Journal of Wildlife Diseases, 37(3), 2001, pp. 459–467

HEMATOLOGICAL EFFECTS AND METAL RESIDUE CONCENTRATIONS FOLLOWING CHRONIC DOSING WITH TUNGSTEN-IRON AND TUNGSTEN-POLYMER SHOT IN ADULT GAME-FARM MALLARDS

R. R. Mitchell,[1] S. D. Fitzgerald,[2,6] R. J. Aulerich,[1,3] R. J. Balander,[1] D. C. Powell,[1] R. J. Tempelman,[1] C. Cray,[4] W. Stevens,[5] and S. J. Bursian[1,3]

[1] Department of Animal Science, [2] Department of Veterinary Pathology, and [3] Institute for Environmental Toxicology, Michigan State University, East Lansing, Michigan 48824, USA
[4] Division of Comparative Pathology, University of Miami School of Medicine, Miami, Florida 33136, USA
[5] Federal Cartridge Company, Anoka, Minnesota 55303, USA
[6] Corresponding author (email: fitzgerald@ahdlms.cvm.msu.edu)

ABSTRACT: The U.S. Fish and Wildlife Service required a chronic dosing study that assessed the health and reproductive effects of tungsten-iron and tungsten-polymer shot in adult game-farm mallards (*Anas platyrhynchos*) prior to granting permanent approval of the shot for waterfowl hunting. Herein, we present the effects of tungsten-iron and tungsten-polymer shot on various hematologic parameters and metal residue concentrations in the femur, liver, kidneys, and gonads. 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) received eight #4 lead shot (positive control) on day 0 of the study. Lead-dosed mallards had significantly decreased hematocrit, hemoglobin concentration, and whole-blood delta aminolevulinic acid dehydratase activity on day 7, as well as significant changes in a number of plasma chemistry parameters compared to ducks in the control, tungsten-iron, or tungsten-polymer groups. Mallards dosed with tungsten-iron or tungsten-polymer shot had occasional significant differences in hematocrit and plasma chemistry values when compared to control mallards over the 150 day period, but these changes were not considered to be indicative of deleterious effects. Low concentrations of tungsten were detected in gonad and kidney samples from males and females and in liver samples from females dosed with tungsten-polymer shot. Tungsten was also detected in femur samples from tungsten-polymer-dosed mallards. Higher concentrations of tungsten were detected in femur, liver, kidney, and gonad samples from tungsten-iron-dosed ducks. Tungsten-iron or tungsten-polymer shot repeatedly administered to adult mallards did not cause adverse hematological effects during the 150 day trial. Concentrations of tungsten in the femur, liver, kidneys, and gonads were generally higher in tungsten-iron-dosed ducks when compared to tungsten-polymer-dosed ducks.

Key words: *Anas platyrhynchos*, delta aminolevulinic acid dehydratase activity, experimental study, hematocrit, hemoglobin concentration, mallard, metal residues, nontoxic shot alternatives, plasma chemistry parameters, toxicity, tungsten-iron shot, tungsten-polymer shot.

INTRODUCTION

Prior to the decision to ban lead shot for waterfowl hunting, there were three general options that were considered as potential solutions to the problem of lead poisoning in waterfowl. These were (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) introduce regulations prohibiting the use of lead shot, combined with the use of alternative, nontoxic shot. 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 two options led to the search for affordable, nontoxic, ballistically-acceptable alternatives to lead (Scheuhammer and Norris, 1995).

Steel shot was the initial alternative to lead, because of its lack of toxicity, ready availability, and relatively low cost (U.S. Department of the Interior, 1986). However, 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.

Ammunition companies continue to search for alternative shot materials that are nontoxic and emulate the ballistic characteristics of lead shot. This continued effort resulted in bismuth shot being the first nontoxic alternative since steel shot to be permanently approved for waterfowl hunting by the United States Fish and Wildlife Service (USFWS, Washington D.C., USA) in 1997 (Kelly et al., 1998). Subsequent nontoxic alternatives to lead shot are tungsten-iron and tungsten-polymer shot, which received conditional approval for use from the USFWS in 1997 (Kelly et al., 1998). In order for the tungsten shot to receive permanent approval, a chronic toxicity test that included assessing effects on mallard (*Anas platyrhynchos*) reproduction as documented in USFWS 50 CFR Part 20.134, Migratory Bird Hunting: Nontoxic Shot Approval Procedure (Federal Register, 1986) was required.

