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A Thirty-Day Dosing Test to Assess the Toxicity of Tungsten-Iron and Tungsten-Polymer Shot in Game-Farm Mallards

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Mary Elissa Kelly

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A THIRTY-DAY DOSING TEST TO ASSESS THE TOXICITY OF TUNGSTEN-IRON AND TUNGSTEN-POLYMER SHOT IN GAME-FARM MALLARDS

By

Mary Elissa Kelly

A THESIS

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ABSTRACT

A THIRTY-DAY DOSING TEST TO ASSESS THE TOXICITY OF TUNGSTEN-IRON AND TUNGSTEN-POLYMER SHOT IN GAME-FARM MALLARDS

By

Mary Elissa Kelly

Groups of eight male and 8 female adult mallards each were dosed with 8 #4 steel shot, 8 #4 lead shot, 8 BBs of tungsten-iron shot, or 8 BBs of tungsten-polymer shot and observed over a 30-day period. An additional 8 males and 8 females received no shot. Mortality, changes in plasma chemistry and whole-blood parameters (Hb, Hct, and ALAD), and histopathological lesions generally occurred in the lead-dosed mallards only. Mild biliary stasis occurred in 5 tungsten-iron birds and 3 tungsten-polymer birds which was considered unremarkable. The results of this study indicate that game-farm mallards dosed with 8 BBs composed of tungsten-iron or tungsten-polymer were not adversely affected during the course of the 30-day trial.

To my parents, Dr. Stephan A. and Susan L. Kelly

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Introduction

Lead poisoning affects every major species of waterfowl in North America and also a wide range of other birds such as predatory species. Within the United States, lead poisoning is common in the mallard (*Anas platyrhynchos*), northern pintail (*Anas acuta*), redhead (*Aythya americana*), scaup (*Aythya marila*), Canada goose (*Branta canadensis*), snow goose(*Anser caerulescens*), and tundra swan (*Olor columbianus*). Friend (1987) reported an estimated annual loss of approximately 2.0 million waterfowl from lead poisoning. This estimate was based on a migratory fall flight of 100 million birds. Lead poisoning has also been the cause of migratory bird mortality in other countries such as Canada, Great Britain, Italy, and New Zealand (Friend, 1987).

Lead has been a major factor in the mortality of North American waterfowl since the late 1800's (Cook and Trainer, 1996). Waterfowl mistakenly ingest spent lead shotgun pellets and lead fishing sinkers that have been deposited on the bottom of lakes, ponds, and marshes as plant food items or grit. Mine wastes, paint pigments, bullets, and other less common lead objects are also ingested by waterfowl. The problem of waterfowl poisoning associated with spent lead shot is the fact that the pellets are usually retained in the gizzard and ground to small lead particles which are then easily absorbed. If a large number of shot have been consumed then acute lead poisoning usually occurs unless the bird eliminates the shot. However, chronic lead poisoning is more commonly seen and occurs when a smaller number of shot is consumed and degrades over a longer period of time (Friend, 1987).

Because of the increasing numbers of birds killed every year due to lead toxicosis, lead shot was banned for waterfowl hunting in 1991 within the United States (Ringelman et al., 1993). Also, a national ban on the use of lead for all migratory game bird hunting will begin at the start of the 1997 season in Canada (Scheuhammer and Norris, 1995). Currently, the only U.S. and Fish and Wildlife Service (USFWS) approved substitute for

lead shot is steel shot (Ringelman et al., 1993). However, a bismuth shot has recently received conditional approval (Ringelman et al., 1993). While steel shot has generally been accepted by hunters, there continues to be an effort to formulate a nontoxic shot with lead's favorable ballistic characteristics.

In order for a candidate shot to be approved for use by the USFWS, it must first undergo a variety of tests to establish that it's nontoxic to waterfowl and other impacted species. A 3-tiered approval process has been designed by the USFWS for candidate shot and shot coatings with shot approval considered after each tier. With the advancement of the next tier, shot testing becomes more demanding. Therefore, a candidate shot or shot coating found to be environmentally nontoxic could be granted approval at the first tier thereby reducing time, expense, and burden on both applicant and the federal government (Federal Register, 1996).

The present study is an acute toxicity test (short-term, 30- day acute toxicity test using commercially available duck food) designed to assess the effects of short-term periodic exposure of waterfowl to 2 different candidate shot, one composed of tungsten (55%) and iron (45%) and the other composed of tungsten (95.5%) and a polymer (4.5%). The protocol for this study was reviewed by the USFWS in 1995 and complies with the general guidelines outlined in the amended test protocol for nontoxic shot approval procedures for shot and shot coatings proposed by the USFWS in 1996.

Objectives

The overall objective of the 30-day dosing trial was to determine if exposure to the 2 different candidate shot, one composed of tungsten (55%) and iron (45%)(TI) and the other composed of tungsten (95.5%) and a polymer (4.5%)(TP), caused any deleterious effects in game farm mallards (*Anas platyrhynchos*). Toxicity of the candidate shot was assessed by:

1) Determination of hematocrit, hemoglobin and whole-blood delta-aminolevulinic acid dehydratase activity values at day 15 and day 30 of the trial.
2) Determination of plasma chemistry values at day 15 and day 30 of the trial.
3) Determination of changes in body weights and organ weights.
4) Determination of metal residue concentrations in the liver, kidney, and femur.
5) Determination of gross and histological changes of selected tissues.
6) Determination of mortality.

Literature Review

The word lead (Pb) is derived from the Latin term *plumbum*. Thus, lead poisoning often called "plumbism". Lead is a soft, malleable heavy metal with a bright bluish tint. It is found naturally at trace concentrations (15 ppm) but rarely occurs in its native form. Instead, lead is usually found in the sulfide form in its chief ore, galena. Other common inorganic salts are insoluble lead carbonate, lead sulfate, and lead chlorophosphate with lead acetate a common soluble form (National Academy of Science, 1980b).

Lead once had a wide range of applications including lead shotshell and fishing sinkers. In 1964, Bellrose estimated that the average hunter fired 5 shots for every duck that was bagged. He also calculated a 12-gauge shell to contain about 280 pellets of # 6 shot. This amounts to approximately 1,400 pellets being deposited on waterfowl hunting grounds for every duck killed. However, because the toxicity of spent lead shot to waterfowl is so great it was banned for use in waterfowl hunting in 1991 in the United States (Ringelman et al., 1993).

Other applications involving lead include storage batteries, gasoline, pigments, ceramics, pesticides, plumbing, and crystal ware (Scheuhammer and Norris, 1995). However, lead was recognized to be one of the major environmental pollutants in air, soil,

and water and thus the use of lead was either banned or severely reduced.

Birds exhibiting clinical signs from lead poisoning were reported as early as 1893-4 (Grinnell, 1901). Grinnell (1901) reported that hunters in the area were noticing wildfowl to be sick with "croup" at Stephensen Lake in Galveston, Texas. Affected birds were lethargic and had a change in tone of call. Yellowish fluid was also noted being eliminated from the bill of the affected birds. Upon dissection of these sick birds, lead shot was found in the gizzards. The inner membranes of the gizzards were cracked and decayed, and yellowish in color. It was concluded from these examinations that the dissected birds had died from chronic lead poisoning.

A case of sick wildfowl near Riverton, Kansas was reported in February of 1923 by Dr. Wetmore. He described progressive paralysis of the legs and wings, ataxia, thin, watery green-stained feces, and a greenish fluid discharge from the tongue and mouth cavity. Post-mortem observations included pale flesh color and distended gall bladder (Phillips and Lincoln, 1930).

The first signs of lead exposure are often depression and anorexia. Birds then become reluctant to fly when approached and may have a marked change in tone of call. As the disease progresses, the wings are held in a "roof-shaped" position which is often followed by wing droop. There may be a fluid discharge from the bill, with swollen or puffy heads in Canada geese from serum-like fluids which accumulate in the tissues of the face. An abundance of bile-stained feces in areas used by waterfowl are also indicative of lead poisoning (Friend, 1987).

Absorbed lead is rapidly distributed to the soft tissues with the highest initial concentrations detected in the liver and kidneys. Over a period of time, lead is redistributed and accumulates in the bone (Goodman and Gilman, 1966). Coburn et al. (1951) conducted a study in adult mallards to obtain information on the absorption and retention of lead. Soluble lead nitrate [Pb $(NO_3)_2$] was administered to all test birds by

gavage into the gizzard. Doses of lead (3, 6, 8, and 12 mg / kg body weight) were given each day with dose adjustments due to body weight variations made every 5 to 7 days. The most significant increase in lead content was noted in the liver with lead poisoned birds having hepatic lead concentrations which were 40 times those of the control birds. Similarly, the skeletal lead concentrations of the lead-dosed birds were 7 times greater than the control concentrations. In a similar study lead was administered to game-farm mallards in doses of 2, 4, or 8 #2 shot. Birds were kept on trial for 30 days. Lead residue concentrations in the liver of lead dosed ducks varied from 50.7 to 84.4 ppm. Lead in muscle ranged from 1.10 ppm to 1.31 ppm, with retention in bone ranging from 3.19 ppm dry weight in the high-dosed ducks to 241 ppm in the low-dosed ducks (Sanderson et al., 1992). Coburn et al (1951) also reported high levels of lead deposition in the bone, liver, feathers, and soft tissues of lead-dosed adult mallard ducks.

Circulating lead combines with erythrocytes and causes increased fragility of red blood cells which results in premature destruction and anemia (Osweiler et al., 1976). Lead can also interfere with the synthesis of heme. Lead blocks the metabolism of aminolevulinic acid (delta-ALA) by inhibiting delta-aminolevulinic acid dehydratase (ALAD) which causes abnormally high concentrations of delta-ALA in the urine and low concentrations in the serum (Osweiler et al., 1976). The detection of abnormal levels of delta-ALA in the serum has been used extensively as a diagnostic tool in waterfowl poisonings.

Bakalli et al. (1995) designed a study to determine the activity of erythrocyte ALAD and the relationship between enzyme activity and tissue lead concentrations in chickens, both during lead intake and after withdrawal of lead from the feed. Enzyme activities in birds dosed with 50 μg lead / gm feed were reduced to 62% of control activity within 24 hours and to 31% of control values after 7 days. Lead was then removed from the feed and after 24 hours enzyme activity increased by 32%, and after 7 days activity

was 90% of control activity. It should be noted that the chicken has been proposed as the model for lead toxicity studies because this species is very sensitive with measurable responses to low concentrations of lead after as little as 24 hours. Serum ALAD activity in chickens may also be used to monitor lead in the environment. Pain and Rattner (1988) reported that ALAD activities in adult black ducks dosed with 1 # 4 lead shot were inhibited by 100% at day one post-dosing with ALAD activities being significantly lower than control activity throughout the experiment ($p=0.000 - 0.035$).

Bates et al. (1968) also reported severe lead-induced changes within the blood. Eight # 6 lead pellets placed in the ventriculus of 10 adult mallard drakes for 25 days caused maturation arrest of promegalobastic-like blood cells in the bone marrow. Coburn et al. (1951) reported a decrease in erythrocyte count during the first 10 to 14 days of a lead dosing trial in mallards. In some cases, erythrocyte counts recovered but when a higher dose of lead was administered the red blood cell pattern changed to one characteristic of acute poisoning. Changes in cell shape were also noted and included dumbbell, bottle, oat, sickle, and tear-shaped forms.

The excretion of lead occurs slowly via the bile and urine. Urinary excretion of inorganic lead under normal conditions is approximately 9% of the amount ingested (Goodman and Gilman, 1966). The rate of urinary excretion depend on the duration of exposure and not necessarily on the absolute body burden. Coburn et al. (1951) dosed adult mallard ducks with lead shot and reported an average excretion rate of 5% of the lead intake (range 1.4-8.2%).

Because elimination of lead from body tissues is slow, acute and chronic exposure to high and low levels of lead, respectively, causes lead poisoning. The toxic dose for wildfowl such as the mallard duck is 8-12 mg / kg body weight for a duration of 19-41 days(National Academy of Science, 1980b). Ducks have also been shown experimentally to be susceptible to poisoning from consumption of marsh soil containing disintegrated lead

shot. Cook and Trainer (1966) exposed Canada geese to a variable number # 4 lead pellets (2-100). Twenty-five or more pellets resulted in death within 10 days while fewer pellets permitted survival for as long as 72 days. Pain and Rattner (1988) dosed adult black ducks with 1 # 4 lead shot and reported 60% mortality at the end of 33 days. All birds that died exhibited signs of acute lead poisoning by day 3 post-dosage. Acute lead poisoning was also reported by Grandy et al. (1968) after 8 # 6 lead shot was administered to mallard drakes. All 15 of the lead-dosed mallards died within 15 days of the study. The average time of death after dosing was 8 days. These ducks lost an average of 22% of their body weight at death and had retained 5 to 8 shot (average 7.2).

Cook and Trainer (1966) dosed pairs of Canada geese with 5, 10, 25, and 100 # 4 lead pellets while single geese received 2 and 50 pellets. The first signs of poisoning, (anorexia and lethargy) occurred 5 days post-dosing. Green diarrhea was observed in one bird as early as day 6, with 4 geese displaying swollen heads on day 6 or 7. Two geese died on day 6 without evidence of lead poisoning. Birds dying from acute exposure lost approximately 19 % of their body weight, while birds dying later in the trial lost 36% of their body weight. The lethal dose of # 4 lead shot was determined to be 4 to 5 pellets for Canada geese under the conditions of this study.

Lead toxicosis not only occur in waterfowl but also in other birds such as mourning doves. Locke and Bagley (1967) collected a sick mourning dove in Maryland which had 2 lead shot in its gizzard. It was extremely emaciated with a pronounced "hatchet" breast that was devoid of any fat. Acid fast intranuclear inclusion bodies were also found in the cells of the proximal convoluted tubules of the kidneys resembling those described for mallards fed lead shot (Locke et al.,1967). However, the morphological characteristics of the bodies appeared as individual granules, flakes, or clumps of granules as opposed to the dumbbell shapes typically seen in the nuclei of kidney tubule cells of mallards.

For areas where bird mortality is high from lead poisoning, two actions can reduce the magnitude of mortality. One practice of control is denying birds the use of problem areas and the other is the collection and proper disposal of dead and moribund birds. This prevents raptures and other scavenger species from ingesting them. Other management practices have also reduced a number of losses due to lead poisoning in site specific areas. These practices include: (1) tillage of surface soil that has embedded lead so that shot is not readily available; and (2) planting foodcrops other than corn and other grains that intensify the toxicity of lead when ingested (Friend, 1987).

Medical treatment of lead poisoned birds is available, but generally not reasonable in the field. However, endangered species or other birds of great importance can be treated with lead-chelating chemicals. One such chemical is disodium or calcium ethylenediaminetetraacetate (CaEDTA). Murase et al. (1992) administered every 12 hours 1 ml of 6% CaEDTA in sterile water via the brachial vein to 27 wild geese suffering from lead poisoning. These birds were treated until radiographic screening indicated that the lead pellets were no longer in the gizzard. In addition to the CaEDTA treatment, birds were also dosed orally with 1 gm of glucose, 0.2 gm of DL-methionine, 2 mg of deoxycycline hydrochloride, 10 ml of vegetable juice, and 10 ml of water every 12 hours. Sixteen of the treated birds died within 4 weeks with none recovering their appetite during the treatment. However, 11 of the 27 birds did recover their appetite after 12-24 days of treatment. The mean duration of the treatment period for these recovered birds was 31.1 days (range, 21-58 days).

Tungsten, the major component of the 2 candidate shot presently tested, is a relatively rare element, occurring in the earth's crust at concentrations averaging 5 ppm (Standen, 1970). It is found in the form of tungstate ores such as wolframite [(Fe, Mn) WO_4], scheelite (Ca WO_4), ferberite ($FeWO_4$) and hubnerite ($MnWO_4$). Major uses of tungsten include incorporation into cutting and wear-resistant materials, mill products,

specialty steels, tools, alloys, and chemicals. Tungsten has a molecular weight of 183.85, specific gravity of 19.35, melting point of 3,410°C and boiling point of 5,660°C. Tungsten metal is insoluble in aqueous solutions while forms such as sodium tungstate (Na_2WO_42H2O) and ammonium paratungstate [(NH4)6W7O2,6H_2O] are variably soluble in water (Stokinger, 1978).

The tungstate ion (WO_4^{2-}) is the most soluble and the most frequently occurring form of the metal in biological systems. Radiotracer studies utilizing this form of tungsten have indicated relatively rapid absorption of the compound with most of it being eliminated within a few days. For example, Wase (1956) reported that mice administered K_2WO_4 (15 mg / kg) by intraperitoneal injection eliminated 78% of the dose via the feces after 24 hours and 98% after 96 hours. Ballou (1960) reported that 40% of an orally administered dose of labeled tungsten in rats was eliminated in the urine after 24 hours, while 58% was eliminated via the feces or remained unabsorbed in the gut. Only 2% of the dose remained in the tissue. Kaye (1968) administered labeled K_2WO_4 to rats and reported that 17% of the dose was present in the carcass 1 hour post-dosing which indicated rapid absorption through the gastrointestinal tract into the systemic circulation. Twenty-four hours after dosing, 40% of the compound had been eliminated via the urine and 20% via the feces. At 72 hours post-dosing, 97% of the tungstate had been cleared from the body. Bell and Sneed (1970) dosed swine with a tracer dose of $(NH_4)_2WO_4$ by gavage or intravenously and reported that most of the radioactivity was eliminated via the urine in 24 hours. In contrast, these same authors reported that sheep administered a tracer dose of $(NH4)_2WO_4$ by capsule or by injection into the abomasum eliminated only 15% of the dose.

