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ACUTE EFFECTS OF LEAD, STEEL, TUNGSTEN-IRON, AND TUNGSTEN-POLYMER SHOT ADMINISTERED TO GAME-FARM MALLARDS

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ABSTRACT: Sixteen-bird groups (sexes equal) of adult mallards (*Anas platyrhynchos*) were orally dosed with eight #4 steel shot, eight #4 lead shot, eight BB-size tungsten-iron shot, eight BB-size tungsten-polymer shot, or were sham-dosed and maintained for 30 days (16 January 1996 to 15 February 1996). Half of the lead-dosed ducks (five males, three females) died during the study, whereas no ducks died in the other dosage groups. For lead-dosed ducks, hematocrit and hemoglobin concentration were decreased on day 15 of the trial, but not on day 30. Delta aminolevulinic acid dehydratase activity in lead-dosed ducks was lower when compared to steel-dosed ducks only. Plasma activities of selected enzymes were elevated in lead-dosed ducks when compared to enzyme activities of ducks in the other groups. For lead-dosed ducks, relative heart, liver, and kidney weights increased in comparison to relative weights of those organs of ducks in other groups. Histology of tissues indicated that renal nephrosis accompanied by biliary stasis was present in the eight lead-dosed ducks that died. For the eight lead-dosed ducks that survived, six had mild to severe biliary stasis. Mild biliary stasis was noted in five tungsten-iron dosed ducks and three tungsten-polymer dosed ducks. Amounts of lead in the femur, liver, and kidneys were higher in lead-dosed ducks than in ducks of the other four groups. Small amounts of tungsten were detected in the femur and kidneys of two tungsten-polymer dosed ducks. Higher concentrations of tungsten were detected in the femur, liver, and kidneys of all tungsten-iron dosed ducks. The rate of shot erosion was highest (80%) for the tungsten-polymer shot, followed by tungsten-iron (55%), lead (50%), and steel shot (33%). Results indicated that tungsten-iron or tungsten-polymer shot (8 shot/duck) orally administered to mallards did not adversely affect them during a 30-day trial.

Key words: *Anas platyrhynchos*, lead shot, mallard, steel shot, toxicity, tungsten-iron shot, tungsten-polymer shot.

INTRODUCTION

Lead poisoning affects every major species of waterfowl in North America and a wide range of avian predator species that feed on lead-poisoned waterfowl. Lead has caused mortality of waterfowl in North America since the late 1800's (Cook and Trainer, 1966). Bellrose (1959) estimated the yearly loss of waterfowl in North America to be between two and three percent of the population. Based on a migratory fall flight of 100 million birds, Friend (1987) estimated an annual loss of two million waterfowl from lead poisoning.

Lead poisoning occurs when waterfowl ingest spent lead shotgun pellets and fishing sinkers while feeding or obtaining grit on the bottom of lakes, ponds, and marshes. Ingested pellets are usually retained in the bird's gizzard for sometime and ground into small particles, which are easily absorbed. If a large number of shot have been consumed (≥10), acute lead poisoning can occur and the bird dies within a few days. Chronic lead poisoning, resulting from ingestion of a smaller number of shot, is more common. Clinical signs characteristic of lead poisoning appear gradually and affected birds die 2 to 3 wk after exposure (Scheuhammer and Norris, 1995).

Because of the increasing numbers of birds killed every year due to lead toxicosis, lead shot was banned for waterfowl hunting within the United States in 1991

(Ringelman et al., 1993). Currently, the only U.S. Fish and Wildlife Service (USFWS; Washington D.C., USA) approved substitutes for lead shot are steel shot and, more recently, bismuth shot (Ringelman et al., 1993). Although steel shot has been tolerated by hunters, efforts continue to formulate a nontoxic shot having lead's favorable ballistic characteristics. Recently, shot composed primarily of tungsten has been examined as a possible nontoxic alternative.

Tungsten is a relatively rare element that most frequently occurs as the tungstate ion (WO_4^{2-}) in biological systems (Stokinger, 1978). Absorbed tungsten accumulates in relatively few tissues with chief deposition sites in animals being bone, spleen, kidney, and liver based on studies with rats (Kinard and Aull, 1945; Kaye, 1968), mice (Wase, 1956), swine, sheep (Bell and Sneed, 1970), and dogs (Aamodt, 1975). Toxicity of tungsten is dependent upon the solubility of the form administered; soluble tungstate ion is considerably more toxic than less soluble forms, i.e., metallic tungsten. Clinical signs resulting from acute exposure of animals to lethal or near-lethal doses of tungsten include nervous prostration, diarrhea, anorexia, and ultimately death preceded by coma caused by respiratory paralysis. When rats (Kinard and Van de Erve, 1941, 1943; Schroeder and Mitchner, 1975) and chickens (Higgens et al., 1956; Teekell and Watts, 1959; Leach et al., 1962; Nell et al., 1980) have been administered doses of tungsten compounds that do not result in mortality, usually in excess of several thousand ppm, the effects are often slight.

For a candidate shot to be approved for use by the USFWS, it must undergo a variety of tests as documented in USFWS 50 CFR Part 20.134, Migratory Bird Hunting: Nontoxic Shot Approval Procedure (Federal Register, 1986) to establish that it is nontoxic to waterfowl and other exposed species. We present results from an acute toxicity test designed to assess effects of short-term periodic exposure of mallards to two candidate shot, one composed of 55% tungsten and 45% iron and the other composed of 95.5% tungsten and 4.5% of the polymer nylon 6. Our objective was to determine if exposure of game-farm mallards (*Anas platyrhyncos*) to the two candidate shot caused any deleterious effects. We assessed shot toxicity by measuring mortality, determining hematocrit (HCT), determining the concentration of hemoglobin (Hb), measuring whole-blood delta-aminolevulinic acid dehydratase (ALAD) activity, and determining plasma chemistries. We monitored body weight at days 15 and 30 of the trial and evaluated organ weights, gross pathology and histopathology of selected tissues, and determined metal residues in liver, kidneys, and femur when we terminated the study.

