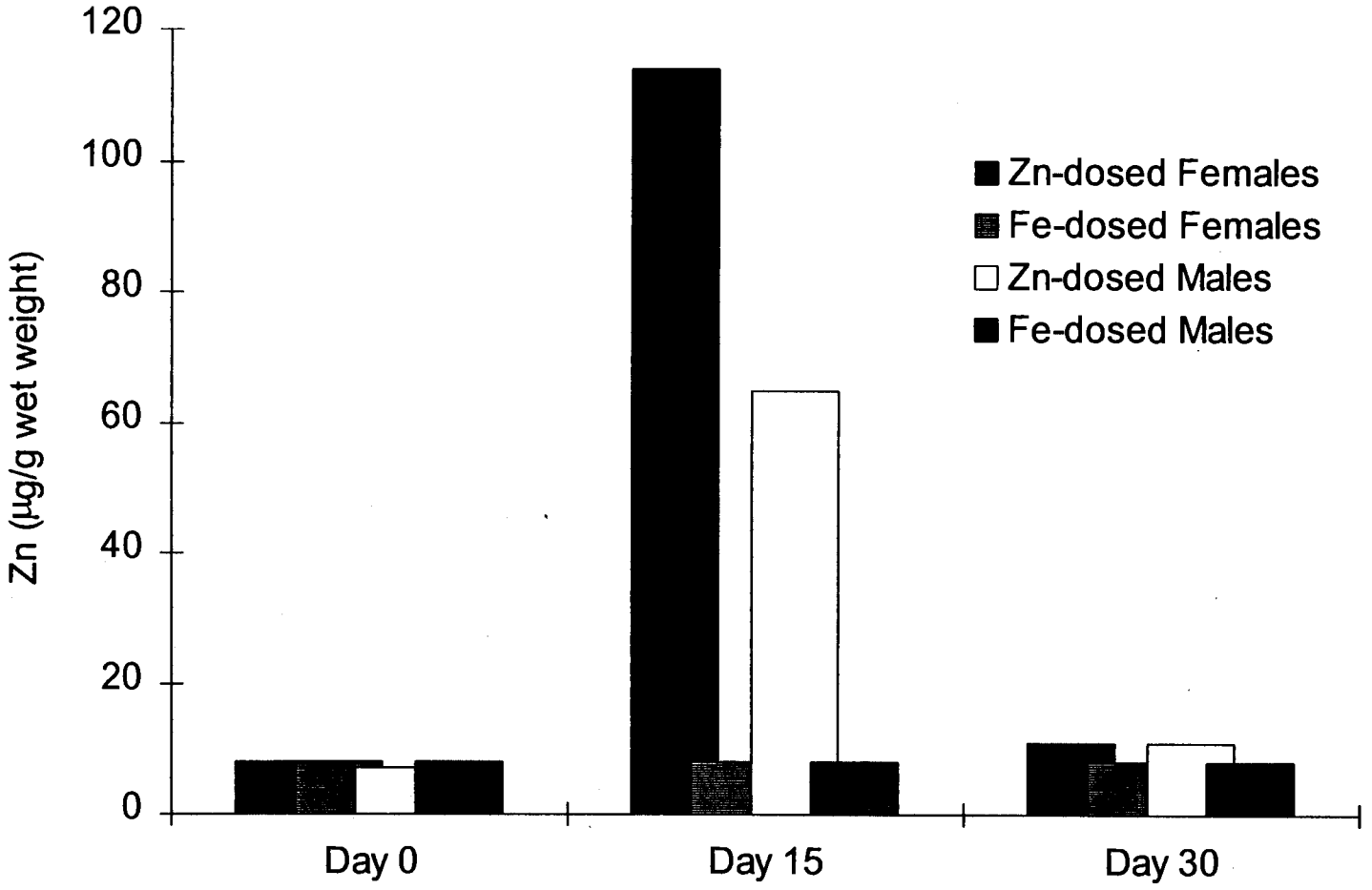

Figure 10. Zn concentrations in erythrocytes of game-farm mallards dosed with 6 No. 4 Zn or 6 No. 4 Fe shot. Sample size for each group equals 20 except as follows: Zn-dosed males and females Day 15, n=16 and 12, respectively; Zn-dosed males and females day 30, n= 11 and 4, respectively.

