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CHRONIC DOSING STUDY TO ASSESS THE HEALTH AND REPRODUCTIVE EFFECTS OF TUNGSTEN-IRON AND TUNGSTEN-POLYMER SHOT ON GAME-FARM MALLARDS

By

Rachel Rebecca Mitchell

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ABSTRACT

CHRONIC DOSING STUDY TO ASSESS THE HEALTH AND REPRODUCTIVE EFFECTS OF TUNGSTEN-IRON AND TUNGSTEN-POLYMER SHOT ON GAME-FARM MALLARDS

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Sixteen male and 16 female adult mallards were orally dosed with 8 #4 steel shot, 8 #4 tungsten-iron shot, or 8 #4 tungsten-polymer shot on days 0, 30, 60, 90, and 120 of a 150-day trial. An additional 6 male and 6 female mallards received 8 #4 lead shot on day 0 of the study. During the first 60 days of the trial, mallards were fed a nutritionally deficient diet (shelled corn) and maintained in a cold environment. Ducks were then switched to commercial layer ration for the subsequent 90 days during which reproductive performance was examined. All lead-dosed ducks died by day 25 of the study, whereas no ducks died in the other dosage groups. Lead-dosed mallards had significantly decreased hematocrit, hemoglobin concentration and whole-blood delta aminolevulinic dehydratase activity on day 7. Exposure to lead shot caused significant changes in a number of plasma chemistry parameters compared to exposure to steel, tungsten-iron, or tungsten-polymer shot at day 7. Mallards dosed with tungsten-iron or tungsten-polymer shot had occasional significant differences in hematocrit and plasma chemistry values when compared to steel-dosed mallards over the 150-day period, but these values were within the normal range reported for mallards and not considered to be indicative of deleterious effects. Relative kidney, heart, brain and gizzard weights of lead-dosed ducks were

significantly greater in comparison to the relative weights of those organs of ducks in the other 3 groups. Histological examination of kidneys and liver indicated renal nephrosis and hepatocellular biliary stasis in the lead-dosed ducks. Significant liver hemosiderosis was present in all steel- and tungsten-iron-dosed males examined, in 5 of 8 steel- and 3 of 8 tungsten-iron-dosed females examined, and in 1 tungsten-polymer-dosed male examined. Concentrations of lead in the femur, gonads, kidneys, and liver were higher in lead-dosed ducks than in ducks of the other 3 groups. Small amounts of tungsten were detected in gonad and kidney samples from males and females, in femur samples from males, and in liver samples from females dosed with tungsten-polymer shot. Higher concentrations of tungsten were detected in femur, gonad, kidney, and liver samples from tungsten-iron-dosed ducks. The rate of shot erosion was highest for tungsten-polymer shot (99%), followed by tungsten-iron (72%), steel (55%), and lead (37%). There were no significant differences in percent egg production, and percent fertility and hatchability of eggs from tungsten-iron- and tungsten-polymer-dosed ducks when compared to steel-dosed ducks. Egg weight and shell thickness of eggs from tungsten-iron-dosed ducks were greater when compared to steel-dosed ducks. Concentrations of tungsten were highest in the shell of eggs from tungsten-iron-dosed ducks than from the eggs of tungsten-polymer-dosed ducks. There were no biological differences in percent survivability and body weight of ducklings from tungsten-iron and tungsten-polymer ducklings when compared to ducklings from steel-dosed ducks. The hematocrit of ducklings from tungsten-iron-dosed ducks was slightly but significantly lower when compared to ducklings from steel-dosed ducks. Relative kidney weight of ducklings from tungsten-polymer-dosed ducks was significantly greater than relative kidney weight of

ducklings from steel-dosed ducks. Histological examination of duckling kidneys and liver indicated no abnormalities. Tungsten was detected in 25%, 9%, and 13% of the femur, kidney, and liver samples, respectively, from ducklings of the tungsten-iron and tungsten-polymer groups. Results of this study indicated that tungsten-iron or tungsten-polymer shot repeatedly administered to adult mallards did not adversely affect them or the offspring they produced during the 150-day trial.

To my husband, Andrew Thomas Mitchell
Where our world is just "ducky"!

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Introduction

In 1991, the United States banned the use of lead shot for waterfowl hunting because of its toxic effects on waterfowl and other wildlife species upon ingestion. Steel and bismuth shot are used as nontoxic alternatives to lead, but there has been a continual effort to develop shot compositions that emulate the ballistic characteristics of lead.

In order for a candidate shot to receive permanent approval for use by the U.S. Fish and Wildlife Service (USFWS), it must undergo a variety of tests as documented in USFWS 50 CFR Part 20.134, Migratory Bird Hunting: Nontoxic Shot Approval Procedure (Federal Register, 1986) to establish that it is nontoxic to waterfowl and other impacted species. The approval procedure is a 3-tiered approach. In Tier 1, the applicant must provide statements of use, chemical characterization, volume of use of the material requested to be approved, and samples of the candidate shot. In addition, the toxicological data for the shot coating and/or shot pertaining to mammals, birds, fish, amphibians, and reptiles should be summarized. The applicant must also provide information on the environmental fate and transport of the shot and shot coatings. In Tier 2, providing that the results from the Tier 1 information is inconclusive, the applicant will conduct a short-term (30-day) acute toxicity test using game-farm mallards provided a diet of commercially available duck food. In Tier 3, a chronic toxicity test is to be conducted. This test utilizes game-farm mallards fed a nutritionally-deficient diet of corn and maintained in a cold environment for 60 days. Mallards are then switched to a breeder diet and reproductive parameters are assessed for the subsequent 90 days. Shot composed of tungsten-iron (55% tungsten and 45% iron) and tungsten-polymer (95.5% tungsten and 4.5% of the polymer nylon 6) were given conditional approval for waterfowl

hunting by the USFWS based partly on the results of a 30-day acute toxicity trial utilizing mallards (Tier 2) (Kelly et al., 1998).

The present study is a 150-day dosing test designed to assess the effects of long-term periodic exposure of waterfowl to 2 candidate shot types composed of 55% tungsten and 45% iron, and 95.5% tungsten and 4.5% of the polymer nylon 6. The study was conducted in 2 phases. The first phase consisted of maintaining mallards, dosed with candidate shot every 30 days, on a nutritionally-deficient diet (shelled corn) in a minimally heated environment with a constant photoperiod of 8 hours light:16 hours dark per day for 60 days. In the second phase of the study, the mallards were switched to a commercial layer ration while dosing with candidate shot continued every 30 days, the photoperiod was increased in increments to 18 hours light:6 hours dark, and reproductive performance was assessed during the subsequent 90 days. The protocol for this study was reviewed by the USFWS in 1997 and complies with the general guidelines outlined in the amended test protocol for nontoxic shot approval procedures for shot and shot coatings proposed by USFWS in 1996 (Tier 3).

Objectives

The overall objective of the 150-day dosing trial was to determine if exposure to 2 candidate shot types, composed of 55% tungsten and 45% iron, or 95.5% tungsten and 4.5% of the polymer nylon 6, caused any deleterious effects in game-farm mallards. Toxicity of candidate shot was assessed by:

1) Determination of hemoglobin (Hb) concentrations and whole-blood delta aminolevulinic acid dehydratase (ALAD) activities on day 7 of the trial.

2) Determination of hematocrit (HCT) on days 7, 30, 60, 90, 120, and 150.

3) Determination of plasma chemistries on days 7, 30, 60, 90, 120 and 150.

4) Determination of egg production, fertility, hatchability, and duckling survivability.

5) Determination of changes in body weights and organ weights.

6) Determination of metal residue concentrations in the liver, kidneys, femur, and gonads of adults, in the liver, kidneys, and femur of ducklings, and in the contents and shell of eggs.

7) Determination of gross and histological changes in selected tissues.

8) Determination of mortality.

Literature Review

Lead is the most ubiquitous toxic metal and is detectable in practically all phases of the inert environment and in all biological systems. This heavy, pliable metal has a bright, bluish color and rarely occurs in the native form, but is usually found in nature as its sulfide, the mineral galena. Because lead is toxic to most living things at high concentrations and because there is no demonstrated biological need for it, the major issue regarding lead is determining the dose at which it becomes toxic (Goyer, 1996).

Lead has been known to man for about 7000 years, and lead poisoning has occurred for at least 2500 years (Eisler, 1988). Ancient Egyptians used lead in the production of paints, pottery glazing, weights, coins, net sinkers, piping, and cooking utensils (Eisler, 1988). Later, Romans used lead in construction of water pipes, in cosmetics, and even as a sweetner in the preparation of wines. The decline of the Roman Empire may have been accelerated by endemic lead poisoning. This theory was later supported by the high concentrations of lead found in the bones of Roman aristocrats

(Eisler, 1988). During the Middle Ages, there was considerable use of lead in paints, weights, and in the preparation of stained glass windows for cathedrals. Later, following the introduction of gunpowder, the need for a projectile made of malleable material resulted in the production of lead shot and lead cannon balls. Today, domestic lead consumption is 1.3 million tons annually, of which half is used in the production of storage batteries and until recently, of gasoline antiknock compounds, specifically tetraethylead and tetramethylead (Eisler, 1988).

The traditional use of lead shot for waterfowl hunting has been the preferred metal for centuries because of its widespread availability, low price, ease of manufacturing, and chemical stability (Thomas, 1997). However, the primary source of lead poisoning in wild waterfowl has been the ingestion of shotgun pellets. The amount of ingested lead that will produce toxicosis and fatalities of waterfowl varies according to nutritional and physiological conditions of birds. A single ingestion of 0.2-2.0 grams of lead shot may prove acutely fatal to most waterfowl (Pain and Rattner, 1988; Rattner et al., 1989). Yet, each year about 3000 tons of lead shot are deposited in the wetlands of North America by waterfowl hunters alone (Thomas, 1997). Given that lead shot has been accumulating for at least 200-300 years, and that it erodes slowly (Jorgensen and Willems, 1987), there is a great risk that waterfowl will develop lead poisoning, both at present and in the future. Other less common sources of lead poisoning in waterfowl include lead fishing sinkers, mine wastes, paint pigments, bullets, and other lead objects that are swallowed.

Since the first report of Grinnell (1894), the typical signs and lesions of lead poisoning in waterfowl have been extensively documented in every North American waterfowl flyway (Bellrose, 1959; Wobeser, 1981; Sanderson and Bellrose, 1986; Friend,

1987; Eisler, 1988). Waterfowl that are well advanced in lead intoxication usually exhibit the following signs: varying degrees of emaciation (loss of up to 40% of the original body weight, and a prominent keel bone), reduced activity with reluctance to fly, lowered food intake, palsy (wing droop), bile staining of vent area, tendency to seek isolation and cover, and loss of ability to stand, walk, or fly. The internal lesions associated with lead poisoning in waterfowl include: lack of fat, atrophy of striated muscle, excess fluid in pericardial sac, distended gallbladder, atrophied gizzard with grinding pads hardened and bile stained, and anemia and paleness of the whole body. It was the extent of the threat lead posed to waterfowl that led the United States government to ban the use of lead shot for waterfowling in 1991.

Prior to the decision of the ban on lead shot for waterfowl hunting, there were 3 general options that were considered as potential solutions to the problem of lead shot poisoning waterfowl: (1) manipulation of the habitat to reduce the availability and/or toxicity of spent shot; (2) coating, plating, or otherwise altering lead shot pellets to reduce toxicity; and (3) regulations prohibiting the use of lead shot, combined with the use of alternative, nontoxic shot (Scheuhammer and Norris, 1995). Manipulation of waterfowl habitat required actions that were expensive, labor-intensive, of questionable effectiveness, and inappropriate as general solutions to the lead shot problem. The attempt to retain the ballistic qualities of lead, but to reduce its toxicity to waterfowl by coating lead shot with other metals or nonmetallic materials, resulted in mortality of waterfowl after ingestion of the modified shot types that was equal to or greater than mortality caused by pure lead shot. The lack of success of the first 2 options led to the search for affordable, nontoxic, ballistically-acceptable alternatives to lead.

Steel shot was found to be the preferred alternative to lead, considering its lack of toxicity, ready availability, and relatively low cost (U.S. Department of the Interior, 1986). One of the major concerns surrounding the phase-out of lead shot has been that the exclusive use of steel shot could lead to a dramatic increase in the proportion of game birds injured but not killed by hunters (crippling rate). The ultimate effect might be that increased losses of birds through crippling would surpass the number of birds saved by the elimination of lead shot (Scheuhammer and Norris, 1995). For this reason, hunters have been reluctant to accept the steel shot regulations. However, between 1950 and 1984, 16 published shooting tests comparing the effectiveness of lead and steel shot were conducted in the United States. The results of these tests were equivocal: 3 of the tests favored lead , 2 favored steel, 2 reported mixed results, and 8 showed no statistically differences in crippling between the 2 shot types (Morehouse, 1992). It has been argued that the crippling of waterfowl is a function of the skill of the shooter rather than the type of ammunition used.

Since lead was banned from the North American marshlands in 1991, ammunition companies have searched for ways to improve the performance of steel loads and to emulate the ballistic characteristics of lead shot. Bismuth shot, the first nontoxic alternative to steel shot, was the first nontoxic alternative to receive permanent approval by the United States Fish and Wildlife Service in 1997 (Kelly et al., 1998). The second nontoxic alternatives are tungsten-iron and tungsten-polymer shot, which received conditional approval for use from the U.S. Fish and Wildlife Service in 1997 (Kelly et al., 1998).

Tungsten 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, alloys, chemicals and tools. 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 ($Na_2WO_4 \cdot 2H_2O$) and ammonium paratungstate [$(NH_4)_6W_7O_{24}.6H_2O$] 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 $(NH_4)_2WO_4$ 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 that 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 a 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 mammals have been administered doses of tungsten compounds that do not result in mortality, 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). The toxicity of

soluble tungsten compounds is determined by measuring xanthine dehydrogenase activity in the liver. Xanthine dehyrdogenase is an enzyme involved in purine metabolism and in the conversion of nitrogenous compounds to uric acid (Nell et al., 1980). In the following studies, tungsten was shown to reduce xanthine dehydrogenase activity when fed to breeder hens and chicks. 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. 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 xanthine dehydrogenase activities. Chicks fed diets containing 1,000 ppm tungsten for 4 weeks had increased hepatic concentrations of tungsten and decreased activities of xanthine dehydrogenase. 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 xanthine dehydrogenase activities. The decrease in tissue xanthine dehydrogenase activities paralleled increases in plasma concentrations of uric acid, xanthine, and hypoxanthine.

In a study that served as the basis for the present test, Kelly et al. (1998) dosed mallards with 8 BBs of tungsten-iron or tungsten-polymer shot and monitored them for 30 days. All mallards survived the 30-day trial with a slight increase in body weight. No statistical differences were observed in HCT, Hb concentrations, and ALAD activities in the 2 tungsten shot-dosed groups when compared to control and steel-dosed groups. Similarly, no changes were detected in selected plasma chemistry variables. The mallards appeared normal at the time of necropsy on day 30 of the trial, and no changes were detected in weights of organs. Five of 16 tungsten-iron-dosed ducks and 3 of 16 tungsten-polymer-dosed ducks manifested a mild hepatocellular biliary stasis, which was not considered deleterious. This condition, however, was not observed in the control and steel-dosed ducks. No other histopathological lesions were noted. Tungsten residues were detected in the femur, liver and kidneys of the tungsten-iron ducks. Concentrations of tungsten only slightly above detection limits were detected in the femur and kidneys of 2 mallards dosed with tungsten-polymer shot. In a similar study, Ringelman et al. (1993) dosed mallards with 12-17 pellets of shot composed of 39% tungsten, 44.5% bismuth, and 16.5% tin and monitored the ducks 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.

Nylon 6 is the other significant component of the tungsten-polymer shot, comprising 4.5% of the total product. Nylon 6 is the commercially important homopolymer of caprolactam. Most completely polymerized materials are physiologically inert, regardless of the toxicity of the monomer from which it's made (Peterson, 1977). Thus, few data exist relative to the toxicity of nylon 6 in animals. Most of the toxicity studies that have been conducted relate to thermal degradation products that are not relevant to the exposure of wildlife to shot containing nylon. One animal study reported in Montgomery (1982) indicated that nylon 6 fed to rats at a level of 25% of the diet (250,000 ppm) for 2 weeks caused a slower rate of weight gain, presumably due to the decrease in food consumption and feed efficiency. There were no anatomic injuries attributable to the feeding of nylon 6 in this study. According to Montgomery (1982), there are no known reports that attribute any metastatic carcinogenic potential to nylon. No studies examining the effects of nylon 6 in avian species were found in the literature.

Materials and Methods

Fifty-four male and 54 female 5-month-old game-farm mallards (*Anas platyrhynchos*) (hatched 28 July 1997) with plumage and body conformation that resembled wild mallards were purchased from Whistling Wings, Inc. (Hanover, Illinois). The ducks arrived by truck at the Michigan State University (MSU) Poultry Science Research and Teaching Center (PSRTC) on 30 December 1997. The ducks were

removed from the transport cages and weighed, and the flight feathers were clipped. The ducks were then randomly assigned as male-female pairs to individual cages.

Cages (0.914 m L x 0.914 m W x 0.457 m H) were constructed from vinyl-coated wire (14 gauge, 2.54 cm mesh) and suspended 61.0 cm from the floor in an enclosed pole barn-type building. Wood shavings were placed underneath the cages to absorb excreta and water. Shavings were replaced every two weeks.

A gas brooder was utilized to keep the room temperature above 0°C. Room temperature and humidity were monitored by an LCD digital thermometer/hygrometer that displayed the current temperature/humidity in addition to the high and low temperature/humidity readings during the previous 24-hour period.

Incandescent bulbs controlled by a timer provided light. Lights were maintained at 8 hours light:16 hours dark during the 26-day acclimation period (30 December 1997 – 25 January 1998).

Food and water were available ad libitum during the acclimation period. The diet during the acclimation period was a commercial pelleted ration (Purina Duck Grower W/O, St. Louis, Missouri; Batch #8858; crude protein $\geq$ 16.0%, lysine $\geq$ 0.63%, methionine $\geq$ 0.30%, crude fat $\geq$ 3.0%, crude fiber $\leq$ 5.0%, calcium 0.40-0.90%, phosphorus $\geq$ 0.55%, sodium chloride 0.20-0.70%). Water was obtained from a university well. Crocks containing drinking water were replenished twice daily and feed was added to the feed crocks as needed (usually every other day).

Each cage contained a nest box consisting of a 5-gallon plastic pail that was secured in a horizontal position in a rear corner of the cage. Attached to the bottom front

of the pail was a 5.08 cm high vinyl-coated wire fence to prevent eggs from rolling out of the nest box. A rubber mat was placed inside the pail to provide a cushion for the eggs and to facilitate cleaning of the nest boxes, which was done on a weekly basis.

On 26 January 1998 (day 0), ducks were randomly assigned to 4 treatment groups and identified by metal leg bands (size 14; National Band and Tag Co., Newport, Kentucky) bearing a unique number and color-coded by treatment. The treatment groups were a lead group (6 males and 6 females receiving 8 pellets of #4 lead shot on day 0), a steel group (16 males and 16 females receiving 8 pellets of #4 steel shot on days 0, 30, 60, 90, and 120), a tungsten-iron group (16 males and 16 females receiving 8 #4 tungsten-iron shot composed of 55% tungsten and 45% iron on days 0, 30, 60, 90, and 120), and a tungsten-polymer group (16 males and 16 females receiving 8 #4 tungsten-polymer shot composed of 95.5% tungsten and 4.5% nylon on days 0, 30, 60, 90, and 120). Each cage was identified with a color-coded card bearing the cage number, the pair's individual band numbers, and the treatment. For record keeping purposes, the ducks were identified by a 4-digit number. The first 2 digits designated the treatment (10 = lead, 20 = steel, 30 = tungsten-iron, 40 = tungsten-polymer), and the last 2 digits were the duck's individual band number.

Mallards were switched to a shelled corn diet on day 0. Each duck was weighed and dosed with the appropriate shot. Prior to dosing, pellets were weighed and placed in groups of 8 into individual plastic vials that were identified by the duck's 4-digit number, cage number, treatment, sex, and day of dosing. Pellets were introduced into the

proventriculus by means of a funnel and a 21.60 cm latex tube through the esophagus. Approximately 5 mls of water helped to flush the pellets into the proventriculus.

All ducks 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. Any duck that died before day 150 was weighed and taken to MSU's Animal Health Diagnostic Laboratory for necropsy as described below. In addition to these observations, feed and water were checked twice daily and the room temperature/humidity was recorded at each entry during the 150-day period. Photoperiod was maintained at 8 hours light:16 hours dark for the duration of the 60-day phase of the 150-day trial.

