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

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ABSTRACT: Tungsten-iron and tungsten-polymer shot were given conditional approval for waterfowl hunting by the U.S. Fish and Wildlife Service based partly on the results of a 30-day acute toxicity trial utilizing mallards (*Anas platyrhynchos*). Final approval of the two tungsten-containing shot was contingent on the results of a 150-day study that assessed the health and reproductive effects of tungsten-iron and tungsten-polymer shot in adult mallards. Reproductive data are presented in this paper. Sixteen male and 16 female 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). Reproductive performance was assessed during the last 90 days (day 61 to day 150) of the trial. There were no significant differences in egg production and fertility and hatchability of eggs from tungsten-iron- and tungsten-polymer-dosed ducks compared to control ducks. There was no evidence of differences in percent survivability and body weight of ducklings from tungsten-iron and tungsten-polymer mallards compared to ducklings from control ducks. Tungsten-iron or tungsten-polymer shot repeatedly administered to adult mallards during the 150 day trial did not adversely affect reproduction or their offspring.

Key words: *Anas platyrhynchos*, duckling survivability, experimental study, mallard, non-toxic shot alternatives, reproduction, toxicity, tungsten-iron shot, tungsten-polymer shot.

INTRODUCTION

Steel shot was the first nontoxic alternative to lead shot for waterfowl hunting in the United States and bismuth shot was approved as the second alternative shot by the United States Fish and Wildlife Service (USFWS) in 1997 (Kelly et al., 1998). Tungsten-iron and tungsten-polymer shot were conditionally approved for waterfowl hunting in 1997 based in part on the results of a 30-day acute dosing study with game-farm mallards (*Anas platyrhynchos*) (Kelly et al., 1998). Kelly et al. (1998) dosed mallards with 8 BB's of tungsten-iron or tungsten-polymer shot and monitored them for 30 days. Results of the study indicated that tungsten-containing shot caused no adverse effects over the 30 day period. Toxicity was assessed by mortality, changes in body and organ weights, hematological effects, metal residues in the femur, liver, and kidneys, and histological effects. Prior to being permanently approved by the USFWS, the two types of tungsten shot had to be evaluated for their potential effects on mallard reproduction (Federal Register, 1986).

A 150 day study was conducted to determine the health and reproductive effects of tungsten-iron and tungsten-polymer shot in adult mallards. Health and hematological effects and metal residue concentrations are reported in Mitchell et al. (2001a, b).

In the present paper, we report the effects of repeated dosing of mallards with tungsten-iron or tungsten-polymer shot on reproduction and on duckling survivability. We assessed egg production, fertility and hatchability of eggs, egg weight, and shell thickness. We also determined duckling growth and survivability through 14 days of age, as well as duckling hematocrit (HCT), organ weights, and tissue metal residues.

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.

The study was conducted at the Michigan State University (MSU) Poultry Science Research and Teaching Center from 26 January 1998 through 25 June 1998. Sixteen male/female pairs of adult mallards purchased from Whistling Wings (Hanover, Illinois, USA) that were two generations removed from wild stock were individually dosed with eight pellets of #4 steel (100% iron), tungsten-iron (55% tungsten and 45% iron), or tungsten-polymer (95.5% tungsten and 4.5% nylon 6) shot on days 0, 30, 60, 90, and 120 of a 150-day trial. The USFWS considered the steel-dosed mallards as the negative control group (hereafter referred to as the control group). The dosing procedure is described in Kelley et al. (1998) and the husbandry is described in Mitchell et al. (2001a).

On day 61 (28 March 1998) of the 150-day trial, all surviving mallards were switched from a shelled corn diet to a commercial layer ration (Mazuri Waterfowl Breeder, Brentwood, Missouri, USA) for the subsequent 90 days. Photoperiod was increased on a weekly basis over 6 wks from 21 April 1998 to 1 June 1998 to provide 18 hr light:6 hr dark. Eggs were collected twice daily from each pair of mallards throughout the 90 day reproduction phase.