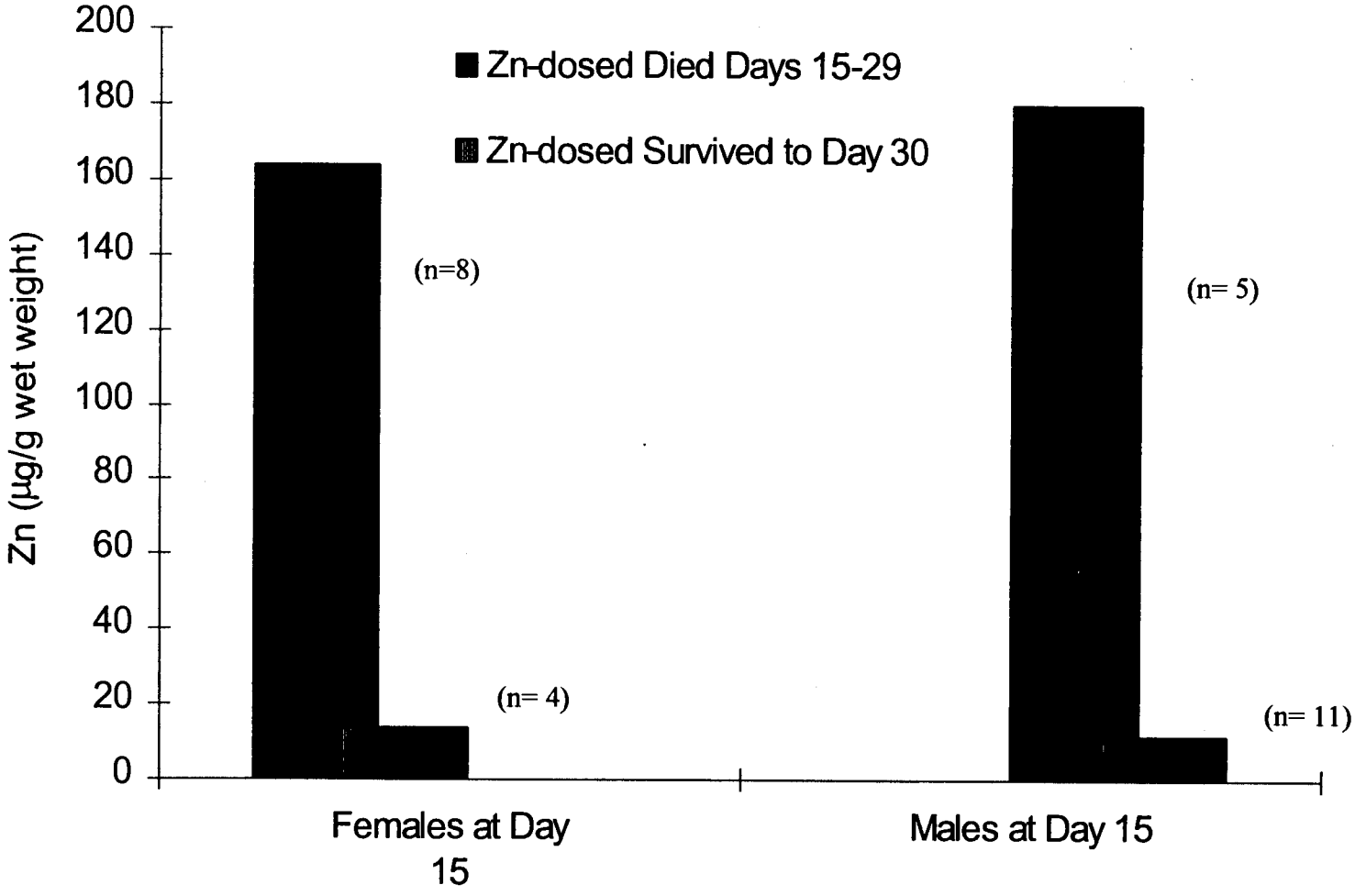

Figure 11. Zn concentrations at Day 15 in erythrocytes of game-farm mallards dosed with 6 No. 4 Zn shot.

