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HEALTH EFFECTS FOLLOWING CHRONIC DOSING WITH TUNGSTEN-IRON AND TUNGSTEN-POLYMER SHOT IN ADULT GAME-FARM MALLARDS

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ABSTRACT: Permanent approval of shot composed of tungsten-iron and tungsten-polymer for waterfowl hunting by the U.S. Fish and Wildlife Service was pending the results of the present study that examined the health and reproductive effects of the two shot types on mallards (Anas platyrhynchos) over a 150-day period. We collected data pertaining to the effects of tungsteniron and tungsten-polymer shot on mortality, body weight, organ weight, tissue pathology, and shot erosion. Thirty-two bird groups (sexes equal) of adult mallards were dosed orally with eight #4 steel shot (control), eight #4 tungsten-iron shot, or eight #4 tungsten-polymer shot on days 0, 30, 60, 90, and 120 of a 150-day trial (26 January 1998 to 25 June 1998). An additional 12 mallards (sexes equal) were dosed orally with eight #4 lead shot (positive control) on day 0 of the study. All lead-dosed ducks died by day 25, whereas no ducks died in the other treatment groups. Significant liver hemosiderosis was present in all control and tungsten-iron-dosed males, in five of eight control and three of eight tungsten-iron-dosed females, and in one tungsten-polymerdosed male examined. The rate of shot erosion was highest for tungsten-polymer shot (99%), followed by tungsten-iron (72%), and steel (55%) shot. Tungsten-iron or tungsten-polymer shot repeatedly administered to adult mallards did not have deleterious health effects during the 150day trial based on mortality, body weights, organ weights, and histology of the liver and kidneys.

Key Words: Anas platyrhynchos, experimental study, mallard, nontoxic shot alternative, toxicity, tungsten-iron shot, tungsten-polymer shot.

INTRODUCTION

Lead shot was preferred for waterfowl hunting for many years because of lead's widespread availability, low price, ease of manufacturing, and chemical stability (Thomas, 1997). However, the primary cause of lead poisoning in wild waterfowl has been the ingestion of shotgun pellets (Friend, 1987). Since first reported by Grinnell (1894), lead poisoning in waterfowl has been documented extensively in every North American waterfowl flyway (Bellrose, 1959; Wobeser, 1981; Sanderson and Bellrose, 1986; Friend, 1987). By the mid-1900's, annual losses of waterfowl attributed to lead poisoning were estimated to be 2-3% of the fall populations in North America (Bellrose, 1959). The United States banned the use of lead shot for waterfowl hunting in 1991.

Steel and bismuth shot are used as nontoxic alternatives to lead, but efforts continue to develop shot compositions that better emulate the ballistic characteristics of lead. 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 approved conditionally for waterfowl hunting by the U.S. Fish and Wildlife Service (USFWS, Washington, D.C., USA) in 1997 based partly on a 30-day acute toxicity trial utilizing mallards (Anas platyrhynchos) (Kelly et al., 1998). That study indicated tungsten-iron or tungsten-polymer shot did not affect game-farm mallards adversely. In order for tungsten shot to be permanently approved by the USFWS, a chronic toxicity test that included assessing effects on mallard reproduction as documented in USFWS 50 CFR Part 20.134, Migratory Bird Hunting: Nontoxic Shot Approval Procedure (Federal Register, 1986) was required.

In the present report, we summarize the

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health effects of long-term periodic exposure of mallards to two candidate shot types composed of 55% tungsten and 45% iron, and 95.5% tungsten and 4.5% of the polymer nylon 6. We assessed health effects by determining mortality, changes in body weights and organ weights, and gross and histological changes in the liver, kidneys, and gonads. Hematological and reproductive effects and metal residue concentrations are reported elsewhere (Mitchell et al., 2001a, b).

MATERIALS AND METHODS

The study design was based on a published protocol (Federal Register, 1986) and modified as requested by the USFWS. The Michigan State University All University Committee on Animal Use and Care (East Lansing, Michigan, USA) approved the final protocol.

Fifty-four male and 54 female 5-mo-old game-farm mallards (two generations removed from wild stock) with plumage and body conformation resembling wild mallards were obtained from Whistling Wings, Inc. (Hanover, Illinois, USA). Ducks were housed randomly as male-female pairs in a minimally heated pole barn in cages measuring 0.914 m long \times 0.914 m wide \times 0.457 m high from 30 December 1997 to 25 June 1998 at Michigan State University's (MSU) Poultry Science Research and Teaching Center (East Lansing, Michigan, USA). The 26-day acclimation period was from 30 December 1997 to 25 January 1998.