We conducted a study to determine if exposure to tungsten-iron and tungsten-polymer shot caused deleterious effects on the health and reproduction of game-farm mallards over a 150–day period. The present report summarizes the effects of exposure to tungsten-iron and tungsten-polymer shot on hematological parameters and concentrations of metal residues in the femur, liver, kidneys, and gonads of adult game-farm mallards. Health and reproductive effects are reported elsewhere (Mitchell et al., 2001a, b).

MATERIALS AND METHODS

The study 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.

Sixteen male/female pairs of adult mallards (Whistling Wings, Hanover, Illinois, USA; two generations removed from wildstock) each were dosed orally 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). Six male/female pairs were administered by gavage eight pellets of #4 lead shot (97% lead, 3% antimony; positive control) on day 0. The dosing procedure was described in Kelly et al. (1998) and the husbandry was described in Mitchell et al. (2001a).

On day 7 of the trial, we collected blood from the brachial vein into two microhematocrit capillary tubes (Drummond Scientific, Broomall, Pennsylvania, USA), one 2-ml Vacutainer® tube (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA) containing EDTA and two 2 ml Vacutainer® tubes containing sodium heparin. Microhematocrit tubes were centrifuged in an IEC MB microhematocrit centrifuge (Needham, Massachusetts, USA) for 5 min. Hematocrit (HCT) was measured using an IEC MB microcapillary reader (Needham, Massachusetts, USA). We shipped cooled samples of whole-blood and samples of frozen plasma to the Division of Comparative Pathology, University of Miami School of Medicine (Miami, Florida, USA) for determination of hemoglobin (Hb) concentration, whole-blood delta aminolevulinic acid dehydratase (ALAD) activity, and plasma clinical chemistries as described in Kelly et al. (1998). On days 30, 60, 90, 120, and 150, we collected blood from all the ducks in the control, tungsten-iron, and tungsten-polymer groups for determination of HCT and from eight pairs in the same treatment group for determination of plasma chemistries.

On day 150 of the trial, all surviving ducks were killed by cervical dislocation and necropsied as described in Mitchell et al. (2001a). The right femur and portions of the liver, kidneys, and testes or ovary were frozen and shipped to

CT&E Environmental Services (Ludington, Michigan, USA) for analysis of iron and tungsten. Samples analyzed included: individual liver samples from eight males and eight females in the control, tungsten-iron, and tungsten-polymer groups and individual testis samples from the eight males in the control, tungsten-iron, and tungsten-polymer groups; 16 (eight male and eight female) pooled kidney and femur samples, each consisting of tissues from two ducks in the control, tungsten-iron, and tungsten-polymer groups and eight ovary samples each consisting of tissues from two female ducks in the control, 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 SW-836 Method 6010, revision 2.0 (U.S. Environmental Protection Agency, 1996). A matrix spike was prepared and analyzed with each digestion batch. The ranges of percent recoveries of iron and tungsten in the process spikes were 102 to 116% and 1 to 81%, respectively, and in the matrix spike, the ranges of percent recoveries of iron and tungsten were 79 to 186% and 78 to 98%, respectively.

Statistical analyses were performed using SAS® software (SAS; Statistical Analysis Systems, Release 6.12, Cary, North Carolina, USA). Hematocrit, Hb concentration, ALAD activity and plasma chemistries for ducks in all four treatment groups at day 7 were analyzed using a two-way analysis of variance (ANOVA) model involving the factors treatment and sex. Subsequent HCT and plasma chemistries for ducks in the control, tungsten-iron, and tungsten-polymer groups were analyzed by ANOVA involving the factors treatment and sex, with repeated measurements on ducks, over a third factor, days. All two-way interactions between treatment, sex, and days were also modeled. SAS® PROC MIXED was used to model a first-order autoregressive correlation structure for repeated measurements over days within animals, as residuals involving measurements taken at adjacent time periods are more likely to have a higher correlation than measurements taken further apart in time (Gill, 1990). The post-day 7 HCT and plasma chemistries were analyzed separately over two different time periods (days 30 through 60 and days 90 through 150) due to differences in the reproductive status of the ducks. Concentrations of metal residues in tissues of the adults were also analyzed using a two-way ANOVA model. 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 tissue metal residue concentrations of adults indicated increasing variability with higher responses; therefore those data were log transformed to stabilize variance. The reported means and 95% confidence intervals for treatment means of plasma chemistries and tissue metal residue concentrations were back (anti-log) transformed to the scale of observation. Treatment group means were reported as the least square 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 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