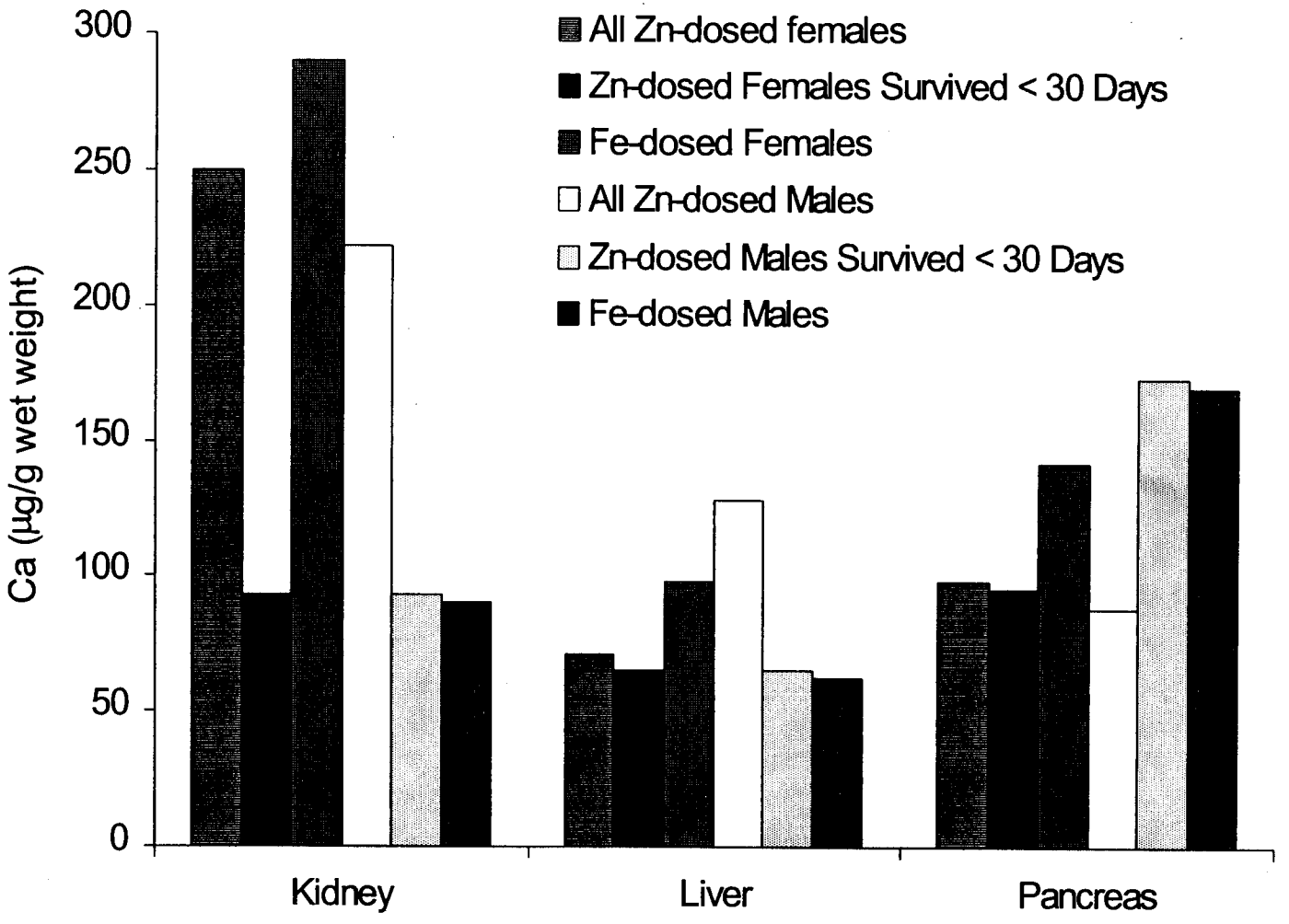

Figure 12. Ca concentrations in organs of game-farm mallards dosed with 6 No. 4 Zn or 6 No. 4 Fe shot. Sample size for each group equals 20 except as follows: Zn-dosed males and females died before Day 30, n= 9 and 16, respectively.

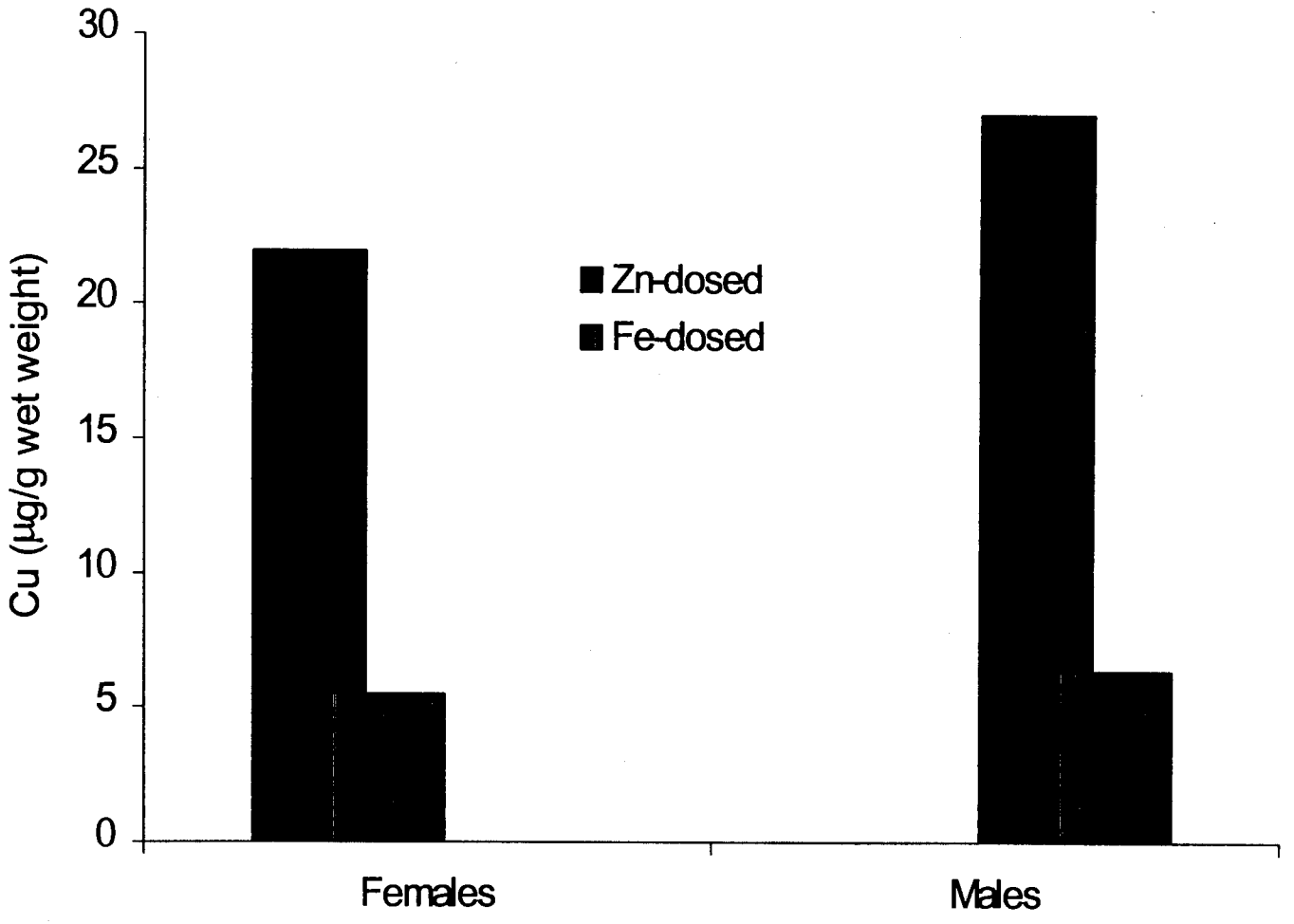

Figure 13. Cu concentrations in kidneys of game-farm mallards dosed with 6 No. 4 Zn or 6 No. 4 Fe shot. Sample size equals 20 for each group.

Figure 14. Zn concentrations in organs of game-farm mallards dosed with 6 No. 4 Zn or 6 No. 4 Fe shot. Sample size equals 40 for each group.