The 11th 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 six locations (two on the pointed end, two on the blunt end and two on the equator) on each egg with an Ames 25 ME Digimatic Outside Micrometer (Waltham, Massachusetts, USA) and the six measurements were averaged. Shells and egg contents were shipped to CT&E Environmental Services (Ludington, Michigan, USA) for analysis of metals as previously described (Mitchell et al., 2001b). The ranges of percent recoveries of iron and tungsten in the process spikes were 106 to 111% and 16 to 43%, respectively, and in the matrix spike, the ranges of percent recoveries of iron and tungsten were 101 to 111% and 52 to 72%, respectively.

Eggs, except the 11th egg from each hen, were set on a weekly basis and incubated with their blunt end up in a Petersime poultry incubator (Gettysburg, Ohio, USA) 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 two hours. Embryo fertility and viability were determined by candling eggs on incubation days 7, 14 and 21. All viable eggs were transferred on day 23 to pedigree hatching baskets that were placed in a Sure-pip hatcher (Agro Environmental Systems Inc., Dallas, Georgia, USA). The temperature in the hatcher was 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 opened and the embryos were examined for deformities and their age determined.

Ducklings were removed from the incubator (within 18 hr after hatching), weighed, and identified with a Swiftak identification tag (Heartland Animal Health, Inc., Fair Play, Missouri, USA). They were housed in heated floor pens (3.05 m W $\times$ 2.29 ml) with water and starter mash (Purina Mills, St. Louis, Missouri, USA) provided ad libitum.

At 14 days of age, each duckling was weighed and blood was collected from the brachial vein into microhematocrit capillary tubes (32 $\times$ 0.8 mm) (Drummond Scientific, Broomall, Pennsylvania, USA) for determination of HCT as previously described (Mitchell et al., 2001b). Ducklings from the first 21 eggs (except the 11th egg) from each hen, if available, were euthanized by cervical dislocation and necropsied. The brain, heart, liver, spleen, kidneys, and bursa were removed and weighed. Gonads were examined to determine sex. Samples of the liver and kidneys from eight male and eight female ducklings in each treatment were stored in 10% formalin-saline (10% formalin in 0.9% sodium chloride) to examine for histopathology. Histology sections were assessed without knowledge of treatment. The right femur and the remaining portions of the liver and kidneys from each necropsied duckling were frozen and shipped to CT&E Environmental Services (Ludington, Michigan, USA) for analysis of metals as previously described (Mitchell et al., 2001b). The samples analyzed included 16 (eight male and eight female) pooled liver, kidney, and femur samples, each consisting of tissues from three male or three female ducklings from the same hen in the control, tungsten-iron, or tungsten-polymer groups. The ranges of percent recoveries of iron and tungsten in the process spikes were 104 to 113% and 1 to 63%, respectively and in the matrix spike the ranges of percent recoveries of iron and tungsten were 84 to 106% and 71 to 110%, respectively.

All statistical analyses were performed using SAS® software (SAS; Statistical Analysis Systems, Release 6.12, Cary, North Carolina, USA). Duckling body weights, HCT, organ weights, and tissue concentrations of metal residues were analyzed by a two-way analysis of

TABLE 1. 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
Control	36	94^{AB}	61
	(24.2–49.5)	(81.6–99.7)	(41.4–79.5)
Tungsten-iron	46	99^{A}	78
	(33.2–59.4)	(90.4–99.5)	(59.0–91.7)
Tungsten-polymer	39	79^{B}	63
	(26.6–52.2)	(60.7–91.9)	(42.5–80.5)

[a] Data are presented as mean (95% confidence interval). Sample size is 14, 16, and 13 for the control, tungsten-iron, and tungsten-polymer, respectively. Percent egg production is the total number of eggs per hen divided by 90 days, % fertility is the number of fertile eggs divided by the number of eggs set, % hatchability is the number of eggs hatched divided by the number of fertile eggs. Means with different capital letter superscripts are significantly different within the column ($P < 0.05$).