On day 7 (2 February 1998), blood was collected from the brachial vein of each duck using a 22 gauge needle. Blood was placed into 2 microhematocrit capillary tubes (75 x 1.2 mm), 1 2-ml Vacutainer tube (Becton Dickinson, Franklin Lakes, New Jersey) containing EDTA (lavender stopper) and 2 2-ml Vacutainer tubes containing sodium heparin (green stopper). Each Vacutainer tube was labeled with the duck's 4 digit number, cage number, treatment, sex and the date of collection.

The microhematocrit capillary tubes were sealed and transported to a small laboratory adjacent to the building where the ducks were housed. Tubes were centrifuged in an IEC MB microhematocrit centrifuge (International Equipment Co., Boston, Massachusetts) and hematocrits were measured with an IEC MB microcapillary reader.

The Vacutainer tube containing EDTA and 1 Vacutainer tube containing sodium heparin from each duck were gently rotated for 1 minute and then refrigerated until all blood samples were collected over a 4-hour period. Since mallards were bled in order of

their cage number rather than by treatment, blood samples from all 4 treatments were collected throughout the period. When blood collection was completed, samples were packed unfrozen in coolers containing U-Tek polyfoam refrigerant packs (Polyfoam Packers, Wheeling, Illinois) and shipped by overnight express to the Division of Comparative Pathology at the University of Miami, Miami, Florida.

The second Vacutainer tube containing sodium heparin from each duck was used for separation of plasma from whole-blood. Refrigerated tubes were transported to the Toxicology Laboratory in Anthony Hall (4 miles from the PSTRC) and spun in a GLC-4 General Laboratory centrifuge (Sorvall Instruments, Newtown, Connecticut) at 50 x g for 5 minutes. Plasma was removed from the Vacutainer tube by a glass Pasteur pipet and transferred to a labeled 1-dram glass vial. Plasma vials were stored in a cooler containing dry ice until all plasma samples had been collected. Vials were then transferred to an ultracold freezer (-72°C) until shipping the next day. Plasma samples were sent on dry ice by overnight express to the Division of Comparative Pathology, University of Miami, Miami, Florida.

Within 1 hour of arrival of the whole-blood and plasma samples at the University of Miami, the tubes and vials were unpacked, separated by container type, and arranged sequentially by the ducks' 4 digit numbers. Tubes and vials 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. EDTA-containing tubes were at room temperature prior to determination of Hb concentration. Tubes containing sodium heparin were stored at 4°C for 3 hours prior to analysis of ALAD activity. Plasma samples were kept frozen prior to determining plasma chemistries.

Hemoglobin was determined by removing 100 µl whole-blood from the Vacutainer tube containing EDTA and placing it in a plastic 96-well microtiter plate. Fifty µl of lysis solution (ammonium chloride) was added to each well and the solutions mixed by automatic pipet for 10 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 (Buffalo, New York). Hemoglobin was quantitated as g/dL (x 1.5 for dilution factor). ALAD (expressed in ALAD units) was measured according to the protocol of Burch and Siegel (1971) and Dieter and Finley (1979). ALAD units equal (corrected absorbance x 12,500)/HCT. Plasma samples were analyzed using a Johnson and Johnson 700XR automated analyzer (Rochester, New York). Control sera samples were run daily prior to analysis to maintain a check on instrument calibration.

On day 9 (4 February 1998), half of the mallards in each treatment group, and on day 11 (6 February 1998), the remaining ducks in each treatment group were transported (12 ducks/crate) to the MSU Large Animal Veterinary Clinic for fluoroscopy by radiologist Dr. Russell Stickle to determine retention of shot. All ducks 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 monitor. Each radiograph was identified by the duck's 4-digit number.

On days 30 (25 February 1998) and 60 (27 March 1998), mallards were weighed and redosed with 8 pellets of their respective shot. Blood was collected from all ducks for HCT determination and from 8 males and 8 females in each treatment group for

determination of plasma chemistries. Hematocrits were determined at MSU and the Division of Comparative Pathology, University of Miami, Miami, Florida assessed plasma chemistries, as described above. Fluoroscopies were performed as previously described on days 37 (4 March 1998) and 39 (6 March 1998), and on days 70 (6 April 1998) and 72 (8 April 1998).

On day 61 (28 March 1998), all surviving mallards were switched to a commercial layer ration (Mazuri Waterfowl Breeder, Brentwood, Missouri; Batch #5640; crude protein ≥ 17.0%, crude fat ≥ 2.5 %, crude fiber ≤ 6.0 %, ash ≤ 10.0%, added minerals ≤ 5.2%) for the next 90 days (reproduction trial). Photoperiod was increased on a weekly basis over 6 weeks beginning on 21 April 1998 and ending on 1 June 1998 to achieve 18 hours light:6 hours dark. Ducks were weighed and redosed with 8 pellets of the appropriate shot, and blood samples taken for HCT and plasma chemistries on days 90 (27 April 1998) and 120 (26 May 1998). Mallards were fluoroscoped on days 100 (6 May 1998) and 102 (8 May 1998) and on day 130 (5 June 1998).

When egg laying began, cages were checked twice daily and all eggs were collected from each pair throughout the 90-day reproduction phase. Eggs were removed, dated, identified by the respective hen's 4-digit number and sequential egg number, weighed, and held for up to 1 week in a cooler at temperatures between 55°- 60°F with 75% relative humidity.

The 11^{th} egg laid by each female was used for determination of shell thickness and for elemental analysis of shell and contents. Measurements of shell thickness were taken

at 6 locations (2 on the pointed end, 2 on the blunt end and 2 on the equator) on each egg with an Ames 25 ME Digimatic Outside Micrometer (Waltham, Massachusetts) and the 6 measurements were averaged. Shells were stored at room temperature in individually labeled plastic bags and the contents were stored in individually labeled I-Chem jars (Nalge, New Castle, Delaware) in a freezer (-4°C).

All eggs, except the 11th egg, were set on a weekly basis and incubated with their blunt end up in a Petersime poultry incubator (Gettysburg, OH) for up to 30 days. Conditions in the incubator were standard for commercial operations, 99.0-99.5°F with wet bulb readings of 83-85°F to yield approximately 60% relative humidity. Eggs were automatically rotated every 2 hours. Embryo fertility was determined by candling eggs on incubation days 7, 14 and 21, and infertile eggs were removed. On incubation day 22, embryo viability was assessed with an embryo viability detector (EVD) that was provided by USFWS. The EVD detects vibrations within the egg and changes the vibrations to sound waves that can be heard in headphones attached to the EVD (Mineau and Pedrosa, 1986). All viable eggs were then transferred to pedigree hatching baskets that were placed in a Sure-pip hatcher (Agro Environmental Systems Inc., Dallas, Georgia) 4 days prior to hatching. The temperature in the hatcher was maintained at 99.0°F with a wet-bulb reading of 89.0°F to yield approximately 70% relative humidity. Eggs were kept in the hatcher until hatching or until day 30 of incubation. Eggs not hatching were identified as shell-less, cracked, dead non-pipped, live non-pipped, dead pipped or live pipped. The eggs were then opened, examined for deformities, and the approximate age of embryos at death was determined.

Ducklings were removed from the incubator (within 18 hours after hatching), weighed, and identified with a Swiftak identification tag (Heartland Animal Health, Inc., Fair Play, Missouri). They were housed in heated floor pens (3.05 m W x 2.29 m L) with water and starter mash (Purina Duck Starter W/O, Batch #8855; crude protein ≥ 20.0%, lysine ≥ 0.95%, methionine ≥ 0.40%, crude fat ≥ 3.0%, crude fiber ≤ 6.0%, calcium 0.60-1.10%, phosphorus ≥ 0.60%, sodium chloride 0.20-0.70%) being provided ad libitum. Water was available in Plasson waterers and feed was placed in metal feeders that were refilled at least twice daily. Shavings were placed on the floor to absorb excreta and water and were replaced on a weekly basis.

At 14 days of age, each duckling was weighed, and blood was collected from the brachial vein into microhematocrit capillary tubes (32 x 0.8 mm) for determination of HCT. Tubes were sealed and centrifuged in a Drummond Scientific microhematocrit centrifuge (Broomall, Pennsylvania). Hematocrits were measured with a Drummond Scientific microcapillary reader. Ducklings from eggs number 1-10 and 12-21 from each hen, if available, were euthanized by cervical dislocation and necropsied. The brain, heart, liver, spleen, kidneys, and bursa were removed for weighing. The gonads were examined to determine sex. Small samples of the liver and kidneys were placed in individually labeled plastic vials containing a 10% formalin-saline solution for subsequent histopathological examination. Additionally, the right femur and the remaining portions of the liver and kidneys from each necropsied duckling were placed in individually labeled plastic bags and frozen for subsequent elemental analysis.

On day 150 of the trial, all surviving adult mallards were weighed, bled as previously described, killed by cervical dislocation, and subjected to necropsy. The

necropsy procedure included a complete gross examination of all body cavities and organs by Dr. Scott Fitzgerald, board-certified veterinary pathologist. Gizzards were opened for inspection of cracked and discolored mucosa and retention of shot. Shot were counted and placed into individually labeled plastic vials for subsequent cleaning and weighing for determination of shot erosion. The brain, gizzard, heart, liver, spleen, kidneys, testes/ovary were removed and weighed. Small samples of the liver, kidneys and testes/ovary from each duck were placed in labeled glass jars containing a 10% formalin-saline solution for subsequent histopathological examination. The right femur and remaining portions of the liver, kidneys, and testes/ovary were placed in individually labeled plastic bags and frozen for subsequent elemental analysis.

Histological examination of tissues was performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist. Liver and kidney samples from 8 male and 8 female ducklings in each treatment (excluding lead) were assessed as were liver, kidney, and ovary/testes samples from 8 male and 8 female adult mallards from the steel, tungsten-iron and tungsten-polymer groups and from the 6 males and 6 females in the lead group. Tissues for microscopic examination were fixed in 10% formalin and embedded in paraffin. Tissue sections were trimmed to 8 microns and stained with hematoxylin and eosin. Selected liver sections from steel-, tungsten-iron- and tungsten-polymer-dosed mallards were stained with Prussian blue for determination of iron pigment (Mallory, 1942).

Elemental analysis of tissues was performed by CT&E Environmental Services (Ludington, Michigan). Frozen samples were transported by ground courier from MSU to Ludington. All tissues were stored frozen until sample preparation and analysis.

Samples analyzed included: individual liver, kidney, femur, and gonad samples from the 12 lead-dosed adults; individual liver samples from 8 adult males and 8 adult females in the steel, tungsten-iron and tungsten-polymer groups and individual testis samples from 8 adult males in the steel, tungsten-iron and tungsten-polymer groups; 16 pooled kidney and femur samples (8 male and 8 female), each consisting of tissues from 2 adult males or 2 adult females in the steel, tungsten-iron and tungsten-polymer groups and 8 pooled ovary samples from 2 adult females in the steel, tungsten-iron and tungsten-polymer groups; the shell and contents of the 11^{th} egg from each hen, if available; 16 pooled liver, kidney, and femur samples (8 male and 8 female), each consisting of tissues from 3 male or 3 female ducklings from the same hen, in the steel, tungsten-iron, and tungsten-polymer groups. Tissues were digested using EPA method 200.3 (U.S. Environmental Protection Agency, 1991). Iron and tungsten were analyzed by Inductively Coupled Argon Emission Plasma Spectroscopy (ICAP) following EPA method SW-836 Method 6010, revision 2.0 (U.S. Environmental Protection Agency, 1996) and lead was analyzed by Graphite Furnace Atomic Absorption (GFAA) based on EPA method SW-846 Method 7421 (U.S. Environmental Protection Agency, 1986). A matrix spike was prepared and analyzed with each digestion batch. When the matrix spike recoveries were outside of quality control acceptance criteria, an analytical spike or post-digestion spike was performed. All matrix spike and/or analytical spike recoveries were within quality control acceptance criteria with the exception of tungsten in batch 8829 that yielded recoveries of 70% and 72% for the matrix spike and analytical spike, respectively. Selected tissues from lead-dosed and steel-dosed ducks were re-analyzed because tungsten was reported in those tissues. Upon reanalysis, tungsten was not detected in any

of the samples in question, with the exception of 3 kidney samples from lead-dosed adults and 2 kidney samples from steel-dosed adults. There was not a sufficient amount of material left to reanalyze these 5 samples. Average percent recovery of iron, tungsten, and lead were 100%, 92%, and 97%, respectively.

All statistical analyses were performed using SAS® software (SAS, 1997). Adult body weights, plasma chemistries, and hematocrits were analyzed by analysis of variance (ANOVA) involving the factors treatment and sex, with repeated measurements on animals, when applicable, over a third factor, days. SAS® PROC MIXED was used to model a first-order autoregressive correlation structure for repeated measurements over days within animals, as residuals involving measurements taken at adjacent time periods are likely to be more correlated than measurements taken further apart in time (Gill, 1990). Body weights were analyzed based on the status of the ducks on the specific days of measurement. Body weights were analyzed separately over three different time points due to differences in status over these periods. First, body weights of ducks from the lead-dosed group were extrapolated to day 30 since all of the lead-dosed ducks died by day 25. Body weight difference from day 0 to day 30 was then analyzed with mean weight differences compared among the 4 treatment groups. Second, at day 60, the body weights of adult ducks in the steel, tungsten-iron and tungsten-polymer groups were analyzed at this single time point because none of the ducks were yet reproductively active. Finally, adult body weights were analyzed over the time period that ducks were reproductively active (days 90, 120, and 150). Hematocrits and plasma chemistries of adult ducks in the steel, tungsten-iron and tungsten-polymer groups were analyzed over

the time period that ducks were not yet reproductively active (days 30 and 60) and over the time period that they were reproductively active (days 90, 120, and 150).

Hematocrit, Hb concentration, ALAD activity and plasma chemistries for all 4 treatment groups at day 7 were analyzed under a two-way ANOVA model involving the factors treatment and sex. Duckling body weights and hematocrits, adult and duckling organ weights, concentrations of metal residues in adult and duckling tissues, and percent shot erosion were also analyzed under a two-way ANOVA model.

Egg production, hatchability, fertility, egg weights, eggshell thickness, concentrations of metal residues in egg shell and egg contents, and duckling survivability were analyzed under a one-way ANOVA model involving the factor treatment.

Residual plots were used to check for homogeneity of variance and for aberrant values. Residual plots for plasma chemistry parameters at days 7, 30, 60, 90, 120, and 150 and adult elemental analysis indicated aberrant values, therefore, those data were log transformed to normalize data. The reported means and 95% confidence intervals for treatment means of plasma chemistries and adult elemental analysis were back (anti-log) transformed to the scale of observation. Percent shot erosion, adult and duckling relative organ weights, egg production, hatchability, fertility, and duckling survivability were percentage data subjected to arcsine, square root transformation prior to statistical analysis. The reported means and 95% confidence intervals for treatment means of percent shot erosion, adult and duckling relative organ weights, egg production, hatchability, and fertility were back $[(\sin(x))^2]$ transformed to the scale of observation.

Treatment group means were reported as the least square mean plus or minus the standard error. Since variability was homogenous across days, all standard error

computations were based on a pooled estimate of residual variance. Therefore, the standard errors of means for a particular parameter were the same unless the sample sizes were not equal. Treatment means were reported separately for each sex, and/or day, if treatment by sex and/or treatment by day interactions, respectively, were statistically significant. Otherwise, reported treatment means and differences were based on pooling information over the sexes and/or days. To control for experimental error rates, a Tukey adjustment was used to test comparisons between means based on the total number of pairwise comparisons. Differences between treatment group means were statistically significant based on a Type I error rate of 0.05.

Results

Adult Mortality

All mallards dosed with lead shot died within the first 25 days of the 150-day trial (Table 1). The average time to death was 16.7 days for males and 11.0 days for females with a range of 9 to 25 days for both sexes. The average weight loss of those mallards dying was 61%. No ducks in the steel-, tungsten-iron-, or tungsten-polymer-dosed groups died during the 150-day trial.

Adult Clinical Signs

Lead-dosed mallards were the only ducks that had obvious clinical signs during the trial. All of them had green-stained excreta within 24 hours of dosing. By day 5, all

Table 1. The effect of treatment shot on percent mortality, time to death (days), and percent weight lost at death of mallards on a 150-day dosing test[a].

Treatment	% Mortality	Time to death	% Weight loss at death
Males			
Steel	-	-	-
Lead	100 (6/6)	16.7 ± 5.25 (9-25)	60.3 ± 4.93 (55.0-69.0)
Tungsten-iron	-	-	-
Tungsten-polymer	-	-	-
Females			
Steel	-	-	-
Lead	100 (6/6)	11.0 ± 0.26 (9-12)	61.6 ± 7.84 (54.0-72.7)
Tungsten-iron	-	-	-
Tungsten-polymer	-	-	-

[a] Data presented as mean ± standard error of the mean. Numbers in parentheses represent number of birds dying/number of birds per group for % mortality, range for time to death, and range for % weight loss at death.

lead-dosed mallards had marked tail and wing droop. Prior to death, ducks were emaciated, lethargic and ataxic.

Adult Body Weights

By day 30 of the trial, lead-dosed ducks lost approximately 530 grams of body weight, while steel-, tungsten-iron-, and tungsten-polymer-dosed ducks lost 73 to 79 grams of body weight (Table 2). Because the lead-dosed ducks died by day 25, body weights of all lead-dosed ducks were extrapolated to day 30 based on body weight taken at time of death to allow for statistical comparison.

From day 30 through day 60, there were no treatment by sex interaction or treatment by day interaction, thus data were combined for both sexes over days 30 and 60. There were no significant differences in body weight between the steel, tungsten-iron and tungsten-polymer groups (Table 3). From day 90 through day 150, there were no treatment by sex or treatment by day interactions, thus data were combined for both sexes over days 90, 120 and 150. Mallards in the tungsten-polymer group had significantly greater body weight when compared to ducks in the steel-dosed group (Table 4).

Adult HCT, Hb Concentration, ALAD Activity

Lead-dosed mallards had significantly lower HCT, Hb concentration, and whole-blood ALAD activity at day 7 when compared to mallards in the steel, tungsten-iron, and tungsten-polymer groups. In contrast, ducks in the tungsten-polymer group had significantly higher ALAD activity than mallards in the other 3 treatment groups (Table 5). From day 30 to day 60, there were no significant differences in HCT between ducks

Table 2. The effect of treatment shot on body weight (gm) loss of mallards from day 0 to day 30 of a 150-day dosing test[a].

Treatment	Body weight loss[b]
Steel	-73.3[A]
Lead	-527.5[B]
Tungsten-iron	-78.6[A]
Tungsten-polymer	-77.5[A]

[a] Data presented as mean of body weight loss. Sample size is 32 for all groups except lead, which is 12. Means with different superscripts are significantly different within the column ($p < 0.5$).

[b] Body weights at day 30 for lead-dosed ducks only were derived by linear extrapolation.

Table 3. The effect of treatment shot on body weight (gm) of mallards from day 30 through day 60 of a 150-day dosing test[a].

Treatment	Body weight
Steel	966.1 ± 18.70
Tungsten-iron	1024.1 ± 18.70
Tungsten-polymer	1017.0 ± 18.70

[a] Data presented as mean ± standard error of the mean. Sample size is 32 for all groups.

Table 4. The effect of treatment shot on body weight (gm) of mallards from day 90 through day 150 of a 150-day dosing test[a].

Treatment	Body weight
Steel	1122.1 ± 19.03^{A}
Tungsten-iron	1172.3 ± 19.06^{AB}
Tungsten-polymer	1204.5 ± 18.78^{B}

[a] Data presented as mean ± standard error of the mean. Sample size is 32 for all groups. Means with different superscripts are significantly different within the column ($p < 0.5$).

Table 5. The effect of treatment shot on whole-blood parameters of mallards on day 7 of a 150-day dosing test[a].

Treatment	Hematocrit	Hemoglobin	ALAD
Steel	49.3 ± 0.65^{A}	15.6 ± 0.28^{A}	53.1 ± 2.86^{A}
Lead	26.4 ± 1.06^{B}	10.3 ± 0.46^{B}	25.2 ± 4.67^{B}
Tungsten-iron	49.7 ± 0.65^{A}	16.2 ± 0.28^{A}	45.5 ± 2.86^{A}
Tungsten-polymer	48.9 ± 0.65^{A}	15.6 ± 0.28^{A}	66.1 ± 2.86^{C}

[a] Data presented as mean ± standard error. Sample size is 32 for all groups except lead, which is 12. Hematocrit is expressed as percentage of packed red blood cell volume; hemoglobin is expressed as g/dL; ALAD refers to delta 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 ($p < 0.5$).

in the steel, tungsten-iron, and tungsten-polymer groups (Table 6). Between day 90 and day 150, there was a significant treatment by sex interaction for HCT. There were no significant differences between HCT of steel-, tungsten-iron-, and tungsten-polymer-dosed males, but HCT of tungsten-polymer-dosed females was significantly lower than HCT of steel- and tungsten-iron–dosed females (Table 7).