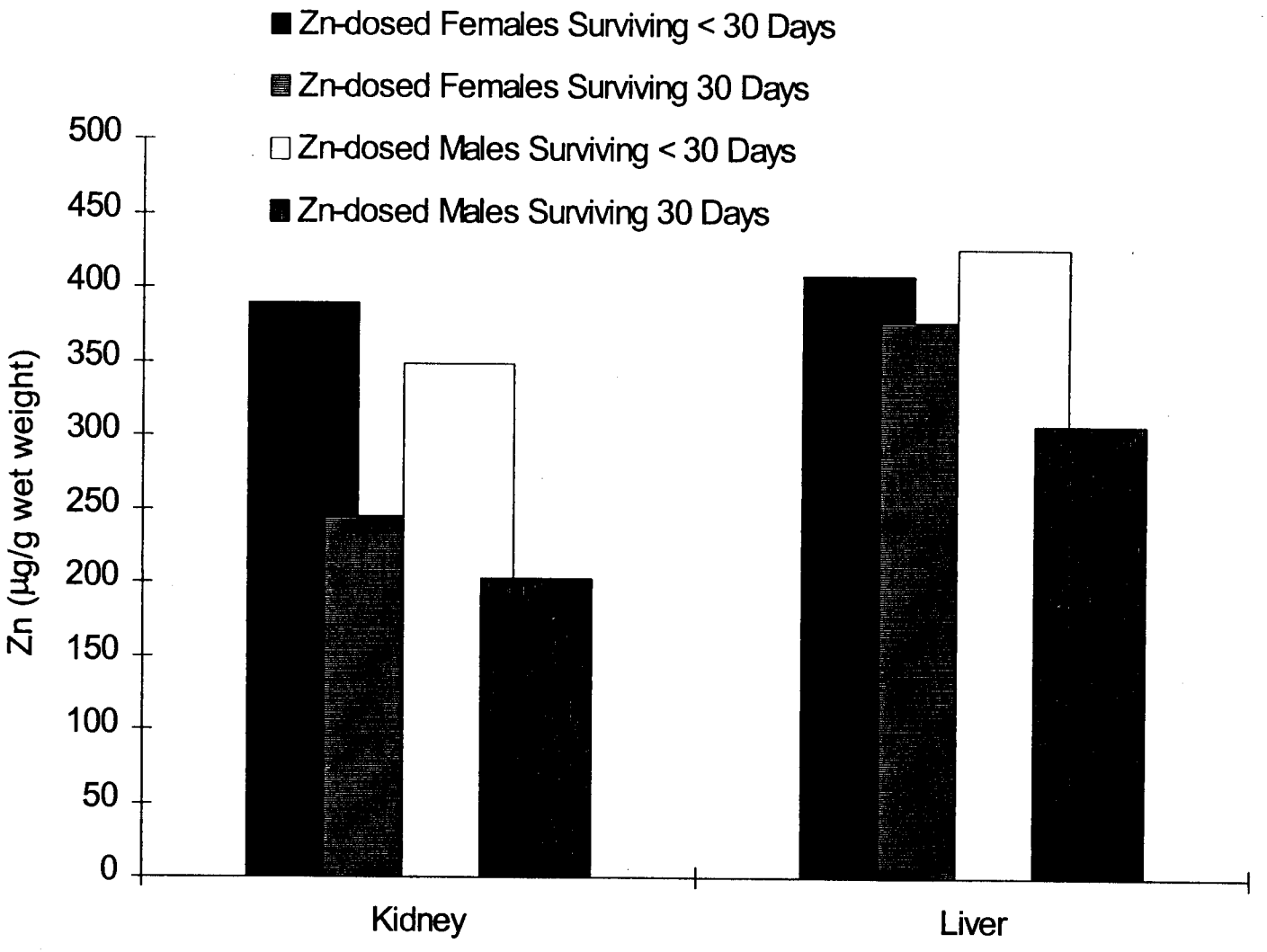

Figure 15. Zn concentrations in kidneys and livers of game-farm mallards dosed with 6 No. 4 Zn shot. Sample sizes were as follows: males surviving, n= 11; males dying, n= 9; females surviving, n= 4; females dying, n= 16.