Distribution of absorbed tungsten is limited to relatively few tissues. Kinard and Aull (1945) fed rats tungsten as the metal (20,000 and 100,000 ppm), tungsten oxide (1,000 ppm tungsten), sodium tungstate (1,000 ppm tungsten) or ammonium paratungstate (5,000 ppm tungsten). They reported that the chief sites of deposition were bone and

spleen with smaller quantities found in the skin, kidney, and liver. This distribution pattern was not dependent on the type of compound administered. Wase (1956) reported that 8 hours after dosing mice with K_2WO_4, the highest concentrations of tungsten were detected in the bone and gastrointestinal tract. Similarly, Kaye (1968) reported that bone was the principle site of tungsten deposition in rats which were administered a tracer dose of K_2WO_4. In the study conducted by Bell and Sneed (1970), the principle sites of tungsten deposition in swine were, in descending order, kidney, bone, liver, and muscle, while in sheep tungsten was found primarily in the kidney followed by the liver, bone and muscle, respectively. Following inhalation of a radiolabeled tungsten oxide aerosol by dogs, the highest concentrations of activity 165 days after exposure were in the lung and kidney with smaller concentrations in bone, gall bladder, liver , and spleen. In terms of organ burden, most of the activity was associated with bone (37% of the body burden), lung (31%), kidney (15%), and liver (9.7%) (Aamodt, 1975).

Tungsten is eliminated in both the urine and feces, the predominant route apparently being dependent on species, type of tungsten compound, and the route of administration. Wase (1956) reported that mice dosed intraperitoneally with K_2WO_4 eliminated 78-98% of the compound via the feces from 24-96 hours post-dosing. Kaye (1968) reported that 40% of an orally administered dose of K_2WO_4 was eliminated in the urine and 20% in the feces at 24 hours post-dosing. Dogs administered an intravenous tracer dose of Na_2WO_4 eliminated 91% of the tungsten via the urine (Aamodt, 1973). Similarly, Bell and Sneed (1970) reported that most of a tracer dose of $(NH_4)_2WO_4$ administered to swine either by intravenous injection or by gavage appeared in the urine within 24 hours post-dosing. In the same study, sheep orally administered $(NH_4)_2WO_4$ excreted 44% and 42% of the radioactivity in the urine and feces, respectively, while $(NH_4)_2WO_4$ introduced into the abomasum resulted in 65% being eliminated in the urine and 17% in the feces.

The biological half-life of tungsten is relatively short, depending upon the tissue being examined. Kaye (1968) reported that the half-life of orally administered K_2WO_4 in rats was approximately 10 hours for the initial fast component of the elimination curve. In general, elimination of tungsten from soft tissue was rapid, but the half-life of tungsten in the spleen was 44 days and that in bone was 1,100 days. Nell et al. (1980) reported an hepatic half-life of 27 hours for Na_2WO_4 injected intraperitoneally into broiler cockerels.

The toxicity of tungsten is dependent upon the solubility of the form administered, with the soluble forms usually being considerably more toxic than the less soluble forms. For example, Frederick and Bradley (1946) determined an LD_{50} for insoluble tungsten metal powder injected intraperitoneally in the rat of 5,000 mg/kg body weight, whereas when the soluble Na_2WO_4 was injected subcutaneously, an LD_{50} of 140-160 mg tungsten/kg body weight (223-255mg Na_2WO_4/kg body weight) was determined (Kinard and Van de Erve, 1940). Pham-Huu-Chanh (1965) reported LD_{50} values of 112 mg/kg body weight and 79 mg/kg body weight when sodium tungstate was administered by intraperitoneal injection to rats and mice, respectively. However, there are exceptions to this relationship between solubility and toxicity. Kinard and Van de Erve (1941) reported that diets containing 5.0% (50,000 ppm) tungsten as the relatively insoluble ammonium paratungstate, 3.96% (39,600 ppm) tungsten as the insoluble tungstic oxide, or 2% (20,000 ppm) tungsten as the soluble sodium tungstate produced 100% mortality in rats while a diet containing 2% tungsten as ammonium paratungstate resulted in 80% mortality. When rats were fed diets containing 0.5% (5,000 ppm) tungsten in different forms, tungstic oxide caused 82% mortality, sodium tungstate caused 58% mortality, and ammonium paratungstate resulted in no deaths. Nell et al. (1980) administered broiler cockerels soluble sodium tungstate via daily injection at 5 mg tungsten from day 1 to day 11, 10 mg from day 12 to day 21, and 20 mg from day 22 to day 35. Four of 40 birds died on trial and all deaths occurred on day 29.

Clinical signs resulting from acute exposure of mammals to lethal or near-lethal doses of the more toxic tungsten compounds via oral and parenteral routes have been summarized by Stokinger (1978). These include nervous prostration, diarrhea, and death preceded by coma due to respiration paralysis. Clinical signs reported by Nell et al. (1980) for chickens dying of exposure to soluble sodium tungstate included anorexia, reduced weight gain, diarrhea, and labored breathing within an hour of death. On gross examination of these birds, muscles and liver were dark red due to extensive hemorrhaging and petechial hemorrhages were observed on the gizzard and proventriculus. Hemorrhages were also observed in the brain, heart, and kidney.

When animals have been administered doses of tungsten compounds which do not result in mortality (in excess of several thousand ppm), effects are often slight. Selle (1942) injected male and female rats daily with 92 mg tungsten/kg body weight as sodium tungstate and reported weight loss of 11 and 26%, respectively. No effects were noted when the same dose was administered daily by oral gavage. Kinard and Van de Erve (1941) reported that when growing rats were administered a diet containing 1,000 ppm (0.1%) tungsten as tungstic oxide or sodium tungstate, or 5,000 ppm (0.5%) tungsten as ammonium paratungstate, the only effect observed was a similar and slight growth depression after 70 days. Kinard and Van de Erve (1943) reported that feeding tungsten metal to rats at concentrations of 25,000 ppm and 100,000 ppm for 70 days resulted in a 15% decline in body weight gain of the females. Schroeder and Mitchner (1975) administered 5 ppm sodium tungstate to rats via the drinking water throughout their lifetime and reported a somewhat shortened lifespan in male rats (983 days vs 1,126 days for controls).

As with mammals, studies in birds have indicated relatively few effects as a result of exposure to moderate concentrations of soluble tungsten compounds (Higgens et al., 1956; Teekell and Watts, 1959; Leach et al., 1962; Nell et al., 1980). In most of these

studies, the effects of tungsten supplementation of the diet have been looked at in conjunction with low dietary concentrations of molybdenum. Molybdenum is an essential component of a number of enzymes important in avian as well as mammalian metabolism. In particular, xanthine dehydrogenase (and the similar xanthine oxidase in mammals) is involved in purine metabolism and the conversion of nitrogenous compounds to uric acid. Because the tungstate ion, WO_4^{2-}, is isomorphic with the molybdate ion, MoO_4^{2-}, tungsten antagonizes the normal metabolic action of molybdenum in its role as a metal carrier and thus decreases the activity of xanthine oxidase/xanthine dehydrogenase (DeRenzo, 1954) as well as other molybdenum-containing enzymes such as sulfite oxidase, aldehyde oxidase, and nitrate reductase (Stokinger 1978).

Higgens et al. (1956) utilized dietary sodium tungstate to produce a molybdenum deficiency in rats and chicks to examine the subsequent effects on xanthine oxidase and xanthine dehydrogenase activity. Three to 4-week-old rats were fed a diet containing 4.5 ppm tungsten (as sodium tungstate) for 7 weeks. This diet provided a tungsten:molybdenum ratio of 100:1 and resulted in reduced xanthine oxidase activity in the liver and intestine as well as a decreased hepatic molybdenum concentration. Rats fed Na_2WO_4 in a 1,000:1 or 2,000:1 ratio of tungsten:molybdenum grew normally and oxidized xanthine to uric acid and allantoin despite the fact that no tissue examined had detectable xanthine oxidase activity or molybdenum. In contrast, chicks fed diets containing sodium tungstate which provided tungsten:molybdenum ratios of 1,000:1 and 2,000:1 experienced depressed growth rates and 25% mortality after 5 weeks. All tissue xanthine dehydrogenase activities and molybdenum concentrations were depleted and 50% of the uric acid normally excreted by chicks was replaced by xanthine and hypoxanthine. Addition of molybdenum to the diet reversed the effects of the 1,000:1 tungsten:molybdenum ratio.

Teekell and Watts (1959) fed chickens sodium tungstate at a concentration of 250

ppm for 10 days and then increased the concentration to 500 ppm for the subsequent 20 days. Incorporation of 250 ppm sodium tungstate had no effect on intestinal and hepatic xanthine dehydrogenase activities, but increasing the dietary sodium tungstate concentration to 500 ppm did cause a steady decline in enzyme activities. Egg production by these hens and subsequent hatchability was not affected. However, those chicks hatched from females fed the sodium tungstate-supplemented diet grew at a slower rate than chicks from control females. Chicks fed a diet containing 500 ppm sodium tungstate for 4 weeks had a slower rate of gain when compared to control chicks, yet tissue xanthine dehydrogenase activities were not affected.

In a study by Leach et al. (1962), the addition of 1,000 ppm tungsten (form not specified) or more to the diets of chicks for 4 weeks resulted in depressed growth rates while concentrations of 500 ppm tungsten or greater caused a marked decrease in hepatic xanthine dehydrogenase activities. Addition of molybdenum to the diets caused a reversal of the tungsten-induced decrease in xanthine dehydrogenase activities but only partially reversed the growth inhibition resulting from 2,000 ppm dietary tungsten.

Nell et al. (1980) examined the effects of both injected and ingested sodium tungstate on xanthine dehydrogenase activity in chicks. Cockerels receiving a single intraperitoneal injection of 20 mg sodium tungstate had increased concentrations of hepatic tungsten but there was no effect on hepatic molybdenum concentrations or xanthine dehydrogenase activities. Chicks fed diets containing 1,000 ppm tungsten for 4 weeks had increased hepatic concentrations of tungsten, reduced concentrations of molybdenum and decreased activities of xanthine dehydrogenase. All of these effects were reversed by supplementation of the diet with molybdenum. In chicks either injected intraperitoneally with sodium tungstate at doses increasing from 5 to 10 to 20 mg at days 12 and 22 of a 35-day period or fed diets containing sodium tungstate at doses which increased from 150 to 600 ppm at day 22 of a 35-day period, mortality was associated with hepatic tungsten

concentrations of 25 ppm as well as decreases in liver molybdenum concentrations and xanthine dehydrogenase activities. The decrease in tissue xanthine dehydrogenase activities paralleled increases in plasma concentrations of uric acid, xanthine, and hypoxanthine.

In a study similar to the present study, Ringelman et al. (1993) dosed mallards with 12-17 pellets of shot (equivalent in mass to 5 # 4 lead shot) composed of 39% tungsten, 44.5% bismuth, and 16.5% tin and monitored the birds for the subsequent 32 days. Based on the lack of effects on mortality, behavior, feed consumption, body weight gain, and blood parameters as well as the absence of gross and histological lesions, and no detectable concentrations of tin and tungsten in the liver and kidney, these authors concluded that the ingested candidate shot had no ill effects on the mallards over the 32-day period.

The other major component of one of the tungsten candidate shot in the present study is iron (Fe). Iron is the fourth most abundant element of the earth's surface (5%) existing as hematite (Fe_2O_3), limonite ($Fe_2O_3 \cdot 3H_2O$), magnetite (Fe_3O_4), taconite, and siderite [$Fe(CrO_2)_2$]. It has a molecular weight of 55.85, a specific gravity of 7.86, a melting point of 1535°C and a boiling point of 2750°C. Iron metal is insouble in alkaline solutions, alcohol, and ether (Stokinger, 1978).

Unlike tungsten, which is not essential for animals, iron is a required nutrient. Iron is found primarily in the ferrous (Fe^{+2}) and ferric (Fe^{+3}) states. Oral doses of iron salts of both valence states are not necessarily toxic. However, iron salts, especially ferrous salts, introduced directly into the bloodstream are highly and almost instantaneously toxic (Stokinger, 1978).

Iron is primarily absorbed in the small intestine as ferrous iron which is oxidized to the ferric state in the blood plasma. Iron functions in the transport of oxygen and is associated predominately with hemoglobin (70%) and the iron-storage proteins ferritin and hemosiderin (26%), with lesser amounts in muscle myoglobin (3.5%), and other iron-

containing enzymes distributed throughout the body (Venugopal and Luckey, 1978; National Academy of Science, 1980a). Iron is eliminated primarily via the bile, small amounts may be ejected by way of sloughed cells from duodenal villi as well as via the sweat and urine. However, it should be noted that the overall rate of metabolism of absorbed iron is very slow thereby limiting the actual amount of excreted iron.

Clinical signs of acute iron toxicity occur in 5 phases subsequent to ingestion of the iron compound (Rumack and Lovejoy, 1991). The first phase is apparent from 30 minutes to 2 hours after the ingestion of large amounts of iron. Clinical signs are lethargy, restlessness, hematemesis, abdominal pain, and bloody diarrhea. Iron can have a corrosive effect on the gastrointestinal mucosa which could in turn cause severe hemorrhagic necrosis with development of shock. However, iron that is absorbed through intact mucosa may also cause shock. The second phase is a transitional period which appears as a recovery period, but in fact progresses to the third phase. The third phase (2-12 hours after phase 1) is characterized by the onset of shock, acidosis, cyanosis, and fever. Acidosis is the result of hydrogen ion release from the conversion of ferric (Fe+3) iron to ferrous (Fe+2) iron and the accumulation of lactic and citric acids. The fourth phase (2-4 days post-ingestion) involves the development of hepatic necrosis. This is theoretically due to a direct toxic action of iron on mitochondria. The fifth and final phase (2-4 weeks after ingestion) occurs with the onset of gastrointestinal obstruction which is secondary to gastric or pyloric scarring and healed tissue. Histological evidence of acute toxicity include vascular congestion of the gastrointestinal tract, liver, kidneys, heart, lungs, spleen, brain, adrenals, and thymus. Smaller amounts of iron ingested over a longer period of time can cause chronic symptoms which include hemorrhagic necrosis of the gastrointestinal tract, hepatotoxicity, metabolic acidosis, increased blood-clotting time, and elevation of plasma concentrations of serotonin and histamine (Venugopal and Luckey, 1978). McGhee et al. (1965) evaluated the effects of various concentrations of copper and

iron on growth, body weight gains, and survival of chickens. One hundred and twenty birds were divided into 3 groups with 6 treatments per group. Each group had varied amounts of copper and iron ($CuSO_4 \cdot 5H_2O$ and $Fe\ SO_4 \cdot 7H_2O$, respectively) mixed into the basal diets. Results indicated that when copper was held constant at 5 ppm and iron varied from 50 to 1,600 ppm (Group 1), increasing levels of iron decreased average body weight. Mortality was 10% at 200 ppm iron and 5 ppm copper. Group 3 had more noticeable growth depressions at iron concentrations of 800 to 1,600 ppm and copper concentrations of 80 to 160 ppm. Overall, iron suppressed growth at 4 weeks of age at concentrations from 50 to 1,600 ppm when fed 5 ppm copper. However, no other toxic effects were seen at 4 weeks of age.

Other signs of chronic iron toxicosis may involve decreased feed intake, growth rate, and feed efficiency. The maximum tolerable level of iron in poultry is reported to be 1,000 ppm with oral doses exceeding 1.5 mg / kg being considered excessive and possibly leading to iron toxicosis (National Academy of Science, 1980a).

Materials and Methods

Thirty-two male and 32 female 6-month-old game farm mallards (hatched 12 June 1995) with plumage and body conformation resembling wild mallards were purchased from Whistling Wings, Inc. (Hanover, Illinois). The birds arrived by truck at the Michigan State University Poultry Teaching and Research Center (PTRC) on 20 December 1995. The birds were randomly removed from the transport cages, identified with a uniquely numbered metal leg band, and weighed. Flight feathers were clipped and the birds were assigned to individual cages.

Cages (0.914 m L x 0.914 m W x 0.457 m H) were constructed of vinyl-coated wire (14 gauge, 2.54 cm mesh) and suspended 60.96 cm off the floor of an enclosed pole

barn-type building. Wood shavings were placed underneath the cages to absorb excreta and water. Shavings were replaced on a weekly basis.

A gas brooder was utilized to keep the room temperature above 0°C. Room temperature was continuously recorded on graph paper by a thermohydrograph. High and low room temperatures during the 30-day test are presented in Table 1. Light was provided by incandescent bulbs controlled by a timer such that lights went on at sunrise and off at sunset. The timer was adjusted weekly to mimic within 15 minutes the natural photoperiod appropriate for Lansing, Michigan (Table 1).

Each cage contained a food and water crock so that food and water were available ad libitum during the acclimation period (20 December 1995-15 January 1996) and the 30-day dosing trial (16 January-15 February 1996). The diet was a commercial pelleted ration (Purina Duck Grower W/O; Batch #8858; crude protein ≥ 16.0%, crude fat ≥ 3.0%, crude fiber ≤ 5.0%, calcium 0.40-0.90%, phosphorus ≥0.55%, sodium chloride 0.20-0.70%) and drinking water was obtained from a university well. The opened feed bags were stored in a covered container to keep them dry and pest-free. Water was changed on a daily basis and feed was added to cleaned crocks as needed (usually every other day).