MATERIALS AND METHODS

Forty male and 40 female 6-mo-old game-farm mallards with plumage and body conformation resembling wild mallards were randomly housed in a minimally heated pole barn in individual cages measuring 0.914 m long × 0.914 m wide × 0.457 m high from 20 December 1995 to 15 February 1996 at Michigan State University's Poultry Science Research and Teaching Center (East Lansing, Michigan, USA). The 3-wk acclimation period was from 20 December 1995 to 15 January 1996 and the 30-day trial was from 16 January 1996 to 15 February 1996. We provided pelleted duck grower ration (Purnia Mills, St. Louis, Missouri, USA) and water *ad libitum*. The temperature, which was continuously monitored, was maintained above 0 C by a propane gas heater suspended from the ceiling in the middle of the room. Photoperiod was controlled by a timer on lights and was adjusted weekly to mimic the natural photoperiod for the experimental study site.

Sixteen ducks (eight males and eight females per group) were randomly assigned to one of five treatments (shot types); control, steel, lead, tungsten-iron, and tungsten-polymer. On the first day of the dosing, each duck was weighed to the nearest gram. Ducks dosed with steel and lead received eight #4 pellets and ducks dosed with tungsten-iron and tungsten-polymer received eight BB-size pellets. Pellets were introduced into the proventriculus by means of a funnel and 21.6 cm long latex tube (0.953 cm I.D.) through the esophagus. About 5 ml of water helped flush shot into the proventriculus.

Control ducks were sham-dosed with the same technique.

We observed all ducks twice daily to assess well-being. We noted in the daily log clinical signs, including inappetence, apparent weight loss, ataxia, lethargy, and discolored excreta. We cleaned screens suspended underneath each cage daily, and we examined excreta for expelled shot.

On day seven of the trial (23 January 1996), we transported the ducks to the Michigan State University Large Animal Veterinary Clinic (East Lansing, Michigan, USA) for fluoroscopy to determine shot retention. We collected blood from the brachial vein into one 3 ml Vacutainer® tube (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA) containing EDTA and two 3 ml Vacutainer® tubes containing sodium heparin on days 15 and 30 (31 January and 15 February 1996) of the trial. We shipped cooled samples of whole blood and samples of frozen plasma to the Division of Comparative Pathology (University of Miami School of Medicine, Miami, Florida, USA) for determination of HCT, Hb concentration, whole-blood ALAD activity, and plasma clinical chemistries. HCT was determined by drawing approximately 50 µl whole blood from the Vacutainer® tube containing EDTA into a microhematocrit tube. Tubes were sealed and centrifuged in an IEC MB microhematocrit centrifuge (Needham, Massachusetts, USA) for 5 min. HCT was measured using an IEC microcapillary reader (Needham, Massachusetts, USA). Hemoglobin was determined by removing 100 µl whole blood from the Vacutainer® tube containing EDTA and placing it in a plastic 96-well microtiter plate. Fifty µl of lysis solution (ammonium chloride) was added to each well and the solutions mixed by automatic pipet for 10 sec. After incubation at room temperature for 1 min, the plate was centrifuged at 1,200 rpm for 10 min to pellet red blood cell nuclei and other debris. The supernatant was removed and hemoglobin was measured using a Leica hemoglobinometer (Buffalo, New York, USA). Hemoglobin was quantitated as g/dl (× 1.5 for dilution factor). ALAD (expressed in ALAD units) was measured according to the protocol of Burch and Siegel (1971) and Dieter and Finley (1979). ALAD units equal (corrected absorbance × 12,500)/HCT. Plasma samples were analyzed using a Johnson and Johnson 700XR automated analyzer (Rochester, New York, USA). Control sera samples were run daily prior to analysis to maintain a check on instrument calibration.

On day 30 of the trial, ducks still alive were weighed, and we collected a blood sample as described. We then killed ducks by cervical dislocation and performed necropsies on each. The necropsy procedure included a gross examination of all body cavities and organs. We opened gizzards to inspect for cracked and discolored mucosa and to recover shot. We counted and stored retrieved shot to later weigh and determine the amount of shot erosion. We removed the brain, heart, liver, spleen, kidneys, and testes and weighed them. We collected and stored samples of liver and kidneys from each duck in a 10% formalin-saline solution to examine them for histopathology. The right femur and the remaining portions of the liver and kidneys were frozen and shipped to Anatech Laboratories (Ludington, Michigan, USA) for analyses of metals. Tissues were digested using EPA method 200.3 (U.S. Environmental Protection Agency, 1991). Iron and tungsten were analyzed by Inductively Coupled Argon Emission Plasma Spectroscopy (ICAP) following EPA method 200.7, revision 4.4 (U.S. Environmental Protection Agency, 1994) and lead was analyzed by Graphite Furnace Atomic Absorption (GFAA) based on EPA method 239.2 (U.S. Environmental Protection Agency, 1979). A matrix spike was performed for every 20 liver samples. A matrix spike as well as an analytical spike were performed for every 20 femur samples because the matrix spikes were performed on chicken femurs instead of mallard femurs. Only analytical spikes were performed for kidney samples because there was not enough tissue to run matrix spikes. Average percent recovery of iron, tungsten, and lead were 103%, 96%, and 98%, respectively.