Ducks dosed with lead had significantly lower HCT, Hb concentration, and whole-blood ALAD activity at day 7 compared to ducks in the control, tungsten-iron, and tungsten-polymer groups. In contrast, ducks in the tungsten-polymer group had significantly higher ALAD activity than mallards in the other three treatment groups (Table 1). From day 30 to day 60, there was no evidence of significant differences in HCT among ducks in the control, tungsten-iron, and tungsten-polymer groups. Between day 90 and day 150, there was a significant treatment by sex interaction for HCT. There was no evidence of significant differences among HCT of males, but HCT of tungsten-polymer-dosed females (mean ± SE = 40 ± 0.8) was significantly lower than HCT of control (44 ± 0.8) and tungsten-iron-dosed females (44 ± 0.8).

There were a number of significant differences in plasma chemistry parameters at day 7 (Table 2). Alkaline phosphatase activity was significantly lower in lead-dosed ducks compared to ducks in the other three groups. Uric acid concentration

TABLE 1. 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
Control	$49^{A} \pm 0.7$	$15.6^{A} \pm 0.28$	$53.1^{A} \pm 2.86$
Lead	$26^{B} \pm 1.1$	$10.3^{B} \pm 0.46$	$25.2^{B} \pm 4.67$
Tungsten-iron	$50^{A} \pm 0.7$	$16.2^{A} \pm 0.28$	$45.5^{A} \pm 2.86$
Tungsten-polymer	$49^{A} \pm 0.7$	$15.6^{A} \pm 0.28$	$66.1^{C} \pm 2.86$

[a] Data are 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 × 12,500)/HCT. Means with different capital letter superscripts are significantly different within the column ($P < 0.05$).

and activities of alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase were significantly higher in lead-dosed ducks compared to ducks in the control, tungsten-iron- and tungsten-polymer-dosed groups. There was a significant treatment by sex interaction for creatine phosphokinase activity at day 7 (Table 3). Both lead-dosed males and females had significantly higher creatine phosphokinase activities compared to their counterparts in the other three groups.

Between days 30 and 60, tungsten-polymer-dosed ducks had significantly higher aspartate aminotransferase activity (U/L) than control ducks (31.7, 23.56–42.58 [mean, 95% confidence interval] versus 17.9, 13.29–24.02). There was a significant treatment by sex interaction for lactate dehydrogenase activity (U/L). Lactate dehydrogenase activity in tungsten-iron-dosed males was significantly lower compared to control males (826.6, 689.66–990.59 [mean, 95% confidence interval] versus 1111.4, 927.32–1331.95). From day 90 through day 150, there was no evidence of significant differences in plasma chemistries.

There was a significant treatment by sex interaction for iron and tungsten concentrations in the femur (Table 4). Iron concentrations in the femur of tungsten-polymer-dosed male and female mallards were significantly lower compared to control males and females, respectively. Tungsten was detected in all of the femur samples from the tungsten-iron-dosed males and females and in five of eight samples from tungsten-polymer-dosed females. Tungsten-iron-dosed females had a significantly higher femur tungsten concentration compared to tungsten-polymer-dosed females.

Iron concentrations in the testes were not significantly different among the three groups (Table 5). Tungsten was detected in the testes of five of eight tungsten-iron-dosed males and two of eight tungsten-polymer-dosed males. Tungsten-polymer-dosed female mallards had significantly lower concentrations of iron in the ovary compared to controls. Tungsten was present in six of eight ovary samples from tungsten-iron-dosed females and in one of eight samples from tungsten-polymer-dosed females (Table 5).

Tungsten was detected in two of 16 kidney samples from the control group, 13 of 16 kidney samples from the tungsten-iron-dosed ducks, and seven of 16 kidney samples from the tungsten-polymer-dosed ducks (Table 6). There was a significant treatment by sex interaction for iron concentration in the kidney samples. Tungsten-polymer-dosed males had a significantly lower concentration of iron in the kidneys compared to controls and tungsten-iron-dosed males. Tungsten-iron- and tungsten-polymer-dosed females had significantly lower concentrations of iron compared to the controls (Table 6).