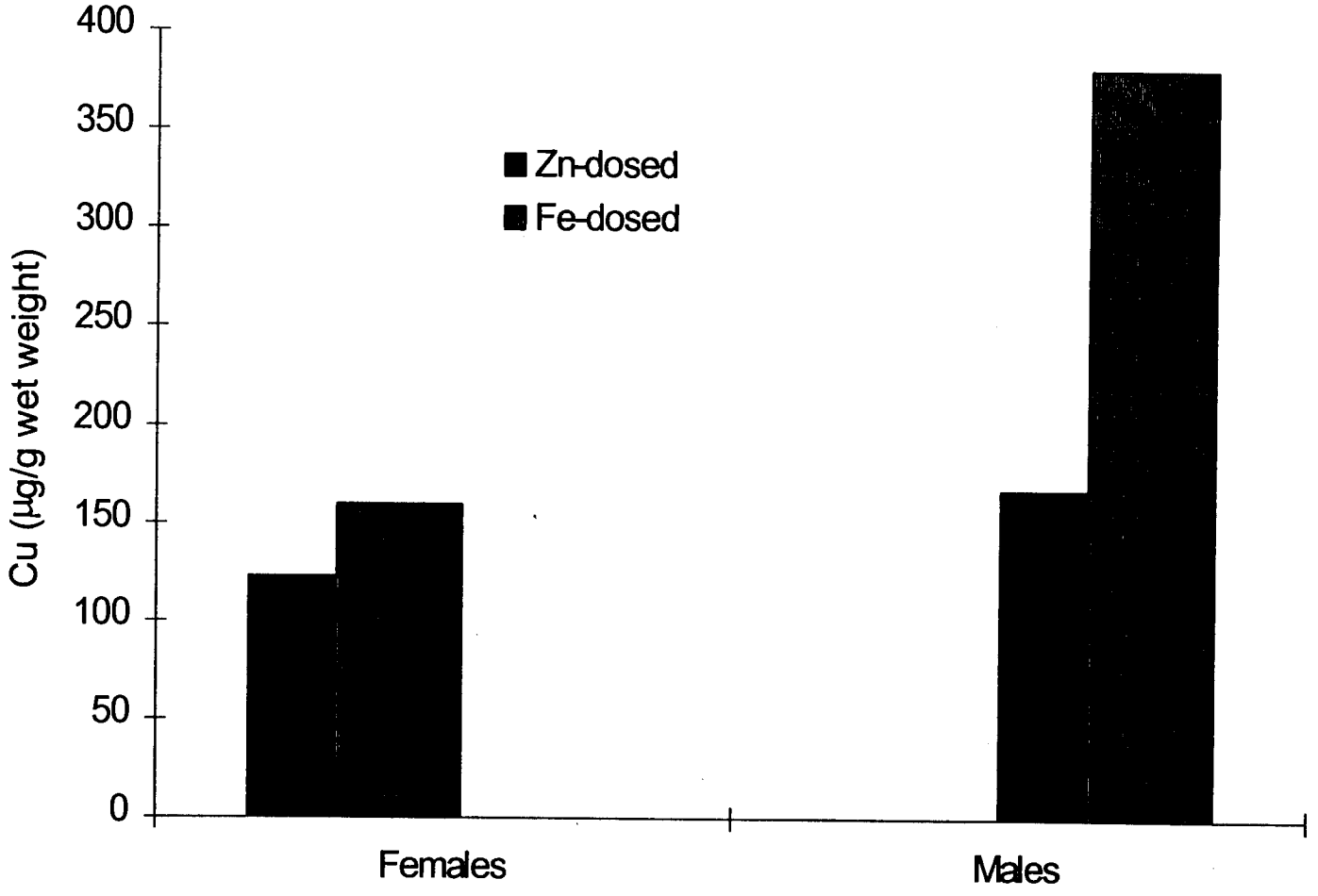

Figure 16. Cu concentrations in livers of game-farm mallards dosed with 6 No. 4 Zn or 6 No. 4 Fe shot. Sample size equals 20 for each group.

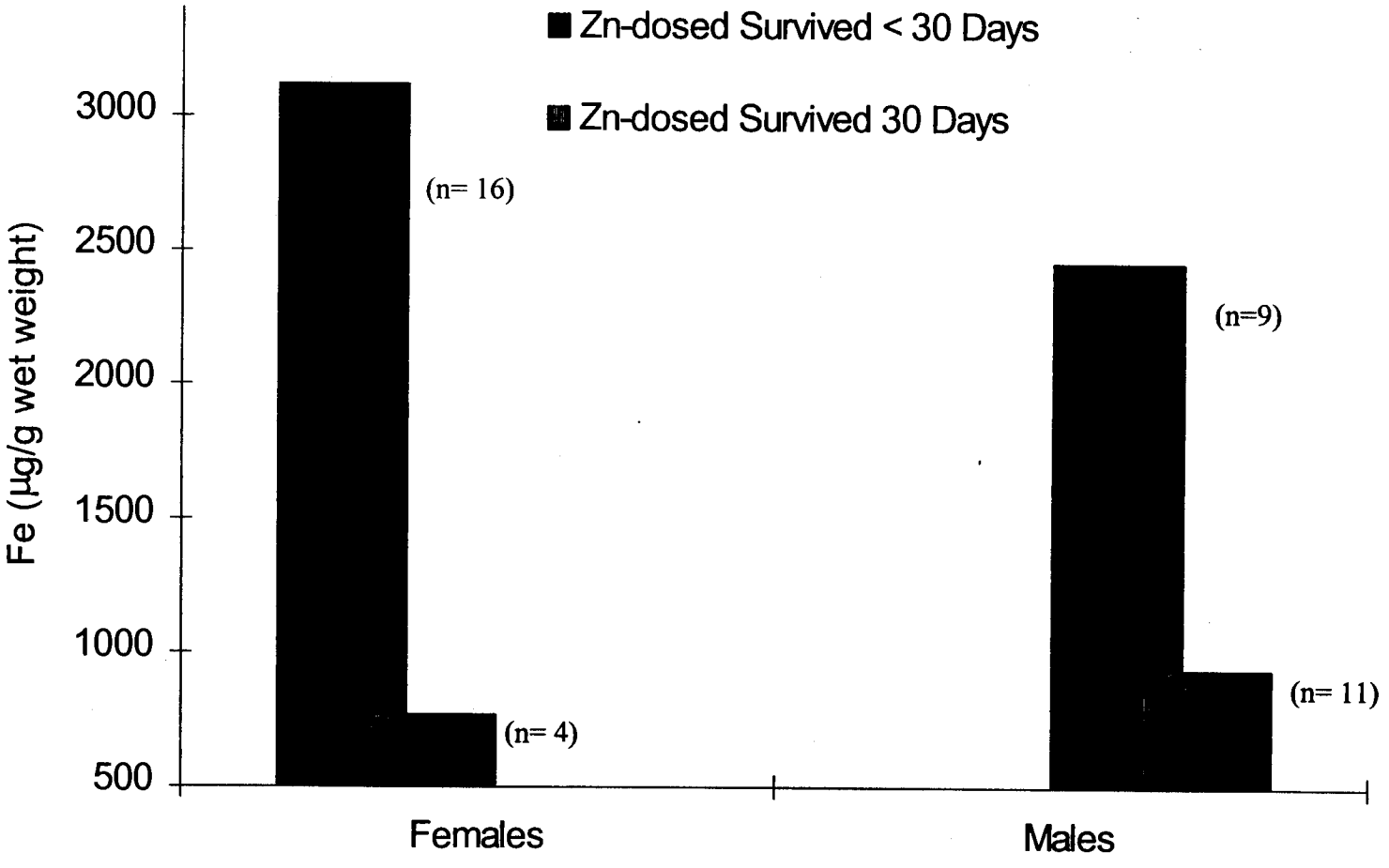

Figure 17. Fe concentrations in livers of game-farm mallards dosed with 6 No. 4 Zn shot.