variance (ANOVA) model involving the factors treatment and sex. Egg production, hatchability and fertility, egg weight, eggshell thickness, concentrations of metal residues in eggshell and egg contents, and duckling survivability were analyzed by a one-way ANOVA model involving the factor treatment. Residual plots were used to check for homogeneity of variance and for aberrant values. Egg production, hatchability and fertility, duckling survivability, and relative organ weights were percentage (p) data subjected to arcsine, square root transformation [$x = \sin^{-1}(\sqrt{p})$] prior to statistical analysis. The reported means and 95% confidence intervals for treatment means of duckling relative organ weights and egg production, hatchability, and fertility were back [$p = (\sin(x))^2$] transformed to the scale of observation. Treatment group means were reported as the least squares 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. 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 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, references to significant differences (whether higher or lower) across compared values indicate statistical differences at $P \leq 0.05$.

RESULTS

Tungsten-polymer-dosed females began laying eggs approximately seven days earlier than females in the control and tungsten-iron groups, which began laying around day 92 of the study. Females in all three groups required 24 to 25 days to lay 21 eggs. There were two control females and three tungsten-polymer-dosed females that did not lay any eggs. Of those ducks that laid eggs, there were one control female, two tungsten-iron-dosed females, and one tungsten-polymer-dosed female that did not lay at least 21 eggs.

Percent egg production (total number of eggs per hen divided by 90 days) was similar among groups and ranged from 36% to 46% (Table 1). Tungsten-polymer-dosed females had a significantly lower percentage of fertile eggs compared to tungsten-iron-dosed females, but percentage of eggs hatching from fertile eggs had no evidence of differences among the control, tungsten-iron, and tungsten-polymer groups.

Eggs laid by tungsten-iron-dosed females were significantly heavier than eggs laid by control or tungsten-polymer-dosed females and the shells of eggs laid by tungsten-iron-dosed females were significantly thicker compared to shells of eggs laid by controls (Table 2).

Iron was detected in two of 14, five of 16, and two of 13 shells of eggs laid by control, tungsten-iron-, and tungsten-polymer-dosed females, respectively, and in the contents of all eggs analyzed (Table 3).

TABLE 2. The effect of treatment shot on weight (g) and shell thickness (mm) of eggs from mallards on a 150-day dosing test.[a]

Treatment	Egg weight	Eggshell thickness
Control	$61.3^{A} \pm 0.24$ [552]	$0.372^{A} \pm 0.012$ [14]
Tungsten-iron	$62.7^{B} \pm 0.22$ [667]	$0.412^{B} \pm 0.011$ [16]
Tungsten-polymer	$61.2^{A} \pm 0.23$ [611]	$0.385^{AB} \pm 0.012$ [13]

[a] Data are presented as mean ± standard error of the mean. Numbers in brackets refer to sample size. Means with different capital letter superscripts are significantly different within the column ($P < 0.05$).

TABLE 3. The effect of treatment shot on concentrations (mg/kg dry weight) of iron and tungsten in the contents and shell of eggs from mallards on a 150-day dosing test.[a]

Treatment	Iron	Tungsten
	Eggshell	
Control	2.8 ± 2.11 [12]	ND
Tungsten-iron	3.0 ± 1.98 [11]	1.9 ± 0.27 [7]
Tungsten-polymer	3.5 ± 2.19 [11]	0.6 ± 0.30 [10]
	Egg contents	
Control	84.4 ± 5.22	ND
Tungsten-iron	78.4 ± 4.88	2.4 ± 0.59 [10]
Tungsten-polymer	70.2 ± 5.42	ND

[a] Data are presented as mean ± standard error of the mean. Sample size for the control, tungsten-iron, and tungsten-polymer is 14, 16, and 13, respectively. Numbers in brackets refer to the number of eggs having a concentration below detection limits. ND refers to not detected. Tungsten detection limit is 1.5 mg/kg dry weight.