All statistical analyses were performed using SAS® software (SAS; Statistical Analysis Systems, Release 612, Cary, North Carolina, USA). Differences between treatment group means were statistically significant based on a Type I error rate of 0.05. A Fisher's exact test was used to assess mortality associations with treatment. Body weights, plasma, and whole-blood parameters were analyzed under a three-way ANOVA involving the factors treatment and sex, with repeated measurements on animals over a third factor, days. SAS PROC MIXED was used to model a first-order autoregressive correlation structure for repeated measurements over days within animals, as residuals involving measurements taken at adjacent time periods are more likely to be closely related than measurements taken further apart in time (Gill, 1990). Organ weights, concentrations of metal residues in tissues, and percent shot erosion were analyzed under a two-way ANOVA model involving the factors treatment and sex. Treatment means were reported separately for each sex, and/or day, if treatment by sex and/or treatment by day interactions were statistically significant. Oth-

TABLE 1. The effect of treatment shot on body weights (g) of mallards on a 30-day dosing test.[a]

Treatment	Day 0	Day 15	Day 30	% Change[b]
Control	1,122 ± 27	1,136 ± 27^{A}	1,173 ± 27^{A}	+4.5
Steel	1,050 ± 27	1,030 ± 27^{A}	1,059 ± 27^{AB}	+0.9
Lead	1,072 ± 27	899 ± 27^{B}	961 ± 32^{B}	−10.3
Tungsten-Iron	1,069 ± 27	1,052 ± 27^{A}	1,099 ± 27^{AB}	+2.3
Tungsten-Polymer	1,070 ± 27	1,078 ± 27^{A}	1,109 ± 27^{A}	+3.6

[a] Data presented as mean ± SE. Sample size is 16 for all groups except for lead on day 15, which is 13, and lead on day 30, which is 8. Means with different superscripts (A, B) are statistically different within the column ($P < 0.05$).
[b] Change in body weight is presented as the mean change in body weight from day 0 to day 30.

erwise, reported treatment means and differences were based on pooling information over the sexes and/or days. To control for experimental error rates, a Bonferroni adjustment was used to test comparisons between means based on the total number of pairwise comparisons. Homogeneity of variance was assessed by residual plots. Residual plots for plasma chemistries indicated heterogeneity of variance; therefore those data were log-transformed and the heterogeneity of variance subsequently was alleviated. The reported means and 95% confidence intervals for treatment means of plasma chemistries were back (anti-log) transformed to the scale of observation. In the following sections reference to significant differences (whether higher or lower) across compared values indicate statistical differences at $P \leq 0.05$, unless otherwise indicated.

RESULTS

Mortality

Eight of 16 (50%) mallards, including five (63%) males and 3 (38%) females, dosed with lead shot died during the 30 day study. Average time to death was 18 ± 2.1 ($\bar{x}$ ± SE) days for males and 15 ± 2.6 days for females; range 10 to 25 days for sexes combined. Average weight loss of ducks that died was slightly over 30%. The mortality rate of lead-dosed ducks was significantly higher than for ducks in the other treatment groups, which experienced no mortality.

Clinical signs

Only lead-dosed ducks manifested obvious clinical signs of poisoning during the trial. Green-stained excreta was noted first and was apparent in 50% of the ducks within 24 hr of dosing. By four days post-dosing, all lead-dosed ducks were eliminating green-stained excreta. Of the five males that died, the three that died the earliest (days 11, 15, and 17) had no other clinical signs. The two males that died on days 20 and 25 developed progressive ataxia 2 to 4 days before they died. The three surviving males appeared normal during the last 9 days of the trial. Two of three lead-dosed females that died (days 10 and 15) had no clinical signs other than green-stained excreta before they died. The female that died on day 21 appeared ataxic the day before it died. Of the five females that survived the trial, two appeared to be normal by day 15 with the exception of an occasional appearance of green-stained excreta. The other three females had varying degrees of ataxia, which became increasingly severe; their condition seemed to improve near the end of the study.

Body weights

Body weights of lead-dosed mallards were significantly lower than those of ducks in the control, steel, tungsten-iron, and tungsten-polymer treatment groups at day 15 and significantly lower than body weights of ducks in the control and tungsten-polymer groups at day 30 (Table 1). No differences in body weights at day zero were detected. During the trial, control ducks and ducks receiving steel shot, tungsten-iron shot, and tungsten-polymer shot gained a slight amount of weight. Lead-dosed ducks that survived the trial lost 10% of their pre-dosing body weight.

TABLE 2. The effect of treatment shot on whole-blood parameters of mallards on a 30-day dosing test.[a]

Treatment	Hematocrit		Hemoglobin		ALAD
	Day 15	Day 30	Day 15	Day 30	
Control	45.1 ± 1.4 (16)	34.6 ± 1.7* (12)	14.1 ± 0.4^{A} (16)	11.1 ± 0.5* (12)	74.8 ± 4.0^{AB} (26)
Steel	44.9 ± 1.4 (16)	38.1 ± 1.5* (14)	14.4 ± 0.4^{A} (16)	12.3 ± 0.5* (14)	80.0 ± 3.8^{A} (29)
Lead	34.5 ± 1.7^{B} (12)	35.4 ± 2.2 (7)	11.2 ± 0.5^{B} (12)	11.9 ± 0.7 (7)	57.5 ± 5.3^{B} (16)
Tungsten-Iron	46.8 ± 1.4^{A} (16)	36.1 ± 1.4* (16)	14.6 ± 0.4^{A} (16)	12.0 ± 0.4* (16)	73.6 ± 3.7^{AB} (30)
Tungsten-Polymer	46.9 ± 1.5^{A} (14)	35.8 ± 1.4* (16)	15.0 ± 0.5^{A} (14)	11.8 ± 0.4* (16)	75.4 ± 3.8^{AB} (29)

[a] Data presented as mean ± SE. Numbers in parentheses refer to sample size. Hematocrit (HCT) is expressed as percentage of packed red blood cell volume; hemoglobin is expressed as g/dL; ALAD refers to δ-aminolevulinic acid dehydratase, which is expressed as ALAD units of activity = (corrected absorbance × 12,500)/HCT. Means with different letter superscripts (A, B) are statistically different within the column ($P < 0.05$). Superscript * refers to means that are statistically different from the mean for the previous day ($P < 0.05$).