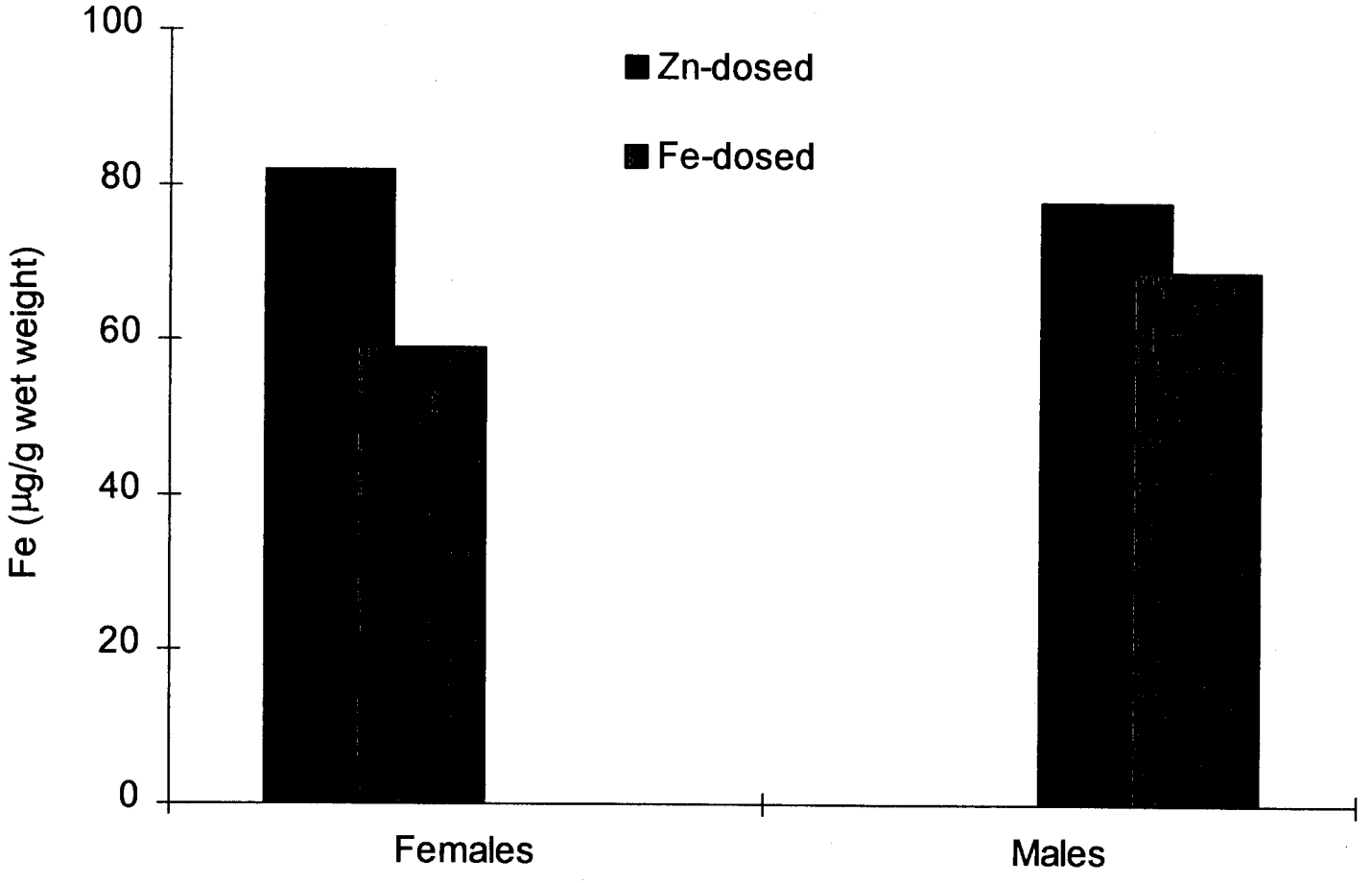

Figure 18. Fe concentrations in pancreases of game-farm mallards dosed with 6 No. 4 Zn or 6 No. 4 Fe shot. Sample size equals 20 for each group.