We provided feed and water ad libitum throughout the trial. We fed a pelleted duck grower ration (Purina Mills, St. Louis, Missouri, USA) during the acclimation period, shelled corn during the first 60 days of the trial (26 January 1998 to 27 March 1998), and a layer mash (Mazuri, Brentwood, Missouri, USA) during the subsequent 90-day reproduction phase of the trial (28 March to 25 June 1998). The temperature, which was continuously monitored, was maintained above 0 C by a propane gas heater suspended from the ceiling in the middle of the room. Photoperiod was controlled by a timer on incandescent lights and maintained at 8 hr light:16 hr dark through the first 60 days of the trial and increased in increments over six weeks to 18 hr light:6 hr dark during the last 90 days of the study.

Pairs of ducks were assigned randomly to one of four treatments (shot types); steel (100% iron), lead (97% lead, 3% antimony), tungsteniron (55% tungsten, 45% iron), and tungstenpolymer (95.5% tungsten, 4.5% nylon 6). The

USFWS considered the steel-dosed mallards (hereafter referred to as controls) as the negative control group and the lead-dosed ducks as the positive control group. On days of dosing, each duck was weighed to the nearest gram. Ducks dosed with steel, tungsten-iron, and tungsten-polymer (16 pairs each) received eight #4 pellets on days 0, 30, 60, 90, and 120 and ducks dosed with lead (6 pairs) received eight #4 pellets on day 0. Pellets were administered as described previously (Kelly et al., 1998).

We observed all ducks twice daily for general well-being. Clinical signs including inappetence, apparent weight loss, ataxia, lethargy, and discolored excreta were recorded. Ducks that died before day 150 were weighed and taken to the MSU Animal Health Diagnostic Laboratory (East Lansing, Michigan, USA) for necropsy. Approximately 10 days after each dosing, we transported the ducks to the MSU Large Animal Veterinary Clinic (East Lansing, Michigan, USA) for fluoroscopy to verify the presence of pellets in the gizzard.

On day 150 of the trial, all surviving mallards were weighed, killed by cervical dislocation, and subjected to necropsy. We opened gizzards for inspection of mucosal lining and presence of shot. We counted and weighed the shot in each gizzard for determination of shot retention and shot erosion, respectively. Shot retention could not be determined from the radiographs because it was difficult to count individual pellets after the second dosing on day 30. Shot erosion was determined by dividing initial average individual pellet weight by final average individual pellet weight. The identification of recovered pellets as to the day of dosing could not be done because of the large number of doses. We removed and weighed the brain, gizzard, heart, liver, spleen, kidneys, and testes or ovary. We collected and stored samples of the liver, kidneys, and testes/ovary from each duck in 10% formalin-saline solution (10% formalin in 0.9% sodium chloride) for histopathology. Liver, kidney, and ovary/testes samples from 16 (sexes equal) mallards in the control, tungsten-iron, and tungsten-polymer groups and from the 12 mallards in the lead group were assessed without knowledge of treatment. Tissues were embedded in paraffin, trimmed to 8 μm, and stained with hematoxylin and eosin. Selected liver sections from control, tungsteniron-, and tungsten-polymer-dosed mallards were stained with Prussian blue for determination of iron pigment (Mallory, 1942).

All statistical analyses were performed using SAS® software (SAS; Statistical Analysis Systems, Release 6.12, Cary, North Carolina, USA). Since all lead-dosed mallards died by

day 25 of the 150-day trial, their data were not included in the statistical analyses. Body weights were analyzed by analysis of variance (ANOVA) involving the factors treatment and sex, with repeated measurements on ducks, 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 ducks, as residuals involving measurements taken at adjacent time periods are more likely to be highly correlated than measurements taken further apart in time (Gill, 1990). Where applicable, all two-way interactions between treatment, sex, and days were modeled. Body weights were analyzed separately over two time periods due to changes in reproductive status over these periods. Body weight differences from day 0 to day 60 were analyzed with mean weight differences compared among the three treatment groups. Next, body weight differences were analyzed over the time period that ducks were reproductively active (day 90 through day 150). Organ weights and percent shot erosion were also analyzed using an ANOVA involving the factors treatment and sex. Percent shot erosion and adult relative organ weights were percentage (p) data subjected to arcsine, square root transformation $[\vec{x} = \sin^{-1}(\sqrt{p})]$ prior to statistical analysis. As standard errors are not readily back-transformed, the reported means and 95% confidence intervals for treatment means of percent shot erosion and adult relative organ weights were back-transformed $[p = (\sin(x))^2]$ to the scale of observation. Residual plots were used to check for homogeneity of variance and for aberrant values. Treatment group means were reported as the least squares mean plus or minus the standard error. 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 mean differences were based on pooling information over the sexes and/or days. To control for experimental Type 1 error rates, a Fisher's protected least significant difference (LSD) was used to test comparisons between means based on the total number of pairwise comparisons. In the following sections reference to significant differences (higher or lower) across compared values indicate statistical differences at $P \le 0.05$.