There was no evidence of significant differences in iron concentration of the eggshell or contents among the three groups. Tungsten was detected in the shells of nine of 16 and three of 13 eggs laid by tungsten-iron- and tungsten-polymer-dosed females, respectively, and in the contents of six of 16 eggs laid by tungsten-iron-dosed females. There was no evidence of significant differences in tungsten concentration in the eggshell or contents.

Survivability of ducklings (the number of ducklings alive on day 14 of age divided by the number of hatchlings for each female) was equivalent for all three groups (Table 4). Body weight over the 14-day period also was similar for ducklings in the three groups. Hematocrit of ducklings in the tungsten-iron group was significantly lower than for ducklings in the control group.

Relative (expressed as a percent of body weight) kidney weight of ducklings in the tungsten-polymer group was significantly higher compared to ducklings in the control and tungsten-iron groups (mean [95% confidence interval]; 1.27 [1.245–1.290]; 1.20 [1.169–1.218]; 1.21 [1.194–1.232], respectively). Relative weights of the liver, spleen, bursa, heart, and brain were equivalent across the three groups.

Liver samples from ducklings in the control, tungsten-iron, and tungsten-polymer groups had mild to moderate diffuse hepatocellular vacuolation with the exception of samples from one female in the control group and one female in the tungsten-polymer group. No kidney lesions were observed.

Concentrations of iron in the femur, kidneys, and liver of ducklings were similar across the three treatment groups (Table 5). Tungsten was detected in four of 16 femur samples from both tungsten groups. Tungsten also was detected in two of 16 kidney samples from the tungsten-iron and one of 16 kidney samples from the tungsten-polymer group. Two of 16 liver samples from the tungsten-iron and tungsten-polymer groups contained tungsten. There was no evidence of significant differences in tungsten concentrations in femur, liver, and kidney samples among the three groups.

DISCUSSION

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. Individual variation could be the reason that four egg-laying females (one control, two tung-

TABLE 4. The effect of treatment shot on duckling survivability, body weight (g) from day 1 through day 14 of age, and hematocrit on day 14 of age.[a]

Treatment	% Survivability	Body weight	Hematocrit
Control	99	165 ± 2.1	40^{A} ± 0.2
	(96.31–100.00)		
	[339]	[156]	[156]
Tungsten-iron	98	165 ± 1.8	39^{B} ± 0.2
	(94.52–99.80)		
	[463]	[202]	[202]
Tungsten-polymer	96	167 ± 2.2	39^{AB} ± 0.3
	(90.31–98.97)		
	[396]	[135]	[135]

[a] Data for % survivability are presented as mean (95% confidence interval). Data for body weight and hematocrit presented as mean ± standard error of the mean. Numbers in brackets refer to sample size. Percent survivability is the number of ducklings alive on day 14 of age divided by the number of hatchlings for each female. Means with different capital letter superscripts are significantly different within the column ($P < 0.05$).

sten-iron-dosed, one tungsten-polymer-dosed) did not lay 21 eggs. The removal of eggs from incubating mallards generally causes continuation of egg laying whereas retention of the clutch will terminate laying. It is possible that the four females mimicked the behavior seen in wild mallards, which terminate egg-laying after producing a clutch of nine to 12 eggs (Alisanskus and Ankney, 1992). In the present study, egg-laying began on day 92, 92, and 85 of the 150-day study and the mean number of days required to lay 21 eggs was 25, 24, and 26 for the control, tungsten-iron-, and tungsten-polymer-dosed females, respectively. These results agreed with those of Sanderson et al. (1997) who reported that egg-laying in non-dosed mallards began on study day 84, on study day 94 for iron-dosed females, and on study day 92 for bismuth-dosed females. The mean range of days required to lay 21 eggs in the Sanderson et al. (1997) study was 26 to 27 days for the three groups.