The ducks were randomly assigned to 5 treatment groups (no-shot, steel shot, lead shot, tungsten-iron shot, and tungsten-polymer shot) with 16 ducks (8 males and 8 females) per group. The evening before the shot was to be administered, feed was removed from the birds to facilitate dosing. On the first day of the 30-day dosing trial (16 January 1996), each bird was weighed and then dosed with shot. The birds in the steel and

Table 1. High and low room temperatures and natural photoperiod during the 30-day dosing test for candidate shot (16 January 1996 to 15 February 1996).

Date	Temperature				Photoperiod (EST)	
	High		Low		Sunrise	Sunset
	F°	C°	F°	C°		
1/16/96	48	9	40	4	0805	1730
1/17/96	56	13	49	9	0805	1732
1/18/96	63	17	56	13	0804	1733
1/19/96	60	16	41	5	0804	1734
1/20/96	49	9	40	4	0803	1735
1/21/96	47	8	44	7	0802	1737
1/22/96	53	12	44	7	0802	1738
1/23/96	54	12	51	11	0801	1739
1/24/96	53	12	44	7	0800	1740
1/25/96	47	8	40	4	0759	1742
1/26/96	53	12	40	4	0759	1743
1/27/96	42	6	36	2	0758	1744
1/28/96	46	8	40	4	0757	1746
1/29/96	45	7	36	2	0756	1747
1/30/96	46	8	36	2	0755	1748
1/31/96	53	12	34	1	0754	1750

Table 1 continued.	High and low room temperatures and natural photoperiod during the 30-day dosing test for candidate shot (16 January 1996 to 15 February 1996).					
Date	Temperature				Photoperiod (EST)	
	High		Low		Sunrise	Sunset
	F°	C°	F°	C°		
2/1/96	44	7	26	-3	0753	1751
2/2/96	45	7	39	4	0752	1752
2/3/96	47	8	36	2	0751	1754
2/4/96	45	7	38	3	0750	1755
2/5/96	46	8	29	-2	0748	1756
2/6/96	49	9	40	4	0747	1758
2/7/96	51	11	43	6	0746	1759
2/8/96	57	14	51	11	0745	1800
2/9/96	70	21	52	11	0744	1801
2/10/96	59	15	40	4	0742	1803
2/11/96	55	13	39	4	0741	1804
2/12/96	44	7	35	2	0740	1805
2/13/96	47	8	39	4	0738	1807
2/14/96	53	12	49	9	0737	1808
2/15/96	53	12	40	4	0736	1809

lead groups received 8 #4 pellets and the birds in the tungsten-iron and tungsten-polymer group received 8 BBs. Prior to dosing, individual shot or BBs were weighed and placed in groups of 8 into individual glass vials which were identified according to shot type, cage number, and the bird's individual band number. At dosing, each vial containing shot or BBs was matched to the appropriate bird. Pellets were introduced into the proventriculus by inserting a latex tube (0.953 cm inner diameter) 21.60 cm through the esophagus into the proventriculus. A plastic funnel was attached to the end of the tube so that the pellets or BBs could be flushed down the tube with approximately 5 ml of water. Between birds, the tube and funnel were placed in a bucket of water to keep the tube moist for easy insertion. Control ducks were sham-dosed identically to treatment birds except for the presence of shot. All birds were observed twice daily for assessment of general well-being. Any clinical signs including, but not limited to, inappetence, apparent weight loss, ataxia, lethargy, and discolored excreta were noted in the daily log. Screens suspended underneath each cage were cleaned on a daily basis and the excreta was examined for any expelled shot or BBs. In the event expelled shot or BBs was recovered, it was to be rinsed off and placed back into the appropriate glass vial for subsequent weighing. Presence or absence of expelled shot on each screen was recorded daily. In addition to these observations, feed and water were checked daily and the room temperature was recorded at each entry.

On day 7 of the trial (23 January 1996), the ducks were transported (10 birds/crate) to the Michigan State University Large Animal Veterinary Clinic (4 miles from the PTRC) for fluoroscopy by radiologist Dr. Russell Stickle to determine retention of shot. Control

birds were also fluoroscoped to insure that no shot had been ingested prior to arrival at Michigan State University. All birds were manually immobilized on their side on the examination table and slowly rotated by hand until the greatest number of shot could be observed on the viewing screen. At this point, a radiograph identified by the last 3 numbers of the bird's leg band was taken.

Blood samples were collected on days 15 and 30 (31 January and 15 February 1996) of the trial after the birds were weighed. Birds were held manually on an examination table on their backs with one wing extended. A 21-gauge, 3.81 cm Vacutainer® multiple sample needle was inserted into the brachial vein and blood was sequentially collected into 1 3-ml Vacutainer • tube containing liquid EDTA (lavender top) and 2 3-ml Vacutainer • tubes containing sodium heparin (green top). Each tube was labeled with the bird's band number, treatment, and the date of collection.

The lavender top tube and one green top tube from each bird were gently rotated for 1 minute and then refrigerated until all blood samples were collected over a 6-hour period. Since birds were bled in order of their cage number rather than by treatment, blood samples from all treatments were collected throughout the day. When blood collection was completed, the samples were packed in styrofoam coolers containing U-Tek• polyfoam refrigerant packs and shipped by overnight express to the Division of Comparative Pathology of the University of Miami, Miami, Florida. These blood samples were processed upon receipt for determination of hematocrit (HCT), and hemoglobin (Hb) concentration (lavender top tube) and red blood cell delta-aminolevulinic acid dehydratase (ALAD) activity (green top tube).

The other green top tube from each bird was used for separation of plasma from whole blood. Tubes were transported in groups of 12 to a laboratory adjacent to the building containing the birds and spun in a centrifuge at 50 x g for 5 minutes. Plasma was removed from the Vacutainer® tube by a glass Pasteur pipet and transferred to an identically labeled 1-dram glass vial. Plasma vials were stored in a styrofoam cooler containing dry ice until all plasma samples had been collected. Vials were then transferred to an ultracold freezer (-72°C) until shipping at the end of the 30-day trial. Plasma samples were sent on dry ice by overnight express to the Division of Comparative Pathology, University of Miami, for determination of albumin, albumin/globulin ratio, alkaline phosphatase activity, amylase activity, aspartate aminotransferase activity, blood urea nitrogen, blood urea nitrogen/creatine ratio, calcium, chloride, cholesterol, CO_2, creatine phosphokinase activity, creatinine, direct bilirubin, total bilirubin, gamma glutamyl transpeptidase activity, glucose, lactate dehydrogenase activity, phosphorus, potassium, sodium, total protein, triglycerides, and uric acid.

Within 1 hour of arrival of the whole-blood samples at the University of Miami, the tubes were unpacked, separated by tube type, and arranged sequentially by the bird's band number on each tube. Tubes were then assigned a second number (1,2,3,etc.). The quality of each sample was grossly examined and noted on the log-in worksheet. Lavender top tubes were at room temperature prior to determination of Hb and HCT. Green top tubes were stored at 4°C for 3 hours prior to analysis for ALAD.

Hemoglobin was determined by removing 100 μl of whole blood from the lavender top tubes by an automatic pipet and placing it in a plastic 96-well microtiter plate. Fifty

μl of lysis solution (Hematall LA-Hgb, Fisher Scientific) was added to each well. The solutions were then mixed by automatic pipet for 10 seconds. After incubation at room temperature for 1 minute, the plate was centrifuged at 1,200 rpm for 10 minutes to pellet red blood cell nuclei and other debris. The supernatant was removed and hemoglobin was measured using a Leica hemoglobinometer. Hemoglobin was quantitated as g/dL (x 1.5 for dilution factor). The hematocrit was determined by drawing approximately 50 μl of blood from the lavender top tube by capillary action into a microhematocrit tube. Tubes were then sealed at one end using hematocrit clay and centrifuged in a standard hematocrit centrifuge (IEC MB microhematocrit centrifuge) for 5 minutes. HCT was determined by a manual hematocrit reader (IEC microcapillary reader #2201). ALAD activity (expressed in ALAD units) was measured according to the procedures of Burch and Siegel (1971) and Dieter and Finley (1979). Samples, which were run in triplicate, were brought to room temperature after 1 hour of rocking on a standard tube rocker. Twenty μl of blood from the green top tube was placed in a 96-well microtiter plate and 130 μl Triton-X and 100 μl ALA-S were added to each well and mixed by automatic pipet. One hundred μl of this solution was removed and then incubated in a 96-well format at 37°C for 1 hour. For a control, an additional 100 μl aliquot was mixed with 100 μl trichloroacetic acid. The sample was then centrifuged for 10 minutes at 1,200 rpm. After centrifugation, 100 μl of the supernatant was removed for later measurement. The incubated mixture went through the same procedure (addition of trichloroacetic acid and subsequent centrifugation). One hundred μl of Ehrlich's reagent was then added to the experimental and control supernatants. After a 13 minute room temperature incubation, the plates were read at 555

nm using a Molecular Devices plate reader. Means for each determination were calculated and control absorbance was subtracted. Samples with a standard deviation greater than 10% were repeated. Final ALAD calculations were done as a function of HCT where ALAD units of activity equal (Corrected Absorbance x 12,500)/HCT.

Upon arrival at the University of Miami, frozen plasma samples were quickly arranged sequentially by the bird's band number on each tube, assigned a second number (1,2,3 etc.), and placed in a freezer until analysis. Twenty-five samples were thawed at a time and assessioned 1 hour prior to analysis. The quality of each sample was grossly examined and noted in the log-in work sheet. Samples were analyzed using a Kodak 750 chemistry analyzer. Control sera samples were run daily prior to analysis of test samples to maintain a check on instrument calibration. Plasma chemistry analyses were performed over a 7-day period.

On day 30 of the trial, birds which had not died were weighed, bled as previously described, killed by cervical dislocation, and subjected to necropsy in a laboratory adjacent to the building housing the ducks. The necropsy procedure included a complete gross examination of all body cavities and organs by Dr. Scott Fitzgerald, diplomate of the American College of Veterinary Pathologists (ACVP). Gizzards were opened for inspection of cracked and discolored mucosa and retention of shot. Shot were counted and placed back into the appropriate glass vial for subsequent cleaning and weighing for determination of shot erosion. The femur, spleen, heart, liver, kidneys, brain, and testes were removed and weighed. Small samples of the liver and kidneys from each bird were placed in labeled plastic containers containing formalin for subsequent histopathological

examination by Dr. Scott Fitzgerald. Lesions in the liver and kidney were scored on a 4-point scale where 0=normal and 3=severe. The femur and the remaining portions of the liver and kidneys were placed in individual labeled plastic bags and frozen on dry ice until all tissues were obtained. The 3 tissues from each bird were then placed in a larger labeled plastic bag and packed in a styrofoam cooler containing dry ice. The cooler was then placed in a chest freezer (-72°C) until it was picked up by a representative of Anatech Laboratories on 19 February 1996. The cooler was repacked with dry ice and transported by car to the Anatech facility in Ludington, MI. Tissues were stored frozen until sample preparation and analysis. Sample preparation/digestion was performed according to EPA procedures for iron, tungsten, molybdenum (by Inductively Coupled Argon Emission Plasma Spectroscopy or ICP) and lead (by Graphite Furnace Atomic Absorption or GFAA). Blanks, calibration verification, duplicate analysis, and spike analysis were performed and processed with the quality control (QC) package.

All statistical analyses were performed on the SAS statistical package (version 6.22; Cary NC). Significance was set at $p < 0.05$. Males and females were analyzed separately. Body and organ weights from those birds dying prior to the end of the trial were excluded from statistical analysis. Data from blood samples which had clotted were also removed from the analysis.

Qualitative data (mortality and histopathological lesions) were analyzed using the Pearson Chi-Square Test. Quantitative data (plasma and whole-blood parameters, body weights, organ weights, tissue metal concentrations, and percent shot erosion) were analyzed using one-way analysis of variance (ANOVA). When statistically significant

differences were detected in the overall ANOVA, Tukey's Studentized Range Test was used to determine differences among groups.

Results

Mortality

The effect of treatment shot on mortality is presented in Table 2. Eight of 16 (50%) mallards dosed with lead shot died during the course of the 30-day study. Of the 8 birds dying, 5 (62.5%) were males and 3 (37.5%) were females. The average time to death was 17.6 days for males and 15.3 days for females with a range of 10 to 25 days for both sexes. The average weight loss of those birds dying was slightly over 30%. No ducks in the other 4 treatment groups (no-shot, steel, tungsten-iron and tungsten-polymer) died during the 30-day trial.

Clinical Signs

The lead-dosed birds were the only ones which had obvious clinical signs during the course of the 30-day trial. Green excreta was the first abnormality noted, being apparent in 50% of the males and females within 24 hours of dosing. By 4 days post-dosing, all lead-dosed birds were eliminating green excreta. Of the 5 males which died, the 3 which died the earliest (days 11, 15, and 17) had no other clinical signs. The 2 males which died on days 20 and 25 developed progressive ataxia from 2 to 4 days prior to death. The 3 surviving males appeared normal during the last 9 days of the trial. Two of the 3 lead-dosed females which died (days 10 and 15) had no clinical signs other than green excreta prior to death. The female which died on day 21 appeared ataxic the day before it died. Of the 5 females which survived the 30-day trial, 2 appeared to be normal

Table 2. The effect of treatment shot on percent mortality, time to death (days), and percent weight lost at death of mallards on a 30-day dosing test[a].

	% Mortality	Time to Death	% Weight Lost at Death
Males			
No-Shot	-	-	-
Steel	-	-	-
Lead	62.5 (5/8)	17.6 (11-25)	31.2 (22.4-37.9)
Tungsten-Iron	-	-	-
Tungsten-Polymer	-	-	-
Females			
No-Shot	-	-	-
Steel	-	-	-
Lead	37.5 (3/8)	15.3 (10-21)	32.7 (17.1-46.9)
Tungsten-Iron	-	-	-
Tungsten-Polymer	-	-	-

[a] Numbers in parentheses represent number of birds dying/number of birds per group for percent mortality, range of time to death, and range of percent weight lost.

by day 15 with the exception of an occasional appearance of green excreta. The other 3 females had varying degrees of ataxia which became increasingly severe and then the condition seemed to improve somewhat near the end of the study.

Body Weights

The effects of treatment shot on body weights of mallards surviving the 30-day test period are presented in Table 3. Significant differences in male body weights were detected at day 15. Lead male body weights were significantly lower than no-shot and tungsten-polymer body weights, but similar to steel and tungsten-iron body weights. There were no significant differences in body weight at days 0 and 30 for either sex. During the 30-day test period, birds receiving no shot gained a slight amount of weight (5.8% for males and 2.8% for females) as did those birds receiving tungsten-iron (5.0% for males and 0.7% for females) and tungsten-polymer (3.7% for males and 3.8% for females). Steel-dosed males also gained a small amount of weight (2.2%) while the females maintained the same weight. Lead-dosed males and females that survived the 30-day trial lost approximately 6.0% of their body weight.

Hematocrit, Hemoglobin Concentration, and ALAD Activity

The effects of treatment shot on hematocrit, hemoglobin concentration, and ALAD activity at day 15 and day 30 of the dosing test are presented in Tables 4 (males) and 5 (females). When blood samples were taken on day 15 of the trial, it was apparent that the blood taken from a number of the lead-dosed birds had a fluorescent-like cast and was less viscous. These observations were substantiated by both hematocrit and hemoglobin concentrations. In lead-dosed males, hematocrit concentrations were significantly lower

Table 3. The effect of treatment shot on body weights (gm) of mallards on a 30-day dosing test[a].				
	Day 0	Day 15	Day 30	% Change[b]
Males				
No-Shot	1230.3 ± 51.29	1248.3 ± 68.36^{B}	1305.8 ± 73.77	+5.8
Steel	1126.8 ± 26.95	1115.3 ± 30.63^{AB}	1149.6 ± 27.53	+2.2
Lead	1134.1 ± 29.42	947.8 ± 59.05^{A}	1058.0 ± 38.04	- 6.5
Tungsten-Iron	1113.1 ± 26.64	1106.5 ± 26.28^{AB}	1166.9± 29.54	+5.0
Tungsten-Polymer	1132.0 ± 25.77	1150.6 ± 40.50^{B}	1175.8 ± 49.90	+3.7
Females				
No-Shot	1014.5 ± 29.95	1025.0 ± 45.20	1041.1 ± 35.55	+2.8
Steel	974.0 ± 45.38	945.5 ± 41.28	969.3 ± 42.70	-0.4
Lead	1010.5 ± 30.64	853.3 ± 64.88	962.4 ± 76.71	-5.9
Tungsten-Iron	1024.6 ± 41.14	996.5 ± 34.55	1030.3 ± 36.08	+0.7
Tungsten-Polymer	1008.5 ± 36.01	1005.1 ± 23.24	1041.5 ± 30.94	+3.8

[a] Data presented as mean ± standard error of the mean. Sample size is 8 for all groups except for day 15 lead females which is 6, day 30 lead females which is 5, day 15 lead males which is 7 and day 30 lead males which is 3. Means with different superscripts are significantly 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.