RESULTS

All mallards dosed with lead shot died within the first 25 days of the 150-day trial. Average time to death was 16.7 days for males and 11.0 days for females; range of

Table 1. The effect of treatment shot on body weight (g) of mallards from day 0 to day 60 and from day 90 to day 150 of a 150-day dosing test. $^{\rm a}$

	Body weight			
Treatment	Day 0-day 60	Day 90-day 150		
Control	987 ± 18.6	$1,081^{A} \pm 17.8$		
Tungsten-iron	$1,048 \pm 18.6$	$1{,}132^{AB} \pm 17.8$		
Tungsten-polymer	$1,040 \pm 18.6$	$1,167^{\mathrm{B}} \pm 17.8$		

^a Data are presented as mean \pm standard error of the mean. Sample size is 32 for all groups. Means with different capital letter superscripts are significantly different within the column (P < 0.05).

9 to 25 days for both sexes. No ducks in the control, tungsten-iron-, or tungstenpolymer-dosed groups died during the 150-day trial.

Only lead-dosed mallards had obvious clinical signs of poisoning during the trial. Green-stained excreta was apparent in these ducks within 24 hr of dosing. By day 5, all lead-dosed mallards had marked tail and wing droop. Prior to death, ducks were emaciated, lethargic, and ataxic.

The average weight loss of ducks that died was 61%. There was no evidence of significant differences in body weight among the control, tungsten-iron, and tungsten-polymer groups from day 0 through day 60. Tungsten-polymer-dosed mallards were significantly heavier than control mallards from day 90 through day 150 (Table 1).

Breast muscle of all lead-dosed mallards was atrophied severely and there was minimal subcutaneous or abdominal fat. The gizzard of six lead-dosed ducks (three males, three females) had a discolored mucosal lining. The vent area of two male and one female lead-dosed mallards was stained with bile and the gallbladder of one male and two female ducks was enlarged. One lead-dosed female had urate crystals surrounding the heart, while one 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 (Mitchell et al., 2001b), two control and three tungsten-polymer-dosed

TABLE 2. The effect of treatment shot on organ weights expressed as percent body weight of mallards on a 150-day dosing test.^a

Treatment	Spleen	Gizzard
Control	0.042^{AB}	2.20 ^A
	(0.0369 – 0.0482)	(2.084 – 2.324)
Tungsten-iron	0.049^{A}	2.13^{AB}
	(0.0428 - 0.0550)	(2.017 - 2.253)
Tungsten-polymer	0.038^{B}	1.98 ^B
	(0.0328 - 0.0436)	(1.867-2.094)

^a Data are presented as means (95% confidence intervals). Sample size for all parameters is 32. Means with different capital letter superscripts are significantly different within the column (P < 0.05).

females did not lay any eggs. One control female had a small egg blocking the lumen of the magnum and the other control duck had scar tissue obstructing the oviduct. There was egg yolk peritonitis in two tungsten-polymer-dosed ducks, whereas the third tungsten-polymer-dosed female appeared normal. All other ducks in the control, tungsten-iron-, and tungsten-polymer-dosed groups appeared normal except for one tungsten-polymer female that had a fibrous tag on a focal area of the liver.

There were no significant differences in relative (expressed as a % of body weight) heart, kidney, brain, liver, and testis or ovary weights among the control, tungsteniron, and tungsten-polymer groups. Tungsten-iron-dosed ducks had significantly higher relative spleen weights than tungsten-polymer-dosed ducks. The relative gizzard weight of tungsten-polymer-dosed mallards was significantly lower compared to controls (Table 2).