Adult Plasma Chemistries

There were a number of significant differences in plasma chemistry parameters at day 7 (Tables 8 and 9). Sodium concentration in lead-dosed mallards was significantly lower when compared to the other 3 groups and significantly lower in the tungsten-polymer-dosed mallards compared to steel-dosed ducks. Conversely, potassium concentration in lead-dosed ducks was higher when compared to tungsten-iron-dosed mallards. Concentrations of blood urea nitrogen and creatinine were significantly higher in lead-dosed ducks compared to the other 3 groups. The blood urea nitrogen/creatinine ratio was significantly lower in lead-dosed ducks compared to the other 3 groups. Total protein and albumin concentrations were significantly lower in lead-dosed ducks when compared to the other 3 groups. Albumin/globulin ratio, concentrations of total bilirubin and uric acid, and activities of alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase were all significantly higher in lead-dosed ducks when compared to steel-, tungsten-iron- and tungsten-polymer-dosed groups. Phosphorus concentration was significantly higher in lead-dosed ducks as compared to steel- and tungsten-iron-dosed groups and significantly higher in tungsten-polymer-dosed ducks as compared to the tungsten-iron-dosed ducks. Alkaline phosphatase activity was significantly lower in

Table 6. The effect of treatment shot on hematocrit of mallards from day 30 through day 60 of a 150-day dosing test[a].

Treatment	Hematocrit
Steel	50.8 ± 0.45
Tungsten-iron	51.1 ± 0.45
Tungsten-polymer	50.5 ± 0.45

[a] Data presented as mean ± standard error. Sample size is 32 for all groups. Hematocrit is expressed as percentage of packed red blood cell volume. Means with different superscripts are significantly different within the column ($p < 0.5$).

Table 7. The effect of treatment shot on hematocrit of male and female mallards from day 90 through day 150 of a 150-day dosing test[a].

Treatment	Hematocrit
Males	
Steel	47.8 ± 0.77
Tungsten-iron	47.9 ± 0.77
Tungsten-polymer	47.2 ± 0.77
Females	
Steel	43.5 ± 0.77^{A}
Tungsten-iron	44.0 ± 0.78^{A}
Tungsten-polymer	40.4 ± 0.78^{B}

[a] Data presented as mean ± standard error. Sample size is 16 for all groups. Hematocrit is expressed as percentage of packed red blood cell volume. Means with different superscripts are significantly different within the column ($p < 0.5$).

Table 8. The effect of treatment shot on plasma chemistry parameters of mallards on day 7 of a 150-day dosing test[a].					
Parameter	Units	Steel	Lead	Tungsten-iron	Tungsten-polymer
Glucose	mg/dL	216.1 (208.51 - 224.06)	209.6 (197.61 - 222.25)	222.9 (214.97 - 231.02)	220.2 (212.43 - 228.26)
Sodium	mmol/L	147.6^{A} (146.01 - 149.25)	128.7^{B} (126.37 - 130.97)	145.6^{AC} (144.01 - 147.20)	144.4^{C} (142.86 - 146.03)
Potassium	mmol/L	3.7^{AB} (3.45 - 3.93)	4.3^{B} (3.84 - 4.74)	3.5^{A} (3.29 - 3.75)	3.8^{AB} (3.52 - 4.00)
Carbon Dioxide	mmol/L	18.5 (17.44 - 19.65)	16.9 (15.34 - 18.64)	17.5 (16.53 - 18.62)	17.5 (16.53 - 18.62)
Blood Urea Nitrogen	mg/dL	3.0^{A} (2.81 - 3.20)	3.7^{B} (3.31 - 4.09)	3.0^{A} (2.81 - 3.20)	3.0^{A} (2.81 - 3.20)
Creatinine	mg/dL	0.2^{A} (0.20 - 0.23)	0.5^{B} (0.44 - 0.57)	0.2^{A} (0.22 - 0.26)	0.2^{A} (0.21 - 0.25)
Blood Urea Nitrogen/ Creatinine		12.2^{A} (11.46 - 12.99)	9.2^{B} (8.26 - 10.13)	11.6^{A} (10.94 - 12.40)	11.6^{A} (10.90 - 12.35)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 32 for all groups except lead, which is 12 or the number indicated in brackets.

Table 8 continued. The effect of treatment shot on plasma chemistry parameters of mallards on day 7 of a 150-day dosing test[a].					
Parameter	Units	Steel	Lead	Tungsten-iron	Tungsten-polymer
Total Protein	gm/dL	4.7^{A} (4.54 - 4.85)	3.6^{B} (3.43 - 3.82)	4.6^{A} (4.47 - 4.78)	4.5^{A} (4.40 - 4.70)
Albumin	gm/dL	2.0^{A} (1.93 - 2.07)	1.7^{B} (1.58 - 1.76)	2.0^{A} (1.93 - 2.07)	2.0^{A} (1.91 - 2.04)
Albumin/Globulin		0.8^{A} (0.73 - 0.78)	0.9^{B} (0.80 - 0.91)	0.8^{A} (0.74 - 0.80)	0.8^{A} (0.73 - 0.79)
Total Bilirubin	mg/dL	0.1^{A} (0.11 - 0.17)	0.3^{B} (0.23 - 0.46)	0.1^{A} (0.11 - 0.16)	0.1^{A} (0.11 - 0.16)
Direct Bilirubin	mg/dL	0.1 (0.06 - 0.28)	0.3 (0.16 - 0.49)	0.2 (0.10 - 0.38)	0.0 [31]
Phosphorus	mg/dL	3.9^{AC} (3.59 - 4.30)	5.1^{BC} (4.36 - 5.86)	3.7^{A} (3.36 - 4.03)	4.4^{C} (4.01 - 4.81)
Uric Acid	mg/dL	3.2^{A} (2.83 - 3.71)	6.6^{B} (5.30 - 8.26)	3.4^{A} (2.95 - 3.88)	3.4^{A} (2.96 - 3.88)
Alkaline Phosphatase	U/L	67.1^{A} (55.55 - 81.07) [29]	29.7^{B} (22.13 - 39.83)	61.5^{A} (51.34 - 73.57)	49.0^{A} (40.91 - 58.62)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 32 for all groups except lead, which is 12 or the number indicated in brackets.

Table 8 continued. The effect of treatment shot on plasma chemistry parameters of mallards on day 7 of a 150-day dosing test[a].					
Parameter	Units	Steel	Lead	Tungsten-iron	Tungsten-polymer
Alanine Aminotransferase	U/L	3.6^{A} (3.10 - 4.17)	33.9^{B} (26.66 - 43.22)	3.1^{A} (2.65 - 3.57)	3.0^{A} (2.59 - 3.48)
Aspartate Aminotransferase	U/L	17.9^{A} (15.44 - 20.82)	71.4^{B} (55.92 - 91.13)	22.1^{A} (19.04 - 25.68)	20.9^{A} (18.00 - 24.27)
Lactate Dehydrogenase	U/L	956.7^{A} (801.27 - 1142.30) [31]	3030.6^{B} (2279.25 - 4029.11)	1181.6^{A} (971.46 - 1437.27) [26]	1085.6^{A} (911.87 - 1292.46)
Gamma Glutamyl Transpeptidase	U/L	5.2 (4.73 - 5.83) [29]	6.5 (5.49 - 7.610	6.0 (5.47 - 6.67)	5.5 (4.97 - 6.07)
Cholesterol	mg/dL	244.4 (230.81 - 258.76)	223.1 (203.22 - 244.91)	243.6 (230.07 - 257.93)	239.0 (225.77 - 253.08)
Triglycerides	mg/dL	120.4^{A} (104.50 - 138.78)	72.3^{B} (57.34 - 91.14)	113.3^{A} (98.28 - 130.53)	109.3^{A} (94.80 - 125.91)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 32 for all groups except lead, which is 12 or the number indicated in brackets.

Table 9. The effect of treatment shot on plasma chemistry parameters of male and female mallards on day 7 of a 150-day dosing test[a].

Parameter	Units	Steel	Lead	Tungsten-iron	Tungsten-polymer
			Males		
Chloride	mmol/L	118.2^{A} (111.82 - 116.66)	100.6^{B} (97.15 - 104.13)	114.2^{A} (111.82 - 116.66)	113.9^{A} (111.54 - 116.37)
Calcium	mg/dL	11.5^{A} (11.04 - 11.990 [15]	10.1^{BC} (9.45 - 10.77)	10.5^{C} (10.11 - 10.950	11.3^{A} (10.87 - 11.78)
Creatinine Phosphokinase	U/L	144.3^{A} (94.47 - 220.46) [15]	1487.7^{B} (761.36 - 2907.36)	242.1^{A} (158.46 - 369.78) [15]	135.8^{A} (90.12 - 204.71)
Amylase	U/L	1387.1^{A} (1298.03 - 1482.37)	1143.1^{B} (1025.67 - 1274.11)	1277.6^{AB} (1186.78 - 1375.13) [13]	1363.1^{A} (1275.38 - 1456.66)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 32 for all groups except lead, which is 12 or the number indicated in brackets.

Table 9 continued. The effect of treatment shot on plasma chemistry parameters of male and female mallards on day 7 of a 150-day dosing test[a].

Parameter	Units	Steel	Lead	Tungsten-iron	Tungsten-polymer
			Females		
Chloride	mmol/L	117.9^{A} (115.49 - 120.34)	92.6^{B} (89.51 - 95.72)	114.7^{AC} (112.34 - 117.04)	111.4^{C} (109.15 - 113.73)
Calcium	mg/dL	11.3^{A} (10.84 - 11.80)	9.4^{B} (8.76 - 10.05)	11.3^{A} (10.82 - 11.77)	10.8^{A} (10.40 - 11.31)
Creatinine Phosphokinase	U/L	181.6^{A} (145.79 - 226.24)	711.7^{B} (497.15 - 1018.82)	156.2^{A} (125.40 - 194.59)	186.6^{A} (149.81 - 232.46)
Amylase	U/L	1262.1^{AC} (1156.67 - 1377.05)	849.4^{B} (736.64 - 979.26)	1367.2^{A} (1249.38 - 1495.92) [15]	1115.4^{C} (1022.29 - 1217.07)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 32 for all groups except lead, which is 12 or the number indicated in brackets.

lead-dosed ducks when compared to ducks in the steel, tungsten-iron, and tungsten-polymer groups. Lead-dosed ducks had significantly lower triglyceride concentration when compared to the other 3 groups.

There was a significant treatment by sex interaction for calcium and chloride concentrations and creatinine phosphokinase and amylase activities at day 7 (Table 9). Lead-dosed males had significantly lower chloride concentration when compared to the other 3 groups. Calcium concentration in lead-dosed males was significantly lower compared to steel- and tungsten-polymer-dosed males and tungsten-iron-dosed males had significantly lower calcium concentration than steel-dosed males. Lead-dosed males had significantly higher creatinine phosphokinase activity compared to the other 3 groups. Amylase activity in lead-dosed males was significantly lower compared to steel-and tungsten-polymer-dosed males. Lead-dosed females had significantly lower chloride concentration when compared to steel-, tungsten-iron, and tungsten-polymer-dosed females and tungsten-polymer-dosed females had significantly lower chloride concentrations than steel-dosed females. Calcium concentration was significantly lower and creatinine phosphokinase activity was significantly higher in lead-dosed females when compared to the other 3 groups. Amylase activity in lead-dosed females was significantly lower when compared to the other 3 dose groups and tungsten-iron-dosed females had significantly higher amylase activity than tungsten-polymer-dosed females.

There were no significant treatment by day or treatment by sex interactions for most of the plasma chemistry parameters between days 30 and 60 (Table 10). Tungsten-iron- and tungsten-polymer-dosed mallards had significantly lower carbon dioxide

Table 10. The effect of treatment shot on plasma chemistry parameters of mallards from day 30 through day 60 of a 150-day dosing test[a].

Parameter	Units	Steel	Tungsten-iron	Tungsten-polymer
Glucose	mg/dL	213.2^{AB} (206.31 - 220.28)	208.2^{B} (201.54 - 215.16)	221.7^{A} (214.56 - 229.09)
Sodium	mmol/L	143.4^{AB} (142.05 - 144.78)	141.3^{B} (139.97 - 142.65)	144.9^{A} (143.51 - 146.26)
Potassium	mmol/L	2.6 (2.46 - 2.77)	2.7 (2.50 - 2.82)	2.6 (2.63 - 2.97)
Chloride	mmol/L	114.4^{AB} (111.89 - 114.56)	112.3^{A} (110.97 - 113.62)	114.9^{B} (113.59 - 116.30)
Carbon Dioxide	mmol/L	20.2^{A} (19.21 - 21.24)	18.4^{B} (17.51 - 19.37)	17.5^{B} (16.63 - 18.39)
Blood Urea Nitrogen[b]	mg/dL	3.0	3.0	3.0
Creatinine	mg/dL	0.2 (0.22 - 0.26)	0.2 (0.21 - 0.25)	0.2 (0.22 - 0.26)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 32 for all groups or the number indicated in brackets.

[b] All values reported for blood urea nitrogen at days 30 and 60 were 3.0. Therefore, a 95% confidence interval was not reported because there is no variation within the data.

Table 10 continued. The effect of treatment shot on plasma chemistry parameters of mallards from day 30 through day 60 of a 150-day dosing test[a].

Parameter	Units	Steel	Tungsten-iron	Tungsten-polymer
Total Protein	gm/dL	4.4^{AB} (4.29 - 4.60)	4.2^{B} (4.08 - 4.37)	4.5^{A} (4.35 - 4.67)
Albumin	gm/dL	1.9 (1.83 - 1.95)	1.8 (1.75 - 1.87)	1.9 (1.85 - 1.98)
Albumin/Globulin		0.7 (0.72 - 0.76)	0.8 (0.73 - 0.77)	0.7 (0.72 - 0.76)
Direct Bilirubin	mg/dL	0.0	0.0	0.0 [31]
Calcium	mg/dL	11.3 (10.84 - 11.89)	11.0 (10.46 - 11.47)	11.4 (10.93 - 11.99)
Phosphorus	mg/dL	3.8^{AB} (3.38 - 4.21)	3.3^{A} (2.96 - 3.69)	4.1^{B} (3.69 - 4.60)
Alkaline Phosphatase	U/L	64.5 (51.75 - 80.48)	64.0 (51.33 - 79.83)	58.9 (47.27 - 73.51)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 32 for all groups or the number indicated in brackets.

Table 10 continued. The effect of treatment shot on plasma chemistry parameters of mallards from day 30 through day 60 of a 150-day dosing test[a].

Parameter	Units	Steel	Tungsten-iron	Tungsten-polymer
Alanine Aminotransferase	U/L	4.1 (3.51 - 4.72)	3.7 (3.18 - 4.27)	3.5 (3.06 - 4.12)
Aspartate Aminotransferase	U/L	17.9^{A} (13.29 - 24.02)	20.0^{AB} (14.90 - 26.93)	31.7^{B} (23.56 - 42.58) [29]
Creatinine Phosphokinase	U/L	198.6 (164.07 - 240.33)	183.7 (151.78 - 222.32)	200.1 (164.75 - 243.03) [31]
Cholesterol	mg/dL	214.7 (198.07 - 232.73)	211.7 (195.31 - 229.50)	229.0 (211.24 - 248.22)
Triglycerides	mg/dL	83.3 (71.11 - 97.49)	82.9 (70.79 - 97.05)	85.0 (72.61 - 99.54)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 32 for all groups or the number indicated in brackets.

concentration when compared to the steel-dosed group. Tungsten-polymer-dosed ducks had significantly higher aspartate aminotransferase activity than steel-dosed ducks. Glucose, sodium, chloride, total protein, and phosphorus concentrations in tungsten-polymer-dosed ducks were significantly higher than in tungsten-iron-dosed ducks.

There was a significant treatment by sex interaction for total bilirubin concentration and lactate dehydrogenase, amylase, and gamma glutamyl transpeptidase activities from day 30 through day 60 (Table 11). Tungsten-iron-dosed males had slightly, but significantly higher total bilirubin concentration then steel- and tungsten-polymer-dosed ducks. Lactate dehydrogenase activity in male tungsten-iron-dosed ducks was significantly lower when compared to steel-dosed males. Amylase activity in tungsten-iron-dosed males was significantly lower when compared to steel-dosed males and was significantly higher in tungsten-iron-dosed females when compared to steel- and tungsten-polymer-dosed females. Gamma glutamyl transpeptidase activity in tungsten-iron-dosed males was significantly higher when compared to steel- and tungsten-polymer-dosed males.

There was a significant treatment by day interaction for blood urea nitrogen/creatinine ratio and uric acid concentration (Table 12) from day 30 through day 60. At day 30, the blood urea nitrogen/creatinine ratio was significantly higher and uric acid concentration was significantly lower in tungsten-iron-dosed ducks compared to tungsten-polymer-dosed ducks. At day 60, the blood urea nitrogen/creatinine ratio was significantly higher in tungsten-polymer-dosed ducks when compared to the steel-dosed group.

From day 90 through day 150, there were a few differences in plasma chemistry

Table 11. The effect of treatment shot on plasma chemistry parameters of male and female mallards from day 30 through day 60 of a 150-day dosing test[a].

Parameter	Units	Steel	Tungsten-iron	Tungsten-polymer
		Males		
Total Bilirubin	mg/dL	0.10^{AC} (0.09 - 0.12)	0.14^{B} (0.12 - 0.17)	0.09^{C} (0.08 - 0.12)
Lactate Dehydrogenase	U/L	1111.4^{A} (927.32 - 1331.95)	826.6^{B} (689.66 - 990.59)	1007.7^{AB} (840.75 - 1207.61)
Amylase	U/L	1371.8^{A} (1259.66 - 1494.13)	1216.2^{B} (1116.66 - 1324.51)	1347.0^{AB} (1236.82 - 1467.04)
Gamma Glutamyl Transpeptidase	U/L	5.0^{AC} (4.51 - 5.65)	6.4^{B} (5.76 - 7.21)	5.2^{C} (4.61 - 5.77)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 16 for all groups or the number indicated in brackets.

Table 11 continued. The effect of treatment shot on plasma chemistry parameters of male and female mallards from day 30 through day 60 of a 150-day dosing test[a].

Parameter	Units	Steel	Tungsten-iron	Tungsten-polymer
		Females		
Total Bilirubin	mg/dL	0.1 (0.09 - 0.13)	0.1 (0.08 - 0.12)	0.1 (0.09 - 0.13)
Lactate Dehydrogenase	U/L	974.7 (813.30 - 1168.18)	1135.2 (947.29 - 1360.63)	1001.4 (829.98 - 1208.34) [15]
Amylase	U/L	1186.9^{A} (1089.86 - 1292.72)	1389.4^{B} (1275.64 - 1382.85)	1179.0^{A} (1082.47 - 1283.95)
Gamma Glutamyl Transpeptidase	U/L	5.3 (4.74 - 5.93)	5.0 (4.47 - 5.60)	5.4 (4.81 - 6.03)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 16 for all groups or the number indicated in brackets.

Table 12. The effect of treatment shot on plasma chemistry parameters of mallards on day 30 and day 60 of a 150-day dosing test[a].

Parameter	Units	Steel	Tungsten-iron	Tungsten-polymer
		Day 30		
Blood Urea Nitrogen/ Creatinine		11.5^{AB} (10.65 - 12.33)	12.1^{A} (11.24 - 13.01)	10.4^{B} (9.70 - 11.24)
Uric Acid	mg/dL	2.5^{AB} (2.12 - 2.84)	2.2^{A} (1.87 - 2.50)	2.9^{B} (2.48 - 3.32)
		Day 60		
Blood Urea Nitrogen/ Creatinine		11.3^{A} (10.49 - 12.16)	11.7^{AB} (10.88 - 12.60)	12.6^{B} (11.69 - 13.54)
Uric Acid	mg/dL	3.1 (2.66 - 3.55)	2.6 (2.25 - 3.00)	2.6 (2.25 - 3.01)

[a] Data presented as means (95% confidence intervals). Sample size is 32 for all groups. Means with different superscripts are significantly different within the row ($p < 0.5$).

Table 13. The effect of treatment shot on plasma chemistry parameters of mallards from day 90 through day 150 of a 150-day dosing test[a].

Parameter	Units	Steel	Tungsten-iron	Tungsten-polymer
Glucose	mg/dL	208.9 (202.74 - 215.23)	212.2 (205.90 - 218.61)	211.9 (205.70 - 218.37)
Sodium	mmol/L	146.9 (145.24 - 148.50)	147.8 (146.16 - 149.44)	148.9 (147.26 - 150.57)
Chloride	mmol/L	111.8 (110.22 - 113.35)	113.5 (111.96 - 115.13)	114.3 (112.73 - 115.92)
Carbon Dioxide	mmol/L	20.1 (19.03 - 21.29)	19.4 (18.37 - 20.56)	18.4 (17.42 - 19.49)
Blood Urea Nitrogen	mg/dL	3.2 (3.02 - 3.41)	3.4 (3.16 - 3.57)	3.3 (3.08 - 3.48)
Creatinine	mg/dL	0.2 (0.20 - 0.23)	0.2 (0.20 - 0.23)	0.2 (0.18 - 0.21)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 32 for all groups or the number indicated in brackets.