Table 4.	The effect of treatment shot on whole-blood parameters of male mallards on a 30-day dosing test[a].					
	Hematocrit Day 15	Hematocrit Day 30	Hemoglobin Day 15	Hemoglobin Day 30	ALAD Day 15	ALAD Day 30
Males						
No-Shot	42.9 ± 2.16^{AB} (8)	32.8 ± 0.98 (6)	13.86 ± 0.583^{B} (8)	10.67 ± 0.332 (6)	90.63± 7.266 (7)	64.08 ± 7.500 (6)
Steel	43.8 ± 1.37^{B} (8)	39.5 ± 2.42 (6)	14.00 ± 0.384^{B} (8)	12.83 ± 0.712 (6)	84.65 ± 11.437 (8)	80.74 ± 11.581 (5)
Lead	34.3 ± 2.95^{A} (6)	35.0 ± 7.51 (3)	10.87 ±1.211^{A} (6)	12.17 ± 1.633 (3)	54.92 ± 11.595 (5)	70.73 ± 19.527 (3)
Tungsten-Iron	44.8 ± 2.17^{B} (8)	36.1 ±1.70 (8)	13.60 ± 0.606^{AB} (8)	11.84 ± 0.503 (8)	80.51 ± 9.871 (7)	67.75 ± 7.162 (8)
Tungsten-Polymer	45.1 ± 1.42^{B} (8)	34.6 ±1.63 (8)	14.9 ± 0.419^{B} (8)	11.29 ± 0.587 (8)	82.51 ± 9.186 (7)	52.44 ± 8.440 (8)

[a] Data presented as mean ± standard error of the mean. 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 toδ-aminolevulinic acid dehydratase which is expressed as ALAD units of activity=(corrected absorbance x 12,500) / HCT. Means with different superscripts are significantly different within the column ($p \leq 0.05$).

Table 5. The effect of treatment shot on whole-blood parameters of female mallards on a 30-day dosing test[a].						
	Hematocrit Day 15	Hematocrit Day 30	Hemoglobin Day 15	Hemoglobin Day 30	ALAD Day 15	ALAD Day 30
Females						
No-Shot	47.4 ± 1.50[B] (8)	36.0 ± 2.76 (6)	14.33 ± 0.288[B] (8)	11.40 ± 0.987 (6)	73.66 ± 5.447[B] (7)	70.42 ± 10.745 (6)
Steel	46.1 ± 1.26[B] (8)	36.9 ± 2.37 (8)	14.76 ± 0.477[B] (8)	11.86 ± 0.740 (8)	82.08 ± 5.617[B] (8)	70.69 ± 10.664 (8)
Lead	34.7 ± 3.99[A] (6)	35.0 ± 3.94 (4)	11.60 ± 1.303[A] (6)	11.40 ± 1.364 (4)	45.36 ± 7.372[A] (5)	52.73 ± 23.856 (3)
Tungsten-Iron	48.8 ± 1.96[B] (8)	36.0 ± 1.57 (8)	15.60 ± 0.520[B] (8)	12.13 ± 0.509 (8)	76.49 ± 2.956[B] (8)	70.13 ± 8.500 (7)
Tungsten-Polymer	48.7 ± 2.00[B] (6)	36.9 ± 1.76 (8)	15.02 ± 0.240[B] (6)	12.29 ± 0.683 (8)	96.67 ± 9.256[B] (6)	69.39 ± 6.675 (8)

[a] Data presented as mean ± standard error of the mean. 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 x 12,500) / HCT. Means with different superscripts are significantly different within the column ($p \leq 0.05$).

compared to steel, tungsten-iron, and tungsten-polymer birds and hemoglobin concentrations were significantly lower compared to no-shot, steel, and tungsten-polymer birds. Lead-dosed females had significantly lower hematocrit's and hemoglobin concentrations when compared to the other 4 groups. The no-shot, steel, tungsten-iron, and tungsten-polymer-dosed birds were not significantly different from one another in hematocrits and hemoglobin concentrations. In addition to the lead-induced changes in hematocrit and hemoglobin concentration, red blood cell ALAD activity was significantly depressed at day 15 in the lead-dosed females when compared to the other 4 groups. While the 15 day ALAD activity for lead-dosed males was numerically lower than the activities for the other 4 groups, it was not significant. By day 30 of the trial, there were no significant differences in any of the whole-blood parameters between the 5 treatment groups.

Plasma Chemistries

The effects of steel, lead, tungsten-iron and tungsten-polymer shot on day 15 plasma chemistries for males are presented in Table 6. Lead shot resulted in a significant increase in plasma glucose concentration when compared to the other 4 dose groups. The albumin/globulin ratio was significantly increased in both the lead and tungsten-polymer birds when compared to no-shot and steel-dosed birds. The plasma activities of the hepatic enzymes alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and creatine phosphokinase were all significantly elevated in lead-dosed males at day 15 when compared to activities in the other 4 groups. There were no significant differences in plasma chemistry parameters between the no-shot, steel, tungsten-iron, and

Table 6. The effect of treatment shot on plasma chemistry parameters of male mallards on day 15 of a 30-day dosing test[a].						
Parameter	Units	No-Shot	Steel	Lead	Tungsten-Iron	Tungsten-Polymer
Glucose	mg/dL	201.0±7.24[B]	195.8±7.70[B]	234.7±8.56[A]	202.4±6.19[B]	196.8±7.05[B]
Sodium	mmol/L	140.8±1.82	138.0±2.27	135.3±2.77	139.4±1.53	140.8±0.90
Potassium	mmol/L	3.04±0.147	3.19±0.329	3.31±0.367	3.03±0.212	3.46±0.092
Chloride	mmol/L	108.5±1.12	107.0±2.12	100.6±3.92	108.9±0.99	108.3±0.96
Carbon Dioxide	mmol/L	24.1±2.33	22.8±1.10	22.7±2.56	22.9±1.54	20.4±1.24
Blood Urea Nitrogen	mg/dL	1.1±0.12	1.3±0.16	2.0±0.69	1.3±0.16	1.3±0.16
Creatinine	mg/dL	0.14±0.018	0.11±0.001	0.16±0.020	0.13±0.002	0.15±0.019
Blood Urea Nitrogen/Creatinine		10.55±2.026	11.73±0.781	14.80±4.246	11.96±1.143	12.33±1.914
Total Protein	gm/dL	4.51±0.101	4.14±0.202	4.06±0.297	4.31±0.126	4.48±0.206
Albumin	gm/dL	1.89±0.061	1.76±0.103	1.89±0.094	1.89±0.061	2.03±0.122
Albumin/Globulin		0.71±0.012[B]	0.74±0.018[B]	0.84±0.030[A]	0.79±0.023[AB]	0.84±0.026[A]
Total Bilirubin	mg/dL	0.40±0.071	0.46±0.151	0.56±0.107	0.46±0.141	0.40±0.065
Direct Bilirubin	mg/dL	0.16±0.057	0.25±0.153	0.34±0.134	0.25±0.142	0.20±0.078

[a] Data presented as mean ± standard error of the mean. Sample size is 8 for all groups except lead which is 7.
Means with different superscripts are significantly different within the row ($p \leq 0.05$).

Table 6 continued. The effect of treatment shot on plasma chemistry parameters of male mallards on day 15 of a 30-day dosing test[a].

Parameter	Units	No-Shot	Steel	Lead	Tungsten-Iron	Tungsten-Polymer
Calcium	mg/dL	9.40±0.163	9.19±0.198	9.49±0.320	9.51±0.203	12.18±0.450
Phosphorus	mg/dL	3.30±0.371	3.11±0.301	4.53±0.657	3.39±0.302	4.15±0.571
Uric Acid	mg/dL	4.15±0.506	3.83±0.517	6.74±1.264	4.34±0.514	4.79±0.591
Alkaline Phosphatase	U/L	50.1±14.05	67.5±12.40	63.4±17.60	53.6±5.46	67.9±15.31
Alanine Aminotransferase	U/L	6.9±2.44^{B}	9.4±2.70^{B}	54.3±19.82^{A}	7.9±1.26^{B}	7.6±2.66^{B}
Aspartate Aminotransferase	U/L	17.6±0.63^{B}	19.3±2.56^{B}	84.3±27.31^{A}	19.8±2.27^{B}	22.4±4.44^{B}
Lactate Dehydrogenase	U/L	671.8±65.00^{B}	679.5±73.89^{B}	3465.9±917.84^{A}	826.0±107.88^{B}	777.3±83.72^{B}
Creatine Phosphokinase	U/L	116.9±11.27^{B}	89.1±11.49^{B}	1763.3±653.37^{A}	176.4±56.76^{B}	185.6±25.60^{B}
Amylase	U/L	1115.8±70.46	1214.1±110.82	1213.3±178.32	1228.3±131.76	1221.5±28.65
Gamma Glutamyl Transpeptidase	U/L	9.1±1.14	9.0±2.86	6.3±0.19	9.3±2.45	9.4±1.46
Cholesterol	mg/dL	250.9±9.23	216.1±11.05	266.1±32.37	247.0±13.10	235.8±23.06
Triglycerides	mg/dL	114.5±10.38	122.1±17.41	126.4±34.88	119.6±8.62	233.8±102.49

[a] Data presented as mean ± standard error of the mean. Sample size is 8 for all groups except lead which is 7. Means with different superscripts are significantly different within the row ($p \leq 0.05$).

tungsten-polymer males at day 15 with the exception of the elevated albumin/globulin ratio in the tungsten-poylmer birds.

The effects of steel, lead, tungsten-iron and tungsten-polymer shot on day 15 plasma chemistries for females are presented in Table 7. Exposure of female mallards to lead shot caused a significant decrease in plasma sodium concentration compared to the no-shot, tungsten-iron, and tungsten-polymer birds and a significant decrease in chloride concentration when compared to the other 4 treatment groups. Plasma creatinine concentrations were significantly higher in the lead-dosed females when compared to the no-shot, steel and tungsten-polymer groups. The activities of alanine aminotransferase and lactate dehydrogenase were significantly higher in lead-dosed females when compared to values for the other 4 treatment groups.

By day 30, there were no differences in male plasma parameters (Table 8). In the lead-dosed females, chloride concentrations continued to be depressed when compared to tungsten-polymer birds. In addition, the concentration of carbon dioxide was significantly higher in lead-dosed females at day 30 when compared to tungsten-polymer birds. Alanine aminotransferase continued to be significantly increased in the lead-dosed females when compared to steel and tungsten-iron-dosed females (Table 9).

Gross Pathology

A summary of gross necropsy observations is presented in Tables 10 (males) and 11 (females). Those lead-dosed birds that died on trial were characterized by either having gizzards with discolored mucosal lining and multiple linear erosions (3 males and 2 females) or the birds were severely emaciated with little breast muscle (2 males and 1

Table 7.	The effect of treatment shot on plasma chemistry parameters of female mallards on day 15 of a 30-day dosing test[a] .					
Parameter	Units	No-Shot	Steel	Lead	Tungsten-Iron	Tungsten-Polymer
Glucose	mg/dL	200.1± 6.77	195.1± 7.41	222.5± 37.70	197.1± 5.27	199.0±6.92
Sodium	mmol/L	142.1± 1.56^{B}	141.3± 0.61^{AB}	134.5± 2.72^{A}	142.4± 1.77^{B}	142.5±1.46^{B}
Potassium	mmol/L	3.61± 0.259	3.16± 0.168	2.98± 0.098	4.08± 0.909	3.55±0.135
Chloride	mmol/L	107.8 ±1.11^{B}	108.6± 0.96^{B}	100.0± 3.21^{A}	109.1± 0.89^{B}	107.9±1.09^{B}
Carbon Dioxide	mmol/L	23.6± 2.28	22.0± 2.30	23.8± 3.40	21.5± 1.63	24.6±2.58
Blood Urea Nitrogen	mg/dL	1.1± 0.12	1.1± 0.12	1.2± 0.17	1.1± 0.12	1.0±0.00
Creatinine	mg/dL	0.11± 0.012^{B}	0.10± 0.000^{B}	0.18± 0.031^{A}	0.14± 0.018^{AB}	0.11±0.012^{B}
Blood Urea Nitrogen/Creatinine		12.34± 0.945	11.54± 0.767	9.02± 1.685	11.75± 1.504	11.36±0.688
Total Protein	gm/dL	4.70± 0.164	4.68± 0.146	3.97± 0.286	4.95± 0.495	4.61±0.130
Albumin	gm/dL	2.11± 0.123	2.03± 0.088	1.73± 0.088	2.20± 0.244	2.03±0.094
Albumin/Globulin		0.81± 0.058	0.76± 0.033	0.82± 0.060	0.79± 0.029	0.78±0.037
Total Bilirubin	mg/dL	0.53± 0.135	0.44± 0.068	0.35± 0.050	1.08± 0.692	0.54±0.115
Direct Bilirubin	mg/dL	0.35± 0.143	0.20± 0.073	0.17± 0.071	0.88± 0.705	0.35±0.126

[a] Data presented as mean ± standard error of the mean. Sample size is 8 for all groups except lead which is 6. Means with different superscripts are significantly different within the row ($p \leq 0.05$).

Table 7 continued. The effect of treatment shot on plasma chemistry parameters of female mallards on day 15 of a 30-day dosing test[a].

Parameter	Units	No-Shot	Steel	Lead	Tungsten-Iron	Tungsten-Polymer
Calcium	mg/dL	12.36± 0.945	10.76± 0.644	10.22± 1.017	11.26±1.297	12.05±0.896
Phosphorus	mg/dL	4.19±0.940	3.63±0.642	3.85±0.508	3.85±0.559	4.25±0.647
Uric Acid	mg/dL	4.33±0.603	4.99±0.932	5.20±0.831	3.81±0.548	3.18±0.373
Alkaline Phosphatase	U/L	196.6±74.93	52.8±10.41	80.5±35.01	94.6±21.24	101.5±28.24
Alanine Aminotransferase	U/L	9.4±2.08^{B}	4.6±1.10^{B}	22.7±4.84^{A}	5.0±1.05^{B}	5.5±1.07^{B}
Aspartate Aminotransferase	U/L	21.5±3.34	21.1±1.73	31.8±3.51	25.6±9.09	21.1±4.06
Lactate Dehydrogenase	U/L	888.1±132.09^{B}	873.4±90.72^{B}	1871.1±411.28^{A}	908.8±242.10^{B}	827.1±110.82^{B}
Creatine Phosphokinase	U/L	803.4±669.47	288.3±94.60	1022.5±590.92	172.4±47.26	184.4±38.12
Amylase	U/L	1224.3±99.22	1180.8±135.70	1098.7±120.76	1082.4±50.40	1037.9±80.82
Gamma Glutamyl Transpeptidase	U/L	12.3±3.49	10.3±1.77	6.2±0.17	16.5±7.88	13.3±2.49
Cholesterol	mg/dL	154.6±25.13	211.3±17.59	241.3±18.29	182.5±17.98	181.3±22.56
Triglycerides	mg/dL	286.3±53.76	158.4±15.80	115.8±29.47	192.5±42.15	299.0±99.59

[a] Data presented as mean ± standard error of the mean. Sample size is 8 for all groups except lead which is 6. Means with different superscripts are significantly different within the row ($p \leq 0.05$).

Table 8. The effect of treatment shot on plasma chemistry parameters of male mallards on day 30 of a 30-day dosing test[a].						
Parameter	Units	No-Shot	Steel	Lead	Tungsten-Iron	Tungsten-Polymer
Glucose	mg/dL	214.0±4.71	217.0±9.09	214.0±14.57	210.4±5.21	210.5±6.82
Sodium	mmol/L	141.0±1.75	144.1±0.95	145.0±3.22	141.1±0.99	142.6±1.21
Potassium	mmol/L	2.10±0.132	2.56±0.134	2.03±0.338	2.05±0.163	2.48±0.110
Chloride	mmol/L	111.5±1.50	114.1±0.86	114.0±1.15	111.3±0.90	112.8±1.21
Carbon Dioxide	mmol/L	18.9±1.26	20.1±1.03	16.7±1.20	21.4±1.09	18.6±2.51
Blood Urea Nitrogen	mg/dL	1.3±0.16	1.0±0.00	1.0±0.00	1.3±0.16	1.3±0.16
Creatinine	mg/dL	0.19±0.012	0.15±0.019	0.20±0.058	0.16±0.018	0.15±0.019
Blood Urea Nitrogen/Creatinine		8.16±0.375	8.88±0.480	7.53±1.475	9.66±0.606	9.16±0.992
Total Protein	gm/dL	3.86±0.087	3.96±0.118	4.40±0.643	3.90±0.136	3.80±0.127
Albumin	gm/dL	1.68±0.041	1.66±0.033	1.83±0.233	1.69±0.055	1.70±0.050
Albumin/Globulin		0.80±0.027	0.76±0.037	0.73±0.033	0.78±0.031	0.81±0.023
Total Bilirubin	mg/dL	0.20±0.019	0.23±0.016	0.20±0.058	0.26±0.042	0.20±0.019
Direct Bilirubin	mg/dL	0.01±0.012	0.00±0.000	0.04±0.000	0.04±0.026	0.00±0.000

[a] Data presented as mean ± standard error of the mean. Sample size is 8 for all groups except lead which is 3. Means with different uperscripts are significantly different within the row ($p \leq 0.05$).