Iron concentration in the liver of tungsten-polymer-dosed mallards was significantly lower compared to ducks in the other two groups. Tungsten was detected in

TABLE 2. The effect of treatment shot on plasma chemistry parameters of mallards on day 7 of a 150-day dosing test.[a]

Parameter	Units	Control	Lead	Tungsten-iron	Tungsten-polymer
Uric Acid	mg/dl	3.2^{A}	6.6^{B}	3.4^{A}	3.4^{A}
		(2.83–3.71)	(5.30–8.26)	(2.95–3.88)	(2.96–3.88)
Alkaline Phosphatase	U/L	67.1^{A}	29.7^{B}	61.5^{A}	49.0^{A}
		(55.55–81.07) [29]	(22.13–39.83)	(51.34–73.57)	(40.91–58.62)
Alanine Aminotransferase	U/L	3.6^{A}	33.9^{B}	3.1^{A}	3.0^{A}
		(3.10–4.17)	(26.66–43.22)	(2.65–3.57)	(2.59–3.48)
Aspartate Aminotransferase	U/L	17.9^{A}	71.4^{B}	22.1^{A}	20.9^{A}
		(15.44–20.82)	(55.92–91.13)	(19.40–25.68)	(18.00–24.27)
Lactate Dehydrogenase	U/L	956.7^{A}	$3{,}030.6^{B}$	$1{,}181.6^{A}$	$1{,}085.6^{A}$
		(801.27–1,142.30) [31]	(2,279.25–4,029.11)	(971.46–1,437.27) [26]	(911.87–1,292.46)

[a] Data are presented as mean (95% confidence interval). Sample size is 32 for all groups except lead, which is 12, unless indicated otherwise by the number in brackets. Means with different capital letter superscripts are significantly different within the row ($P < 0.05$).

the liver of all tungsten-iron-dosed mallards and in two of 16 tungsten-polymer-dosed mallards (Table 7).

DISCUSSION

Depressions in HCT, Hb concentration and ALAD activity are consistent with lead toxicity. 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 can be caused by inhibition of a number of enzymes including ALAD. ALAD is a key enzyme in the synthesis of heme, which is an integral component of hemoglobin (Goyer, 1996).

In our study, HCT, Hb concentration and ALAD activity were significantly depressed in lead-dosed mallards at day 7. These results agree with other studies in which mallards were exposed to lead. Pain and Rattner (1988) reported that HCT and Hb concentrations were significantly depressed in black ducks administered one #4 shot within six days of dosing, but recovery was apparent by 30 days post-dosing. ALAD activity was inhibited by 100% at one day post-dosing, increased slightly between three and nine days post-dosing (approximately 70% inhibition) and then declined again until the end of the 30-day study. Kelly et al. (1998) reported significant decreases in HCT and Hb concentration at day 15, but not at day 30 in mallards dosed with eight #4 pellets of lead shot. ALAD activity was also significantly depressed over the 30-day trial.

Since the inhibition of ALAD activity is 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 was not thought to be treatment related. Hematocrits measured during this time were

TABLE 3. The effect of treatment shot on plasma creatine phosphokinase activities of male and female mallards on day 7 of a 150-day dosing test.[a]

Parameter	Units	Control	Lead	Tungsten-iron	Tungsten-polymer
			Males		
Creatine Phosphokinase	U/L	144.3[A] (94.47–220.46) [15]	1,487.7[B] (761.36–2,907.36)	242.1[A] (158.46–369.78) [15]	135.8[A] (90.12–204.71)
			Females		
Creatine Phosphokinase	U/L	181.6[A] (145.79–226.24)	711.7[B] (497.15–1,018.82)	156.2[A] (125.40–194.59)	186.6[A] (149.81–232.46)

[a] Data are presented as means (95% confidence interval). Sample size is 32 for all groups except lead, which is 12, unless indicated otherwise by the number in brackets. Means with different capital letter superscripts are significantly different within the row ($P < 0.05$).

lower than hematocrits measured during the first 60 days of the trial when birds were not reproductively active. 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 eight #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).

The administration of lead shot caused

TABLE 4. 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	
Control	96.2[A] (87.99–105.24)	ND
Tungsten-iron	88.9[AB] (81.26–97.20)	23.5 (18.30–31.45)
Tungsten-polymer	81.0[B] (74.05–88.57)	ND
	Females	
Control	220.5[A] (181.80–267.35)	ND
Tungsten-iron	162.7[A] (134.17–197.31)	34.0[A] (28.60–40.33)
Tungsten-polymer	99.0[B] (81.61–120.00)	5.1[B] (4.12–6.36) [3]

[a] Data are presented as mean (95% confidence interval). Sample size is 8 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. Means with different capital letter superscripts are significantly different within the column ($P < 0.05$).