Table 13 continued. The effect of treatment shot on plasma chemistry parameters of mallards from day 90 through day 150 of a 150-day dosing test[a].				
Parameter	Units	Steel	Tungsten-iron	Tungsten-polymer
Total Protein	gm/dL	5.1[AB] (4.89 - 5.32)	5.0[A] (4.80 - 5.22)	5.4[B] (5.18 - 5.63)
Albumin	gm/dL	2.1 (2.07 - 2.22)	2.1 (2.05 - 2.20)	2.2 (2.15 - 2.31)
Albumin/Globulin		0.7 (0.70 - 0.77)	0.8 (0.72 - 0.79)	0.7 (0.66 - 0.73)
Total Bilirubin	mg/dL	0.2 (0.16 - 0.21)	0.2 (0.18 - 0.23)	0.2 (0.16 0 0.21)
Direct Bilirubin	mg/dL	0.1 (0.10 - 0.18)	0.2 (0.14 - 0.20)	0.1 (0.11 - 0.18)
Calcium	mg/dL	16.6 (15.05 - 18.31) [31]	15.8 (14.37 - 17.45)	17.3 (15.72 - 19.09)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 32 for all groups or the number indicated in brackets.

Table 13 continued. The effect of treatment shot on plasma chemistry parameters of mallards from day 90 through day 150 of a 150-day dosing test[a].

Parameter	Units	Steel	Tungsten-iron	Tungsten-polymer
Phosphorus	mg/dL	4.7 (4.22 - 5.31) [31]	4.7 (4.23 - 5.30)	4.9 (4.39 - 5.50)
Uric Acid	mg/dL	4.4 (3.86 - 4.93)	4.5 (4.00 - 5.12)	4.5 (3.94 - 5.04)
Alkaline Phosphatase	U/L	74.9 (59.85 - 93.65)	68.0 (54.39 - 85.11)	68.9 (55.08 - 86.19)
Alanine Aminotransferase	U/L	9.0 (7.31 - 11.18)	9.2 (7.47 - 11.43)	8.6 (6.97 - 10.67)
Aspartate Aminotransferase	U/L	23.9 (20.87 - 27.44)	26.9 (23.47 - 30.85)	21.6 (18.81 - 24.72)
Lactate Dehydrogenase	U/L	1220.5 (1066.57 - 1396.75)	1247.0 (1089.75 - 1427.10)	1109.5 (969.62 - 1269.78)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 32 for all groups or the number indicated in brackets.

Table 13 continued. The effect of treatment shot on plasma chemistry parameters of mallards from day 90 through day 150 of a 150-day dosing test[a].				
Parameter	Units	Steel	Tungsten-iron	Tungsten-polymer
Creatinine Phosphokinase	U/L	191.3 (158.11 - 231.57)	188.0 (155.34 - 227.51)	160.2 (132.38 - 193.91)
Amylase	U/L	1448.1 (1291.81 - 1623.27)	1399.1 (1248.13 - 1568.38)	1460.5 (1302.71 - 1637.13)
Gamma Glutamyl Transpeptidase	U/L	5.6 (5.17 - 6.07) [31]	6.1 (5.66 - 6.64)	5.6 (5.18 - 6.06)
Cholesterol	mg/dL	159.8^{AB} (149.20 - 171.09)	155.7^{A} (145.37 - 166.70)	178.4^{B} (166.57 - 191.01)
Triglycerides	mg/dL	247.3 (201.04 - 304.17)	237.4 (193.02 - 292.01)	279.4 (227.12 - 343.64)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 32 for all groups or the number indicated in brackets.

parameters (Table 13). Total protein and cholesterol concentrations were significantly higher in tungsten-polymer-dosed ducks when compared to the tungsten-iron-dosed group. There was a significant treatment by day interaction for potassium and blood urea nitrogen/creatinine ratio (Table 14). At day 90, blood urea nitrogen/creatinine ratio was significantly higher in tungsten-polymer-dosed ducks as compared to the other 2 groups. At day 150, potassium concentration was significantly higher in tungsten-iron-dosed ducks when compared to ducks in the steel-dosed group.

Adult Gross Pathology

All lead-dosed mallards had severe atrophy of breast muscle with minimal subcutaneous or abdominal fat with the exception of 1 female, which had moderate breast muscle atrophy (Tables 15 and 16). Three lead-dosed males and females had discoloration of the mucosal lining of the gizzard. The vent areas of 2 male and 1 female lead-dosed mallards were stained with bile and 1 male and 2 female lead-dosed ducks had enlarged gallbladders. One lead-dosed female had urate crystals surrounding the heart, while 1 lead-dosed male had a focal area of the liver with a firm, gray covering on the subcapsular surface.

During the 90-day reproduction phase (day 60 to day 150), there were 2 steel-dosed and 3-tungsten-polymer-dosed females that did not lay any eggs. It was noted that 1 steel-dosed female had a small egg blocking the lumen of the magnum and the other steel-dosed duck had obstructing scar tissue in the oviduct. Of the 3 tungsten-polymer-dosed females that did not lay eggs, 2 had egg yolk peritonitis while the third female

Table 14. The effect of treatment shot on plasma chemistry parameters of mallards on days 90, 120 and 150 of a 150-day dosing test[a].

Parameter	Units	Steel	Tungsten-iron	Tungsten-polymer
		Day 90		
Potassium	mmol/L	2.6 (2.41 - 2.76)	2.7 (2.50 - 2.86)	2.7 (2.49 - 2.86)
Blood Urea Nitrogen/ Creatinine		10.8^{A} (10.65 - 12.33)	11.9^{A} (11.24 - 13.01)	16.2^{B} (9.70 - 11.24)
		Day 120		
Potassium	mmol/L	2.1 (1.99 - 2.28)	2.0 (1.85 - 2.12)	2.1 (2.00 - 2.28)
Blood Urea Nitrogen/ Creatinine		17.4 (10.49 - 12.16)	16.2 (10.88 - 12.60)	18.2 (11.69 - 13.54)
		Day 150		
Potassium	mmol/L	2.6^{A} (2.46 - 2.82)	3.0^{B} (2.76 - 3.17)	2.8^{AB} (2.63 - 3.02)
Blood Urea Nitrogen/ Creatinine		17.1 (14.82 - 19.76)	20.2 (17.51 - 23.36)	17.1 (14.83 - 19.78)

[a] Data presented as means (95% confidence intervals). Means with different superscripts are significantly different within the row ($p < 0.5$). Sample size is 32 for all groups or the number indicated in brackets.

Table 15. The gross necropsy observations of the effect of treatment shot on male mallards on a 150-day dosing test.

ID#	Treatment	Days on Trial	Observation(s)[a]
2001	Steel	150	Normal
2003	Steel	150	Normal
2005	Steel	150	Normal
2007	Steel	150	Normal
2009	Steel	150	Normal
2011	Steel	150	Normal
2013	Steel	150	Normal
2015	Steel	150	Normal
2017	Steel	150	Normal
2019	Steel	150	Normal
2021	Steel	150	Normal
2023	Steel	150	Normal
2025	Steel	150	Normal
2027	Steel	150	Normal
2029	Steel	150	Normal
2031	Steel	150	Normal

[a] Gross necropsy observations performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 15 continued. The gross necropsy observations of the effect of treatment shot on male mallards on a 150-day dosing test.			
ID#	Treatment	Days on Trial	Observation(s)[a]
1001	Lead	9	Severe breast muscle atrophy with minimal subcutaneous or abdominal fat Discolored mucosal lining of the gizzard Enlarged gallbladder
1003	Lead	25	Severe breast muscle atrophy with minimal subcutaneous or abdominal fat
1005	Lead	16	Severe breast muscle atrophy with minimal subcutaneous or abdominal fat Discolored mucosal lining of the gizzard
1007	Lead	21	Vent area stained with bile Severe breast muscle atrophy with minimal subcutaneous or abdominal fat Focal area of the liver with a firm gray covering on the subcapsular surface
1009	Lead	15	Severe breast muscle atrophy with minimal subcutaneous or abdominal fat
1011	Lead	14	Vent area stained with bile Severe breast muscle atrophy with minimal subcutaneous or abdominal fat Mild, discolored mucosal lining of the gizzard

[a] Gross necropsy observations performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 15 continued. The gross necropsy observations of the effect of treatment shot on male mallards on a 150-day dosing test.

ID#	Treatment	Days on Trial	Observation(s)[a]
3001	Tungsten-iron	150	Normal
3003	Tungsten-iron	150	Normal
3005	Tungsten-iron	150	Normal
3007	Tungsten-iron	150	Normal
3009	Tungsten-iron	150	Normal
3011	Tungsten-iron	150	Normal
3013	Tungsten-iron	150	Normal
3015	Tungsten-iron	150	Normal
3017	Tungsten-iron	150	Normal
3019	Tungsten-iron	150	Normal
3021	Tungsten-iron	150	Normal
3023	Tungsten-iron	150	Normal
3025	Tungsten-iron	150	Normal
3027	Tungsten-iron	150	Normal
3029	Tungsten-iron	150	Normal
3031	Tungsten-iron	150	Normal

[a] Gross necropsy observations performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 15 continued. The gross necropsy observations of the effect of treatment shot on male mallards on a 150-day dosing test.

ID#	Treatment	Days on Trial	Observation(s)[a]
4001	Tungsten-polymer	150	Normal
4003	Tungsten-polymer	150	Normal
4005	Tungsten-polymer	150	Normal
4007	Tungsten-polymer	150	Normal
4009	Tungsten-polymer	150	Normal
4011	Tungsten-polymer	150	Normal
4013	Tungsten-polymer	150	Normal
4015	Tungsten-polymer	150	Normal
4017	Tungsten-polymer	150	Normal
4019	Tungsten-polymer	150	Normal
4021	Tungsten-polymer	150	Normal
4023	Tungsten-polymer	150	Normal
4025	Tungsten-polymer	150	Normal
4027	Tungsten-polymer	150	Normal
4029	Tungsten-polymer	150	Normal
4031	Tungsten-polymer	150	Normal

[a] Gross necropsy observations performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 16. The gross necropsy observations of the effect of treatment shot on female mallards on a 150-day dosing test.

ID#	Treatment	Days on Trial	Observation(s)[a]
2002	Steel	150	Normal
2004	Steel	150	Small egg blocking lumen of the magnum
2006	Steel	150	Normal
2008	Steel	150	Normal
2010	Steel	150	Normal
2012	Steel	150	Normal
2014	Steel	150	Normal
2016	Steel	150	Normal
2018	Steel	150	Normal
2020	Steel	150	Normal
2022	Steel	150	Normal
2024	Steel	150	Normal
2026	Steel	150	Normal
2028	Steel	150	Obstructing scar tissue in the oviduct
2030	Steel	150	Normal
2032	Steel	150	Normal

[a] Gross necropsy observations performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 16 continued. The gross necropsy observations of the effect of treatment shot on female mallards on a 150-day dosing test.

ID#	Treatment	Days on Trial	Observation(s)[a]
1002	Lead	12	Vent area stained with bile Severe breast muscle atrophy with minimal subcutaneous or abdominal fat Mild, discolored mucosal lining of the gizzard
1004	Lead	12	Severe breast muscle atrophy with minimal subcutaneous or abdominal fat
1006	Lead	9	Severe breast muscle atrophy with minimal subcutaneous or abdominal fat Discolored mucosal lining of the gizzard Enlarged gallbladder
1008	Lead	10	Moderate breast muscle atrophy Enlarged gallbladder Urate crystals surrounding the heart
1010	Lead	12	Severe breast muscle atrophy with minimal subcutaneous or abdominal fat Discolored mucosal lining across entire surface of the gizzard
1012	Lead	11	Severe breast muscle atrophy with minimal subcutaneous or abdominal fat

[a] Gross necropsy observations performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 16 continued. The gross necropsy observations of the effect of treatment shot on female mallards on a 150-day dosing test.

ID#	Treatment	Days on Trial	Observation(s)[a]
3002	Tungsten-iron	150	Normal
3004	Tungsten-iron	150	Normal
3006	Tungsten-iron	150	Normal
3008	Tungsten-iron	150	Normal
3010	Tungsten-iron	150	Normal
3012	Tungsten-iron	150	Normal
3014	Tungsten-iron	150	Normal
3016	Tungsten-iron	150	Normal
3018	Tungsten-iron	150	Normal
3020	Tungsten-iron	150	Normal
3022	Tungsten-iron	150	Normal
3024	Tungsten-iron	150	Normal
3026	Tungsten-iron	150	Normal
3028	Tungsten-iron	150	Normal
3030	Tungsten-iron	150	Normal
3032	Tungsten-iron	150	Normal

[a] Gross necropsy observations performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 16 continued. The gross necropsy observations of the effect of treatment shot on female mallards on a 150-day dosing test.

ID#	Treatment	Days on Trial	Observation(s)[a]
4002	Tungsten-polymer	150	Normal
4004	Tungsten-polymer	150	Normal
4006	Tungsten-polymer	150	Normal
4008	Tungsten-polymer	150	Fatty liver
4010	Tungsten-polymer	150	Normal
4012	Tungsten-polymer	150	Egg yolk peritonitis
4014	Tungsten-polymer	150	Normal
4016	Tungsten-polymer	150	Normal
4018	Tungsten-polymer	150	Normal
4020	Tungsten-polymer	150	Normal
4022	Tungsten-polymer	150	Normal
4024	Tungsten-polymer	150	Egg yolk peritonitis
4026	Tungsten-polymer	150	Normal
4028	Tungsten-polymer	150	Normal
4030	Tungsten-polymer	150	Focal area of liver with fibrous tag
4032	Tungsten-polymer	150	Normal

[a] Gross necropsy observations performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

appeared normal. All other ducks in the steel-, tungsten-iron- and tungsten-polymer-dosed groups appeared normal except for 1 tungsten-polymer female that had a fibrous tag on a focal area of the liver.

Adult Organ Weights

Spleen and heart weights of lead-dosed mallards were significantly lower when compared to the other 3 groups (Table 17). There was a significant treatment by sex interaction for liver and gonad weights (Table 18). Testes weight of lead-dosed males and liver weight of lead-dosed females were significantly lower when compared to the steel-, tungsten-iron-, and tungsten-polymer-dosed groups.

When organ weight was expressed as a percent of body weight, the relative weights of kidneys, heart, brain and gizzard of lead-dosed mallards were significantly higher when compared to the other 3 groups (Table 19). Relative spleen weight of lead-dosed ducks was significantly lower than relative spleen weights of steel-dosed and tungsten-iron-dosed mallards and relative spleen weight of tungsten-iron-dosed ducks was significantly higher compared to tungsten-polymer-dosed ducks. There was a significant treatment by sex interaction for relative liver weights (Table 20). Relative liver weight of lead-dosed males was significantly higher compared to the other 3 groups. Relative testis and ovary weights were analyzed separately because of the anatomical difference (Table 20). Testis weight of lead-dosed males and ovary weight of lead-dosed females were significantly lower when compared to the other 3 groups.

Table 17. The effect of treatment shot on organ weights (gm) of mallards on a 150-day dosing test[a].					
Treatment	Spleen	Kidneys	Heart	Brain	Gizzard
Steel	0.471 ± 0.0343^{A}	6.238 ± 0.2047	8.967 ± 0.2382^{A}	4.794 ± 0.0720	23.425 ± 0.7115
Lead	0.159 ± 0.0561^{B}	6.591 ± 0.3342	7.753 ± 0.3890^{B}	4.981 ± 0.1176	24.818 ± 1.1618
Tungsten-iron	0.569 ± 0.0343^{A}	6.558 ± 0.2047	9.321 ± 0.2382^{A}	4.947 ± 0.0720	23.937 ± 0.7115
Tungsten-polymer	0.448 ± 0.0343^{A}	6.721 ± 0.2047	9.145 ± 0.2382^{A}	4.953 ± 0.0720	22.741 ± 0.7115

[a] Data presented as mean ± standard error of the mean. Sample size for all parameters is 32 except for lead, which is 12. Means with different superscripts are significantly different within the column ($p < 0.5$).

Table 18. The effect of treatment shot on organ weights (gm) of male and female mallards on a 150-day dosing test[a].		
Treatment	Liver	Gonads
Males		
Steel	15.141 ± 0.6035	33.581 + 2.4213[A]
Lead	13.685 ± 0.9855	0.512 + 3.9539[B]
Tungsten-iron	15.823 ± 0.6035	37.124 + 2.4213[A]
Tungsten-polymer	15.014 ± 0.6035	37.355 + 2.4213[A]
Females		
Steel	24.791 ± 1.6871[A]	24.422 + 4.7301
Lead	13.089 ± 2.7551[B]	0.566 + 7.7242
Tungsten-iron	25.371 ± 1.6871[A]	22.116 + 4.7301
Tungsten-polymer	25.276 ± 1.6871[A]	22.901+ 4.7301

[a] Data presented as mean ± standard error of the mean. Sample size for all parameters is 16 except for lead, which is 6. Means with different superscripts are significantly different within the column ($p < 0.5$).

Table 19. The effect of treatment shot on organ weights expressed as percent body weight of mallards on a 150-day dosing test[a].

Treatment	Spleen	Kidneys	Heart	Brain	Gizzard
Steel	0.042^{AC} (0.0369 - 0.0478)	0.584^{A} (0.5555 - 0.6134)	0.840^{A} (0.8007 - 0.8810)	0.454^{A} (0.4323 - 0.4740)	2.204^{A} (2.0621 - 2.3469)
Lead	0.026^{B} (0.0193 - 0.0335)	1.067^{B} (1.0046 - 1.1299)	1.240^{B} (1.1619 - 1.3212)	0.851^{B} (0.7707 - 0.8606)	4.021^{B} (3.7128 - 4.3374)
Tungsten-iron	0.049^{C} (0.0428 - 0.0547)	0.582^{A} (0.5540 - 0.6118)	0.835^{A} (0.7953 - 0.8754)	0.446^{A} (0.4245 - 0.4658)	2.134^{A} (1.9945 - 2.2747)
Tungsten-polymer	0.038^{AB} (0.0331 - 0.0433)	0.581^{A} (0.5525 - 0.6103)	0.792^{A} (0.7550 - 0.8331)	0.434^{A} (0.4142 - 0.4536)	1.978^{A} (1.8463 - 2.1165)

[a] Data presented as means (95% confidence intervals). Sample size for all parameters is 32 except for lead, which is 12. Means with different superscripts are significantly different within the column ($p < 0.5$).

Table 20. The effect of treatment shot on organ weights expressed as percent body weight of male and female mallards on a 150-day dosing test[a].

Treatment	Liver	Gonads
	Males	
Steel	1.398[A] (1.3053 - 1.4931)	3.080[A] (2.7044 - 3.4783)
Lead	2.085[B] (1.9033 - 2.2747)	0.079[B] (0.0096 - 0.2151)
Tungsten-iron	1.407[A] (1.3167 - 1.5029)	3.297[A] (2.9091 - 3.7091)
Tungsten-polymer	1.285[A] (1.1964 - 1.3743)	3.104[A] (2.7271 - 3.5040)
	Females	
Steel	2.344 (2.0792 - 2.6239)	1.764[A] (1.0026 - 2.7336)
Lead	2.293 (1.8706 - 2.7532)	0.094[B] (0.0534 - 0.7090)
Tungsten-iron	2.245 (1.9861 - 2.5194)	1.496[A] (0.8025 - 2.3986)
Tungsten-polymer	2.183 (1.9279 - 2.4540)	1.520[A] (0.8204 - 2.4293)

[a] Data presented as means (95% confidence intervals). Sample size for all parameters is 16 except lead, which is 6. Means with different superscripts are significantly different within the column ($p < 0.5$).

Histopathology of Adult Liver, Kidneys, and Gonads

All lead-dosed mallards had kidney nephrosis ranging from mild to moderate with the exception of 1 female that had a normal kidney. All lead-dosed mallards had mild to moderate liver biliary stasis (Tables 21-24). Mallards dosed with steel, tungsten-iron, or tungsten-polymer shot that were examined had normal kidneys and no indication of liver biliary stasis. However, all of the steel- and tungsten-iron-dosed males examined as well as 1 tungsten-polymer-dosed male had liver hemosiderosis ranging from mild to moderate. Five of 8 steel-dosed females and 3 of 8 tungsten-iron-dosed females that were examined had liver hemosiderosis ranging from mild to moderate. Diffuse hepatocellular vacuolation was apparent in ducks of all 4 groups, but this condition was judged not to be treatment related. The testes and ovary in the lead-dosed mallards were inactive, while these tissues in steel- tungsten-iron- and tungsten-polymer-dosed ducks that were examined appeared normal.