Table 8 continued.	The effect of treatment shot on plasma chemistry parameters of male mallards on day 30 of a 30-day dosing test[a] .					
Parameter	Units	No-Shot	Steel	Lead	Tungsten-Iron	Tungsten-Polymer
Calcium	mg/dL	9.44±0.124	9.49±0.081	9.90±0.379	9.60±0.113	9.70±0.151
Phosphorus	mg/dL	2.40±0.307	1.81±0.167	2.53±0.484	2.21±0.414	2.00±0.375
Uric Acid	mg/dL	5.31±0.783	2.99±0.275	3.93±0.722	4.21±0.451	4.78±0.783
Alkaline Phosphatase	U/L	52.1±99.93	81.0±20.38	45.67±6.64	63.3±6.95	60.3±6.94
Alanine Aminotransferase	U/L	5.8±1.37	8.4±2.27	9.7±3.38	8.1±2.17	7.9±2.29
Aspartate Aminotransferase	U/L	24.1±36.71	29.1±4.71	59.3±34.84	31.5±14.82	26.1±3.85
Lactate Dehydrogenase	U/L	729.3±39.15	858.6±58.92	1115.3±180.01	774.5±96.76	835.1±71.79
Creatine Phosphokinase	U/L	211.4±243.43	187.5±21.27	534.3±295.86	257.8±105.70	213.5±32.74
Amylase	U/L	1312.8±134.31	1448.1±162.33	1529.3±361.90	1377.0±151.67	1446.9±159.04
Gamma Glutamyl Transpeptidase	U/L	6.0±0.00	6.0±0.00	6.0±0.00	6.3±0.25	5.3±0.75
Cholesterol	mg/dL	219.1±9.73	207.4±8.10	206.0±12.16	236.8±9.56	225.6±7.42
Triglycerides	mg/dL	133.5±15.56	97.3±9.91	110.3±22.93	90.9±9.60	103.1±7.28

[a] Data presented as mean ± standard error of the mean. Sample size is 8 for all groups except lead which is 3. Means with different superscripts are significantly different within the row ($p \leq 0.05$).

Table 9.	The effect of treatment shot on plasma chemistry parameters of female mallards on day 30 of a 30-day dosing test[a] .					
Parameter	Units	No-Shot	Steel	Lead	Tungsten-Iron	Tungsten-Polymer
Glucose	mg/dL	215.5±10.66	224.9±6.45	223.4±14.40	217.4±8.83	213.9±4.99
Sodium	mmol/L	145.3±0.82	145.0±0.50	143.2±1.28	146.0±1.53	148.4±1.82
Potassium	mmol/L	2.99±0.157	2.53±0.190	2.74±0.196	2.66±0.129	2.71±0.257
Chloride	mmol/L	111.5 ± 0.76^{AB}	112.1 ± 0.64^{AB}	108.2 ± 1.28^{A}	112.8 ± 1.56^{AB}	114.9 ± 2.17^{B}
Carbon Dioxide	mmol/L	20.1 ± 0.83^{AB}	22.1 ± 0.61^{AB}	24.2 ± 1.59^{A}	21.4 ± 1.20^{AB}	19.1 ± 1.27^{B}
Blood Urea Nitrogen	mg/dL	1.8±0.49	1.1±0.12	1.6±0.25	1.4±0.18	2.0±0.68
Creatinine	mg/dL	0.11±0.013	0.14±0.018	0.14±0.025	0.14±0.181	0.11±0.012
Blood Urea Nitrogen/Creatinine		15.56±4.278	10.59±0.654	10.14±4.977	11.50±1.545	22.63±6.248
Total Protein	gm/dL	4.41±0.356	4.26±0.107	4.24±0.515	4.03±0.150	4.48±0.216
Albumin	gm/dL	1.95±0.158	1.84±0.060	1.82±0.211	1.79±0.081	2.05±0.128
Albumin/Globulin		0.81±0.035	0.76±0.018	0.76±0.040	0.83±0.031	0.83±0.037
Total Bilirubin	mg/dL	0.25±0.033	0.21±0.023	0.28±0.038	0.26±0.042	0.20±0.027
Direct Bilirubin	mg/dL	0.08±0.042	0.01±0.012	0.10±0.045	0.06±0.037	0.06±0.026

[a] Data presented as mean ± standard error of the mean. Sample size is 8 for all groups except lead which is 5. Means with different superscripts are significantly different within the row ($p \leq 0.05$).

Table 9 continued.	The effect of treatment shot on plasma chemistry parameters of female mallards on day 30 of a 30-day dosing test[a].					
Parameter	Units	No-Shot	Steel	Lead	Tungsten-Iron	Tungsten-Polymer
Calcium	mg/dL	15.19±2.361	11.26±0.636	13.96±3.876	13.08±1.313	16.98±3.111
Phosphorus	mg/dL	4.18±0.839	1.78±0.290	3.98±1.170	2.48±0.500	4.05±0.706
Uric Acid	mg/dL	4.29±0.730	4.34±0.598	5.76±0.878	4.65±0.711	3.08±0.578
Alkaline Phosphatase	U/L	591.5±241.30^{A}	54.0±6.90^{B}	129.8±58.87^{AB}	175.8±84.23^{AB}	239.5±117.47^{AB}
Alanine Aminotransferase	U/L	17.1±3.69^{AB}	5.9±2.08^{B}	21.8±6.86^{A}	5.6±1.59^{B}	10.5±2.43^{AB}
Aspartate Aminotransferase	U/L	59.3±19.31	33.0±6.30	90.8±36.35	33.4±8.20	41.1±10.27
Lactate Dehydrogenase	U/L	1463.3±366.93	1024.9±161.90	2185.0±1045.38	975.6±96.95	1012.0±210.33
Creatine Phosphokinase	U/L	1281.3±629.57	196.5±28.46	1535.2±1117.19	483.5±194.65	975.3±757.84
Amylase	U/L	1552.6±203.59	1211.6±86.41	1103.8±62.24	1232.0±167.90	1230.4±152.22
Gamma Glutamyl Transpeptidase	U/L	7.3±0.82	6.0±0.00	6.2±0.20	6.4±0.37	6.3±0.16
Cholesterol	mg/dL	148.8±24.17	197.6±13.21	195.4±16.51	155.3±17.84	129.4±22.49
Triglycerides	mg/dL	393.3±104.61	133.6±12.85	443.8±317.54	188.5±33.38	280.4±75.68

[a] Data presented as mean ± standard error of the mean. Sample size is 8 for all groups except lead which is 5. Means with different superscripts are significantly different within the row ($p \leq 0.05$).

Table 10 . The gross necropsy observations from the effects of treatment shot on male mallards on a 30-day dosing test for candidate shot.

Duck ID	Treatment	Days on Trial	Observation(s)[a]
467111	No-Shot	30	Normal
467119	No-Shot	30	Fatty Liver
467128	No-Shot	30	Normal
467135	No-Shot	30	Normal
467155	No-Shot	30	Normal
467163	No-Shot	30	Normal
467172	No-Shot	30	Normal
467180	No-Shot	30	Normal
467107	Steel	30	Normal
467115	Steel	30	Normal
467124	Steel	30	Normal
467141	Steel	30	Normal
467151	Steel	30	Normal
467159	Steel	30	Normal
467168	Steel	30	Normal
467187	Steel	30	Normal

[a] Gross necropsy observations performed by Dr. Scott Fitzgerald.

Table 10 continued. The gross necropsy observations from the effects of treatment shot on male mallards on a 30-day dosing test for candidate shot.

Duck ID	Treatment	Days on Trial	Observation(s)[a]
467109	Lead	25	Discolored mucosal lining of the gizzard with multiple linear erosions
467117	Lead	11	Discolored mucosal lining of the gizzard with linear erosions
467126	Lead	30	Normal
467133	Lead	30	Severe breast muscle atrophy
467153	Lead	30	Normal
467161	Lead	17	Prominent keel bone with no subcutaneous or abdominal fat (emaciation) Moderately thickened koilin layer of the gizzard Small liver (1/2 to 3/4 normal)
467170	Lead	20	Prominent keel bone (emaciation) Multifocal, moderate thickening of the koilin layer of the gizzard Small liver (3/4 normal) with multiple 1 mm white foci scattered throughout the capsular and cut surfaces
467178	Lead	15	Discolored mucosal lining of the gizzard with multiple linear erosions

[a] Gross necropsy observations performed by Dr. Scott Fitzgerald, or in the case of 467170, by Dr. R.M. Fulton, board-certified avian diagnostician.

Table 10 continued. The gross necropsy observations from the effects of treatment shot on male mallards on a 30-day dosing test for candidate shot.

Duck ID	Treatment	Days on Trial	Observation(s)[a]
467103	Tungsten-Iron	30	Normal
467113	Tungsten-Iron	30	Normal
467132	Tungsten-Iron	30	Normal
467139	Tungsten-Iron	30	Normal
467147	Tungsten-Iron	30	Normal
467157	Tungsten-Iron	30	Normal
5417	Tungsten-Iron	30	Normal
467185	Tungsten-Iron	30	Normal
467101	Tungsten-Polymer	30	Normal
467121	Tungsten-Polymer	30	Normal
467130	Tungsten-Polymer	30	Normal
467137	Tungsten-Polymer	30	Normal
467145	Tungsten-Polymer	30	Normal
467165	Tungsten-Polymer	30	Normal
467174	Tungsten-Polymer	30	Normal
467182	Tungsten-Polymer	30	Normal

[a] Gross necropsy observations performed by Dr. Scott Fitzgerald.

Table 11 . The gross necropsy obervations from the effects of treatment shot on female mallards on a 30-day dosing test for candidate shot.

Duck ID	Treatment	Days on Trial	Observation(s)[a]
467112	No-Shot	30	Normal
467120	No-Shot	30	Moderate fatty liver
467127	No-Shot	30	Moderate fatty liver
467136	No-Shot	30	Normal
467156	No-Shot	30	Normal
467164	No-Shot	30	Fatty liver
467171	No-Shot	30	Normal
467179	No-Shot	30	Normal
467108	Steel	30	Normal
467116	Steel	30	Normal
467123	Steel	30	Normal
467142	Steel	30	Normal
467152	Steel	30	Normal
467160	Steel	30	Normal
5418	Steel	30	Normal
467186	Steel	30	Normal

[a] Gross necropsy observations performed by Dr. Scott Fitzgerald.

Table 11 continued. The gross necropsy observations from the effects of treatment shot on female mallards on a 30-day dosing test for candidate shot.

Duck ID	Treatment	Days on Trial	Observation(s)[a]
467110	Lead	30	Normal
5421	Lead	10	Discolored, cracked mucosal lining of the gizzard with linear erosions
467125	Lead	30	Normal
467134	Lead	30	Fatty liver
467154	Lead	30	Normal
467162	Lead	30	Moderately emaciated
467169	Lead	21	Emaciated Air sac granuloma
467177	Lead	15	Discolored mucosal lining of the gizzard with linear erosions

[a] Gross necropsy observations performed by Dr. Scott Fitzgerald.

Table 11 continued. The gross necropsy observations from the effects of treatment shot on female mallards on a 30-day dosing test for candidate shot.

Duck ID	Treatment	Days on Trial	Observation(s)[a]
467106	Tungsten-Iron	30	Normal
467114	Tungsten-Iron	30	Normal
467131	Tungsten-Iron	30	Normal
467140	Tungsten-Iron	30	Normal
467150	Tungsten-Iron	30	Normal
467158	Tungsten-Iron	30	Normal
5419	Tungsten-Iron	30	Normal
467184	Tungsten-Iron	30	Normal
467102	Tungsten-Polymer	30	Normal
467122	Tungsten-Polymer	30	Normal
467129	Tungsten-Polymer	30	Normal
467138	Tungsten-Polymer	30	Normal
5436	Tungsten-Polymer	30	Normal
467166	Tungsten-Polymer	30	Normal
467173	Tungsten-Polymer	30	Normal
467181	Tungsten-Polymer	30	Normal

[a] Gross necropsy observations performed by Dr. Scott Fitzgerald.

female). With 2 exceptions, gizzard erosion occurred in those birds dying during the first half of the trial and emaciation was characteristic of those birds dying between days 16-25. Of the 8 surviving lead-dosed birds, 1 male and 1 female were emaciated at the time of necropsy, 1 female had a fatty liver while the other 5 birds (2 males and 3 females) appeared normal. Four no-shot birds (1 male and 3 females) had moderately fatty livers while all birds in the steel, tungsten-iron and tungsten-polymer shot groups appeared normal.

Absolute Organ Weights

The effects of steel, lead, tungsten-iron, and tungsten-polymer shot on absolute organ weights of male and female mallards are presented in Table 12 and 13. There were no significant differences between treatments in male organ weights while lead-dosed female liver weight was significantly higher when compared to the average liver weight of steel-dosed females.

Organ Weights as a Percent of Body Weight

The effects of treatment shot on male and female organ weights expressed as a percent of body weight are presented in Table 14 and 15. The only change noted within males was an increase in relative kidney weights in lead-dosed birds when compared to relative kidney weights of no-shot and tungsten-iron males. In females, relative heart weights of the lead-dosed birds were significantly higher when compared to the no-shot and tungsten-polymer birds whereas relative kidney weights of lead-dosed females were significantly greater when compared to the tungsten-iron and tungsten-polymer birds. Relative liver weights were significantly higher in lead-dosed females when compared to

Table 12. The effect of treatment shot on organ weights (gm) of male mallards on a 30-day dosing test[a].						
	Brain	Heart	Kidneys	Liver	Spleen	Testes
Males						
No-Shot	4.012±0.3842	11.129±0.7067	5.998±0.4754	21.595±2.6228	0.499±0.0373	16.714 ± 5.4287
Steel	4.199±0.2858	10.246±0.3406	5.599±0.2561	18.920±1.6676	0.550± 0.1153	16.576 ± 4.7407
Lead	4.251±0.5354	9.823±0.4922	6.256±0.0529	19.992±1.8747	0.462 ± 0.0781	11.241 ± 9.3526
Tungsten-Iron	4.555±0.1958	9.998±0.3081	5.363±0.1873	17.19±1.1538	0.462 ± 0.0753	14.530 ± 3.7839
Tungsten-Polymer	5.637±1.4717	9.596±0.1952	5.693±0.2345	18.43±1.2853	0.431±0.0527	18.207±6.2010

[a] Data presented as mean ± standard error of the mean. Sample size for all parameters is 8 except for no-shot male spleen weight which is 7, lead male brain, heart, kidneys, liver, and spleen weights which is 3, and lead male testes weight which is 2. Means with different superscripts are significantly different within the column ($p \leq 0.05$).

Table 13. The effect of treatment shot on organ weights (gm) of female mallards on a 30-day dosing test[a].						
	Brain	Heart	Kidneys	Liver	Spleen	Testes
Females						
No-Shot	4.521±1.5985	8.489±0.7067	6.262±0.3820	23.437±2.5379^{AB}	0.531±0.0585	.
Steel	4.227±0.2338	8.586±0.3406	5.478±0.2355	15.926±0.6615^{B}	0.382±0.0322	.
Lead	3.540±0.3847	9.479±0.4922	6.858±0.7845	26.749±3.7655^{A}	0.451±0.0483	.
Tungsten-Iron	4.348±0.2185	9.146±0.3081	5.596±0.2895	18.700±1.6558^{AB}	0.417±0.0252	.
Tungsten-Polymer	4.279±0.2636	8.436±0.2751	5.607±0.3158	20.626±2.3818^{AB}	0.442±0.0457	.

[a] Data presented as mean ± standard error of the mean. Sample size for all parameters is 8 except for all lead weights which is 5. Means with different superscripts are significantly different within the column ($p \leq 0.05$).

Table 14.	The effect of treatment shot on organ weights expressed as percent body weight of male mallards on a 30-day dosing test[a] .					
	Brain	Heart	Kidneys	Liver	Spleen	Testes
Males						
No-Shot	0.323 ± 0.0435	0.854 ± 0.0318	0.460 ± 0.0274^{B}	1.641 ± 0.1604	0.040 ± 0.0310	1.229 ± 0.3950
Steel	0.370 ± 0.0312	0.894 ± 0.0371	0.489 ± 0.0212^{AB}	1.640 ± 0.1294	0.049 ± 0.0767	1.403 ± 0.3899
Lead	0.407 ± 0.0636	0.930 ± 0.0513	0.593 ± 0.0177^{A}	1.883 ± 0.1090	0.043 ± 0.0335	1.100 ± 0.9199
Tungsten-Iron	0.394 ± 0.0298	0.864 ± 0.0453	0.459 ± 0.0956^{B}	1.474 ± 0.0961	0.039 ± 0.0668	1.214 ± 0.2945
Tungsten-Polymer	0.479 ±0.1178	0.824 ± 0.0240	0.490 ± 0.0244^{AB}	1.558 ± 0.0578	0.036 ± 0.0375	1.470 ± 0.4848

[a] Data presented as mean ± standard error of the mean. Sample size for all parameters is 8 except for no-shot male spleen weight which is 7, lead male brain, heart, kidneys, liver, and spleen weights which is 3, and lead male testes weight which is 2. Means with different superscripts are significantly different within the column ($p \leq 0.05$).

Table 15. The effect of treatment shot on organ weights expressed as percent body weight of female mallards on a 30-day dosing test[a] .						
	Brain	Heart	Kidneys	Liver	Spleen	Testes
Females						
No-Shot	0.438 ± 0.0183	0.818 ± 0.0237^{B}	0.606 ± 0.0424^{AB}	2.235 ± 0.2052^{AB}	0.051 ± 0.0548	.
Steel	0.441 ± 0.0255	0.889 ± 0.0267^{AB}	0.568 ± 0.0202^{AB}	1.660 ± 0.0857^{B}	0.038 ± 0.0293	.
Lead	0.382 ± 0.0563	1.024 ± 0.1313^{A}	0.726 ± 0.0801^{A}	2.772 ± 0.2642^{A}	0.048 ± 0.0376	.
Tungsten-Iron	0.428 ± 0.0265	0.890 ± 0.0218^{AB}	0.545 ± 0.0204^{B}	1.808 ± 0.1266^{B}	0.038 ± 0.0293	.
Tungsten-Polymer	0.413 ± 0.0312	0.811± 0.0199^{B}	0.538 ± 0.0288^{B}	1.954 ± 0.1855^{B}	0.043 ± 0.0045	.