HCT, Hb concentration, and ALAD activity

Ducks dosed with lead had significantly lower HCT values and Hb concentrations at day 15 when compared to ducks in the other treatment groups, which were not significantly different from one another (Table 2). No significant differences in HCT and Hb concentrations were detected among treatment groups at day 30, but both parameters decreased significantly between day 15 and day 30 in all treatment groups except the lead-dosed group. Whole-blood ALAD activity did not vary statistically over time, thus a single value for each treatment group is presented in Table 2. Lead-dosed ducks had significantly lower ALAD activity compared to the steel-dosed group only.

Plasma chemistries

The plasma activities of creatine phosphokinase and aspartate aminotransferase were significantly higher in lead-dosed ducks compared to ducks in the other groups (Table 3). Lactate dehydrogenase activity was significantly higher at day 15 in ducks dosed with lead when compared to the other groups, but not at day 30 (Ta-

TABLE 3. The effect of treatment shot on creatine phosphokinase and aspartate aminotransferase of mallards on a 30-day dosing test.[a]

Treatment	Creatine phosphokinase (U/L)	Aspartate aminotransferase (U/L)
Control	233.6^{A} (168.8–323.2)	24.0^{A} (19.2–30.1)
Steel	155.8^{A} (112.6–215.6)	23.2^{A} (18.6–29.0)
Lead	616.9^{B} (408.4–931.9)	46.3^{B} (34.9–61.6)
Tungsten-Iron	178.4^{A} (128.9–246.9)	21.7^{A} (17.3–27.1)
Tungsten-Polymer	195.6^{A} (141.3–270.6)	23.4^{A} (18.7–29.3)

[a] Data presented as means (95% confidence intervals). Sample size is 32 for all groups except lead, which is 21. Means with different superscripts (A, B) are statistically different within the column ($P < 0.05$).

TABLE 4. The effect of treatment shot on plasma lactate dehydrogenase and alanine aminotransferase of mallards on a 30-day dosing test.[a]

Treatment	Lactate dehydrogenase (U/L) Day 15	Lactate dehydrogenase (U/L) Day 30		
Control	733.0[A] (591.5–908.3)	949.8 (766.4–1,176.9)		
Steel	741.1[A] (598.0–918.3)	894.2 (721.6–1,108.1)		
Lead	2,078.3[B] (1,637.1–2,638.4)	1,368.0 (1,007.9–1,856.6)		
Tungsten-Iron	778.0[A] (627.9–964.1)	835.6 (674.3–1,035.5)		
Tungsten-Polymer	758.3[A] (612.0–939.7)	862.4 (695.9–1,068.6)		
Treatment	Alanine aminotransferase (U/L) Day 15	Alanine aminotransferase (U/L) Day 30	Alanine aminotransferase (U/L) Males	Alanine aminotransferase (U/L) Females
Control	6.23[A] (4.32–8.99)	8.12 (5.63–11.71)	4.93[A] (3.26–7.46)	10.25[A] (6.78–15.50)
Steel	5.27[A] (3.65–7.60)	5.33 (3.70–7.70)	6.63[AB] (4.38–10.02)	4.24[A] (2.80–6.41)
Lead	26.83[B] (17.84–40.35)	11.90 (7.02–20.16)	17.66[B] (10.31–30.26)	18.08[B] (11.02–29.65)
Tungsten-Iron	5.53[A] (3.83–7.97)	5.31 (3.68–7.66)	6.58[AB] (4.35–9.96)	4.45[A] (2.94–6.74)
Tungsten-Polymer	5.14[A] (3.56–7.41)	7.07 (4.90–10.20)	5.69[A] (3.76–8.61)	6.38[A] (4.22–9.65)

[a] Data presented as means (95% confidence intervals). Sample size for lactate dehydrogenase and alanine aminotransferase (days) is 16 for all groups except lead, which is 13 for day 15 and 8 for day 30. Sample size for alanine aminotransferase (sexes) is 16 for all groups except lead, which is 10 for males and 11 for females. Means with different superscripts (A, B) are statistically different within the column ($P < 0.05$).

ble 4). Significant treatment by day and treatment by sex interactions existed for plasma alanine aminotransferase activity. Enzyme activity was significantly higher in lead-dosed ducks at day 15 but not at day 30. In males dosed with lead, alanine aminotransferase activity was significantly higher when compared to male ducks in the control and tungsten-polymer groups, whereas enzyme activity in females dosed with lead was significantly higher compared to enzyme activity for females in the control, steel, tungsten-iron, and tungsten-polymer groups.

Gross pathology

Gizzards of lead-dosed ducks that died had either discolored mucosal linings or multiple linear erosions (three males and two females) and, except for two ducks, gizzard erosion occurred in ducks that died during the first one-half of the trial. Ducks that died between days 16 to 25 were emaciated and had little breast muscle (two males and one female). Of the eight surviving lead-dosed ducks, one male and one female were emaciated at the time of necropsy, one female had a fatty liver, whereas the other five ducks (two males and three females) appeared normal. Four control ducks (one male and three females) had moderately fatty livers, whereas all ducks in the steel, tungsten-iron, and tungsten-polymer shot groups appeared normal.

Relative organ weights

Lead-dosed ducks had statistically higher relative heart weights compared to ducks in the tungsten-polymer group (Ta-

ble 5). Relative kidney weights were significantly higher in the lead-dosed ducks compared to relative kidney weights in the other treatment groups and relative liver weights were significantly higher in the ducks dosed with lead compared to ducks in the steel, tungsten-iron, and tungsten-polymer groups.

Histopathology of kidney and liver

Eight lead-dosed ducks developed renal nephrosis ranging from mild to severe with accompanying mild to severe hepatic biliary stasis. All of these ducks died during the trial (days 10 to 25). The kidneys of the eight surviving lead-dosed ducks were normal. Six of the eight surviving ducks dosed with lead had mild to severe hepatic biliary stasis. Five tungsten-iron dosed ducks and three tungsten-polymer dosed ducks developed mild hepatic biliary stasis.