Metal Residues in Tissues of Adults

Lead-dosed mallards had a significantly higher concentration of lead in the femur when compared to the steel-, tungsten-iron- and tungsten-polymer-dosed groups (Table 25). There was a significant treatment by sex interaction for iron and tungsten concentrations in the femur (Table 26). Iron concentrations in the femur samples of lead- and tungsten-polymer-dosed female mallards were significantly lower when compared to steel- and tungsten-iron-dosed females. Tungsten was detected in all of the femur samples from the tungsten-iron-dosed males and females and in 5 of 8 samples from the tungsten-polymer-dosed females. Tungsten-iron-dosed females had a significantly higher femur tungsten concentration compared to tungsten-polymer-dosed females.

Table 21. The histopathological effects of treatment shot on the liver and kidneys of male mallards on a 150-day dosing test.		
ID#	Treatment	Observation(s)[a]
2001	Steel	Mild hemosiderosis
2003	Steel	Mild hemosiderosis
2013	Steel	Mild hemosiderosis
2015	Steel	Mild hemosiderosis
2021	Steel	Mild hemosiderosis
2023	Steel	Moderate hemosiderosis
2025	Steel	Mild hemosiderosis
2027	Steel	Mild hemosiderosis

[a] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 21 continued. The histopathological effects of treatment shot on the liver and kidneys of male mallards on a 150-day dosing test.		
ID#	Treatment	Observation(s)[a]
1001	Lead	Moderate, diffuse acute hepatocellular and cholangial biliary stasis Mild, diffuse acute degeneration and vacuolation of proximal convoluted tubule epithelium
1003	Lead	Mild, diffuse acute hepatocellular and cholangial biliary stasis Mild, diffuse hepatocellular vacuolation Moderate, diffuse acute proximal convoluted tubule epitheliular necrosis with pyknosis
1005	Lead	Moderate, diffuse acute hepatocellular and cholangial biliary stasis Mild, diffuse acute degeneration and vacuolation of proximal convoluted tubule epithelium
1007	Lead	Moderate, diffuse acute hepatocellular and cholangial biliary stasis Mild, diffuse hepatocellular vacuolation Focal, hepatocellular parenchymal necrosis Moderate, diffuse acute proximal convoluted tubule epitheliular necrosis with pyknosis
1009	Lead	Moderate, diffuse acute hepatocellular and cholangial biliary stasis Mild, diffuse acute degeneration and vacuolation of proximal convoluted tubule epithelium
1011	Lead	Mild, diffuse acute hepatocellular and cholangial biliary stasis Mild, diffuse hepatocellular vacuolation Moderate, diffuse acute proximal convoluted tubule epitheliular necrosis with pyknosis

[a] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 21 continued. The histopathological effects of treatment shot on the liver and kidneys of male mallards on a 150-day dosing test.		
ID#	Treatment	Observation(s)[a]
3005	Tungsten-iron	Mild hemosiderosis
3007	Tungsten-iron	Moderate hemosiderosis
3009	Tungsten-iron	Mild hemosiderosis
3011	Tungsten-iron	Mild hemosiderosis
3017	Tungsten-iron	Mild hemosiderosis
3019	Tungsten-iron	Mild hemosiderosis
3029	Tungsten-iron	Mild hemosiderosis
3031	Tungsten-iron	Mild hemosiderosis
4001	Tungsten-polymer	Normal
4003	Tungsten-polymer	Mild, diffuse hepatocellular vacuolation
4013	Tungsten-polymer	Normal
4015	Tungsten-polymer	Normal
4021	Tungsten-polymer	Normal
4023	Tungsten-polymer	Normal
4025	Tungsten-polymer	Mild hemosiderosis
4027	Tungsten-polymer	Normal

[a] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 22. The histopathological effects of treatment shot on the liver and kidneys of female mallards on a 150-day dosing test.		
ID#	Treatment	Observation(s)[a]
2004	Steel	Mild, diffuse hepatocellular vacuolation
2006	Steel	Mild hemosiderosis
2018	Steel	Mild hemosiderosis
2014	Steel	Mild hemosiderosis
2018	Steel	Mild, diffuse hepatocellular vacuolation
2020	Steel	Normal
2028	Steel	Moderate hemosiderosis Mild, diffuse hepatocellular vacuolation
2030	Steel	Mild hemosiderosis Mild, diffuse hepatocellular vacuolation

[a] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 22 continued.		The histopathological effects of treatment shot on the liver and kidneys of female mallards on a 150-day dosing test.
ID#	Treatment	Observation(s)[a]
1002	Lead	Moderate, diffuse acute hepatocellular and cholangial biliary stasis Kidney - normal
1004	Lead	Moderate, diffuse acute hepatocellular and cholangial biliary stasis Mild, diffuse acute degeneration and vacuolation of proximal convoluted tubule epithelium
1006	Lead	Moderate, diffuse acute hepatocellular and cholangial biliary stasis Moderate, diffuse hepatocellular vacuolation Mild, diffuse acute degeneration and vacuolation of proximal convoluted tubule epithelium
1008	Lead	Mild, diffuse acute hepatocellular and cholangial biliary stasis Mild, diffuse hepatocellular vacuolation Multifocal hepatocellular necrosis Moderate, diffuse acute proximal convoluted tubule epitheliular necrosis with pyknosis
1010	Lead	Mild, diffuse acute hepatocellular and cholangial biliary stasis Mild, diffuse acute degeneration and vacuolation of proximal convoluted tubule epithelium
1012	Lead	Moderate, diffuse acute hepatocellular and cholangial biliary stasis Mild, diffuse hepatocellular vacuolation Mild, diffuse acute degeneration and vacuolation of proximal convoluted tubule epithelium

[a] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 22 continued	The histopathological effects of treatment shot on the liver and kidneys of female mallards on a 150-day dosing test.	
ID#	Treatment	Observation(s)[a]
3002	Tungsten-iron	Normal
3004	Tungsten-iron	Mild hemosiderosis
3014	Tungsten-iron	Mild, diffuse hepatocellular vacuolation
3016	Tungsten-iron	Mild, diffuse hepatocellular vacuolation
3022	Tungsten-iron	Moderate hemosiderosis
3024	Tungsten-iron	Normal
3026	Tungsten-iron	Mild hemosiderosis
3028	Tungsten-iron	Moderate, diffuse hepatocellular vacuolation
4010	Tungsten-polymer	Normal
4012	Tungsten-polymer	Mild, diffuse hepatocellular vacuolation
4014	Tungsten-polymer	Mild, diffuse hepatocellular vacuolation
4016	Tungsten-polymer	Normal
4018	Tungsten-polymer	Normal
4024	Tungsten-polymer	Moderate, diffuse hepatocellular vacuolation
4028	Tungsten-polymer	Mild, diffuse hepatocellular vacuolation
4030	Tungsten-polymer	Mild, diffuse hepatocellular vacuolation

[a] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 23. The severity of testis, liver and kidney lesions induced by treatment shot in male mallards on a 150-day dosing test[a]

ID#	Trt	Days on Trial	Testes	Kidney nephrosis	Liver hemosiderosis	Liver biliary stasis
2001	Steel	150	0	0	1	-
2003	Steel	150	0	0	1	-
2013	Steel	150	0	0	1	-
2015	Steel	150	0	0	1	-
2021	Steel	150	0	0	1	-
2023	Steel	150	0	0	2	-
2025	Steel	150	0	0	1	-
2027	Steel	150	0	0	1	-
1001	Lead	9	Inactive	1	-	2
1003	Lead	25	Inactive	2	-	1
1005	Lead	16	Inactive	1	-	2
1007	Lead	21	Inactive	2	-	1
1009	Lead	15	Inactive	1	-	2
1011	Lead	14	Inactive	2	-	1

[a] Lesion scores: 0 = normal; 1 = mild; 2 = moderate; 3 = severe.

Table 23 continued. The severity of testis, liver and kidney lesions induced by treatment shot in male mallards on a 150-day dosing test[a]

ID#	Trt	Days on Trial	Testes	Kidney nephrosis	Liver hemosiderosis	Liver biliary stasis
3005	T-iron	150	0	0	1	-
3007	T-iron	150	0	0	2	-
3009	T-iron	150	0	0	1	-
3011	T-iron	150	0	0	1	-
3017	T-iron	150	0	0	1	-
3019	T-iron	150	0	0	1	-
3029	T-iron	150	0	0	1	-
3031	T-iron	150	0	0	1	-
4001	T-polymer	150	0	0	0	-
4003	T-polymer	150	0	0	0	-
4013	T-polymer	150	0	0	0	-
4015	T-polymer	150	0	0	0	-
4021	T-polymer	150	0	0	0	-
4023	T-polymer	150	0	0	0	-
4025	T-polymer	150	0	0	1	-
4027	T-polymer	150	0	0	0	-

[a] Lesion scores: 0 = normal; 1 = mild; 2 = moderate; 3 = severe.

Table 24. The severity of ovary, liver and kidney lesions induced by treatment shot in female mallards on a 150-day dosing test[a]

ID#	Trt	Days on Trial	Ovary	Kidney nephrosis	Liver hemosiderosis	Liver biliary stasis
2004	Steel	150	0	0	0	-
2006	Steel	150	0	0	1	-
2008	Steel	150	0	0	1	-
2014	Steel	150	0	0	1	-
2018	Steel	150	0	0	0	-
2020	Steel	150	0	0	0	-
2028	Steel	150	0	0	2	-
2030	Steel	150	0	0	1	-
1002	Lead	12	Inactive	0	-	2
1004	Lead	12	Inactive	1	-	2
1006	Lead	9	Inactive	1	-	2
1008	Lead	10	Inactive	2	-	1
1010	Lead	12	Inactive	1	-	1
1012	Lead	11	Inactive	1	-	2

[a] Lesion scores: 0 = normal; 1 = mild; 2 = moderate; 3 = severe.

Table 24 continued. The severity of ovary, liver and kidney lesions induced by treatment shot in female mallards on a 150-day dosing test[a]						
ID#	Trt	Days on Trial	Ovary	Kidney nephrosis	Liver hemosiderosis	Liver biliary stasis
3002	T-iron	150	0	0	0	-
3004	T-iron	150	0	0	1	-
3014	T-iron	150	0	0	0	-
3016	T-iron	150	0	0	0	-
3022	T-iron	150	0	0	2	-
3024	T-iron	150	0	0	0	-
3026	T-iron	150	0	0	1	-
3028	T-iron	150	0	0	0	-
4010	T-polymer	150	0	0	0	-
4012	T-polymer	150	0	0	0	-
4014	T-polymer	150	0	0	0	-
4016	T-polymer	150	0	0	0	-
4018	T-polymer	150	0	0	0	-
4024	T-polymer	150	0	0	0	-
4028	T-polymer	150	0	0	0	-
4030	T-polymer	150	0	0	0	-
[a] Lesion scores: 0 = normal; 1 = mild; 2 = moderate; 3 = severe.						

Table 25. The effect of treatment shot on concentration (mg/kg dry weight) of lead in the femur of mallards on a 150-day dosing test[a].

Treatment	Lead
Steel	4.5^{A} (2.66 - 7.47)
Lead	311.3^{B} (171.48 - 564.87)
Tungsten-iron	4.9^{A} (2.94 - 8.25)
Tungsten-polymer	4.1^{A} (2.46 - 6.91)

[a] Data presented as means (95% confidence intervals). Sample size is 16 for all groups except for lead, which is 12. Means with different superscripts are significantly different within the column ($p < 0.5$).

Table 26. The effect of treatment shot on concentrations (mg/kg dry weight) of iron and tungsten in the femur of male and female mallards on a 150-day dosing test[a].

Treatment	Iron	Tungsten
Males		
Steel	96.2 (80.53 - 115.00)	ND
Lead	90.8 (73.94 - 111.58)	ND
Tungsten-iron	88.9 (74.37 - 106.20)	23.5
Tungsten-polymer	81.0 (67.78 - 96.78)	ND
Females		
Steel	220.5^{A} (181.28 - 268.00)	ND
Lead	91.1^{B} (72.70 - 114.13)	ND
Tungsten-iron	162.7^{A} (133.85 - 197.79)	34.0^{A} (28.60 - 40.33)
Tungsten-polymer	99.0^{B} (81.41 - 120.30)	5.1^{B} (4.12 - 6.36) [3]

[a] Data presented as means (95% confidence intervals). Sample size is 8 for all groups except for lead, which is 6. Means with different superscripts are significantly different within the column ($p < 0.5$). Numbers in brackets refer to the number of pooled samples having a tissue concentration below detection limits. ND refers to not detected. Tungsten detection limit is 3 mg/kg dry weight.

Iron concentrations in the testes were significantly higher in lead-dosed males compared to the other 3 groups (Table 27). Lead was detected in the testes of 1 of 8 tungsten-polymer-dosed males. Tungsten was detected in the testes of 5 of 8 tungsten-iron-dosed males and 2 of 8 tungsten-polymer-dosed males. Tungsten-polymer-dosed female mallards had significantly lower concentrations of iron in the ovary when compared to steel-dosed females. Lead-dosed females had a significantly higher concentration of lead in the ovary when compared to the other 3 groups. Lead was detected in the ovary of 1 of 8 samples from steel-dosed females, 7 of 8 samples from lead-dosed females, and 2 of 8 samples from tungsten-polymer-dosed females. Tungsten was present in 6 of 8 ovary samples from tungsten-iron-dosed females and in 1 of 8 samples from tungsten-polymer-dosed females.

Lead-dosed mallards had a significantly higher concentration of lead in the kidneys when compared to the other 3 groups (Table 28). Lead was detected in the kidney of 4 of 16 samples from steel-dosed mallards, 3 of 16 samples from tungsten-iron-dosed ducks, and 5 of 16 samples from tungsten-polymer-dosed ducks. Tungsten was detected in 2 of 16 kidney samples from the steel-dosed group, 3 of 12 kidney samples from the lead-dosed ducks, 13 of 16 kidney samples from the tungsten-iron-dosed ducks, and 7 of 16 kidney samples from the tungsten-polymer-dosed ducks. Tungsten-iron-dosed ducks had a significantly higher concentration of tungsten when compared to the other 3 groups.

There was a significant treatment by sex interaction for iron concentration in the kidney samples (Table 29). Lead-dosed and tungsten-polymer-dosed males had significantly lower concentrations of iron in the kidneys when compared to steel-dosed

Table 27. The effect of treatment shot on concentrations (mg/kg dry weight) of iron, lead, and tungsten in the gonads of male and female mallards on a 150-day dosing test[a].

Treatment	Iron	Lead	Tungsten
Males			
Steel	58.5^{A} (45.11 - 75.80)	4.3 (0.10 - 189.92)	ND
Lead	2225.7^{B} (167.27 - 304.60)	12.2 (2.59 - 57.16)	ND
Tungsten-iron	56.3^{A} (43.40 - 72.94)	ND	5.6 (3.13 - 9.99) [3]
Tungsten-polymer	67.8^{A} (52.27 - 87.85)	0.5 (0.01 - 22.09) [7]	7.0 (2.80 - 17.53) [6]
Females			
Steel	488.5^{A} (326.98 - 729.68)	0.7^{A} (0.34 - 1.44) [7]	ND
Lead	206.0^{AB} (123.98 - 342.20) [1]	13.4^{B} (9.72 - 18.54) [1]	ND
Tungsten-iron	354.1^{AB} (237.06 - 529.01)	ND	8.4 (3.69 - 19.14) [2]
Tungsten-polymer	211.8^{B} (141.80 - 316.43)	0.6^{A} (0.36 - 1.00) [6]	8.0 (1.06 - 60.09) [7]

[a] Data presented as means (95% confidence intervals). Sample size is 8 for all groups except for lead, which is 6. Means with different superscripts are significantly different within the column ($p < 0.5$). Numbers in brackets refer to the number of males or pooled samples of females having a tissue concentration below detection limits. ND refers to not detected. Lead and tungsten detection limits are 0.5 and 3.0 mg/kg dry weight, respectively.

Table 28. The effect of treatment shot on concentrations (mg/kg dry weight) of lead and tungsten in the kidneys of mallards on a 150-day dosing test[a].

Treatment	Lead	Tungsten
Steel	NE [12]	4.0 (1.94 - 8.24) [14]
Lead	621.0[B] (488.82 - 789.03)	5.9 (3.18 - 11.10) [9]
Tungsten-iron	NE [13]	9.5 (7.15 - 12.61) [3]
Tungsten-polymer	0.8[A] (0.55 - 1.17) [11]	6.8 (4.46 - 10.48) [9]

[a] Data presented as means (95% confidence intervals). Sample size is 16 for all groups except lead, which is 12. Means with different superscripts are significantly different within the column ($p < 0.5$). Numbers in brackets refer to the number of pooled samples having a tissue concentration below detection limits. NE refers to non-estimable because most values in the data set were 0.0, which is not log transformable.

Table 29. The effect of treatment shot on concentrations (mg/kg dry weight) of iron in the kidneys of male and female mallards on a 150-day dosing test[a].	
Treatment	Iron
Males	
Steel	678.8[A] (591.40 - 778.99)
Lead	484.9[B] (413.56 - 568.50)
Tungsten-iron	563.1[A] (490.63 - 646.26)
Tungsten-polymer	509.0[B] (443.46 - 584.12)
Females	
Steel	825.0[A] (712.44 - 955.37)
Lead	406.7[B] (343.30 - 481.74)
Tungsten-iron	623.6[C] (538.51 - 722.13)
Tungsten-polymer	398.9[B] (344.50 - 461.97)

[a] Data presented as means (95% confidence intervals). Sample size is 8 for all groups except for lead, which is 6. Means with different superscripts are significantly different within the column ($p < 0.5$).

males. In the females, lead-dosed ducks had a significantly lower concentration of iron when compared to steel- and tungsten-iron-dosed ducks. Tungsten-iron- and tungsten-polymer-dosed females had significantly lower concentrations of iron when compared to the steel-dosed females, while tungsten-iron-dosed females had a significantly higher concentration of iron than tungsten-polymer-dosed females.

Iron concentrations in the liver from tungsten-polymer-dosed mallards were significantly lower compared to the other 3 groups. Lead-dosed mallards had a significantly higher concentration of lead in the liver compared to steel-, tungsten-iron-, and tungsten-polymer-dosed ducks, while tungsten-polymer-dosed ducks had a significantly lower concentration of lead than the steel-dosed ducks (Table 30). Lead was detected in 12 of 16 ducks in the steel-dosed group, 9 of 16 ducks in the tungsten-iron-dosed group, and 4 of 16 mallards in the tungsten-polymer-dosed group. Tungsten was detected in the liver of all tungsten-iron-dosed mallards and in 2 of 16 tungsten-polymer-dosed mallards.

Shot Recovery and Percent Shot Erosion

Approximately 90% of the lead pellets administered were recovered when the lead-dosed ducks were necropsied between day 9 and day 25 of the trial (Table 31). Over half of the steel pellets were recovered at day 150 as opposed to less than 3% of the tungsten-polymer shot. Approximately 40% of the tungsten-iron shot was recovered at day 150. Of the 4 shot types, lead shot eroded the least in both males and females, followed by steel, tungsten-iron, and tungsten-polymer shot, respectively. Based on the weight of the pellets recovered, there was nearly complete erosion of the tungsten-polymer shot and 64% and 80% erosion of tungsten-iron shot in males and females,

Table 30. The effect of treatment shot on concentrations (mg/kg dry weight) of iron, lead, and tungsten in the liver of mallards on a 150-day dosing test[a].

Treatment	Iron	Lead	Tungsten
Steel	10128.4^{A} (7599.97 - 13496.69)	1.5^{A} (1.25 - 1.90) [4]	ND
Lead	9897.9^{A} (7109.50 - 13799.67)	218.3^{B} (176.92 - 269.37)	ND
Tungsten-iron	6890.5^{A} (5170.89 - 9182.92)	1.2^{AC} (0.94 - 1.53) [7]	70.4 (50.69 - 97.71)
Tungsten-polymer	1157.2^{B} (868.35 - 1542.25)	0.7^{C} (0.47 - 0.97) [12]	NE [14]

[a] Data presented as means (95% confidence intervals). Sample size is 16 for all groups except lead, which is 12. Means with different superscripts are significantly different within the column ($p < 0.5$). Numbers in brackets refer to the number of mallards having a tissue concentration below detection limits. ND refers to not detected. Tungsten detection limit is 3.0 mg/kg dry weight. NE refers to non-estimable because most values in the data set were 0.0, which is not log transformable.

Table 31. Number of pellets recovered and percent erosion of shot in male and female mallards on a 150-day dosing test[a].