[a] Data presented as mean ± standard error of the mean. Sample size for all parameters is 8 except for all lead female weights which is 5. Means with different superscripts are significantly different within the column ($p \leq 0.05$).

the steel, tungsten-iron and tungsten-polymer groups.

Histopathology of Kidney and Liver

The effects of treatment shot on liver and kidney pathology are presented in Tables 16 (males) and 17 (females). Lesion scores for the liver and kidney are presented in Tables 18 (males) and 19 (females). Five male lead-dosed birds developed renal nephrosis ranging from mild to severe with accompanying mild to severe hepatic biliary stasis. All of these birds died during the course of the trial (days 11-25). The remaining 3 lead-dosed males had normal kidneys and mild to moderate hepatic biliary stasis. These birds survived to the end of the 30-day trial. Two tungsten-iron-dosed males and 3 tungsten-polymer-dosed males developed mild liver biliary stasis. The severity of liver lesions in the lead-dosed males was significant when compared to the other 4 groups while there were no statistical differences in the severity of kidney lesions. Three lead-dosed females had renal nephrosis ranging from mild to moderate with accompanying moderate to severe hepatic biliary stasis. These 3 females died on days 10, 15, and 21 of the trial. Three of the 8 lead-dosed females developed liver biliary stasis only (mild to severe) and they survived the duration of the 30-day trial. Two lead-dosed females had normal liver and kidney tissues. Three tungsten-iron females developed mild biliary stasis with no kidney involvement while the tungsten-polymer females were unaffected. The severity of liver lesions in the lead-dosed females was significant when compared to the other 4 groups. There were no statistical differences in the severity of kidney lesions. Throughout all 5 groups, diffuse hepatocellular vacuolation was apparent, but this condition was judged not to be treatment related.

Table 16 . The histopathological effects of treatment shot on the liver and kidneys of male mallards on a 30-day dosing test for candidate shot.

Duck ID	Trt[a]	AHDL[b] Code	Observation(s)[c]
467111	NS	467-111	Moderate, diffuse hepatocellular vacuolation
467119	NS	196881-14	Severe, diffuse hepatocellular vacuolation
467128	NS	169881-23	Normal
467135	NS	196881-30	Moderate, diffuse hepatocellular vacuolation
467155	NS	196881-45	Normal
467163	NS	196881-52	Mild, diffuse hepatocellular vacuolation
467172	NS	196881-58	Moderate, diffuse hepatocellular vacuolation
467180	NS	196881-62	Normal
467107	S	196881-5	Moderate, diffuse hepatocellular vacuolation
467115	S	196881-12	Moderate, diffuse hepatocellular vacuolation
467124	S	169881-19	Moderate, diffuse hepatocellular vacuolation
467141	S	196881-30	Normal
467151	S	196881-41	Normal
467159	S	196881-49	Mild, diffuse hepatocellular vacuolation
467168	S	196881-56	Normal
467187	S	196881-68	Mild, diffuse hepatocellular vacuolation

[a] NS refers to no-shot and S refers to steel.
[b] AHDL refers to Michigan State University's Animal Health Diagnostic Laboratory where the tissues were prepared and assessed for histopathological changes.
[c] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald.

Table 16 continued. The histopathological effects of treatment shot on the liver and kidneys of male mallards on a 30-day dosing test for candidate shot.

Duck ID	Trt[a]	AHDL[b]Code	Observation(s)[c]
467109	L	196210	Diffuse, acute proximal (mild)and distal (severe) convoluted renal tubule necrosis with pyknosis, vacuolation,and inclusions Moderate, diffuse hepatocellular and cholangial biliary stasis with mild extramedullary hematopoiesis, 25 days on trial
467117	L	195237-1	Mild, diffuse acute proximal and distal renal tubular necrosis with pyknosis and rare inclusions Severe, diffuse hepatocellular and cholangial biliary stasis, 11 days on trial
467126	L	196881-21	Kidney-normal Moderate, diffuse hepatocellular vacuolation Mild hepatocellular biliary stasis
467133	L	196881-28	Kidney-normal Mild, diffuse hepatocellular vacuolation Moderate hepatocellular and cholangial biliary stasis
467153	L	196881-43	Kidney-normal Mild, diffuse hepatocellular vacuolation Mild hepatocellular and cholangial biliary stasis
467161	L	195403-1	Moderate proximal and distal renal tubule necrosis with pyknosis, vacuolation Mild hepatocellular and cholangial biliary stasis, 17 days on trial
467170	L	195549-2	Mild proximal renal tubule necrosis with inclusions Moderate hepatocellular and cholangial biliary stasis with multifocal hepatic abssesses, 20 days on trial
467178	L	195403-1	Moderate proximal and distal renal tubule necrosis with pyknosis, vacuolation, and inclusions Moderate hepatocellular and cholangial biliary stasis, 15 days on trial

[a] L refers to lead.
[b] AHDL refers to Michigan State University's Animal Health Diagnostic Laboratory where the tissues were prepared and assessed for histopathological changes.
[c] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald.

Table 16 continued. The histopathological effects of treatment shot on the liver and kidneys of male mallards on a 30-day dosing test for candidate shot.

Duck ID	Trt[a]	AHDL[b] Code	Observation(s)[c]
467103	TI	196881-3	Mild, periportal hepatocellular vacuolation
467113	TI	196881-10	Normal
467132	TI	169881-27	Moderate, diffuse hepatocellular vacuolation
467139	TI	196881-34	Moderate, diffuse hepatocellular vacuolation
467147	TI	196881-39	Mild hepatocullular vacuolation
467157	TI	196881-47	Mild, diffuse hepatocellular biliary stasis
5417	TI	196881-60	Mild hepatocellular vacuolation Mild hepatocellular and cholangial biliary stasis
467185	TI	196881-66	Normal
467101	TP	196881-1	Moderate, diffuse hepatocellular vacuolation
467121	TP	196881-16	Mild, diffuse hepatocellular vacuolation
467130	TP	196881-25	Moderate, diffuse hepatocellular vacuolation
467137	TP	196881-32	Moderate, diffuse hepatocellular vacuolation
467145	TP	196881-38	Moderate hepatocullular vacuolation Mild hepatocellular biliary stasis
467165	TP	196881-54	Mild hepatocellular biliary stasis
467174	TP	196881-60	Normal
467182	TP	196881-64	Mild, hepatocellular biliary stasis

[a] TI refers to tungsten-iron and TP refers to tungsten-polymer.
[b] AHDL refers to Michigan State University's Animal Health Diagnostic Laboratory where the tissues were prepared and assessed for histopathological changes.
[c] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald.

Table 17 . The histopathological effects of treatment shot on the liver and kidneys of female mallards on a 30-day dosing test for candidate shot.

Duck ID	Trt[a]	AHDL[b] Code	Observation(s)[c]
467112	NS	196881-9	Moderate, diffuse hepatocellular vacuolation
467120	NS	196881-15	Moderate, diffuse hepatocellular vacuolation
467127	NS	196881-22	Severe, diffuse hepatocellular vacuolation
467136	NS	196881-31	Mild hepatocellular vacuolation
467156	NS	196881-46	Moderate, diffuse hepatocellular vacuolation
467164	NS	196881-53	Moderate, diffuse hepatocellular vacuolation
467171	NS	196881-57	Mild, diffuse hepatocellular vacuolation
467179	NS	196881-61	Moderate, diffuse hepatocullular vacuolation
467108	S	196881-6	Mild, diffuse hepatocellular vacuolation
467116	S	196881-13	Mild, diffuse hepatocellular vacuolation
467123	S	169881-18	Moderate, diffuse hepatocellular vacuolation
467142	S	196881-37	Normal
467152	S	196881-42	Normal
467160	S	196881-50	Moderate, diffuse hepatocellular vacuolation
5418	S	196881-70	Mild, diffuse hepatocellular vacuolation
467186	S	196881-67	Mild, diffuse hepatocellular vacuolation

[a] NS refers to no-shot and S refers to steel.
[b] AHDL refers to Michigan State University's Animal Health Diagnostic Laboratory where the tissues were prepared and assessed for histopathological changes.
[c] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald.

Table 17 continued. The histopathological effects of treatment shot on the liver and kidneys of female mallards on a 30-day dosing test for candidate shot.

Duck ID	Trt[a]	AHDL[b] Code	Observation(s)[c]
467110	L	196881-7	Kidney-normal Moderate, diffuse hepatocellular vacuolation Moderate hepatocellular and cholangial biliary stasis
5421	L	195237-2	Diffuse proximal (mild) and distal (moderate) renal tubule necrosis with pyknosis and inclusions Severe hepatocellular and cholangial biliary stasis with mild extramedullary hematopoiesis, 10 days on trial
467125	L	196881-20	Kidney-normal Moderate, diffuse hepatocellular vacuolation Mild hepatocellular and cholangial biliary stasis
467134	L	196881-29	Kidney-normal Severe, diffuse hepatocellular vacuolation
467154	L	196881-44	Kidney-normal Moderate, diffuse hepatocellular vacuolation Moderate hepatocellular and cholangial biliary stasis
467162	L	195881-51	Kidney-normal Mild, diffuse hepatocellular vacuolation
467169	L	195785	Moderate proximal and distal renal tubule necrosis with pyknosis and inclusions Moderate, diffuse hepatocellular vacuolation Moderate hepatocellular and cholangial biliary stasis Air sacculitis/histocytic, 21 days on trial
467177	L	195403-2	Mild proximal and distal renal tubule necrosis with pyknosis Mild, diffuse hepatocellular vacuolation Severe hepatocellular and cholangial biliary stasis, 15 days on trial

[a] L refers to lead.
[b] AHDL refers to Michigan State University's Animal Health Diagnostic Laboratory where the tissues were prepared and assessed for histopathological changes.
[c] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald.

Table 17 continued. The histopathological effects of treatment shot on the liver and kidneys of female mallards on a 30-day dosing test for candidate shot.

Duck ID	Trt[a]	AHDL[b] Code	Observation(s)[c]
467106	TI	196881-4	Moderate, diffuse hepatocellular vacuolation
467114	TI	196881-11	Moderate, diffuse hepatocellular vacuolation Mild, diffuse hepatocellular biliary stasis
467131	TI	169881-26	Mild, diffuse hepatocellular vacuolation Mild hepatocellular biliary stasis
467140	TI	196881-35	Mild, diffuse hepatocellular vacuolation
467150	TI	196881-40	Moderate, diffuse hepatocullular vacuolation Mild hepatocellular biliary stasis
467158	TI	196881-48	Mild, diffuse hepatocellular vacuolation
5419	TI	196881-71	Moderate, diffuse hepatocellular vacuolation
467184	TI	196881-65	Normal
467102	TP	196881-2	Moderate, diffuse hepatocellular vacuolation
467122	TP	196881-17	Moderate, diffuse hepatocellular vacuolation
467129	TP	169881-24	Moderate, diffuse hepatocellular vacuolation
467138	TP	196881-33	Moderate, diffuse hepatocellular vacuolation
5436	TP	196881-72	Normal
467166	TP	196881-55	Normal
467173	TP	196881-59	Normal
467181	TP	196881-63	Moderate, diffuse hepatocellular vacuolation

[a] TI refers to tungsten-iron and TP refers to tungsten-polymer.
[b] AHDL refers to Michigan State University's Animal Health Diagnostic Laboratory where the tissues were prepared and assessed for histopathological changes.
[c] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald.

Table 18. The severity of liver and kidney lesions induced by treatment shot in male mallards on a 30-day dosing test.

Duck ID	Treatment	Days on Trial	Renal Nephrosis[a]	Hepatic Biliary Stasis[a]
467111	No-Shot	30	0	0
467119	No-Shot	30	0	0
467128	No-Shot	30	0	0
467135	No-Shot	30	0	0
467155	No-Shot	30	0	0
467163	No-Shot	30	0	0
467172	No-Shot	30	0	0
467180	No-Shot	30	0	0
467107	Steel	30	0	0
467115	Steel	30	0	0
467124	Steel	30	0	0
467141	Steel	30	0	0
467151	Steel	30	0	0
467159	Steel	30	0	0
467168	Steel	30	0	0
467187	Steel	30	0	0

[a] Lesion scores: 0, normal; +, mild; ++, moderate; +++, severe.

Table 18 continued. The severity of liver and kidney lesions induced by treatment shot in male mallards on a 30-day dosing test.

Duck ID	Treatment	Days on Trial	Renal Nephrosis[a]	Hepatic Biliary Stasis[a]
467117	Lead	11	+	+++
467178	Lead	15	++	++
467161	Lead	17	++	+
467170	Lead	20	+	++
467109	Lead	25	+++	++
467126	Lead	30	0	+
467133	Lead	30	0	++
467153	Lead	30	0	+

[a] Lesion scores: 0, normal; +, mild; ++, moderate; +++, severe.

Table 18 continued. The severity of liver and kidney lesions induced by treatment shot in male mallards on a 30-day dosing test.

Duck ID	Treatment	Days on Trial	Renal Nephrosis[a]	Hepatic Biliary Stasis[a]
467103	Tungsten-Iron	30	0	0
467113	Tungsten-Iron	30	0	0
467132	Tungsten-Iron	30	0	0
467139	Tungsten-Iron	30	0	0
467147	Tungsten-Iron	30	0	0
467157	Tungsten-Iron	30	0	+
5417	Tungsten-Iron	30	0	+
467185	Tungsten-Iron	30	0	0
467101	Tungsten-Polymer	30	0	0
467121	Tungsten-Polymer	30	0	0
467130	Tungsten-Polymer	30	0	0
467137	Tungsten-Polymer	30	0	0
467145	Tungsten-Polymer	30	0	+
467165	Tungsten-Polymer	30	0	+
467174	Tungsten-Polymer	30	0	0
467182	Tungsten-Polymer	30	0	+

[a] Lesion scores: 0, normal; +, mild; ++, moderate; +++, severe.

Table 19. The severity of liver and kidney lesions induced by treatment shot in female mallards on a 30-day dosing test.

Duck ID	Treatment	Days on Trial	Renal Nephrosis[a]	Hepatic Biliary Stasis[a]
467112	No-Shot	30	0	0
467120	No-Shot	30	0	0
467127	No-Shot	30	0	0
467136	No-Shot	30	0	0
467156	No-Shot	30	0	0
467164	No-Shot	30	0	0
467171	No-Shot	30	0	0
467179	No-Shot	30	0	0
467108	Steel	30	0	0
467116	Steel	30	0	0
467123	Steel	30	0	0
467142	Steel	30	0	0
467152	Steel	30	0	0
467160	Steel	30	0	0
5418	Steel	30	0	0
467186	Steel	30	0	0

[a] Lesion scores: 0, normal; +, mild; ++, moderate; +++, severe.

Table 19 continued. The severity of liver and kidney lesions induced by treatment shot in female mallards on a 30-day dosing test.

Duck ID	Treatment	Days on Trial	Renal Nephrosis[a]	Hepatic Biliary Stasis[a]
5421	Lead	10	++	+++
467177	Lead	15	+	+++
467169	Lead	21	++	++
467110	Lead	30	0	++
467125	Lead	30	0	+
467134	Lead	30	0	0
467154	Lead	30	0	++
467162	Lead	30	0	0

[a] Lesion scores: 0, normal; +, mild; ++, moderate; +++, severe.

Table 19 continued. The severity of liver and kidney lesions induced by treatment shot in female mallards on a 30-day dosing test.

Duck ID	Treatment	Days on Trial	Renal Nephrosis[a]	Hepatic Biliary Stasis[a]
467106	Tungsten-Iron	30	0	0
467114	Tungsten-Iron	30	0	+
467131	Tungsten-Iron	30	0	+
467140	Tungsten-Iron	30	0	0
467150	Tungsten-Iron	30	0	+
467158	Tungsten-Iron	30	0	0
5419	Tungsten-Iron	30	0	0
467184	Tungsten-Iron	30	0	0
467102	Tungsten-Polymer	30	0	0
467122	Tungsten-Polymer	30	0	0
467129	Tungsten-Polymer	30	0	0
467138	Tungsten-Polymer	30	0	0
5436	Tungsten-Polymer	30	0	0
467166	Tungsten-Polymer	30	0	0
467173	Tungsten-Polymer	30	0	0
467181	Tungsten-Polymer	30	0	0

[a] Lesion scores: 0, normal; +, mild; ++, moderate; +++, severe.

Tissue Metal Analysis

The concentrations of iron, lead, tungsten, and molybdenum in the femur of male and female mallards are presented in Table 20. Lead-dosed males had significantly higher concentrations of iron in the femur when compared to the no-shot, tungsten-iron and tungsten-polymer birds and significantly higher concentrations of lead than the other 4 groups. Tungsten was detected in the femur of tungsten-iron males only. In females, femur lead concentration was significantly higher in the lead-dosed group when compared to the other 4 treatment groups. Tungsten was detected in both tungsten-iron and tungsten-polymer birds with the concentration in tungsten-iron birds being significantly higher compared to the tungsten-polymer group. Molybdenum was not detected in the femur of any bird.