TABLE 5. The effect of treatment shot on concentrations (mg/kg dry weight) of iron and tungsten in tissues of ducklings.[a]

Treatment	Iron	Tungsten
	Femur	
Control	118.8 ± 3.23	ND
Tungsten-iron	112.1 ± 3.23	1.1 ± 0.59 [12]
Tungsten-polymer	110.1 ± 3.23	1.7 ± 0.59 [12]
	Kidneys	
Control	242.5 ± 6.62	ND
Tungsten-iron	251.3 ± 6.62	1.2 ± 0.56 [14]
Tungsten-polymer	247.5 ± 6.62	0.4 ± 0.56 [15]
	Liver	
Control	463.8 ± 46.06	ND
Tungsten-iron	521.3 ± 46.06	0.6 ± 0.34 [14]
Tungsten-polymer	399.4 ± 46.06	0.6 ± 0.34 [14]

[a] Data are presented as mean ± standard error of the mean. Sample size is 16 for all groups. Numbers in brackets refer to the number of pooled samples having a tissue concentration below the detection limit. ND refers to not detected. Tungsten detection limit is 3 mg/kg dry weight.

In our study, ingestion of tungsten containing shot did not have an apparent effect on the rate of egg production, fertility, or hatchability. 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 sodium tungstate had no adverse effect on rate of egg production or hatchability. The slightly lower fertility of eggs laid by tungsten-polymer-dosed females may be because the females began laying before the males were reproductively active. Four of the 13 tungsten-polymer-dosed females that laid eggs did not begin to lay fertile eggs until after the 17th egg was laid. Sim-

ilarly, there were three control females that did not produce fertile eggs until after the 12th egg was laid.

The weight and shell thickness of eggs from tungsten-iron-dosed ducks were statistically higher compared to eggs from control and tungsten-polymer-dosed females, but the differences were not considered biologically relevant. In our study, the egg weights were 61.3, 62.7, and 61.2 g and shell thicknesses were 0.372, 0.412, and 0.385 mm for the eggs from the control, 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 g and shell thickness of 0.335, 0.338, and 0.335 mm for non-dosed, iron-dosed, and bismuth-dosed mallards, respectively.

Iron concentration in egg contents was highest in the control group, intermediate in the tungsten-iron-dosed group, and lowest in the tungsten-polymer-dosed group. Tungsten was detected in the shells and contents of eggs from tungsten-iron-dosed ducks and in the shells of eggs from tungsten-polymer-dosed ducks. The concentration of tungsten in the eggs followed the same trend as in the adult tissue samples (Mitchell et al., 2001b) in that tungsten was detected in nine shells of eggs from tungsten-iron-dosed females at a concentration higher than the concentration of tungsten detected in three 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; Bell and Sneed, 1970; Aamodt, 1975).

Tungsten-iron and tungsten-polymer shot had no adverse effects on survivability, body weight, or HCT of ducklings. The slightly, but significantly, lower HCT of tungsten-iron ducklings was not considered biologically relevant. Sanderson et al. (1997) reported that bismuth alloy shot caused no adverse effects on duckling survivability, body weight (day 7), or HCT.

Ducklings in the tungsten-polymer group had slightly, but significantly, greater relative kidney weights compared to ducklings in the other two groups. This difference was considered not to be biologically relevant.

The most common finding in the liver of ducklings, regardless of treatment, was mild to moderate hepatocellular vacuolation. 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 in the kidneys.

Iron concentration was highest in the liver, intermediate in the kidneys, and lowest in the femur samples. 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. Tungsten was detected in relatively few samples of the femur, kidneys, and liver from ducklings of tungsten-iron- and tungsten-polymer-dosed females.

In summary, male and female mallards administered 40 #4 tungsten-iron or tungsten-polymer shot and maintained for 150 days were not adversely affected in terms of reproduction based on the variables measured. Offspring of tungsten-dosed mallards had comparable survivability and growth over the first 14 days of age compared to control ducklings.

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