Metal residues in tissues

Lead-dosed ducks had significantly higher concentrations of iron in the femur when compared to ducks in the control and tungsten-polymer groups and significantly higher concentrations of lead in the femur than ducks in the other treatment groups (Table 6). Tungsten was detected in the femur of all the ducks in the tungsten-iron group and in two ducks of the tungsten-polymer group only; the concentration in tungsten-iron dosed ducks was significantly higher than the concentration in tungsten-polymer dosed ducks.

The concentration of hepatic iron was significantly higher in the lead-dosed ducks compared to ducks in the control and tungsten-polymer groups and ducks in the steel and tungsten-iron groups had significantly higher hepatic iron concentrations than ducks in the control group (Table 6). Lead-dosed ducks had significantly higher concentrations of lead in the liver compared to ducks in the other groups. Tungsten was detected in the liver of the tungsten-iron dosed ducks only.

Iron concentrations in the kidneys were significantly higher in steel-dosed ducks

TABLE 5. The effect of treatment shot on relative organ weights of mallards on a 30-day dosing test.[a]

Treatment	Brain	Heart	Kidneys	Liver	Spleen	Testes
Control	0.38 ± 0.03	0.84 ± 0.03^{AB}	0.53 ± 0.02^{A}	1.94 ± 0.10^{AB}	0.046 ± 0.003	1.23 ± 0.40
Steel	0.41 ± 0.03	0.89 ± 0.03^{AB}	0.53 ± 0.02^{A}	1.65 ± 0.10^{A}	0.044 ± 0.003	1.40 ± 0.40
Lead	0.39 ± 0.05	0.98 ± 0.04^{B}	0.66 ± 0.03^{B}	2.33 ± 0.15^{B}	0.046 ± 0.005	1.10 ± 0.80
Tungsten-Iron	0.41 ± 0.03	0.88 ± 0.03^{AB}	0.50 ± 0.02^{A}	1.64 ± 0.10^{A}	0.039 ± 0.003	1.21 ± 0.40
Tungsten-Polymer	0.45 ± 0.03	0.82 ± 0.03^{A}	0.51 ± 0.02^{A}	1.76 ± 0.10^{A}	0.039 ± 0.003	1.47 ± 0.40

[a] Data are organ weights expressed as % body weight and are presented as mean ± SE. Sample size for all parameters is 16 except for control spleen weight, which is 15, lead brain, heart, kidneys, liver, and spleen weights, which is 8, and lead testes weight, which is 2. Means with different superscripts (A, B) are statistically different within the column ($P < 0.05$).

TABLE 6. The effect of treatment shot on metal residues (mg/kg, dry weight) in tissues of mallards on a 30-day dosing test.[a]

Tissue/Treatment	Metal		
	Iron	Lead	Tungsten[b]
Control	78.5 ± 5.8^{A}	3.5 ± 39.0^{A}	ND
Steel	93.8 ± 5.8^{AB}	1.0 ± 39.0^{A}	ND
Lead	107.6 ± 5.8^{B}	250.6 ± 39.0^{B}	ND
Tungsten-Iron	89.3 ± 5.8^{AB}	0.9 ± 39.0^{A}	10.3 ± 0.7^{A}
Tungsten-Polymer	76.8 ± 5.8^{A}	3.5 ± 39.0^{A}	4.3 ± 0.7^{B}
Liver			
Control	892.2 ± 263.2^{A}	0.3 ± 9.7^{A}	ND
Steel	2,006.3 ± 263.2^{BC}	0.1 ± 9.7^{A}	ND
Lead	3,062.6 ± 263.2^{B}	78.3 ± 9.7^{B}	ND
Tungsten-Iron	2,220.6 ± 263.2^{BC}	0.1 ± 9.7^{A}	14.1 ± 0.6
Tungsten-Polymer	1,421.3 ± 263.2^{AC}	0.1 ± 9.7^{A}	ND
Kidneys			
Control	411.9 ± 16.6^{AC}	1.1 ± 22.2^{A}	ND
Steel	481.3 ± 16.6^{B}	0.2 ± 22.2^{A}	ND
Lead	366.9 ± 16.6^{A}	256.3 ± 22.2^{B}	ND
Tungsten-Iron	466.3 ± 16.6^{C}	0.2 ± 22.2^{A}	6.8 ± 0.2^{A}
Tungsten-Polymer	400.0 ± 16.6^{AC}	0.4 ± 22.2^{A}	2.4 ± 0.2^{B}

[a] Data presented as mean ± SE. Sample size is 16 for all groups. Means with different superscripts (A, B, C) are statistically different within the column ($P < 0.05$).

[b] Tungsten detection limit is 4.0, 0.8, and 2.0 mg/kg, dry weight, for the femur, liver, and kidneys, respectively.

compared to the other groups and ducks in the tungsten-iron group had significantly higher renal iron concentrations than lead-dosed ducks (Table 6). Lead-dosed ducks had statistically higher concentrations of lead in the kidneys than did ducks in the other groups. Tungsten was detected in the kidneys of all the ducks dosed with tungsten-iron and in the kidneys of two ducks dosed with tungsten-polymer shot; the concentrations in the tungsten-iron dosed ducks were significantly higher than the concentrations in the ducks dosed with tungsten-polymer shot.