The concentrations of iron, lead, tungsten, and molybdenum in the liver of male and female mallards are presented in Table 21. Hepatic iron concentrations were significantly higher in the lead-dosed males when compared to the no-shot, steel, and tungsten-polymer birds and tungsten-iron birds had significantly higher liver iron concentrations compared to no-shot males. Hepatic lead concentrations were significantly higher in the males receiving lead shot compared to the other 4 groups. Lead-dosed males had significantly higher concentrations of molybdenum when compared to no-shot, steel, and tungsten-polymer birds and tungsten-iron males had a higher molybdenum concentration than no-shot males. Tungsten was detected only in the liver of tungsten-iron males. In females, hepatic iron concentrations were significantly elevated in the lead-dosed birds when compared to the no-shot birds and hepatic lead concentrations were significant

Table 20. The effect of treatment shot on concentrations (mg/kg dry weight) of iron, lead, tungsten, and molybdenum in the femur of mallards on a 30-day dosing test[a].				
	Iron	Lead	Tungsten[b]	Molybdenum[c]
Males				
No-Shot	67.4 ± 2.92[B]	1.778 ± 0.7462[B]	ND	ND
Steel	81.3 ± 2.80[AB]	1.041 ± 0.4381[B]	ND	ND
Lead	108.3 ± 14.24[A]	162.500 ± 24.0349[A]	ND	ND
Tungsten-Iron	75.6 ± 3.89[B]	0.815 ± 0.1233[B]	7.9 ± 0.46	ND
Tungsten-Polymer	69.1 ± 4.09[B]	1.096 ± 0.2259[B]	ND	ND
Females				
No-Shot	89.6 ± 3.73	5.221 ± 2.2745[B]	ND	ND
Steel	106.4 ± 7.56	0.888 ± 0.1156[B]	ND	ND
Lead	107.0 ± 15.09	338.750±172.5589[A]	ND	ND
Tungsten-Iron	102.9 ± 4.93	1.044 ± 0.3182[B]	12.6±2.86[A]	ND
Tungsten-Polymer	84.5 ± 10.22	5.859 ± 4.8792[B]	4.5 ± 0.38[B]	ND

[a] Data presented as mean ± standard error of the mean. Sample size is 8 for all groups. Means with different superscripts are significantly different within the column ($p \leq 0.05$).
[b] Tungsten detection limit for femur is 4.0 mg/kg dry weight.
[c] Molybdenum detection limit for femur is 2.0 mg/kg dry weight.

Table 21. The effect of treatment shot on concentrations (mg/kg dry weight) of iron, lead, tungsten, and molybdenum in the liver of mallards on a 30-day dosing test[a].				
	Iron	Lead	Tungsten[b]	Molybdenum
Males				
No-Shot	913.8 ± 158.07^{C}	0.276 ± 0.1634^{B}	ND	6.16 ± 0.921^{C}
Steel	1775.0 ± 180.03^{BC}	0.145 ± 0.0399^{B}	ND	11.41 ± 1.044^{BC}
Lead	3100.0 ± 407.52^{A}	74.000 ± 19.8107^{A}	ND	19.38 ± 2.096^{A}
Tungsten-Iron	2312.5 ± 339.87^{AB}	0.154 ± 0.0488^{B}	14.56 ± 2.245	13.35± 2.114^{AB}
Tungsten-Polymer	1528.8 ± 221.69^{BC}	0.090 ± 0.0124^{B}	ND	10.38 ± 1.533^{BC}
Females				
No-Shot	870.6 ± 146.43^{B}	0.253 ± 0.1131^{B}	ND	6.79 ± 1.260
Steel	2237.5 ± 197.25^{AB}	0.101 ± 0.0240^{B}	ND	14.31 ± 1.346
Lead	3031.3 ± 899.65^{A}	82.500 ± 38.5377^{A}	ND	17.48 ± 5.468
Tungsten-Iron	2128.8 ± 221.85^{AB}	0.085 ± 0.0156^{B}	13.58 ± 1.445	12.74 ± 1.539
Tungsten-Polymer	1313.8 ± 266.37^{AB}	0.140 ± 0.0279^{B}	ND	8.13 ± 0.751

[a] Data presented as mean ± standard error of the mean. Sample size is 8 for all groups. Means with different superscripts are significantly different within the column ($p \leq 0.05$).
[b] Tungsten detection limit for liver is 0.8 mg/kg dry weight.

higher in the lead-dosed females when compared to birds in the other 4 groups. As with the males, tungsten was detected only in the liver of tungsten-iron-dosed females.

The concentrations of iron, lead, tungsten, and molybdenum in the kidneys of male and female mallards are presented in Table 22. Renal lead concentrations were significantly elevated in lead-dosed males compared to the other 4 groups while tungsten was significantly higher in the kidneys of tungsten-iron males and females compared to tungsten-polymer males and females. The concentrations of renal lead and tungsten were similarly elevated in the lead-dosed and tungsten-polymer-dosed females, respectively. In addition, female mallards dosed with steel had significantly higher kidney concentrations of iron when compared to the no-shot, lead, and tungsten-polymer birds while molybdenum concentrations were highest in the steel-dosed birds, intermediate in the no-shot and tungsten-polymer groups, and lowest in the lead-dosed and tungsten-iron-dosed birds.

Shot Recovered and Percent Shot Erosion

The initial and final weights of treatment shot (on a pellet basis) administered to mallards and the resulting erosion rates are presented in Table 23. Fluoroscopy of the birds on day 7 of the trial indicated that all birds receiving shot had retained the 8 pellets administered on day 1 within the gizzard with the exception of 1 steel-dosed bird which had 6 pellets . Most of the shot administered (at least 75%) was recovered from the gizzard at the time of necropsy regardless of shot type. Only 1 bird, a lead-dosed female, had no shot present at necropsy. No shot was found in the excreta of any of the birds which was examined daily during the course of the trial. Steel shot had the least amount

Table 22. The effect of treatment shot on concentrations (mg/kg dry weight) of iron, lead, tungsten, and molybdenum in the kidney of mallards on a 30-day dosing test[a].				
	Iron	Lead	Tungsten[b]	Molybdenum
Males				
No-Shot	460.0 ± 39.64	1.550 ± 1.3505^{B}	ND	12.9 ± 7.45
Steel	460.0 ± 17.83	0.288 ± 0.0915^{B}	ND	5.4 ± 0.18
Lead	413.8 ± 20.61	248.000 ± 53.1481^{A}	ND	3.8 ± 0.25
Tungsten-Iron	461.3 ± 18.07	0.225 ± 0.0313^{B}	7.3 ± 0.75	4.1 ± 0.23
Tungsten-Polymer	403.8 ± 21.87	0.300 ± 0.0845^{B}	2.4 ± 0.26	5.0 ± 0.19
Females				
No-Shot	363.8 ± 17.31^{C}	0.713 ± 0.4426^{B}	ND	4.8 ± 0.16^{B}
Steel	502.5 ± 25.83^{A}	0.138 ± 0.0183^{B}	ND	5.8 ± 0.16^{A}
Lead	320.0 ± 25.70^{C}	264.500 ± 83.9062^{A}	ND	3.5 ± 0.27^{C}
Tungsten-Iron	471.3 ± 21.08^{AB}	0.150 ± 0.0378^{B}	6.3 ± 0.53	4.0 ± 0.00^{C}
Tungsten-Polymer	396.3 ± 17.92^{BC}	0.463 ± 0.1149^{B}	2.4 ± 0.37	5.0 ± 0.00^{B}

[a] Data presented as mean ± standard error of the mean. Sample size is 8 for all groups. Means with different superscripts are significantly different within the column ($p \leq 0.05$).
[b] Tungsten detection limit for kidney is 2.0 mg/kg dry weight.

Table 23. The mean weight (gm) of individual shot administered, the number and mean weight (gm) of individual shot retrieved from each bird at necropsy, and percent shot erosion during a 30-day dosing test[a].

	Shot Wt. at Day 0	No. Shot at Necropsy	Shot Wt. at Necropsy	Percent Shot Erosion
Males				
No-Shot	-	-	-	-
Steel	0.1497 ± 0.00065	7.1 ± 0.61	0.1053 ± 0.00278	29.72 ± 1.814^{C}
Lead	0.1970 ± 0.00092	6.8 ± 0.90	0.1148 ± 0.01747	41.50 ± 8.801^{BC}
Tungsten-Iron	0.5302 ± 0.00090	8.0 ± 0.00	0.2681 ± 0.01203	49.44 ± 2.266^{B}
Tungsten-Polymer	0.5487 ±0.00316	7.5 ± 0.38	0.0816 ± 0.01473	85.12 ± 2.710^{A}
Females				
No-Shot	-	-	-	-
Steel	0.1492 ± 0.00037	7.8 ± 0.25	0.0965 ± 0.00345	35.28 ± 2.323^{B}
Lead	0.1950 ± 0.00075	6.0 ± 1.21	0.0718 ± 0.02192	63.20 ± 11.232^{A}
Tungsten-Iron	0.5295 ± 0.00083	7.4 ± 0.42	0.2086 ± 0.01070	60.60 ± 2.019^{AB}
Tungsten-Polymer	0.5540 ± 0.00225	7.8 ± 0.25	0.1359 ± 0.03433	75.58 ± 6.161^{A}

[a] Data presented as mean ± standard error of the mean. 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 per bird. Sample size is 8 birds for each parameter. Means with different superscripts are significantly different within the column ($p \leq 0.05$).

of erosion (29.7% for males and 35.3% for females) while tungsten-polymer shot had the most erosion (85% and 76%, respectively, for males and females). In males, the erosion of tungsten-iron shot was significantly greater than steel shot, but significantly less than tungsten-polymer shot while the erosion of lead shot was similar to both steel and tungsten-iron. In females, there was no significant difference in erosion between lead, tungsten-iron, and tungsten-polymer shot which all eroded to a greater extent than steel shot. If erosion of lead shot is considered separately in those birds which died during the trial from those which survived the trial, then percent erosion of shot retrieved from birds dying was 37.0% for lead-dosed males and 30.3% for lead-dosed females compared to 41.8% for surviving males and 83.0% for surviving females.

Shot erosion rates when the total weight of shot administered per bird is compared to the total weight of shot retrieved, regardless of pellet number are presented on Table 24. When expressed in this manner, erosion rate was highest for tungsten-polymer shot in the males (86%) followed by lead (54%), tungsten-iron (49%), and steel (37%). These values were statistically different when tungsten-polymer was compared with the other 3 treatment groups. In the females, tungsten-polymer shot had the highest erosion rate (76%) which is comparable with the lead (64%) and tungsten-iron erosion rate (64%), and significantly greater than the erosion rate of the steel shot (37%). When the erosion rate of lead shot in birds dying on trial was compared to the erosion rate in birds surviving the trial, the erosion of lead shot was considerably more in males surviving the trial (83%) compared to those dying (37%) as it was in females (84% vs 30%).

The steel shot recovered maintained their original round shape while most of the

Table 24. The mean weight (gm) of total shot administered, the mean weight of total shot retrieved from each bird at necropsy, and percent shot erosion during a 30-day dosing test[a].

	Total Shot Wt. at Day 0	Total Shot Wt. at Necropsy	Percent Shot Erosion
Males			
No Shot	-	-	-
Steel	1.1978±0.00518	0.7531±0.07029	37.22±5.765^{B}
Lead	1.5759±0.00732	0.7211±0.14724	54.22±9.327^{B}
Tungsten-Iron	4.2413±0.00722	2.1444±0.09625	49.44±2.266^{B}
Tungsten-Polymer	4.3898±0.02536	0.6098±0.12029	86.10±2.759^{A}
Females			
No Shot	-	-	-
Steel	1.1934±0.00295	0.7500±0.04022	37.16±3.353^{B}
Lead	1.5596±0.00593	0.5687±0.17660	63.58±11.315^{A}
Tungsten-Iron	4.2358±0.06256	1.5449±0.12915	63.53±3.049^{AB}
Tungsten-Polymer	4.4315±0.01796	1.0849±0.27588	75.63±6.190^{A}

[a] Data presented as mean±standard error of the mean. On day 0, male and female mallards were dosed with 8 pellets of the appropriate shot. Values reported reflect the average total weight of all pellets administered per bird in each treatment. Sample size is 8 birds for each parameter. Means with different superscripts are significantly different within the column ($p \leq 0.05$).

lead pellets recovered were flattened and oval in shape. The tungsten-polymer shot were flattened and disk-like in appearance while the tungsten-iron shot were round with small dimples on the surface.

Discussion

Mortality

In the present study, only lead-dosed birds died during the course of the 30-day trial (Table 2). Eight #4 lead shot resulted in 50% mortality (62.5% for males, 37.5% for females) within 10-25 days of dosing (average of 16.8 days). Those birds dying lost an average of 30% of their body weight (17.1-46.9%). 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. It should be pointed out that temperatures were somewhat low, compared to a normal housing temperature of 13°C, during the course of the trial (the average low temperature was 9°C, Table 1) which can be considered an additional stress to the dosed birds. It is thus significant that only lead-dosed birds died and not those birds administered the steel and tungsten shot.

Pain and Rattner (1988) administered 1 #4 lead shot to pen-reared black ducks and reported 60% mortality (4 of 5 males and 2 of 5 females) between 4 to 6 days post-dosing. While mortality in this study was thought to be uncommonly high based on the dose, the authors felt that a number of factors including lack of acclimatization, high ambient temperatures (37.6°C), and frequent handling may have been responsible. Body weight loss was no greater in the lead-dosed birds than in the control birds.

In a subsequent study, Rattner et al. (1989) administered 1 #4 lead shot to pen-reared and wild black ducks and to game-farm and wild mallards maintained on pelleted feed during the winter. In contrast to the previous study, no mortalities were reported during the first 14 days, although transient signs of lead toxicity were noted. Birds were then dosed with either 2 or 4 #4 lead shot. Twenty-eight days after receiving the second dose, the wild mallards experienced 25% mortality and by 49 days post-dosing there was 40% mortality among the wild black ducks and 45% among the wild mallards. When this study was repeated in the summer, a single black duck died 21 days after receiving 1 #4 lead shot followed by 4 #4 lead shot.

Sanderson and associates (1992) dosed mallards with 2, 4, or 8 #2 lead shot or 4 #2 lead shot plus 4 #2 bismuth shot and maintained the birds up to 30 days on a diet of shelled corn. In this study, mortality was 95% with only 2 ducks (2 #2 lead shot) surviving. The average survival times were 19.1, 15.0, 12.6, and 14.4 days for birds receiving 2, 4, and 8 #2 lead shot, and the lead/bismuth combination, respectively. Average body weight loss was 42.2% with a range of 16-56%. The high mortality in the latter study was probably due in part to the shelled corn diet. Diet has been reported to influence the severity of lead poisoning in waterfowl (Kendall et al., 1996).

In the present study, none of the birds dosed with tungsten-iron or tungsten-polymer shot died. In a similar toxicity study in which mallards were dosed with 12-17 pellets (an average of 1.03 gm which is equivalent to 5 #4 lead shot) composed of 39.05% tungsten, 44.49% bismuth, and 16.46% tin, no mortalities were reported during the 32-day trial (Ringelman et al., 1993). In the latter study, birds received approximately 0.4 gm

tungsten while in the present study, birds were dosed with approximately either 4.2 gm tungsten (4.43 gm of TP shot x 95.5% tungsten) or 2.3 gm tungsten (4.24 gm of TI shot x 55% tungsten).

Tungsten has been reported to cause mortality in birds. Nell et al. (1980) administered broiler cockerels sodium tungstate by injection at 5 mg tungsten from day 1 to day 11, 10 mg from day 12 to day 21, and 20 mg from day 22 to day 35. They reported that 4 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 (Tables 21 and 22), then the birds in the present study were exposed to approximately 3.4 gm tungsten. However, if an average erosion rate of 56% for the tungsten-iron shot is used, then the birds were exposed to approximately 1.3 gm tungsten. In the chicken study, however; the tungsten was in a soluble form administered by injection to relatively small birds, all of which would enhance toxicity.

Clinical Signs

Birds receiving lead shot were the only ones which had obvious clinical signs. These signs (green excreta and, in some cases, ataxia) are typical of birds intoxicated with lead (Friend, 1987; Pain and Rattner, 1988; Rattner et al., 1989). Lead-dosed ducks which survived the trial appeared relatively normal at time of necropsy.

Ducks dosed with tungsten-iron and tungsten-polymer shot in the present study appeared normal throughout the 30-day trial which agrees with results reported by Ringelman et al. (1993) for mallards dosed with tungsten-bismuth-tin shot. Clinical signs of acute tungsten poisoning reported for mammals include nervous prostration, diarrhea

and death preceded by coma due to respiratory paralysis (Stokinger, 1978). Clinical signs for birds administered tungsten include anorexia, reduced weight gain, diarrhea and labored breathing within an hour of death (Nell et al., 1980).

Body Weights

Body weights of birds surviving the 30-day trial changed little. No-shot, steel, tungsten-iron and tungsten-polymer-dosed birds gained a slight amount of weight or stayed approximately the same (-0.4 to 5.8%) while lead-dosed ducks lost approximately 6% of their body weight (Table 3).

Pain and Rattner (1988) reported that black ducks which survived dosing with 1 #4 lead shot were similar in body weight to the control birds at the end of the trial. Sanderson et al. (1992) indicated that the 2 mallards which survived dosing with 2 #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 body weight as controls in the study by Ringelman et al. (1993).

Hematocrit, Hemoglobin Concentration, and ALAD Activity

Depressions in hematocrit, hemoglobin concentration and delta-aminolevulinic acid dehydratase (ALAD) activity are all indicators of lead toxicity. Lead interacts with the erythrocyte which results in increased fragility of the membrane, thus shortening the lifespan of the erythrocyte. Additionally, lead inhibits ALAD, a key enzyme in the synthesis of heme which is an integral component of hemoglobin. The combined effect of lead on erythrocyte lifespan and heme synthesis results in lead-induced anemia which

results in decreased hematocrit and hemoglobin (Goyer, 1996).