Treatment	Number of pellets administered	Initial individual pellet wt.	Number of pellets recovered	Final individual pellet wt.	Percent shot erosion
Males					
Steel	40	0.152 ± 0.0003	24.4 ± 1.21	0.077 ± 0.0033	49.5^{A} (44.7 - 54.2)
Lead	8	0.222 ± 0.0025	6.5 ± 0.69	0.172 ± 0.0107	21.7^{B} (15.6 - 28.4)
Tungsten-iron	40	0.208 ± 0.0003	20.0 ± 1.21	0.075 ± 0.0033	63.8^{C} (59.2 - 68.3)
Tungsten-polymer	40	0.186 ± 0.0003	0.6 ± 1.21	0.004 ± 0.0033	99.7^{D} (99.0 - 100.0)
Females					
Steel	40	0.152 ± 0.0003	23.0 ± 1.21	0.061 ± 0.0033	60.0^{A} (53.4 - 66.0)
Lead	8	0.223 ± 0.0025	7.3 ± 0.69	0.186 ± 0.0107	15.2^{B} (8.49 - 23.4)
Tungsten-iron	40	0.208 ± 0.0003	11.4 ± 1.21	0.043 ± 0.0033	80.3^{C} (74.9 - 85.1)
Tungsten-polymer	40	0.186 ± 0.0003	1.3 ± 1.21	0.006 ± 0.0033	99.1^{D} (97.4 - 99.9)

[a] Data for shot weight are presented as mean ± standard error of the mean. Data for shot erosion are presented as means (95% confidence intervals). Sample size is 16 for all groups except for lead, which is 6. Means with different superscripts are significantly different within the column ($p < 0.5$). Percent shot erosion was determined by dividing initial individual pellet weight by final individual pellet weight.

respectively. Fluoroscopy of the mallards during the trial substantiated the relatively rapid erosion of the 2 types of tungsten shot.

Date First Egg was Laid and Number of Days Required to Lay 21 Eggs

Tungsten-polymer-dosed females began laying eggs approximately 7 days earlier than females in the steel and tungsten-iron groups, which began laying around day 92 of the study (Table 32). Females in all 3 groups required 24 to 25 days to lay 21 eggs. There were 2 steel-dosed females and 3 tungsten-polymer-dosed females that did not lay any eggs. Of those ducks that laid eggs, there was 1 steel-dosed female, 2 tungsten-iron-dosed females and 1 tungsten-polymer-dosed female that did not lay at least 21 eggs.

Percent Egg Production, Fertility, and Hatchability

Percent egg production was similar among groups and ranged from 36% to 46% (Table 33). Tungsten-polymer-dosed females had significantly lower percent fertility when compared to tungsten-iron-dosed females, but percent hatchability was not different between the steel, tungsten-iron, and tungsten-polymer groups.

Egg Weight and Shell Thickness

Eggs laid by tungsten-iron-dosed females were significantly heavier than eggs laid by steel- or tungsten-polymer-dosed females and the shells of these eggs were significantly thicker compared to shells of eggs laid by steel-dosed females (Table 34).

Table 32. The day the first egg was laid and the number of days required for mallards to lay 21 eggs[a].

Treatment	Day first egg was laid[b]	Days required to lay 21 eggs[c]
Steel	92.0 ± 1.31 (14)	25.4 ± 0.31 (13)
Tungsten-iron	91.7 ± 0.96 (16)	24.1 ± 0.33 (14)
Tungsten-polymer	84.5 ± 1.02 (13)	25.6 ± 0.45 (12)

[a] Data presented as mean + standard error of the mean.

[b] Numbers in parentheses refer to the number of egg-laying females.

[c] Numbers in parentheses refer to the number of females that laid 21 eggs.

Table 33. The effect of treatment shot on egg production of mallards on a 150-day dosing test and on fertility and hatchability of eggs[a].

Treatment	% Egg production	% Fertility	% Hatchability
Steel	36.4 (24.22 - 49.51)	94.0^{AB} (81.63 - 99.71)	61.4 (41.38 - 79.54)
Tungsten-iron	46.1 (33.18 - 59.37)	98.5^{A} (90.38 - 99.46)	77.5 (58.97 - 91.68)
Tungsten-polymer	39.0 (26.57 - 52.20)	78.5^{B} (60.74 - 91.92)	62.5 (42.54 - 80.48)

[a] Data presented as means (95% confidence intervals). Sample size is 14,16, and 13 for steel, tungsten-iron, and tungsten-polymer, respectively. Percent egg production is equal to the total number of eggs divided by 90 days, % fertility is equal to the number of fertile eggs divided by the number of eggs set, % hatchability is equal to the number of eggs hatched divided by the number of fertile eggs. Means with different superscripts are significantly different within the column ($p < 0.5$).

Table 34. The effect of treatment shot on weight (gm) and shell thickness (mm) of eggs from mallards on a 150-day dosing test[a].		
Treatment	Egg weight	Egg shell thickness
Steel	61.3 ± 0.24^{A} (552)	$0.372 + 0.012^{A}$ (14)
Tungsten-iron	62.7 ± 0.22^{B} (667)	$0.412 + 0.011^{B}$ (16)
Tungsten-polymer	61.2 ± 0.23^{A} (611)	$0.385 + 0.012^{AB}$ (13)

[a] Data presented as mean ± standard error of the mean. Numbers in parentheses refer to sample size. Means with different superscipts are significantly different within the column ($p < 0.5$).

Metal Residues in Egg Shell and Contents

Iron was detected in 2 of 14, 5 of 16, and 2 of 13 shells of eggs laid by steel-, tungsten-iron-, and tungsten-polymer-dosed females, respectively, and in the contents of all eggs analyzed (Table 35). There were no significant differences in iron concentration of the eggshell or contents between the 3 groups. Lead was not detected in either the shell or contents of eggs from the 3 groups. Tungsten was detected in the shell of 9 of 16 and 3 of 13 eggs laid by tungsten-iron- and tungsten-polymer-dosed females and in the contents of 6 of 16 eggs laid by tungsten-iron-dosed females. There were no significant differences in tungsten concentration in the eggshell or contents.

Survivability, Body Weight and Hematocrit of Ducklings

Survivability of ducklings through day 14 of age was equivalent for all 3 groups (Table 36). Body weight over the 14-day period was also similar for ducklings in the 3 groups. Hematocrit of ducklings in the tungsten-iron group was significantly lower when compared to ducklings in the steel group.

Duckling Organ Weights

Absolute and relative kidney weights of ducklings in the tungsten-polymer group were significantly higher when compared to ducklings in the steel and tungsten-iron groups (Tables 37 and 38). Absolute and relative weights of the liver, spleen, bursa, heart, and brain were equivalent across the 3 groups (Tables 37 – 39).

Table 35. The effect of treatment shot on concentrations (mg/kg dry weight) of iron, lead, and tungsten in the contents and shell of eggs from mallards on a 150-day dosing test[a].

Treatment	Iron	Lead	Tungsten
Egg shell			
Steel	2.8 ± 2.11 (12)	ND	ND
Tungsten-iron	3.0 ± 1.98 (11)	ND	1.9 ± 0.27 (7)
Tungsten-polymer	3.5 ± 2.19 (11)	ND	0.6 ± 0.30 (10)
Egg contents			
Steel	84.4 ± 5.22	ND	ND
Tungsten-iron	78.4 ± 4.88	ND	2.4 ± 0.59 (10)
Tungsten-polymer	70.2 ± 5.42	ND	ND

[a] Data presented as mean ± standard error of the mean. Sample size for steel, tungsten-iron, and tungsten-polymer is 14, 16, and 13, respectively. Numbers in parentheses refer to the number of eggs having a concentration below detection limits. ND refers to non-detect. Lead and tungsten detection limits are 0.5 and 1.5, respectively.

Table 36. The effect of treatment shot on duckling survivability, body weight (gm) from day 0 through day 14, and hematocrit on day 14[a].

Treatment	% Survivability	Body weight	Hematocrit
Steel	99.1 (96.31 - 100.00)	165.4 ± 2.08 (156)	39.7 ± 0.24[A] (156)
Tungsten-iron	98.0 (94.52 - 99.80)	165.3 ± 1.82 (202)	38.6 ± 0.21[B] (202)
Tungsten-polymer	95.7 (90.31 - 98.97)	167.0 ± 2.24 (135)	39.1 ± 0.26[AB] (135)

[a] Data for % survivability presented as means (95% confidence intervals). Data for body weight and hematocrit presented as mean ± standard error of the mean. Numbers in parentheses refer to sample size except % survivability. Means with different superscripts are significantly different within the column ($p < 0.5$).

Table 37. The effect of treatment shot on organ weights (gm) of ducklings[a].						
Treatment	Liver	Spleen	Kidneys	Bursa	Heart	Brain
Steel	13.709 ± 0.2592	0.282 ± 0.0118	3.497± 0.0573[A]	0.707 ± 0.0185	2.359 ± 0.0324	2.504 ± 0.0162
Tungsten-iron	13.567 ± 0.2267	0.276 ± 0.0104	3.520 ± 0.0501[A]	0.730 ± 0.0161	2.347 ± 0.0283	2.514 ± 0.0142
Tungsten-polymer	13.779 ± 0.2788	0.265 ± 0.0128	3.734 ± 0.0616[B]	0.684 ± 0.0198	2.437 ± 0.0348	2.537 ± 0.0174

[a] Data presented as mean ± standard error of the mean. Sample sizes for steel, tungsten-iron, and tungsten-polymer are 156, 202, and 135, respectively. Means with different superscripts are significantly different within the column ($p < 0.5$).

Table 38. The effect of treatment shot on liver, spleen and kidneys expressed as percent body weight of ducklings[a].

Treatment	Liver	Spleen	Kidneys
Steel	4.695 (4.5768 - 4.8094)	0.0918 (0.0870 - 0.00967)	1.196^{A} (1.169 - 1.2183)
Tungsten-iron	4.640 (4.5392 - 4.7411)	0.0942 (0.0900 - 0.0986)	1.211^{A} (1.1942 - 1.2315)
Tungsten-polymer	4.661 (4.5392 - 4.7880)	0.090 (0.0847 - 0.0948)	1.267^{B} (1.2447 - 1.2895)

[a] Data presented as means (95% confidence intervals). Sample sizes for steel, tungsten-iron, and tungsten-polymer are 156, 202, and 135, respectively. Means with different superscripts are significantly different within the column ($p< 0.5$).

Table 39. The effect of treatment shot on bursa, heart and brain expressed as percent body weight of ducklings[a].

Treatment	Bursa	Heart	Brain
Steel	0.242 (0.2321 - 0.2528)	0.817 (0.8007 - 0.8313)	0.877 (0.8550 - 0.8998)
Tungsten-iron	0.247 (0.2380 - 0.2558)	0.813 (0.7989 - 0.8258)	0.881 (0.8624 - 0.9017)
Tungsten-polymer	0.230 (0.2198 - 0.2409)	0.833 (0.8168 - 0.8495)	0.881 (0.8587 - 0.9074)

[a] Data presented as means (95% confidence intervals). Sample sizes for steel, tungsten-iron, and tungsten-polymer are 156, 202, and 135, respectively.

Histopathology of Duckling Liver and Kidneys

Liver samples from ducklings in the steel, tungsten-iron, and tungsten-polymer groups had mild to moderate diffuse hepatocellular vacuolation with the exception of samples from 1 female in the steel group and 1 female in the tungsten-polymer group. No kidney lesions were observed (Tables 40 and 41).

Metal Residues in Tissues of Ducklings

Concentrations of iron in the femur, kidneys, and liver of ducklings were similar across the 3 treatment groups (Table 42). Lead was detected in 2 of 16, 3 of 16, and 4 of 16 femur samples in the steel, tungsten-iron, and tungsten-polymer groups, respectively. Lead was also detected in 1 of 16, 4 of 16, and 3 of 16 kidney samples in the steel, tungsten-iron, and tungsten-polymer groups, respectively. Furthermore, lead was detected in 1 of 16, 1 of 16, and 2 of 16 liver samples in the steel, tungsten-iron, and tungsten-polymer groups, respectively. Tungsten was detected in the femur of 4 of 16 samples from tungsten-iron and 4 of 16 samples from tungsten-polymer ducklings. Tungsten was also detected in 2 of 16 kidney samples from tungsten-iron ducklings and 1 of 16 kidney samples from tungsten-polymer ducklings. Two of 16 liver samples from tungsten-iron and tungsten-polymer ducklings contained tungsten. There were no significant differences in lead and tungsten concentrations in the femur, liver, and kidney samples between the 3 groups.

Table 40. The histopathological effects of treatment shot on the liver and kidneys of male ducklings.

ID#	Hen#	Treatment	Observation(s)[a]
Y13	2014	Steel	Mild, diffuse hepatocellular vacuolation
Y15	2002	Steel	Mild, diffuse hepatocellular vacuolation
Y48	2022	Steel	Moderate, diffuse hepatocellular vacuolation
Y129	2010	Steel	Moderate, diffuse hepatocellular vacuolation
Y172	2018	Steel	Moderate, diffuse hepatocellular vacuolation
DSX3869	2008	Steel	Moderate, diffuse hepatocellular vacuolation
Y93	2030	Steel	Moderate, diffuse hepatocellular vacuolation
Y187	2032	Steel	Mild, diffuse hepatocellular vacuolation
DSX3807	3018	Tungsten-iron	Moderate, diffuse hepatocellular vacuolation
DSX3651	3018	Tungsten-iron	Moderate, diffuse hepatocellular vacuolation
B203	3018	Tungsten-iron	Moderate, diffuse hepatocellular vacuolation
B78	3018	Tungsten-iron	Moderate, diffuse hepatocellular vacuolation
B59	3018	Tungsten-iron	Moderate, diffuse hepatocellular vacuolation
B258	3018	Tungsten-iron	Mild, diffuse hepatocellular vacuolation
B171	3018	Tungsten-iron	Mild, diffuse hepatocellular vacuolation
B75	3018	Tungsten-iron	Moderate, diffuse hepatocellular vacuolation

[a] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 40 continued. The histopathological effects of treatment shot on the liver and kidneys of male ducklings.			
ID#	Hen#	Treatment	Observation(s)[a]
P87	4018	Tungsten-polymer	Mild, diffuse hepatocellular vacuolation
DSX3849	4020	Tungsten-polymer	Moderate, diffuse hepatocellular vacuolation
DSX3845	4016	Tungsten-polymer	Moderate, diffuse hepatocellular vacuolation
P164	4008	Tungsten-polymer	Mild, diffuse hepatocellular vacuolation
P144	4030	Tungsten-polymer	Moderate, diffuse hepatocellular vacuolation
DSX3693	4014	Tungsten-polymer	Moderate, diffuse hepatocellular vacuolation
P79	4004	Tungsten-polymer	Moderate, diffuse hepatocellular vacuolation
P171	4026	Tungsten-polymer	Mild, diffuse hepatocellular vacuolation

[a] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 41. The histopathological effects of treatment shot on the liver and kidneys of female ducklings.

ID#	Hen#	Treatment	Observation(s)[a]
Y20	2030	Steel	Moderate, diffuse hepatocellular vacuolation
DSX3803	2014	Steel	Moderate, diffuse hepatocellular vacuolation
Y141	2032	Steel	Mild, diffuse hepatocellular vacuolation
Y84	2010	Steel	Moderate, diffuse hepatocellular vacuolation
DSX3539	2026	Steel	Mild, diffuse hepatocellular vacuolation
Y82	2012	Steel	Normal
Y217	2006	Steel	Moderate, diffuse hepatocellular vacuolation
Y222	2022	Steel	Moderate, diffuse hepatocellular vacuolation
B82	3026	Tungsten-iron	Moderate, diffuse hepatocellular vacuolation
B57	3012	Tungsten-iron	Moderate, diffuse hepatocellular vacuolation
DSX3635	3032	Tungsten-iron	Moderate, diffuse hepatocellular vacuolation
DSX3877	3010	Tungsten-iron	Moderate, diffuse hepatocellular vacuolation
B276	3014	Tungsten-iron	Moderate, diffuse hepatocellular vacuolation
B295	3002	Tungsten-iron	Mild, diffuse hepatocellular vacuolation
B168	3022	Tungsten-iron	Mild, diffuse hepatocellular vacuolation
DSX3625	3018	Tungsten-iron	Mild, diffuse hepatocellular vacuolation

[a] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 41 continued.		The histopathological effects of treatment shot on the liver and kidneys of female ducklings.	
ID#	Hen#	Treatment	Observation(s)[a]
DSX3813	4018	Tungsten-polymer	Moderate, diffuse hepatocellular vacuolation
DSX3545	4020	Tungsten-polymer	Moderate, diffuse hepatocellular vacuolation
P129	4016	Tungsten-polymer	Moderate, diffuse hepatocellular vacuolation
P122	4008	Tungsten-polymer	Normal
P35	4030	Tungsten-polymer	Moderate, diffuse hepatocellular vacuolation
DSX3893	4014	Tungsten-polymer	Moderate, diffuse hepatocellular vacuolation
P13	4004	Tungsten-polymer	Mild, diffuse hepatocellular vacuolation
P173	4026	Tungsten-polymer	Mild, diffuse hepatocellular vacuolation

[a] Histopathological assessment of tissues was performed by Dr. Scott Fitzgerald, board-certified veterinary pathologist

Table 42. The effect of treatment shot on concentrations (mg/kg dry weight) of iron, lead, and tungsten in tissues of ducklings[a].

Treatment	Iron	Lead	Tungsten
Femur			
Steel	118.8 ± 3.23	0.2 ± 0.14 (14)	ND
Tungsten-iron	112.1 ± 3.23	0.2 ± 0.14 (13)	1.1 ± 0.59 (12)
Tungsten-polymer	110.1 ± 3.23	0.3 ± 0.14 (12)	1.7 ± 0.59 (12)
Kidneys			
Steel	242.5 ± 6.62	0.1 ± 0.16 (15)	ND
Tungsten-iron	251.3 ± 6.62	0.4 ± 0.16 (12)	1.2± 0.56 (14)
Tungsten-polymer	247.5 ± 6.62	0.2 ± 0.16 (13)	0.4 ± 0.56 (15)
Liver			
Steel	463.8 ± 46.06	0.03 ± 0.063 (15)	ND
Tungsten-iron	521.3 ± 46.06	0.03 ± 0.063 (15)	0.6 ± 0.34 (14)
Tungsten-polymer	399.4 ± 46.06	0.14 ± 0.063 (14)	0.6 ± 0.34 (14)

[a] Data presented as mean ± standard error of the mean. Sample size is 16 for all groups. Numbers in parentheses refer to the number of pooled samples having a tissue concentration below detection limits. ND refer to non-detect. Tungsten detection limit is 3 mg/kg dry weight.

Discussion

Adult Mortality

Only the lead-dosed ducks died during the 150-day trial and mortality was 100% by day 25 (Table 1). These results suggest that waterfowl fed a nutritionally inadequate diet consisting of corn are more susceptible to the toxic effects of lead than ducks fed a diet high in protein. Jordan and Bellrose (1950) reported that 86% of Pekin ducks (*Anas platyrhynchos*) dosed with 25 #4 lead shot and maintained on a corn diet died within 17 days. In a subsequent study, Jordan and Bellrose (1951) reported that only 1 or 2 #6 lead shot pellets were sufficient to cause lead poisoning in 50% of game-farm mallards fed a whole-corn diet. Grandy et al. (1968) and Longcore et al. (1974) reported 100% mortality within 7 to 28 days in pen-raised mallards that were dosed with 8 #6 lead shot and maintained on corn. Sanderson et al. (1992) dosed mallards with 2, 4, or 8 #2 lead shot or 4 #2 lead shot plus 4 #2 bismuth shot and maintained the ducks for up to 30 days on a diet of shelled corn. Mortality was 95% with only 2 ducks (dosed with 2 #2 lead shot) surviving. In contrast, Rattner et al. (1989) reported no mortality after 14 days in pen-raised and wild black ducks (*Anas rubripes*) and game-farm and wild mallards maintained on duck pellets that were dosed with a single #4 lead shot. The same ducks were then dosed with either 2 or 4 #4 lead shot and maintained on a pellet diet for another 49 days. Mortality of wild black ducks was 40% and that of wild mallards was 45%. Jordan and Bellrose (1950) reported that only 33% of Pekin ducks dosed with 25 #4 lead shot died within 17 days when fed duck pellets. Kelly et al. (1998) reported 50% mortality after 30 days in game-farm mallards dosed with 8 #4 lead shot and maintained on a commercial duck pelleted diet.

In the present study, none of the birds dosed with tungsten-iron or tungsten-polymer shot died. In a similar toxicity study in which game-farm mallards where dosed with 8 BBs of tungsten-iron or tungsten-polymer shot, no mortalities were recorded during the 30-day trial (Kelly et al., 1998). In addition, Ringelman et al. (1993) dosed mallards with 12 to 17 pellets composed of 39% tungsten, 44% bismuth, and 16 % tin, and reported no mortalities during the 32-day trial.