All lead-dosed mallards except one female had renal nephrosis ranging from mild to moderate and all lead-dosed mallards had mild to moderate liver biliary stasis. Mallards dosed with steel, tungsteniron, or tungsten-polymer shot that were examined had normal kidneys and no indication of liver biliary stasis. All of the control and tungsten-iron-dosed males and one tungsten-polymer-dosed male examined had liver hemosiderosis (deposition of iron in the form of hemosiderin) ranging

from mild to moderate. Liver hemosiderosis ranging from mild to moderate was apparent in five of eight control and three of eight tungsten-iron-dosed females. Mallards dosed with steel, lead, tungsten-iron, or tungsten-polymer shot had diffuse hepatocellular vacuolation. The testes and ovary were inactive in the lead-dosed mallards and appeared normal in the control, tungsten-iron-, and tungsten-polymerdosed ducks that were examined.

Approximately 90% of the lead pellets administered were recovered when the ducks were necropsied between day 9 and day 25 of the trial. Erosion of lead shot was 18% (data not shown). Over half of the steel pellets, approximately 40% of the tungsten-iron pellets, and less than 3% of the tungsten-polymer pellets were recovered on day 150 (Table 3). Erosion of steel, tungsten-iron, and tungsten-polymer shot was 55%, 72%, and 99% respectively. Fluoroscopy of the mallards during the trial substantiated the relatively rapid erosion of the two types of tungsten shot compared to steel shot (Fig. 1).

DISCUSSION

Only the lead-dosed ducks died during the 150-day trial (100% mortality by 25 days of dosing). These results agree with those of other studies (Jordan and Bellrose, 1950, 1951; Grandy et al., 1968; Longcore et al., 1974; Sanderson et al., 1992; Kelly et al., 1998) in which lead-dosed ducks maintained on corn were particularly susceptible to the toxic effects of lead. No duck dosed with steel, tungsten-iron or tungsten-polymer shot died. In our previous study, in which game-farm mallards were dosed with eight BB's of tungsten-iron or tungsten-polymer shot, no mortality was recorded during the 30-day trial (Kelly et al., 1998). No mortality was reported in mallards dosed with 12 to 17 pellets composed of 39% tungsten, 44% bismuth, and 16% tin, after 32 days (Ringelman et al., 1993).

Tungsten has been reported to cause mortality in birds. Broiler cockerels (*Gal-lus domesticus*) dosed with soluble sodium

Treatment	Number of pellets admin- istered	Initial individual pellet wt. (g)	Number of pellets recovered/bird	Final individual pellet wt. (g)	Percent shot erosion
			Males		
Control	40	0.152 ± 0.0003	24.4 ± 1.21	0.077 ± 0.0033	49.5 ^A (44.9–54.1)
Tungsten-iron	40	0.208 ± 0.0003	20.0 ± 1.21	0.075 ± 0.0033	63.8 ^B (59.3–68.2)
Tungsten-polymer	40	0.186 ± 0.0003	0.6 ± 1.21	0.004 ± 0.0033	$\begin{array}{c} 99.7^{\mathrm{C}} \\ (99.0100.0) \end{array}$
			Females		
Control	40	0.152 ± 0.0003	23.0 ± 1.21	0.061 ± 0.0033	60.0 ^A (53.6–65.8)
Tungsten-iron	40	0.208 ± 0.0003	11.4 ± 1.21	0.043 ± 0.0033	80.3 ^B (75.1–84.9)
Tungsten-polymer	40	0.186 ± 0.0003	1.3 ± 1.21	0.006 ± 0.0033	99.1 ^C (97.5–99.9)

Table 3. Number of pellets recovered and percent erosion of shot in male and female mallards on a 150-day dosing test.^a

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 exhibited 40% mortality by day 29 (Nell et al., 1980). However, the toxicity of tungsten is dependent on the solubility of the form administered. Soluble forms, such as sodium tungstate, are considerably more toxic than the insoluble tungsten metal (Kinard and Van de Erve, 1940; Frederick and Bradley, 1946).

Lead-dosed mallards were the only ducks that had obvious clinical signs, which were characteristic of lead poisoning (Wobeser, 1981; Friend, 1987; Locke and Thomas, 1996). Mallards dosed with steel, tungsten-iron, and tungsten-polymer shot appeared normal throughout the 150-day trial. These results agree with those reported by Kelly et al. (1998) and Ringelman et al. (1993). Nell et al. (1980) reported that clinical signs in chickens administered tungsten were anorexia, reduced weight gain, diarrhea, and labored breathing before death.