Shot recovered and percent shot erosion

Fluoroscopy of the mallards on day seven of the trial indicated that all ducks receiving shot had retained all eight pellets administered on day one with the exception of one steel-dosed duck that had voided two pellets (Table 7). Most shot administered (≥75%) was recovered at necropsy regardless of type. Only one duck, a lead-

TABLE 7. Erosion of shot during a 30-day dosing test.[a]

Treatment	Shot weight at day 0	Number shot at necropsy	Shot weight at necropsy	Percent shot erosion
Control	—	—	—	—
Steel	0.1495 ± 0.0011^{A}	7.4 ± 0.4	0.1009 ± 0.0122^{A}	32.50 ± 3.90^{A}
Lead	0.1960 ± 0.0011^{B}	6.8 ± 0.4	0.0984 ± 0.0127^{A}	49.87 ± 4.04^{B}
Tungsten-Iron	0.5298 ± 0.0011^{C}	7.7 ± 0.4	0.2383 ± 0.0122^{B}	55.02 ± 3.90^{B}
Tungsten-Polymer	0.5513 ± 0.0011^{D}	7.6 ± 0.4	0.1087 ± 0.0122^{A}	80.35 ± 3.90^{C}

[a] Data presented as mean ± SE. On day 0, male and female mallards were dosed with 8 pellets of the appropriate shot. Values reported reflect the average weight of individual pellets (g) per duck. Sample size is 16 ducks for each parameter. Means with different superscripts (A, B, C, D) are statistically different within the column ($P < 0.05$).

dosed female, had voided all shot at necropsy. No shot was found in the excreta of any ducks that we examined daily during the trial. Steel shot eroded the least when compared statistically to the other groups, whereas, tungsten-polymer shot eroded the most. Lead and tungsten-iron shot eroded significantly less than tungsten-polymer shot, but more than steel shot. If erosion of lead shot is considered separately for those ducks that died during the trial from those that survived, then percent erosion of shot retrieved from ducks that died was 34% compared to 71% for lead-dosed ducks that survived.

The steel shot recovered maintained their original sphericity, whereas most lead pellets were flattened and oval. The tungsten-iron shot were spherical with small dimples on the surface, whereas the tungsten-polymer shot were also flattened and disk-like.

DISCUSSION

Mortality

Only lead-dosed ducks died during the 30-day trial (50% mortality within 10 to 25 days of dosing). These results are generally similar to those reported in other studies involving lead-dosed waterfowl, although differences in shot size, number of shot administered, diet, and environmental conditions preclude direct comparison. Lead-dosed ducks maintained on a pelleted feed, as in our study, tend to have a lower mortality rate than ducks fed a corn diet. Jordan and Bellrose (1950) reported that only 33% of Pekin ducks (*Anas platyrhynchos*) dosed with 25 #4 lead shot died within 17 days when fed pelleted feed. Rattner et al. (1989) reported no mortality after 14 days in pen-raised and wild black ducks (*Anas rubripes*) and game-farm and wild mallards maintained on pelleted food that were dosed with a single #4 lead shot. The same ducks were then dosed with either two or four #4 lead shot and maintained on a pelleted diet for another 49 days. Mortality of wild black ducks was 40% and that of wild mallards was 45%. In contrast, Jordan and Bellrose (1950) reported that 86% of Pekin ducks dosed with 25 #4 shot and maintained on a corn diet died within 17 days. Grandy et al. (1968) and Longcore et al. (1974) reported 100% mortality within 7 to 28 days in pen-raised mallards maintained on corn that were dosed with 8 #6 lead shot. Sanderson et al. (1992) dosed mallards with two, four, or eight #2 lead shot or four #2 lead shot plus four #2 bismuth shot and maintained the ducks for up to 30 days on a diet of shelled corn. Mortality was 95% with only two ducks (dosed with two #2 lead shot) surviving.

In our study, none of the ducks dosed with steel, tungsten-iron, or tungsten-polymer shot died. In a similar toxicity study in which mallards were dosed with 12 to 17 pellets (an average of 1.03 gm which is equivalent to five #4 lead shot) composed of 39% tungsten, 44% bismuth, and 16% tin, no mortalities were reported during the 32-day trial (Ringelman et al., 1993). In the latter study, ducks received approximately 0.4 gm tungsten, whereas in our study, ducks were dosed with an average of either 4.2 gm tungsten (4.43 gm of tungsten-polymer shot × 95.5% tungsten) or 2.3 gm tungsten (4.24 gm of tungsten-iron shot × 55% tungsten).

Tungsten has been reported to cause mortality in birds. Nell et al. (1980) dosed broiler cockerels with sodium tungstate by intramuscular injection at 5 mg tungsten from day one to day 11, 10 mg from day 12 to day 21, and 20 mg from day 22 to day 35. They reported that four of 10 birds died on day 29 of the trial. The total quantity of tungsten administered to the birds over the 35-day period was 0.44 gm. If an average erosion rate of 80% for the tungsten-polymer shot is used, then the ducks in the tungsten-polymer group in our study were exposed to 3.4 gm tungsten. Since the tungsten-iron shot had an erosion rate of 56%, the ducks in the tungsten-iron group were exposed to approximately 1.3 gm tungsten. However, in the study of chickens the tungsten was in a

soluble form injected in animals that were relatively small, which might enhance toxicity.

Clinical signs

Ducks receiving lead shot were the only ones that had obvious clinical signs. These signs (green-stained excreta and, in some instances, ataxia) are typical of birds intoxicated with lead (Friend, 1987; Rattner et al., 1989). Lead-dosed ducks that survived the trial appeared relatively normal at necropsy. Ducks dosed with tungsten-iron and tungsten-polymer shot in our study appeared normal throughout the trial, which agrees with results of Ringelman et al. (1993) for mallards dosed with tungsten-bismuth-tin shot. Clinical signs for chickens administered tungsten include anorexia, reduced weight gain, diarrhea, and labored breathing before death (Nell et al., 1980).

Body weights

Body weights of ducks surviving the 30-day trial changed little. Control, steel, tungsten-iron, and tungsten-polymer dosed ducks gained a slight amount of weight (0.9 to 5.8%), whereas lead-dosed ducks lost approximately 10% of their body weight. Our results are similar to those reported in other studies. Sanderson et al. (1992) indicated that the two mallards that survived dosing with two #2 lead pellets lost 16% of their body weight at the end of the 30-day trial compared to a 4% body weight loss for the non-dosed controls. Mallards dosed with 12 to 17 pellets of tungsten-bismuth-tin shot gained a similar amount of weight as controls (Ringelman et al., 1993).