In the present study, lead-dosed males alive at day 15 had a significantly lower hematocrit (packed erythrocyte volume) concentration than steel, tungsten-iron and tungsten-polymer birds and hemoglobin concentration than the no-shot, steel and tungsten-polymer-dosed birds (Table 4). In the lead-dosed females alive at day 15, hematocrit and hemoglobin concentration were also significantly lower when compared to the other 4 groups (Table 5). ALAD activity was numerically lower in the lead-dosed males at day 15 (63% of average activity in no-shot and steel-dosed birds) but individual variation precluded statistical significance (Table 4). In the females, the lead-dosed birds had significantly lower ALAD activities when compared to the other 4 treatment groups (58% of average activity in no-shot and steel-dosed birds, Table 5). At the end of the 30-day trial, there were no statistical differences in these whole-blood parameters in either sex.

It should be pointed out that blood samples were not obtained from those birds dying on trial unless they were alive at day 15. It is likely that hematocrit, hemoglobin concentration, and ALAD activity were depressed in those birds at time of death. It is somewhat surprising that activities in lead-dosed birds were not inhibited to a greater extent. A possible explanation could be inadequate preservation of the blood sample. In the present study, blood samples used for ALAD analysis were kept cool during storage and shipping rather than being frozen. It is possible that this resulted in lower than normal acitivity for all samples, but it would not account for only moderate inhibition of ALAD activity in lead-dosed birds. Another possibility is that of the 6 male and 6 female lead-dosed birds bled on day 15, the 3 males and 5 females that survived the 30-day trial may

not have experienced lead toxicosis which would account for the relatively high ALAD values as well as the lack of an effect on hematocrit and hemoglobin concentration at day 30.

Pain and Rattner (1988) reported that hematocrit and hemoglobin concentration were significantly depressed in black ducks administered 1 #4 lead shot within 6 days of dosing but recovery was apparent by 30 days post-dosing. ALAD activity was inhibited by 100% at 1 day post-dosing, increased between 3-9 days post-dosing (approximately 70% inhibition) and then declined again until the end of the 30-day study. In a subsequent study, Rattner et al. (1989) reported no change in hematocrit and a transient decrease in ALAD activity (>90%) over 14 days in black ducks and mallards dosed with 1 #4 lead shot. Birds were then re-dosed with either 2 or 4 #4 lead shot and observed for an additional 4 weeks. ALAD activity continued to be inhibited by more than 90%.

In the study by Sanderson et al. (1992) where mallards were dosed with either 2, 4 or 8 #2 lead shot or 4 #2 lead shot plus 4 #2 bismuth shot, hematocrit was significantly depressed in those birds dying (by 36.4% from dosing to the last time they were bled). In the 2 lead-dosed birds which survived the trial, hematocrits had returned to pre-test values.

Bakalli et al. (1995) reported that blood ALAD activity was quickly and significantly depressed in broiler chicks administered 50 ppm lead acetate via the feed for 42 days (38% inhibition within 24 hours, 69% inhibition after 7 days). However, 24 hours after the birds were placed on clean feed, ALAD activity significantly increased by 32% and by 7 days on clean feed, ALAD activity was near normal (90% of control).

In the present study, birds receiving the tungsten-iron or tungsten-polymer shot did not have hematocrits, hemoglobin concentrations or ALAD activities which were significantly different than values for the no-shot and steel-dosed birds at 15 and 30 days post-dosing. These results agree with those reported by Ringelman et al. (1993) who dosed mallards with 12-17 pellets (equivalent in mass to 5 # 4 lead shot) of shot composed of tungsten, bismuth, and tin in that hematocrit and hemoglobin concentrations were unaffected over the 32- day trial. ALAD activity was not assessed in the latter trial.

Plasma Chemistries

The plasma values reported in the present study are reasonably close to values reported for mallards in other studies (Fairbrother et al., 1990; Ringelman et al., 1993) considering the influence of sex, age, reproductive status, and environmental conditions. The administration of lead shot caused a number of changes in 15-day plasma chemistry values in both males and females (Tables 6 and 7).

In the lead-dosed males, glucose concentration was approximately 17% higher when compared to the other 3 groups. While March et al. (1976) have reported a slight hyperglycemia in lead-poisoned geese, the increase reported here is not considered to be biologically relevant. The albumin/globulin ratio in lead-dosed birds and tungsten-polymer birds was elevated by approximately 15% when compared to the no-shot and steel-shot dosed males. While statistically significant, this increase is probably not biologically relevant. Plasma uric acid levels for all 5 groups remained within the range of normal values (Campbell and Coles, 1986). In terms of uric acid, it is significant that there were no changes noted in the tungsten-iron or tungsten-polymer-dosed birds in that high levels

of tungsten will inhibit xanthine oxidase (e.g. Higgens et al., 1956; Teekell and Watts, 1959; Leach et al., 1962; Nell et al., 1980). Xanthine oxidase normally oxidizes xanthine to uric acid. When xanthine oxidase was inhibited in chicks fed diets high in tungsten, increased plasma concentrations of uric acid and xanthine plus hypoxanthine were apparent (Nell et al., 1980). The plasma activities of alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase were significantly elevated in the lead-dosed males when compared to the other 4 groups. While an increase in the plasma value of one of these enzymes would not necessarily be a useful diagnostic tool, the fact that all three are elevated in the lead-dosed birds would strongly suggest significant liver damage (Campbell and Coles, 1986). There was also a dramatic increase in creatine phosphokinase activity (20-fold) in the lead-dosed males compared to the other 4 groups. Increases in the serum activity of this enzyme have been associated with lead toxicity (Campbell and Coles, 1986).

Changes in plasma chemistry values for lead-dosed females at day 15 included decreases in sodium and chloride and increases in creatinine and the activities of alanine aminotransferase and lactate dehydrogenase compared to one or a combination of the other 4 groups. The decrease in plasma sodium and chloride concentrations could be indicative of renal damage (Coles, 1986), but the drop was less than 8% when compared to the other 4 groups. The increase in creatinine could also reflect kidney damage, but Fairbrother et al. (1990) discount the use of this parameter as a diagnostic tool in birds. The increased activity of alanine aminotransferase (4.0 fold) and lactate dehydrogenase (2.2 fold) are suggestive of lead-induced hepatic damage (Campbell and Coles, 1986).

There were no significant differences in plasma parameters between the no-shot, steel, tungsten-iron and tungsten-polymer birds at 15 days post-dosing with the exception of an increased albumin/globulin ratio in tungsten-polymer males. These results agree with 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 over the 32-day test period.

At 30 days post-dosing, there were few changes in plasma parameters and those changes were only associated with the females (Tables 8 and 9). Lead-dosed females at day 30 still had a low chloride concentration and a high concentration of carbon dioxide compared to tungsten-polymer birds. Alanine aminotransferase acitivities were quite variable, but were significantly higher in lead-dosed females compared to the steel and tungsten-iron birds. These differences were not considered to be necessarily indicative of lead toxicity. In general, those lead-dosed birds which survived the 30-day trial had relatively normal plasma values and whole blood parameters when compared to the other 4 groups.

Gross Pathology

Several birds (5 of 16) in the lead-dosed group exhibited discoloration and erosions in the lining of the gizzard (Tables 10 and 11). This effect has been previously described in both naturally occurring and experimentally-induced cases of lead toxicosis (Slauson and Cooper, 1990; Alden and Frith, 1991; Popp and Cattley, 1991). No birds in the other 4 groups exhibited gross lesions within their gizzards. Fatty liver was noted grossly in birds in both the no-shot and lead-dosed groups and was therefore not considered a significant

lesion. Fatty liver is considered a non-specific change, as it may result from hepatocellular damage (ie. toxicosis), mobilization of internal fat stores (ie. inadequate energy intake), or a variety of other metabolic conditions (Slauson and Cooper, 1990). The lack of gross changes in the tungsten-iron and tungsten-polymer birds agrees with results reported by Ringelman et al. (1993) for mallards dosed with tungsten-bismuth-tin shot.

Organ Weights

Organ weights of birds surviving the 30-day trial (Table 12 and 13) were expressed as a percent of body weights (Table 14 and 15) to correct for any differences that might be due to the size and growth of the bird. When expressed on a relative basis, lead-dosed males had higher relative kidney weights compared to no-shot and tungsten-iron males and lead-dosed females had increased relative heart (vs. no-shot and tungsten-polymer) and kidney weights (vs tungsten-iron and tungsten-polymer birds) and increased relative liver weights (vs steel, tungsten-iron and tungsten-polymer birds). Sanderson et al. (1992) reported no differences in absolute liver weights in lead-dosed mallards when compared to control birds except for ducks dosed with 2 #2 lead pellets which had significantly lighter livers. These authors commented, however, that liver weights of lead-poisoned waterfowl are difficult to evaluate because the organ can be enlarged or atrophied.

Histopathology of Kidney and Liver

Microscopic renal lesions were found only in birds from the lead-treated group, and only in those birds which died spontaneously prior to the conclusion of the trial. These renal lesions were characterized by acute tubular necrosis (nephrosis), and were accompanied in most cases by variable numbers of eosinophilic intranuclear inclusions

within tubular epithelial cells. These changes have been previously reported to be associated with lead toxicoses in many different animal species (Alden and Frith, 1991). Since renal lesions were not found in any of the 8 lead-treated birds at the termination of the trial, it appears that toxic nephrosis is an acute or subacute effect of lead toxicosis that occurred while the birds were actively absorbing lead from their gizzard. Whether the tubular necrosis was due to direct toxic effects of lead on the tubular epithelium, or was mediated through hemoglobin released during periods of intravascular hemolysis, or a combination of the two is unknown, as both are recognized to produce this lesion (Alden and Frith, 1991). The red blood cell parameters did indicate anemia at day 15 of the study which may have been due to lead-induced intravascular hemolysis. The absence of renal lesions in either the steel, tungsten-iron or tungsten-polymer dosed birds 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 divided into two distinct groups; non-specific fatty accumulation and more significant biliary stasis. Intrahepatocellular fatty vacuolation was present in over 50% of the birds in each of the 5 experimental groups. As previously discussed, fatty accumulation can be due to 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 due to obstruction of bile ducts, or primary hepatocellular disfunction (Popp and Cattley, 1991). In this study, there was no evidence of cholelithiasis or other obstructive biliary disease, and so biliary stasis was considered evidence of hepatocellular dysfunction. The degree of biliary stasis was

graded, and statistical analysis indicated that only the lead-dosed group had significant elevated amounts of biliary stasis, although several individual birds in the tungsten-iron and tungsten-polymer group also exhibited mild biliary stasis. None of the birds in the no-shot and steel-dosed groups had biliary stasis. There was good correlation between the morphologic appearance of biliary stasis, and the elevated plasma hepatic enzyme activities (indicating hepatocellular damage) at day 15 in the lead-dosed group. The hepatic biliary stasis was considered to be a morphologic indicator of hepatocellular damage in this study, and that significant evidence of this damage was present only in the lead-dosed group. However, since biliary stasis was observed in some of the tungsten-iron and tungsten-polymer birds, but not the no-shot and steel-dosed birds, it is apparent that the experimental shot is inducing a pathological condition, however slight, that is not found in the control birds.

Tissue Metal Analysis

Birds dosed with lead shot tended to have elevated iron concentrations in the femur liver, and kidney (Tables 20-22). Femur iron concentrations were significantly higher in the lead-dosed males compared to the no-shot, tungsten-iron and tungsten-polymer birds, liver iron concentrations were higher in lead-dosed males compared to no-shot, steel, and tungsten-polymer birds and liver iron concentrations were higher in lead-dosed females compared to no-shot females. Tungsten-iron males had higher liver iron concentrations than did the no-shot birds. In the kidney, steel and tungsten-polymer-dosed females had significantly higher concentrations of iron when compared to the no-shot and lead-dosed birds with steel also significantly higher than tungsten-polymer females. The high levels

of iron in the liver of lead-dosed birds 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 was higher when compared to birds not dosed with lead. They attributed the increase to a lead-induced interference of heme synthesis which in turn caused an accumulation of iron in the liver and muscle.

All treatment groups, including the no-shot birds, had detectable concentrations of lead in the femur, liver, and kidney (Tables 20-22). However, concentrations of lead in the birds dosed with lead shot were increased by a factor of 10 when compared to the other 4 groups. Sanderson et al. (1992) reported concentrations of lead in the femurs of all mallards on trial regardless of treatment with concentrations in the birds receiving lead shot the highest. Lead was not detected in the liver and muscle of control birds and birds dosed with steel shot in the latter study.

Tungsten was detected in the femur and kidney of tungsten-polymer-dosed birds at concentrations slightly above detection limits and in the femur, liver, and kidney of tungsten-iron birds (Tables 18-20). The bone, liver, and kidney are principle sites of tungsten disposition in a number of different species (Kinard and Aull, 1945; Wase, 1956; Kaye, 1968; Bell and Sneed, 1970; Aamodt, 1975) with the primary site apparently being species-dependent. In the present study, the highest concentrations of tungsten were detected in the liver. In contrast, Ringelman et al. (1993) who dosed mallards with tungsten bismuth-tin shot did not detect tungsten in the liver and kidney, the only tissues examined. Tungsten was detected in the kidneys of both tungsten-iron and tungsten-polymer males and females tissues. Tungsten residues in the kidney of male and female

tungsten-iron-dosed birds were both significantly higher than residues found in the tungsten-polymer-dosed birds.

Molybdenum concentrations were assessed in the present study because it has been reported that administration of tungsten can cause a decrease in tissue molybdenum concentrations (Higgens et al., 1956; Nell et al., 1980). Of the 3 tissues examined, molybdenum was detected in the liver and kidney. In the liver, the no-shot birds had the lowest concentrations of molybdenum (significant when males were compared to their lead counterparts) while the lead-dosed birds had the highest concentrations (significant in males when compared to no-shot, steel, and tungsten-polymer birds). In the kidney, male no-shot birds had the highest concentration of molybdenum although there were no significant differences between the 5 groups. Steel-dosed females had the highest concentration of renal molybdenum, with no-shot and tungsten-polymer birds containing intermediate concentrations, and lead-dosed and tungsten-iron birds having the lowest concentrations of renal molybdenum. Based on these results it would seem that assessing molybdenum concentration as an indication of tungsten exposure is not warranted.

Shot Recovered and Percent Shot Erosion

Fluoroscopy of the birds on day 7 of the trial indicated that each dosed bird had 8 pellets present in the gizzard with the exception of 1 steel-dosed bird which had 6 pellets. During the course of the trial, the excreta was checked on a daily basis and there was no evidence of shot passage. It is possible, however, that since 100% of the pellets were not recovered at necropsy, some may have been eliminated from the bird and not detected in the excreta.

Males dosed with steel shot retained an average of 89% of the pellets while females retained 98%. Recovery of lead shot was 85% in the males and 75% in the females, the latter being the lowest percent recovery. One hundred percent of the tungsten-iron pellets and 94% of the tungsten-polymer pellets administered were recovered in the males at necropsy while the recovery rate in tungsten-iron and tungsten-polymer females was 93% and 98%, respectively (Table 23). These recovery rates are similar to those reported by Sanderson et al. (1992) for lead and iron shot and 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 steel shot was significantly less than erosion of tungsten-iron and tungsten-polymer in the males and significantly less than both lead and tungsten-polymer in the females (Table 23). The erosion rate of steel, lead, and tungsten-iron shot in females is greater than the erosion rates of the same shot in males. If shot erosion is based on the total weight of the pellets recovered (this would assume that a non-recovered pellet had eroded completely), the erosion rate of tungsten-polymer shot in males is significantly greater than that of steel, lead, and tungsten-iron shot. However, in the females erosion rates of tungsten-polymer shot is only significantly greater when compared to steel shot (Table 24).

Steel pellets recovered at necropsy retained their original round shape. It was sometimes difficult to find the lead, tungsten-iron and tungsten-polymer shot in the 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 8 BB size tungsten-iron and tungsten-polymer shot and maintained over a 30-day period did not experience any adverse effects based on the parameters examined. All birds survived the 30-day trial with a slight increase in body weight. There were no significant differences in hematocrit, hemoglobin concentration, and ALAD activity in the 2 candidate groups when compared to no-shot and steel (control) groups. Similarly, there were no changes in the 25 plasma chemistry parameters except for the elevated albumin/globulin ratio in tungsten-polymer males on day 15. The birds appeared normal at the time of necropsy on day 30 of the trial and there were no changes in organ weights. Five of 16 tungsten-iron birds and only 3 of 16 tungsten-polymer birds had mild hepatocellular biliary stasis which was not considered to be deleterious. However, this condition was not observed in the no-shot and steel-dosed birds. No other histopathological lesions were noted. Tungsten was detected in the femur, liver, and kidney of the tungsten-iron birds and at concentrations only slightly above detection limits in the femur of tungsten-polymer males and in the kidneys of tungsten-polymer-dosed males and females. Even though the erosion rate of tungsten-polymer shot was greater than tungsten-iron shot, the erosion rate was only significant when compared in male birds. Therefore, the results of this study indicate that game farm mallards dosed with either 8 BBs composed of tungsten-iron or tungsten-polymer were not adversely affected during the course of the 30-day trial. However, if the two experimental shot were to be compared tungsten-polymer would be the better shot due to its apparent

low body tissue deposition, low occurrence of biliary stasis, and greater erosion pattern. It seems the tungsten-polymer shot is more environmental friendly than the tungsten-iron shot.

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