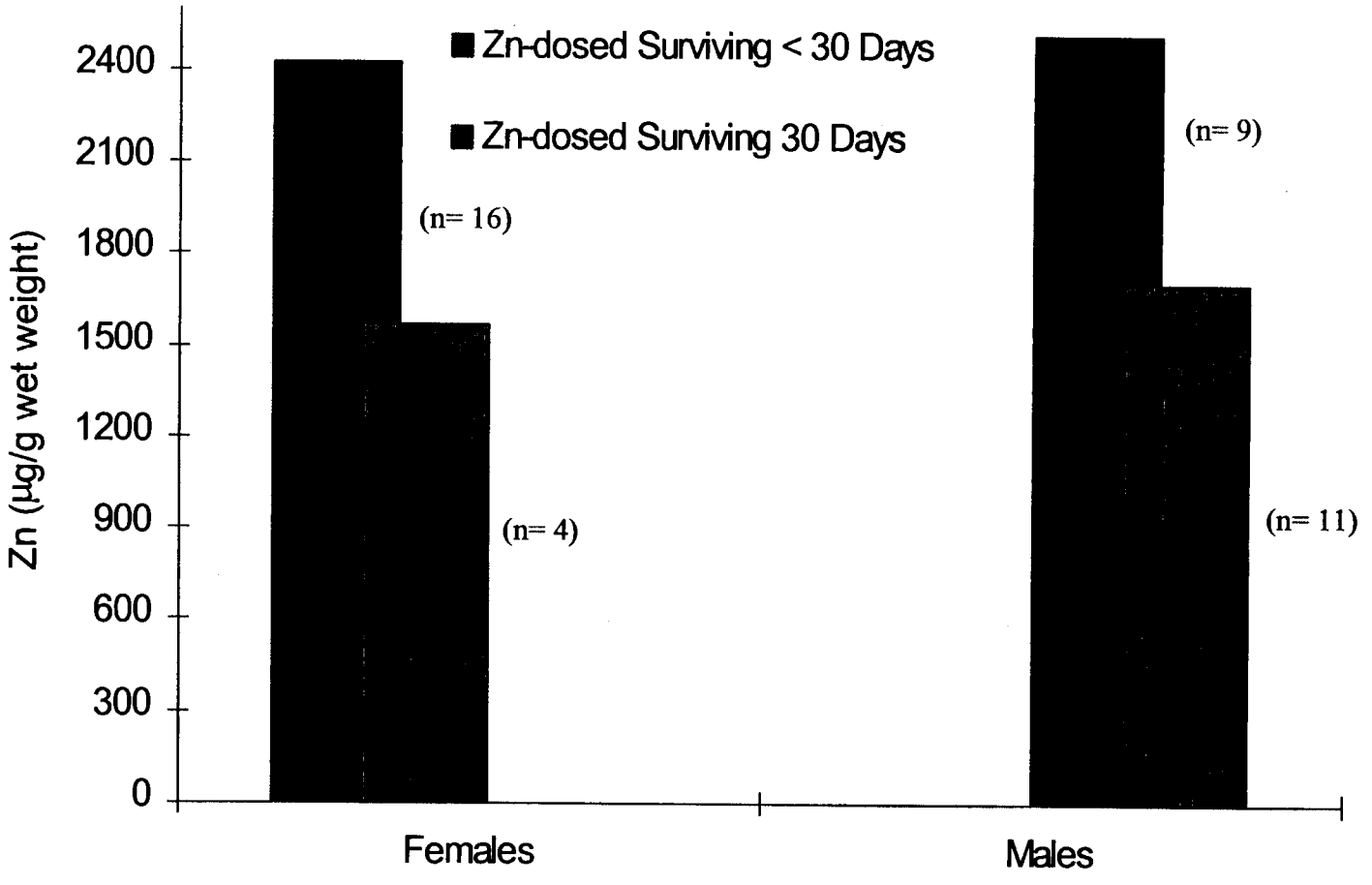

Figure 19. Zn concentrations in pancreases of game-farm mallards dosed with 6 No. 4 Zn shot.

Appendix A

ANALYTICAL REPORT: ACUTE TOXICITY OF ZINC SHOT ON GAME FARM MALLARDS

L. M. Skowron, Illinois State Water Survey

Introduction

The samples were analyzed by the staff of the Office of Analytical and Water Treatment Services at the Illinois State Water Survey in Champaign, Illinois. Reports of the final results are on pages A-8 to A-31. The laboratory is certified for the analysis of environmental samples by the Illinois Environmental Protection Agency, Certificate Number 100202. Provisions for certification are described in the Illinois Administrative Code, Title 35, Subtitle A, Chapter II, Part 183, Joint Rules of the Illinois Environmental Protection Agency, The Illinois Department of Public Health and the Illinois Department of Nuclear Safety: Certification and Operations of Environmental Laboratories. Although this certification is targeted towards the analysis of public drinking water supply samples, the same requirements for personnel, facilities, equipment, methodologies, quality control, etc. are met for the analysis of all other sample types.

Methods

Sample Storage

Samples were inventoried upon receipt and stored frozen at -10° C. The freezer temperature was documented daily. The samples were allowed to thaw to room temperature before preparations for metals analysis. Samples were labeled by tissue type and a number. The sex of the duck and the treatment it received during the study were not identified prior to analysis. There was as occasional problem when the glass test tubes broke during the freezing and thawing of the samples of blood components. The affected samples were transferred to polypropylene test tubes if breakage occurred.

Sample Digestions

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

Digestions for ICP analysis: Sample weights of 0.5 to 1.0 grams were used. A mixed portion of the sample was weighed to 0.1 mg using an electronic top loading balance directly into a tared 50 mL

conically tipped polypropylene centrifuge tube. The tubes were precleaned using a 24 hour 10% nitric acid (HNO_3) soak followed by a deionized water rinse. The samples and tubes were tared, 1.00 of hydrogen peroxide (H_2O_2) added, and the weights recorded. Approximately 20 to 30 mL of an acid and internal standard solution were then added to the sample after taring. The acid concentrations were 2% HNO_3 and 10% hydrochloric acid (HCl). The Be concentration was targeted at 2.00 mg/L. The actual weights used are listed in the tables of Raw Data and Calculations, pages A-32 to A-154.

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 accounted for in the total weight.