The body weight loss of lead-dosed mal-

lards (61%) was in the range for waterfowl that die of chronic lead poisoning (Sanderson and Irwin, 1976; Sanderson et al., 1992). Body weights in the control, tungsten-iron, and tungsten-polymer groups changed little over the 150 day period. In short-term studies (30–32 days), mallards dosed with tungsten-containing shot gained a similar amount of weight as control and non-dosed mallards (Ringelman et al., 1993; Kelly et al., 1998).

Gross lesions in lead-dosed mallards were similar to those reported previously (Slauson and Cooper, 1990; Alden and Frith, 1991; Popp and Cattley, 1991; Kelly et al., 1998). Gross abnormalities in the reproductive tract of two control and two tungsten-polymer-dosed females were probably responsible for their failure to lay eggs during the 90-day reproductive trial (Mitchell et al., 2001b). The lack of gross changes in mallards dosed with tungsteniron and tungsten-polymer shot agrees with findings reported by Ringelman et al. (1993) and Kelly et al. (1998), although ex-

^a Data for shot weight are presented as mean \pm standard error of the mean. Data for shot erosion are presented as mean (95% confidence interval). Sample size is 16 for all groups. Percent shot erosion was determined by dividing mean initial individual pellet weight by the mean final individual pellet weight. Means with different capital letter superscripts are significantly different within the column (P < 0.05).

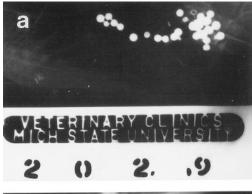






FIGURE 1. Radiographs of steel (a), tungsten-iron (b), and tungsten-polymer (c) pellets in the proventriculus/ventriculus of mallards. The radiographs were taken 10 days after the final administration of eight pellets (40 pellets total). The numbers refer to the duck's individual identification numbers. Notice the extent of erosion of tungsten-iron and particularly tungsten-polymer pellets compared to steel pellets. Bar = $10 \, \text{mm}$.

posure periods in these studies were considerably shorter than in the present study.

The lower relative gizzard weight of tungsten-polymer dosed mallards compared to controls was not considered to be deleterious since the gizzards appeared normal upon gross examination. Kelly et al. (1998) reported no differences in relative organ weights among mallards dosed with steel, tungsten-iron, or tungsten-polymer shot.

Microscopic renal lesions (acute tubular necrosis or nephrosis) were found only in lead-dosed ducks. Acute tubular nephrosis is associated with lead toxicosis in many animal species (Alden and Frith, 1991). The absence of renal lesions in the control, 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 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. The degree of biliary stasis was graded, and only the leaddosed group had detectable biliary stasis. Hemosiderosis was found only in the steel and tungsten-iron groups with the exception of one male from the tungsten-polymer group. Hemosiderosis commonly occurs when ducks are fed iron-containing shot (Locke et al., 1967). Rozman et al. (1974) determined that iron shot-induced hemosiderosis did not cause hepatic damage. Additionally, intrahepatocellular fatty vacuolation was present in ducks in each of the four experimental 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 leaddosed mallards were inactive and no histologic lesions were found. The testes and ovary from control, tungsten-iron, and tungsten-polymer groups were normal.

Recoveries of steel, tungsten-iron, and

tungsten-polymer shot were 59%, 39% and 2% respectively. The low recoveries of tungsten-iron and tungsten-polymer shot were also expected because of their high erosion rates (72% and 99%, respectively). These results were substantiated during fluoroscopy of ducks in that steel pellets were readily visible while the tungsteniron and particularly the tungsten-polymer pellets were often difficult to see because of disintegration. In a 30-day study utilizing the same shot types, Kelly et al. (1998) reported similar results in that erosion rates for steel, tungsten-iron and tungstenpolymer shot were 33%, 55%, and 80%, respectively.

In summary, male and female mallards administered eight #4 tungsten-iron or tungsten-polymer shot at 30-day intervals over 150 days were not adversely affected based on the variables measured. All tungsten-dosed ducks survived the 150 day trial with little change in body weight. The ducks appeared normal at the time of necropsy on day 150 and there was no evidence of mean differences in relative organ weights compared to controls. Liver hemosiderosis, which commonly occurs when ducks are fed iron-containing shot, was present in both tungsten-iron-dosed and control ducks. Essentially all of the tungsten-polymer shot eroded (99%) and the erosion rate for the tungsten-iron shot at 72% was higher than the erosion rate for steel shot (55%).

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