HCT, Hb concentration, and ALAD activity

Depressions in HCT, Hb concentration, and ALAD activity indicate lead toxicity. Lead interacts with the erythrocyte resulting in increased fragility of the membrane and shortening the life of the erythrocyte. Additionally, lead inhibits ALAD, a key enzyme in the synthesis of heme which is an integral component of Hb. The combined effect of lead on erythrocytes and heme synthesis results in lead-induced anemia, which is demonstrated by decreased HCT and Hb concentration (Goyer, 1996).

In our study, HCT and Hb concentration were statistically lower in the lead-dosed ducks compared to ducks in the other groups at day 15 but not day 30. However, HCT and Hb concentration decreased significantly between day 15 and day 30 in all groups except the lead group which may have prevented detecting a lead-induced effect on HCT and Hb concentration at day 30. The decrease in HCT and Hb concentration could have been due to degradation of blood samples during shipping. However, if this was the case, then all blood samples should have been affected in a similar manner and a lead-induced effect would have been detected if present. An alternative explanation is that HCT and Hb concentration in surviving lead-dosed ducks had, in fact, recovered. Sanderson et al. (1992) dosed mallards maintained on corn with either two, four, or eight #2 lead shot or four #2 lead shot plus four #2 bismuth shot and HCT significantly decreased by 36% in ducks that died. In the two lead-dosed ducks that survived the trial, HCT returned to pretest values.

In our study, HCT and Hb concentrations in ducks receiving tungsten-iron or tungsten-polymer shot were not statistically different at 15 and 30 days post-dosing from values for the control and steel-dosed ducks. These results agree with findings of Ringelman et al. (1993), who dosed mallards with 12 to 17 pellets (equivalent in mass to five #4 lead shot) of shot composed of tungsten, bismuth, and tin and HCT and Hb concentrations were unaffected over the 32-day trial. ALAD activity was statistically lower in lead-dosed ducks compared to ducks in the steel-dosed group only. It is somewhat surprising that ALAD activities in lead-dosed ducks were not inhibited to a greater extent. Rattner et al. (1989) reported a transient decrease

in ALAD activity (>90%) over 14 days in black ducks and mallards maintained on pelleted feed dosed with one #4 lead shot. Ducks were then redosed with either two or four #4 lead shot and observed for an additional four weeks. ALAD activity continued to be inhibited by more than 90%. However, Bakalli et al. (1995) reported that in broiler chicks given 50 ppm lead acetate via the feed for 42 days, blood ALAD activity was quickly and statistically depressed (69% after seven days) but returned to 90% of control activity within 7 days of the birds being placed on clean feed. It is possible, in our study, that ALAD activity in lead-dosed ducks was recovering in the surviving ducks at the end of the trial, and thus, masked an effect that might have been seen had we been able to take blood samples from all lead-dosed ducks prior to death.

Plasma chemistries

Lead shot caused significant increases in plasma activities of creatine phosphokinase, aspartate aminotransferase, lactate dehydrogenase and alanine aminotransferase. Although an increase in the plasma value of one of these enzymes is not necessarily diagnostic of an effect, the fact that the activities of all four enzymes were elevated in the lead-dosed ducks suggests substantial liver damage (Campbell and Coles, 1986). No statistical differences existed in plasma parameters among the control, steel, tungsten-iron, and tungsten-polymer groups. The results in our study agree with those of Ringelman et al. (1993), who reported that the administration of shot composed of tungsten-bismuth-tin had no effect on plasma chemistry variables in mallards during a 32-day test.

Gross pathology

The linings of gizzards of several ducks (five of 16) in the lead-dosed group were discolored and eroded. These effects have been described in naturally occurring and experimentally-induced cases of lead toxicosis (Slauson and Cooper, 1990; Alden and Frith, 1991; Popp and Cattley, 1991). No gizzards from ducks in the other four groups had gross lesions. Fatty liver was noted in ducks in both the control and lead-dosed groups and therefore was not considered a diagnostic lesion. Fatty liver is considered a non-specific change, often resulting from hepatocellular damage (i.e., toxicosis), mobilization of internal fat stores (i.e., inadequate energy intake), or a variety of other metabolic conditions (Slauson and Cooper, 1990). The lack of gross changes in mallards dosed with tungsten-iron and tungsten-polymer shot agrees with findings of Ringelman et al. (1993).

Organ weights

In lead-dosed ducks, relative kidney weights were statistically higher when compared to the other groups, relative liver weights were higher when compared to the steel, tungsten-iron, and tungsten-polymer groups, and relative heart weights were higher than the heart weights of tungsten-polymer ducks. Sanderson et al. (1992) reported no differences in absolute liver weights in lead-dosed mallards when compared to control ducks except for ducks dosed with two #2 lead pellets, which had statistically lighter livers. However, these authors commented that liver weights of lead-poisoned waterfowl are difficult to evaluate because the organ can be either enlarged or atrophied.

Histopathology of kidney and liver

Microscopic renal lesions were found only in ducks from the lead-treated group, and only in ducks that died before the trial ended. These renal lesions were characterized by acute tubular necrosis (nephrosis), and usually were accompanied by a variable number of eosinophilic intranuclear inclusions within tubular epithelial cells. Previously, these changes have been reported as associated with lead toxicoses for many animal species (Alden and Frith, 1991). Because renal lesions were not de-

tected in the eight surviving lead-dosed ducks when the trial ended, it seems that toxic nephrosis was an acute or subacute effect of lead toxicosis that occurred while the ducks were actively absorbing lead shot in the gizzard. Whether the tubular necrosis was caused by direct toxic effects of lead on the tubular epithelium, or was mediated through Hb released during periods of intravascular hemolysis, or a combination of the two is unknown; both processes produce this lesion (Alden and Frith, 1991). The red blood cell parameters did indicate anemia at day 15 of the study, which may have resulted from lead-induced intravascular hemolysis. The absence of renal lesions in the steel, tungsten-iron, and tungsten-polymer dosed ducks suggests, either that these metals are non-toxic to the renal tubular epithelium, or that these substances were not absorbed in sufficient quantities to produce renal tubular toxicity.