Tungsten has been reported to cause mortality in birds. Nell et al. (1980) dosed broiler cockerels with sodium tungstate by intramuscular injection at 5 mg tungsten from day 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. However, the tungsten was in a soluble form injected in animals that were relatively small, resulting in a higher exposure rate based on mg/kg body weight, which might enhance toxicity.

Adult Clinical Signs

Lead-dosed mallards were the only ducks that had obvious clinical signs. The classic signs of lead poisoning, seen in more chronic cases, usually develop in the following sequence: anorexia and lethargy; greenish diarrhea that stains the feathers surrounding the vent; muscular weakness first evident as an inability to fly and then as an inability to walk or move; coma; and death. There is a progressive weight loss and atrophy of the breast muscle resulting in a "hatchet-breast" appearance (Wobeser, 1981; Friend, 1987; Locke and Thomas, 1996). Mallards dosed with tungsten-iron and tungsten-polymer shot appeared normal throughout the 150-day trial. These results agree with those reported by Kelly et al. (1998) who dosed mallards with 8 BBs of tungsten-iron or tungsten-polymer shot and Ringelman et al. (1993) who dosed mallards with

tungsten-bismuth-tin shot. Nell et al. (1980) reported that clinical signs in chickens administered tungsten were anorexia, reduced weight gain, diarrhea, and labored breathing before death.

Adult Body Weights

Lead-dosed mallards lost a significant amount of body weight (54 – 73%) after the first 25 days of the trial (Table 1). Generally, waterfowl that die of chronic lead poisoning lose from 40-60% of their body weight before death. Sanderson and Irwin (1976) reported that 8 of 20 male game-farm mallards on a diet of corn and dosed with 5 #4 lead pellets died of acute lead poisoning an average of 7.6 days post-dosing after losing 20.5% of their body weight. The 12 remaining ducks died of chronic lead poisoning an average of 20.7 days post-dosing and lost 47.6% of their body weight. Sanderson et al. (1992) reported the average weight loss of game-farm mallards on a diet of corn and dosed with 2, 4 or 8 #2 lead shot was 42.2 %, with a range of 16% to 56% for individual ducks.

In the present study, there were statistically significant differences in body weights at specific time points between the tungsten-iron- or tungsten-polymer-dosed ducks and steel-dosed ducks (Tables 3 - 4). However, over the 150-day period body weights changed little. In males, there was a 3% drop in body weight in the steel- and tungsten-iron-dosed ducks, while tungsten-polymer-dosed ducks had no change in body weight. Steel-dosed females gained 9%, tungsten-iron-dosed females gained 8% and tungsten-polymer-dosed females gained 14% of their original weight over the 150-day period. The weight gain of the females was probably associated with an increase in food consumption during the reproductive phase of the trial. Sanderson et al. (1997) reported that body

weights of mallards dosed with 8 #4 bismuth alloy shot on days 0, 30, 60, 90 over a 150-day period were similar compared to controls. The females, which were reproductively active, were heavier than males at day 120. Ringelman et al. (1993) reported that mallards dosed with 12 to 17 pellets of tungsten-bismuth-tin shot gained a similar amount of weight as controls over 32 days. Mallards dosed with 8 BBs of tungsten-iron or tungsten-polymer shot gained a slight amount of weight (0.9 to 5.8%) during a 30-day period (Kelly et al., 1998).

Adult HCT, Hb Concentration, ALAD Activity

The low hematocrit, hemoglobin concentration and delta aminolevulinic acid deyhdratase (ALAD) activity in lead-dosed mallards at day 7 are all indicators of lead toxicity (Table 5). Lead poisoning is associated with two basic hematologic defects: shortened erythrocyte lifespan and impairment of heme synthesis. Shortened lifespan of the red blood cell may be due to increased mechanical fragility of the cell membrane. The impairment of heme synthesis is due to the inhibition of ALAD. ALAD is a key enzyme in the synthesis of heme, which is an integral component of hemoglobin (Goyer, 1996). Pain and Rattner (1988) reported that hematocrit and hemoglobin concentrations were significantly depressed in black ducks administered 1 #4 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 slightly between 3-9 days post-dosing (approximately 70% inhibition) and then declined again until the end of the 30-day study. Finley et al. (1976) reported that mallard drakes fed 25 ppm lead exhibited a 40% decrease in blood ALAD activity 3 weeks after post-dosing and enzyme activity remained at this level through the 12-week treatment period. In the same study, ducks fed 5 ppm of lead in the diet for 12

weeks had a 36% decrease in blood ALAD activity. Since the inhibition of ALAD activity has been shown to be a sensitive indicator of lead poisoning, the elevated ALAD activity in tungsten-polymer-dosed ducks at day 7 was considered not to be biologically significant

The slight, but statistically significant, decrease in hematocrit of tungsten-polymer-dosed females from day 90 through day 150 (Table 7) was not thought to be treatment related but rather reflected to the reproductive status of all females in each group. Hematocrits measured during this time were lower than hematocrits measured during the first 60 days of the trial when birds were not reproductively active (Table 6). Bell et al. (1965) and Sturkie (1976) reported that lowered hematocrit was associated with egg production in birds. Similar results were reported by Sanderson et al (1997), in that female mallards repeatedly dosed with 8 #4 bismuth alloy shot had a decline in average hematocrit during reproduction. Tungsten has been shown to have no effect on hematocrit in short-term studies (< 32 days) using game-farm mallards (Ringelman et al, 1993, Kelly et al 1998).

Adult Plasma Chemistries

The administration of lead shot caused a number of changes in day 7 plasma chemistry values. The decrease in sodium concentration in lead-dosed mallards (Table 8) may have been indicative of early renal disease associated with renal tubular damage (Campbell and Coles, 1986). Plasma sodium concentration in the tungsten-polymer-dosed group was statistically lower compared to the steel-dosed group, but within the normal range reported for mallards (Lewandowski et al., 1986; Kelly, 1997).

The elevated concentrations of blood urea nitrogen and creatinine in lead-dosed mallards (Table 8) could indicate one of the following possibilities: pre-renal azotemia (dehydration), renal azotemia (primary renal damage), and post-renal azotemia (obstruction of the ureters) (Campbell and Coles, 1986). Histopathological examination of the kidneys from the lead-dosed ducks suggests that pre-renal and renal azotemia are the causes of the elevation in blood urea nitrogen and creatinine. However, blood urea nitrogen, creatinine, and blood urea nitrogen/creatinine ratio are not considered useful diagnostic tests for renal function in birds (Campbell and Coles, 1986). The depressed plasma protein and albumin concentrations in the lead-dosed mallards (Table 8) was probably associated with early signs of chronic renal disease and malnutrition (Campbell and Coles, 1986). The elevation in albumin/globulin ratio in lead-dosed mallards (Table 8) reflected the depressed total protein and albumin concentrations.

Birds lack the enzyme biliverdin reductase needed to reduce biliverdin to bilirubin, thus bilirubin accounts for only a small percentage of the total bile pigment (Campbell and Coles, 1986). Since histopathological examination of lead-dosed mallards indicated biliary dysfunction rather than biliary obstruction, the elevated concentration of bilirubin in the lead-dosed mallards (Table 8) was associated with biliary dysfunction. Elevated phosphorus concentration may be associated with renal disease, in which concentrations can be 9.5 mg/dL or greater (Campbell and Coles, 1986). In the present study, the significantly elevated phosphorus concentration in lead-dosed mallards (Table 8) was only slightly above concentrations considered to be normal (5.38 vs 2 - 4.5 mg/dL; Campbell and Coles, 1986). The elevated concentration of uric acid in the lead-dosed ducks (Table 8) may be an indication of starvation or renal disease. The increase in uric

acid concentration is thought to be a result of a decreased rate of tubular excretion plus poor nutritional status, which can cause an increase in uric acid production as body proteins are degraded (Campbell and Coles, 1986; March et al., 1976). However, while the lead-dosed mallards had a marked increase in blood uric acid concentration, the value was within the normal range (2 to 15 mg/dL) as reported by Campbell and Coles (1986).

The hepatic enzymes alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase can be useful diagnostic tests to determine lead-poisoning in mallards. The depressed alkaline phosphatase activity observed in the lead-dosed ducks (Table 8) is due to direct inhibition of the enzyme by lead (Rozman et al., 1974). Although increases in the plasma activities of alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase are not specific to liver disease in birds, the increased activities in the lead-dosed ducks (Table 8) were probably associated with hepatocellular damage (Campbell and Coles, 1986). Rozman et al. (1974) and Kelly et al. (1998) reported significant increases in plasma alanine aminotransferase activity in mallards dosed with lead shot.

Triglyceride concentration tends to be low in fasted birds and then increases when birds are refed (Hermier et al., 1984). The decrease in triglyceride concentration in lead-dosed ducks (Table 8) was probably due to inappetence associated with lead poisoning.

Since changes in chloride generally follow those of sodium, the decrease in chloride concentration in male and female lead-dosed mallards (Table 9) was assumed to be associated with the decrease in sodium concentration (Table 8). The chloride concentration for tungsten-polymer-dosed females was within normal range (108 – 112 mmol/l) reported for mallards (Kelly, 1997). Calcium concentrations in male and female

lead-dosed ducks and tungsten-iron-dosed males were significantly decreased at day 7 (Table 9) and while hypocalcemia is associated with renal failure, the lead-dosed mallards and tungsten-iron-dosed males had concentrations within the normal range (8 – 18 mg/dL) reported by Campbell and Coles (1986). Lead-dosed male and female ducks had marked elevated activities of creatinine phosphokinase (Table 9), which has been reported to be associated with lead toxicity (Campbell and Coles, 1986, Kelly et al., 1998). Since a diagnostic use for serum amylase has not been well investigated in birds (Campbell and Coles, 1986), it is not clear what the biological relevance is of the decrease in plasma amylase activity in the lead-dosed males and females (Table 9).

The plasma values reported for steel-, tungsten-iron-, and tungsten-polymer-dosed mallards from day 30 through day 60 (Tables 10 – 12) and from day 90 through day 150 (Tables 13 – 14) are within the range of normal values reported in other studies (Campbell and Coles, 1986; Lewandowski et al., 1986; Fairbrother et al., 1990; Ringelman et al., 1993; Kelly, 1997; Kelly et al. 1998). Thus, the occasional significant difference in values between the tungsten-dosed ducks and the steel-dosed ducks were not considered to be biologically relevant.

Adult Gross Pathology

The linings of the gizzards of 6 of 12 ducks in the lead-dosed group were discolored (Tables 15 and 16). This effect has been described in naturally occurring and experimentally-induced cases of lead toxicosis (Slauson and Cooper, 1990; Alden and Frith, 1991; Popp and Cattley, 1991). No birds in the other 3 groups had gross lesions within their gizzards. Other gross observations noted in the lead-dosed ducks included urate crystals surrounding the heart in one bird, which is consistent with visceral gout,

and enlarged gallbladders. Both of these lesions have been previously associated with lead toxicosis (Slauson and Cooper, 1990; Alden and Frith, 1991; Popp and Cattley, 1991).

During the 90-day reproductive trial, 4 of 5 female ducks that did not lay eggs had abnormalities that probably were responsible for their failure to lay eggs. The lack of gross changes in mallards dosed with tungsten-iron and tungsten-polymer shot agrees with findings reported by Ringelman et al. (1993) and Kelly et al. (1998), although exposure periods in these studies were considerably shorter than in the present study.

Adult Organ Weights

The higher relative kidney, heart, brain, and gizzard weights of lead-dosed ducks (Table 19) are associated with the significant weight loss (-61%) due to chronic lead poisoning. Additionally, lower relative spleen weight in lead-dosed ducks (Table 19) can be attributed to lead-induced atrophy of this organ (Rocke and Samuel, 1991). These results are similar to those in the study by Kelly et al. (1998) who reported that relative kidney and heart weights were significantly higher in mallards dosed with 8 #4 lead shot as compared to control ducks. Sanderson et al. (1997) reported mallards dosed with 8 #4 lead shot had greater gizzard and kidney weights than controls.

The difference in relative gonad weights of lead-dosed male and female mallards compared to the other 3 groups (Table 20) was due to the fact that lead-dosed ducks died before becoming reproductively active. The increase in relative liver weight in lead-dosed males (Table 20) was associated with the marked decrease in body weight. There were no differences in relative liver weights of females. Although the depressed body weight in lead-dosed females caused an increase in their relative organ weights, the liver

weights of the females in the other 3 groups were high because these ducks were reproductively active.

Histopathology of the Adult Gonads, Liver and Kidneys

Microscopic renal lesions (acute tubular necrosis or nephrosis) were found only in lead-dosed ducks (Tables 21 – 24). Acute tubular nephrosis is associated with lead toxicosis in many animal species (Alden and Frith, 1991). The absence of renal lesions in the steel-, tungsten-iron-, and tungsten-polymer-dosed ducks suggested that these metals were non-toxic to the renal tubular epithelium, or that they were not absorbed in sufficient quantities to produce renal tubular toxicity.

The primary hepatic lesions observed (Tables 21-24) were categorized as substantial biliary stasis or liver hemosiderosis. The accumulation of bile within hepatocytes or within canaliculi is somewhat nonspecific, as it may occur because of obstruction of bile ducts, or primary hepatocellular dysfunction (Popp and Cattley, 1991). In the present study, no evidence of cholelithiasis or other obstructive biliary disease was detected, thus biliary stasis was considered evidence of hepatocellular dysfunction. As previously mentioned, the increase found in plasma total bilirubin concentration in lead-dosed mallards at day 7 suggested hepatocellular dysfunction, rather than biliary obstruction. The degree of biliary stasis was graded, and only the lead-dosed group had detectable biliary stasis. Hemosiderosis was only found in the steel and tungsten-iron groups with the exception of one male from the tungsten-polymer group. Hemosiderosis (deposition of iron in the form of hemosiderin) commonly occurs when ducks are fed iron-containing shot (Locke et al., 1967). Additionally, intrahepatocellular fatty vacuolation was present in at least half of the ducks in each of the 4 experimental groups

with the exception of males in the steel, tungsten-iron, and tungsten-polymer groups. Fatty accumulation can be due to a variety of causes and was judged as an incidental finding in this study.

The gonads from the lead-dosed mallards were inactive and no histologic lesions were found. The testes and ovary from steel, tungsten-iron, tungsten-polymer groups were all normal.

Metal Residues in Tissues of Adults

Iron was detected in femur, gonads, kidneys, and liver samples in all treatment groups (Tables 26, 27, 29, 30). In general, the concentration of iron was highest in the tissue samples from the tungsten-iron- and steel-dosed ducks. Moreover, the iron concentrations in samples of the femur, kidneys, and gonads from tungsten-iron- and steel-dosed females were generally higher than in the males. The sex-related difference in iron concentration was related to physiological changes in the female in preparation for the egg-laying season. Underwood (1971) reported a 5-fold increase in iron in the serum of ducks during the egg-laying season. In contrast, the physiological changes due to egg-laying obviously do not apply to lead-dosed mallards because none of these ducks were reproductively active. The elevated concentrations of iron in the lead-dosed females could be attributed to lead-induced interference of heme synthesis, which caused an accumulation of iron in the liver. The high concentration of iron in the liver of lead-dosed ducks agrees with results reported by Sanderson et al. (1992) and Kelly et al. (1998).

The high concentration of iron in liver samples from steel- and tungsten-iron-dosed ducks were associated with the histological findings of hemosiderosis. Locke et

al. (1967) dosed mallards with 8 pellets of iron shot, which resulted in hemosiderosis of the liver and hepatic iron concentrations ranging from 3,185 to 6,131 ppm. Because liver hemosiderosis commonly occurs when ducks are fed iron-containing shot, Rozman et al. (1974) investigated the effects of hemosiderosis on the hepatic enzymes alkaline phosphatase, aspartate aminotransferease, and alanine aminotransferase and found no significant changes of these enzymes in groups of ducks receiving up to 64 #4 steel shot when compared to control ducks. In the present study, the activity of plasma enzymes alkaline phosphatase, aspartate aminotransferease, and alanine aminotransferase had no significant changes in the steel- and tungsten-iron-dosed ducks when values were compared to those of control mallards from Fairbrother et al. (1990).

Lead was generally detected in femur, gonad, kidney, and liver samples from all treatment groups with the exception of gonad samples from the tungsten-iron-dosed group (Table 25, 27, 28, 30). Concentrations of lead in the lead-dosed ducks were approximately 100 to 6000 fold higher when compared to the other 3 groups. Kelly et al. (1998) reported concentrations of lead in the femur, liver, and kidneys of all mallards on trial with the highest concentrations being in the lead-dosed ducks. In the present study, lead concentrations were highest in the kidneys, intermediate in the femur and liver, and lowest in the gonads. In contrast, Havera et al. (1992) reported wild mallards redosed with lead shot had lead concentrations highest in the wing bone, intermediate in the kidney, and lowest in the liver.

In the tungsten-iron-dosed ducks, the number of femur, gonad, kidney, and liver samples that tungsten was detected in and the concentration of tungsten in these tissue samples were substantially greater when compared to the tungsten-polymer-dosed ducks

(Tables 26-28, 30). The bone, liver, and kidneys are principle sites of tungsten deposition in a number of different species (Kinard and Aull, 1945; Wase, 1956; Kaye, 1968; Bell and Sneed, 1970; Aamodt, 1975) and the primary site of tungsten deposition is species-specific. In the present study, the concentration of tungsten was highest in the liver, intermediate in the femur, and lowest in the kidneys and gonads. These results agree with Kelly et al. (1998) who reported tungsten concentrations highest in the liver, intermediate in the femur, and lowest in the kidneys from mallards dosed with tungsten-iron or tungsten-polymer shot. Ringelman et al. (1993) did not detect tungsten in either the liver or kidneys from mallards dosed with tungsten-bismuth-tin shot. However, the proportion of tungsten in the tungsten-bismuth-tin shot was 39%, while in the present study, tungsten concentrations were 55% and 95.5 % for tungsten-iron and tungsten-polymer shot, respectively.

Tungsten was also detected in the kidneys of 2 steel-dosed and 3 lead-dosed ducks (Table 28). It was thought this was due to the normal variance one can expect from readings near the instrument's detection limit that may have been accentuated by "noise" induced by a complex matrix such as animal tissue (personal communication, CT&E Environmental Services).

Shot Recovery and Percent Shot Erosion

Lead-dosed ducks had the highest percent of shot recovered (86%), followed by steel (59%), tungsten-iron (39%), and tungsten-polymer (2%). Since all lead-dosed ducks died by day 25 and the steel-, tungsten-iron-, and tungsten-polymer-dosed groups survived until day 150, the high recovery shot rate seen in lead-dosed ducks was expected.

Percent shot erosion in male ducks dosed with steel, lead, tungsten-iron, and tungsten-polymer shot was 50%, 22%, 64%, and 99%, respectively (Table 31). Percent shot erosion in female ducks dosed with steel, lead, tungsten-iron, and tungsten-polymer shot was 60%, 15%, 80%, 99%, respectively. These results were substantiated during fluoroscopy of ducks in that steel and lead pellets were readily visible while the tungsten-iron and particularly the tungsten-polymer pellets were often difficult to see because of disintegration. Kelly et al. (1998) reported similar findings from a 30-day test with percent shot erosion highest in tungsten-polymer-dosed ducks (80%), intermediate in tungsten-iron- and lead-dosed ducks (55% and 50%, respectively), and lowest in steel-dosed ducks (33%). Furthermore, Kelly et al. (1998) compared the percent shot erosion in the lead-dosed ducks that survived the 30-day trial (71%) to the lead-dosed ducks that died during the 30-day trial (34%). These results for the lead-dosed ducks that died in the Kelly et al. (1998) study are similar to those reported in the present study.

Date First Egg was Laid and Number of Days Required to Lay 21 Eggs

The administration of tungsten-iron or tungsten-polymer shot did not have an effect on the commencement or duration of egg laying by female mallards (Table 32). The fact that 4 egg-laying females (1 steel-dosed, 2 tungsten-iron-dosed, 1 tungsten-polymer-dosed) did not lay 21 eggs may have been the result of individual variation. The removal of eggs from incubating mallards will result in the continuation of egg laying whereas retention of the clutch will terminate egg-laying. It is possible that these 4 females mimicked the behavior seen in wild mallards, which terminate egg laying after a clutch of eggs has been laid. In the present study, egg laying began at day 92, 92, and 85, and the days required to lay 21 eggs were 25, 24, and 26 for the steel-, tungsten-iron-, and

tungsten-polymer-dosed females, respectively. These results agree with those of Sanderson et al. (1997) who reported that egg-laying in control mallards began on day 84, on day 94 for iron-dosed females, and on day 92 for bismuth-dosed females The mean range to lay 21 eggs was 26 to 27 days for the 3 groups in the latter study.