Sample preparations were completed 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 the 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: The instrument used was a Thermo Jarrell Ash (TJA) AtomComp Model 61 vacuum spectrometer. It has a polychromator configured with 44 fixed channels, including analytical lines for high and low concentrations of Ca and Mg. Although results for a limited number of elements were reported, measurements were actually made for 30 analytes to monitor for spectral interferences. No significant spectral interferences were identified. Blank subtraction and background correction were 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 (1) was used. The method was modified in that a different digestion process was used. Beryllium was chosen as an internal standard because it was not present in the samples, there are no spectral or background interferences, and it is very precisely detectable. The following wavelengths, analytical ranges, and calibration concentrations were used:

Analyte	Wavelength, nm	Analytical Range, mg/L	Calibration Standard Concentration, mg/L
Beryllium	313.042	0.003-10.0	6.00
Calcium	393.366	0.03-20.0	10.0

Analyte	Wavelength, nm	Analytical Range, mg/L	Calibration Standard Concentration, mg/L
Calcium	317.933	1.0-800	210
Copper	324.754	0.006-30.0	6.00
Iron	259.94	0.02-200	10.0
Magnesium	279.553	0.010-20	6.00
Magnesium	383.231	1.0-200	100
Phosphorous	214.914	0.40-200	10.0
Tin	189.989	0.10-150	20.0
Zinc	213.856	0.010-30.0	6.00

Quality Control

The instruments were calibrated daily and the standard curve was verified using NIST traceable quality control samples (QCS). Samples (usually ten) 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):

Analyte	SRM	Analyte	SRM
calcium	3109a	phosphorus	3139a
copper	3114	tin	3161
iron	3126a	zinc	3168a
magnesium	3131a		

Summaries of the measurements of these solutions are on pages A-155 to A-175.

Ten percent of the samples were digested and analyzed in duplicate, half of them spiked. The data for these samples are presented on pages A-175 to A-202. Spike solutions were traceable to NIST SRMs.

Digestion blanks and spiked digestion blanks were prepared at a frequency of 10%. They were taken through the complete digestion and analytical process in the same manner as the samples. The data are presented on pages A-202 to A-214.

Method Detection Limits (MDL) were calculated according to Equations 8 and 9. These data are summarized on page A-215 to A-218.

Calculations

The ICP data were saved during analysis into database files using ThermoSpec (TJA) software utilizing Enable OA. These data were then imported into Enable spreadsheets for tabulations and calculations. The Enable spreadsheets were saved in a Lotus 123 format on diskette for delivery to Dr. Levengood for his use. The following equations were used for calculations:

1. Conversion of ICP measured concentration, mg/L, to tissue concentration as $\mu g/g$:

$$Analyte,\ \mu g/g = \frac{A \times Be_{tv} \times \left(W_s + (W_I \times 1.036) + (W_H \times 1.12)\right)}{W_s \times Be_m}$$

Where:
- A = Measured analyte concentration, mg/L
- Be_{tv} = True value of Be internal standard, mg/L
- W_s = Sample weight, g
- W_I = Weight of internal standard solution, g
- W_H = Weight of H_2O_2, g
- Be_m = Measured concentration of Be internal standard, mg/L
- 1.036 = Correction factor for the density of the acid mixture
- 1.12 = Correction factor for the density of H_2O_2.

2. Conversion of GFAA measured concentration, μg/L, to tissue concentration as μg/g:

$$Analyte,\ \mu g/g = \frac{0.05 \times A}{W}$$

Where: 0.05 = Conversion factor
A = Measured concentration, μg/L
W = Weight of sample, g

3. Relative percent difference for sample duplicates:

$$RPD = \frac{(A_1 - A_2) \times 100}{(A_1 + A_2)/2}$$

Where: RPD = Relative percent difference
A_1 = Larger of the two observed values
A_2 = Smaller of the two observed values.

4. Spike solution concentration for ICP measurements:

$$Csa = \frac{S \times V \times Be_m \times 1.036}{(W_t \times Be_{tv})}$$

Where: Csa = Actual concentration of spike added, mg/L
S = Analyte concentration in spike solution, mg/L
V = Volume of spike solution added, mL
Be_{tv} = True value of Be internal standard, mg/L
W_t = Total weight of sample and internal standard solution, g
Be_m = Measured concentration of Be internal standard, mg/L
1.036 = Correction factor for the density of the acid mixture.

5. Percent recovery for spiked sample duplicates:

$$\%R = 100 \times \left(\frac{A - U}{Csa} \right)$$

Where: %R = Percent recovery
A = Measured analyte concentration in spiked aliquot
U = Measured analyte concentration in unspiked aliquot
Csa = Actual concentration of spike added.

6. Percent recovery for spiked digestion blanks :

$$\%R = 100 \times \left(\frac{A}{Csa} \right)$$

Where: %R = Percent recovery
A = Measured analyte concentration in spiked aliquot
Csa = Actual concentration of spike added.

7. Percent recovery for performance check solutions and SRMs:

$$\%R = 100 \times \left(\frac{A}{A_{tv}} \right)$$

Where: %R = Percent recovery
A = Measured analyte concentration
A_{tv} = True value of analyte concentration.

8. Method detection limits for sample measurements as mg/L:

$$MDL = t_{(n-1,\ 1-\alpha\ =\ 0.99)} \times s$$

Where:
- MDL = Method detection limit, mg/L
- s = Standard deviation of the replicate analyses
- $t_{(n-1,\ 1-\alpha\ =0.99)}$ = Student's t-value for a one-sided 99% confidence level and a standard deviation estimate with n-1 degrees of freedom.

9. Method detection limits for analytes in samples as μg/g:

$$MDL_s = \frac{MDL \times Be_{tv} \times \left(W_s + (W_I \times 1.036) + (W_H \times 1.12)\right)}{W_s \times Be_m}$$

Where:
- MDL_S = Method detection limit in samples as μg/g
- MDL = Method detection limit measured in samples calculated according to equation 8
- Be_{tv} = True value of Be internal standard, mg/L
- W_s = Sample weight, g
- W_I = Weight of internal standard solution, g
- W_H = Weight of H_2O_2, g
- Be_m = Measured concentration of Be internal standard, mg/L
- 1.036 = Correction factor for the density of the acid mixture
- 1.12 = Correction factor for the density of H_2O_2.

(1) "Methods for the Determination of Metals in Environmental Samples - Supplement I", EPA-600/R-94-111, May 1994.

(2) *Standard Methods for the Examination of Water and Wastewater*, 18th Edition, American Public Health Association, 1992.

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