The hepatic histologic lesions were categorized as (a) non-specific fatty accumulation or (b) substantial biliary stasis. Intrahepatocellular fatty vacuolation was present in at least half of the ducks in each of the five experimental groups. As previously discussed, fatty accumulation can be from a variety of causes and was judged an incidental finding in this study. The accumulation of bile within hepatocytes or within canaliculi is also somewhat non-specific, as it may occur because of obstruction of bile ducts, or primary hepatocellular disfunction (Popp and Cattley, 1991). In our study, no evidence of cholelithiasis or other obstructive biliary disease was detected, thus biliary stasis was considered evidence of hepatocellular dysfunction. The degree of biliary stasis was graded, and only the lead-dosed group had moderate to severe biliary stasis, although several individual ducks in the tungsten-iron and tungsten-polymer group also exhibited mild biliary stasis. None of the ducks in the control and steel-dosed groups had biliary stasis. Morphologic appearance of biliary stasis and elevated plasma hepatic enzyme activities (indicating hepatocellular damage) were correlated in the lead-dosed group. The hepatic biliary stasis was considered to be a morphologic indicator of hepatocellular damage in our study and significant evidence of this damage was present only in the lead-dosed group. Because biliary stasis was observed in some of the tungsten-iron and tungsten-polymer ducks, but not in the control ducks and ducks dosed with steel shot, it is apparent that the experimental shot was inducing a slight pathological condition, that was not evident in control ducks.

Tissue metal analysis

The concentration of iron in the femur and liver tended to be higher in ducks dosed with lead, steel, and tungsten-iron shot when compared to ducks in the control and tungsten-polymer groups, whereas the concentration of iron in the kidneys was elevated in the ducks dosed with steel and tungsten-iron. The high levels of iron in the liver of lead-dosed ducks agrees with results reported by Sanderson et al. (1992), who indicated that the concentrations of iron in the liver and muscle of lead-dosed mallards were higher when compared to ducks not dosed with lead. They attributed the increase to a lead-induced interference of heme synthesis, which caused an accumulation of iron in the liver and muscle.

All treatment groups, including the ducks that were sham-dosed, had detectable concentrations of lead in the femur, liver, and kidneys. Concentrations of lead in the ducks dosed with lead shot, however, were 10 fold higher when compared to the other four groups. Sanderson et al. (1992) reported concentrations of lead in the femur of all mallards on trial regardless of treatment with the highest concentrations observed in the ducks receiving lead shot. Lead was not detected in the liver and muscle of either control ducks or ducks dosed with steel shot in the latter study.

Tungsten was detected in the femur, liv-

er, and kidneys of all the ducks dosed with tungsten-iron shot and in the femur and kidneys of only two ducks dosed with tungsten-polymer shot. The bone, liver, and kidneys are principle sites of tungsten deposition in a number of different species (Kinard and Aull, 1945; Wase, 1956; Kaye, 1968; Bell and Sneed, 1970; Aamodt, 1975). The primary site of tungsten deposition is evidently species-specific. In our study, the highest concentrations of tungsten were detected in the liver, whereas, Ringelman et al. (1993), who dosed mallards with tungsten-bismuth-tin shot, did not detect tungsten in either the liver or kidneys, which they examined.

Shot recovered and percent shot erosion

Recovery of shot from ducks dosed with steel, lead, tungsten-iron, and tungsten-polymer shot was 93%, 85%, 96%, and 95%, respectively. These rates are similar to the findings of Sanderson et al. (1992) for steel and lead shot but higher than the retention rate of the bismuth shot. Ringelman et al. (1993) indicated that by 11 days post-dosing, only 44% of the tungsten-bismuth-tin shot had been retained. If shot erosion is based on the average weight of the pellets actually recovered, then the erosion of the tungsten-polymer shot was substantially greater than the erosion of the steel, lead, and tungsten-iron shot. Steel pellets recovered at necropsy retained their original sphericity. Lead, tungsten-iron, and tungsten-polymer shot were sometimes difficult to find in gizzard contents because of their smaller size and, in the case of the lead and tungsten-polymer shot, their flattened, disk-like appearance.

CONCLUSIONS

Male and female mallards administered eight BB-size tungsten-iron and tungsten-polymer shot and maintained for 30 days were not adversely affected based on the variables measured. All ducks survived the 30-day trial with a slight increase in body weight. No significant differences were observed in HCT, Hb concentration, and ALAD activity in the two tungsten shot groups when compared to control and steel-dosed groups. Similarly, no changes were detected in selected plasma chemistry variables. The ducks appeared normal at the time of necropsy on day 30 of the trial, and no changes were detected in weights of organs. Five of 16 tungsten-iron dosed ducks and three of 16 tungsten-polymer dosed ducks manifested a mild hepatocellular biliary stasis, which was not considered deleterious. However, this condition was not observed in the control and steel-dosed ducks. No other histopathological lesions were noted. Tungsten residues were detected in the femur, liver, and kidneys of the tungsten-iron ducks. Concentrations of tungsten only slightly above detection limits were detected in the femur and kidneys of two ducks dosed with tungsten-polymer shot. The erosion rate of tungsten-polymer shot was 25% greater than the erosion rate of tungsten-iron shot, which was 5% and 22% greater than those of lead and steel shot, respectively. Therefore, the results of our study indicate that eight BB-size shot composed of either tungsten-iron or tungsten-polymer did not adversely affect game-farm mallards during the 30-day trial. Because the two formulations of tungsten-shot are non-toxic to mallards after 30 days exposure, they have potential use for waterfowl hunting. Of the two shot tested, tungsten-polymer shot seems preferable because of its high erosion rate and its low concentrations in selected tissues.

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