Percent Egg Production, Fertility, and Hatchability

In our study, tungsten did not have an apparent effect on the rate of egg production, fertility, or hatchability (Table 33). These findings are similar to those of Teekell and Watts (1959) who reported that supplementation of the diet of breeder hens with 250 or 500 ppm tungsten had no adverse effect on rate of egg production or hatchability. The slight decrease in percent fertility of eggs laid by tungsten-polymer-dosed females may be because tungsten-polymer-dosed females became reproductively active earlier than tungsten-polymer-dosed males. Four of the 13 tungsten-polymer-dosed females that laid eggs did not begin to lay fertile eggs until after the 17^{th} egg was laid. Similarly, there were 3 steel-dosed females that did not produce fertile eggs until after the 12^{th} egg was laid.

Egg Weight and Shell Thickness

The weight and shell thickness of eggs from tungsten-iron-dosed ducks were statistically greater compared to eggs from steel- and tungsten-polymer-dosed females (Table 34), but the difference was not considered biologically relevant. In our study, the egg weights were 61, 63, and 61 grams and shell thicknesses were 0.372, 0.412, and 0.385 mm for the eggs from the steel-, tungsten-iron-, and tungsten-polymer-dosed ducks, respectively. Sanderson et al. (1997) reported similar findings with egg weights of 61.2,

61.2, and 61.3 grams and shell thickness of 0.335, 0.338, and 0.335 mm for control, iron-dosed and bismuth-dosed mallards, respectively.

Metal Residues in Egg Shell and Contents

Iron concentration in egg contents was highest in the steel-dosed group, intermediate in the tungsten-iron-dosed group, and lowest in the tungsten-polymer-dosed group (Table 35). The presence of iron in the contents of eggs is associated with a 5-fold increase of iron in the serum during the egg-laying season in ducks (Underwood, 1971).

Tungsten was detected in the shell and contents of eggs from tungsten-iron-dosed ducks and in the shell of eggs from tungsten-polymer-dosed ducks (Table 35). The concentration of tungsten in the eggs followed the same trend as in the adult tissue samples. Tungsten was detected in 9 shells of eggs from tungsten-iron-dosed females at a concentration that was higher compared to the concentration of tungsten detected in 3 shells of eggs from tungsten-polymer-dosed females. The presence of tungsten in shells can be attributed to the fact that calcium-containing tissues are among the principle sites of tungsten deposition (Kinard and Aull, 1945; Wase, 1956; Kaye, 1968; Bell and Sneed, 1970; Aamodt, 1975).

Survivability, Body Weight, and Hematocrit of Ducklings

The administration of tungsten-iron and tungsten-polymer shot had no adverse effects on the survivability, body weight, or hematocrit of ducklings (Table 36). The slight but significant decrease in hematocrit of tungsten-iron ducklings was not considered biologically relevant. Sanderson et al. (1997) reported bismuth alloy shot caused no adverse effects on duckling survivability, body weight (day 7), or hematocrit.

Duckling Organ Weights

Ducklings in the tungsten-polymer group had slightly, but significantly greater absolute and relative kidney weights compared to ducklings in the other 2 groups (Tables 37, 38). This difference was considered not to be biologically relevant.

Histopathology of Duckling Liver and Kidneys

The most common finding in the liver of ducklings in all treatment groups was mild to moderate hepatocellular vacuolation (Tables 40 and 41). Based on the ducklings' young age, this condition was considered normal and was due primarily to hepatic glycogen accumulation. Sanderson et al. (1997) reported a similar condition in the liver of ducklings from a reproduction study that assessed the effects of bismuth alloy shot. There were no histologic lesions present in the kidneys of the ducklings.

Metal Residues in Tissues of Ducklings

Iron concentration was highest in the liver, intermediate in the kidneys, and lowest in the femur samples from ducklings (Table 42). Sanderson et al. (1997) reported similar findings in that iron concentration was highest in the liver and lowest in the kidney from ducklings of mallards dosed with bismuth alloy shot. Lead was detected in trace amounts in the femur, kidneys, and liver samples from the ducklings. Sanderson et al. (1997) also reported the presence of lead in the liver and kidneys of the ducklings. Tungsten was detected in relatively few samples of the femur, kidneys, and liver from ducklings of tungsten-iron- and tungsten-polymer-dosed females.

Conclusions

Male and female mallards administered 40 #4 tungsten-iron or tungsten-polymer shot and maintained for 150 days were not adversely affected based on the variables measured. All ducks, with the exception of lead-dosed mallards, survived the 150-day

trial. No significant differences were observed in HCT, Hb concentration, and ALAD activity at day 7 in the 2 tungsten shot groups when compared to the steel-dosed group. The differences in hematocrit and plasma chemistry variables that occurred from day 30 through day 150 were within the normal range for mallards and thus were not considered biologically relevant. The ducks appeared normal at the time of necropsy on day 150 of the trial, and no deleterious changes were detected in weights of organs. Three of 8 tungsten-iron-dosed females, 8 of 8 tungsten-iron-dosed males, and 1 of 8 tungsten-polymer-dosed males manifested mild to moderate liver hemosiderosis, which was not considered deleterious. Similarly, liver hemosiderosis was present in 5 of 8 steel-dosed females and 8 of 8 steel-dosed males. No other histopathological lesions were noted. Tungsten residues were generally detected in the femur, gonads, kidneys, and liver of tungsten-iron- and tungsten-polymer-dosed ducks. Concentrations of tungsten were generally higher and occurred in more tissue samples from the tungsten-iron-dosed ducks compared to tungsten-polymer-dosed mallards. The erosion rate of tungsten-polymer shot was 28% greater than the erosion rate of tungsten-iron shot, which was 35% and 17% greater than the erosion rates of lead and steel shot, respectively. There were no significant differences in percent egg production, fertility and hatchability in the 2 tungsten-dosed groups when compared to the steel-dosed group. Similarly, there were no relevant differences in egg weight and shell thickness of eggs from tungsten-iron and tungsten-polymer-dosed ducks. Tungsten-residues were detected in the shell of 9 of 16 eggs and in the contents of 6 of 16 eggs from tungsten-iron-dosed females. The concentration of tungsten was slightly above the detection limit in the shell of 3 of 13 eggs from tungsten-polymer-dosed ducks. No relevant differences were observed in

duckling survivability, body weight, or hematocrit when compared to ducklings from steel-dosed ducks. Absolute and relative kidney weights of ducklings from tungsten-polymer ducks were slightly greater when compared to ducklings from steel-dosed ducks. No other significant differences in duckling organ weights were observed. All ducklings had mild to moderate hepatocellular vacuolation, which was considered normal. No other histopathological lesions were noted. Tungsten residues were detected in the femur of 4 of 16 tungsten-iron and tungsten-polymer ducklings, in the kidney of 2 of 16 and 1 of 16 tungsten-iron and tungsten-polymer ducklings, and in the liver of 2 of 16 tungsten-iron and tungsten-polymer ducklings. Because the two formulations of tungsten-shot were non-toxic to mallards after 150 days exposure, they have potential for permanent use for waterfowl hunting. If one were to compare steel, tungsten-iron, and tungsten-polymer shot, tungsten-polymer shot seems preferable because of its high erosion rate and its low concentrations in selected tissues, when compared to steel and tungsten-iron shot.

References

Aamodt, R. L. 1973. Retention and excretion of injected ^{181}W labeled sodium tungstate by beagles. Health Phys. 24:519-524.

Aamodt, R. L. 1975. Inhalation of ^{181}W labeled tungstic oxide by six beagle dogs. Health Phys. 28:733-742.

Alden, C. L. and C.H. Frith. 1991. Urinary system. In Handbook of Toxicologic Pathology (W. M. Haschek and C. G. Rousseaux, Eds.) pp.334-344. Academic Press, San Diego.

Ballou, J. E. 1960. Metabolism of ^{185}W in the rat. USAEC Document HW-64112.

Bell, D.J., T.P. Bird, and W.M. McIndoe. 1965. Changes in erythrocyte levels and the mean corpuscular haemoglobin concentration in hens during the laying cycle. Comp. Biochem. Phsiol. 50(A):415-417.

Bell, M. C. and N.N. Sneed. 1970. Metabolism of tungsten by sheep and swine. In Trace Element Metabolism in Animals (C. F. Mills, Ed.) pp.70-72. E. and S. Livingstone, London.

Bellrose, F.C. 1959. Lead poisoning as a mortality factor in waterfowl populations. Illinois Nat. Hist. Surv. Bull. 27:236-288.

Burch, H. B. and A.L. Siegel. 1971. Improved method for measurement of delta-aminolevulinic acid dehydratase activity of human erythrocytes. Clin. Chem. 17:1038-1041.

Campbell, T. W. and E.H. Coles. 1986. Avian clinical pathology. In Veterinary Clinical Pathology (E. H. Coles, Ed.) pp.279-301. W. B. Saunders, Philadelphia.

Dieter, M. P. and M.T. Finley. 1979. δ-Aminolevulinic acid dehydratase enzyme activity in blood, brain, and liver of lead-dosed ducks. Environ. Res. 19:127-135.

Eisler, R. 1988. Lead hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish Widl. Serv. Biol. Rep. 85 (1.14), Washington.

Fairbrother, A., M.A. Craig, K. Walker, and D. O'Loughlin. 1990. Changes in mallard (Anas platyrhynchos) serum chemistry due to age, sex, and reproductive condition. J. Wildl. Dis. 26:67-77.

Federal Register. 1986. Nontoxic shot approval procedures for shot 51: 42098-42102.

Finley, M.T., M.P. Dieter, and L.N. Locke. 1976. Sublethal effects of chronic lead ingestion in mallard ducks. J. Toxicol. Environ. Hlth. 1:929-937.

Frederick, W. G. and W.R. Bradley. 1946. Toxicity of some materials used in the manufacture of cemented tungsten carbide tools. Ind. Med. 15:482-483.

Friend, M. 1987. Lead poisoning. In Field Guide to Wildlife Diseases, Volume 1, General Field Procedures and Diseases of Migratory Birds. (M. Friend, Ed.) pp.175-189. U.S. Department of Interior, Fish and Wildlife Service, Washington, DC.

Gill, J.L. 1990. Repeated measurements on animals: Why not assume autocorreclation? J. Animal Breed. Gene. 107:89-95.

Goyer, R. A. 1996. Toxic effects of metals. In Casarett and Doull's Toxicology (C. D. Klaassen, Ed.) pp 691-736. McGraw-Hill, New York.

Grandy, J.W., L.N. Locke, and G.E. Bagley. 1968. Relative toxicity of lead and five

proposed substitute shot types to pen-reared mallards. J. Wildl. Mgmt. 32:483-488.

Grinnell, G.B. 1894. Lead poisoning. Forest and Stream 42:117-118.

Havera, S.P., S.G. Wood, and M.M. Georgi. 1992. Blood and tissue parameters in wild mallards redosed with lead shot. Bull. Environ. Contam. Toxicol. 49:238-245.

Hermier, D., M.J. Chapman, and B. Leclercq. 1984. Plasma lipoprotein profile in fasted and refed chickens of two strains selected for high or low adiposity. J. Nutr.114:1112-1121.

Higgens, E. S., D.A. Richert, and W.W. Westerfeld. 1956. Molybdenum deficiency and tungstate inhibition studies. J. Nutr. 59:539-559.

Jordan, J.S. and F.C. Bellrose. 1950. Shot alloys and lead poisoning in waterfowl. Transactions of the 15th North American Wildlife Conference. 15:155-168.

Jordan, J.S. and F.C. Bellrose. 1951. Lead poisoning in wild waterfowl. Illinois Natural History Survey Biological Notes 26. 27 pp.

Jorgensen, S.S. and M. Willems. 1987. The fate of lead in soils the transformation of lead pellets in shooting-range soils. Ambio 16:11-15.

Kaye, S. V. 1968. Distribution and retention of orally administered radiotungsten in the rat. Health Phys. 15:398-417.

Kelly. M.E. 1997. A thirty-day dosing test to assess the toxicity of tungsten-iron and tungsten-polymer shot in game-farm mallards. M.S. Thesis. Michigan State University, East Lansing, MI. 95 pp.

Kelly, M.E., S.D. Fitzgerald, R.J. Aulerich, R.J. Balander, D.C. Powell, R.L. Stickle, W. Stevens, C. Cray, R.J. Tempelman, and S.J. Bursian. 1998. Acute effects of lead, steel, tungsten-iron, and tungsten-polymer shot administered to game-farm mallards. J. Wildl. Dis. 34:673-687.

Kinard, F. W. and J. Van de Erve. 1940. Rat mortality following sodium tungstate injection. Amer. J. Med. Sci. 199:688-690.

Kinard, F. W. and J. Van de Erve. 1941. The toxicity of orally-ingested tungsten compounds in the rat. J. Pharmacol. Exp. Ther. 72:196-201.

Kinard, F. W. and J. Van de Erve. 1943. Effect of tungsten metal diets in the rat. J. Lab. Clin. Med. 28:1541-1543.

Kinard, F. W. and J.C. Aull. 1945. Distribution of tungsten in the rat following ingestion of tungsten compounds. J. Pharmacol. Expl Ther. 72:53-55.

Leach, R. M., Jr., D.E. Turk, T.R. Zeigler, and L.C. Norris. 1962. Studies on the role of molybdenum in chick nutrition. Poultry Sci. 41:300-304.

Lewandowski, A.H., T.W. Campbell, and G.J. Harrison. 1986. Clinical chemistries. In Clinical Avian Medicine and Surgery. (G.J. Harrison and L.R. Harrison, Eds). Pp. 192-200. W.B. Saunders, Philadelphia, PA.

Littell, R.C., P.R. Henry, and C.B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76:1216-1231.

Locke, L.N., H.D. Irby, and G.E. Bagley. 1967. Histopathology of mallards dosed with lead and selected substitute shot. Bulletin of Wildlife Disease Assoc. 3:143-147.

Locke, L.N. and N.J. Thomas. 1996. Lead poisoning of waterfowl and raptors. In Noninfectious Diseases of Wildlife. (A. Fairbrother, L.N. Locke, and G.L. Hoff, Eds) pp. 108-117. Iowa State University Press, Ames, Iowa. pp. 108-117.

Longcore, J.R., R. Andrews, L.N. Locke, G.E. Bagley, and L.T. Young. 1974. Toxicity of lead and proposed substitute shot to mallards. U.S. Fish and Wildlife Service Special Scientific Report-Wildlife No. 183, U.S. Fish and Wildlife Service, Washington, D.C., 23 pp.

Mallory, F.B. 1942. Pathological Technique. p 137.W.B. Saunders, Philadelphia.

March, G. L., T.M. John, B.A. McKeon, L.Sileo, and J.C. George. 1976. The effects of lead poisoning on various plasma constituents in the Canada goose. J. Wildl. Dis. 12:14-19.

Mineau, P. and M. Pedrosa. 1986. A portable device for non-destructive determinations of avian embryonic viability. J. Field Ornithol. 57:53-56.

Montgomery, R. R. 1982. Polymers. In Patty's Industrial Hygiene and Toxicology, Vol. IIIA (G. D. Clayton and F. E. Clayton, Eds.) pp. 4209-4526. John Wiley and Sons, New York.

Morehouse, K. 1992. Cripplin gloss and shot-type. The United States Experience.In Lead poisoning in waterfowl. (D.J. Pain ed.) pp. 32-37. IWRB Spec. Publ. No. 16, Slimbirdge, UK.

Nell, J. A., E.F. Annison, and D. Balnave. 1980. The influence of tungsten on the molybdenum status of poultry. Br. Poult. Sci. 21:193-202.

Pain, D. J. and B.A. Rattner. 1988. Mortality and hematology associated with the ingestion of one number four lead shot in black ducks, Anas rubripes. Bull. Environ. Contam. Toxicol. 40:159-164.

Peterson, J. E. 1977. Industrial Health. Prentice-Hall, Englewood Cliffs, NJ.

Pham-Huu-Chanh. 1965. The comparative toxicity of sodium chromate, molybdate, tungstate, and metavanadate. Arch. Int. Pharmacodyn. 154:243-249.

Popp, J. A. and R.C. Cattley. 1991. Hepatobiliary system. In Handbook of Toxicologic Pathology (W. M. Haschek and C. G. Rousseaux, Eds) pp.289-290. Academic Press, San Diego.

Rattner, B. A., W.J. Fleming, and C.M. Bunck. 1989. Comparative toxicity of lead shot in black ducks (Anas rubripes) and mallards (Anas platyrhynchos). J. Wildl. Dis. 25:175-183.

Ringelman, J. K., M.W. Miller, and W.F. Andelt. 1993. Effects of ingested tungsten-bismuth-tin shot on captive mallards. J. Wildl. Manage. 57:725-732.

Rocke, T.E. and M.D. Samuel. 1991. Effects of lead shot ingestion on selected cells of the mallard immune system. J. Wildl. Dis. 27: 1-9.

Rozman, R.S., L.N. Locke, and S.F. McClure, III. 1974. Enzyme changes in mallard ducks fed iron or lead shot. Avian Dis. 18:435-444.

Sanderson, G.C. and J.C. Irwin. 1976. Effects of various combinations and numbers of lead:iron pellets dosed in wild-type captive mallards. Final Report. Contract No. 14-16-0008-914. U.S. Fish and Wildlife Service and Illinois Natural History Survey. 67 pp.

Sanderson, G.C. and F.C. Bellrose. 1986. A review of the problem of lead poisoning in waterfowl. Illinois Nat. Hist. Surv. 4:1-34.

Sanderson, G. C., S.G. Wood, G.L. Foley, and J.D. Brawn. 1992. Toxicity of bismuth shot compared with lead and steel shot in game-farm mallards. Transactions of the 57th North American Wildlife and Natural Resource Conference. pp. 526-540.

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

SAS. 1997. Statistical Analysis Systems, Release 6.12, Cary, North Carolina, USA.

Scheuhammer, A.M., and S.L. Norris. 1995. A review of the environmental impacts of lead shotshell ammunition and lead fishign weights in Canada. Canadian Wildlife Service. Occasional Paper No. 88 (3-54).

Schroeder, H. A. and M. Mitchener. 1975. Life-term studies in rats: effects of aluminum, barium, beryllium, and tungsten. J. Nutr. 105:421-427.

Selle, R. M. 1942. Effects of subcutaneous injections of sodium tungstate on the rat. Fed. Proc. 1:165.

Slauson, D. O. and B.J. Cooper. 1990. Disease at the cellular level. In Mechanisms of Disease. A Textbook of Comparative General Pathology (J. W. Pine, Jr., Ed.) pp.72-75. Williams and Wilkins, Baltimore.

Standen, A. 1970. Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 22. John Wiley and Sons, New York.

Stokinger, H. E. 1978. Tungsten, W. In Patty's Industrial Hygiene and Toxicology, Vol. IIA (G. D. Clayton and F. E. Clayton, Eds.) pp.1981-1994. John Wiley and Sons, New York.

Sturkie, P.D. 1976. Avian Physiology. 3rd Edition. Springer-Verlag, New York. 400 pp.

Teekell, R. A. and A.B. Watts. 1959. Tungsten supplementation of breeder hens. Poultry. Sci. 38:741-794.

Thomas, V.G. 1997. Attitudes and issues preventing bans on toxic lead shot and sinkers in North America and Europe. Env. Values 6:185-199.

Underwood, E.J. 1971. Trace Elements in Human and Animal Nutrition. 3rd Edition. Academic Press, New York and London. 543 pp.

United States Department of the Interior. 1986. Final supplemental environmental impact statement: use of lead shot for hunting migratory birds in the United States. U.S. Fish and Widl. Serv. Off. Migr. Bird Manage., Washington, D.C. 549 pp.

United States Environmental Protection Agency. 1986. Method 7421: Atomic absorption furnace technique, revision 0. EPA-SW-846/Vol.1A, Chp. 3, Sect 3.3. Environmental Protection Agency, Washington, D.C.

United States Environmental Protection Agency. 1991. Method 200.3: Sample preparation procedure for spectrochemical determination of total recoverable

elements in biological tissues, revision 1.0. EPA-600/4-91-010. Environmental Protection Agency, Washington, D.C.

United States Environmental Protection Agency. 1996. Method 6010: Inductively coupled plasma atomic emmission spectometry, revision 2.0. EPA-SW-836/Vol.1A, Chp. 3, Sect. 3.3. Environmental Protection Agency, Washington, D.C.

Wase, A. W. 1956. Absorption and distribution of radio-tungstate in bone and soft tissues. Arch. Biochem. Biophys. 61:272-277.

Wobeser, G.A. 1981. Lead and other metals. In Diseases of Wild Waterfowl. (G.M. Wobesor, Ed). Pp. 151-159. Plenum Press, New York and London.