TABLE 5. The effect of treatment shot on concentrations (mg/kg dry weight) of iron and tungsten in the gonads of male and female mallards on a 150-day dosing test.[a]

Treatment	Iron	Tungsten
	Males	
Control	58.5 (44.57–76.69)	ND
Tungsten-iron	56.3 (42.90–73.79)	5.6 (3.13–9.99) [3]
Tungsten-polymer	67.8 (51.67–88.87)	7.0 (2.80–17.53) [6]
	Females	
Control	488.5[A] (347.55–686.53)	ND
Tungsten-iron	354.1[AB] (251.96–497.71)	8.4 (3.69–19.14) [2]
Tungsten-polymer	211.8[B] (150.71–297.71)	8.0 (1.06–60.09) [7]

[a] Data are presented as mean (95% confidence interval). Sample size is 8 for all groups. Numbers in brackets refer to the number of males or pooled samples of females having a tissue concentration below the detection limit. ND refers to not detected. Tungsten detection limit is 3.0 mg/kg dry weight. Means with different capital letter superscripts are significantly different within the column ($P < 0.05$).

TABLE 6. The effect of treatment shot on concentrations (mg/kg dry weight) of iron and tungsten in the kidneys of male and female mallards on a 150-day dosing test.[a]

Treatment	Iron	Tungsten
	Males	Sexes combined
Control	678.8[A]	4.0
	(591.87–778.39)	(1.94–8.24)
Tungsten-iron	563.1[AB]	[14]
	(491.01–645.73)	9.5
Tungsten-polymer	509.0[B]	(7.15–12.61)
	(443.81–583.67)	[3]
		6.8
		(4.46–10.48)
		[9]
	Females	
Control	825.0[A]	
	(731.64–930.27)	
Tungsten-iron	623.6[B]	
	(553.05–703.19)	
Tungsten-polymer	398.9[C]	
	(353.79–449.84)	

[a] Data are presented as mean (95% confidence interval). Sample size for iron and tungsten concentrations is 8 and 16, respectively. 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 3.0 mg/kg dry weight. Means with different capital letter superscripts are significantly different within the column ($P < 0.05$).

TABLE 7. The effect of treatment shot on concentrations (mg/kg dry weight) of iron and tungsten in the liver of mallards on a 150-day dosing test.[a]

Treatment	Iron	Tungsten
Control	10,128.4[A]	ND
	(7,486.75–13,701.56)	
Tungsten-iron	6,890.5[A]	70.4
	(5,093.67–9,322.01)	(50.69–97.71)
Tungsten-polymer	1,157.2[B]	NE
	(855.43–1,565.52)	[14]

[a] Data are presented as mean (95% confidence interval). Sample size is 16 for all groups. Numbers in brackets refer to the number of mallards having a tissue concentration below the detection limit. 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. Means with different capital letter superscripts are significantly different within the column ($P < 0.05$).

a number of changes in day 7 plasma chemistry values. The elevated concentration of uric acid 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 (March et al., 1976; Campbell and Coles, 1986). Renal tubular damage was documented in the kidneys from lead-dosed ducks in the present study (Mitchell et al., 2001a). 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 could be due to 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 were probably associated with hepatocellular damage (Campbell and Coles, 1986). Kelly et al. (1998) reported significant increases in plasma alanine and aspartate aminotransferase and lactate dehydrogenase activities in mallards dosed with lead shot. Lead-dosed male and female ducks had marked elevated activities of creatine phosphokinase, which has been reported to be associated with lead toxicity (Campbell and Coles, 1986, Kelly et al., 1998).

Tungsten-polymer-dosed mallards had a significantly increased plasma aspartate aminotransferase activity from day 30 through day 60 compared to controls. Since avian aspartate aminotransferase activity greater than 230 IU/L is considered abnormal (Campbell and Coles, 1986), the slight increase reported here was considered to be biologically irrelevant. The significant decrease in lactate dehydrogenase activity in tungsten-iron males from day 30 through day 60 was also considered to be biologically irrevelant because only increases in enzyme activity have been as-

sociated with hepatic abnormalities (Campbell and Coles, 1986).

Iron was detected in femur, gonad, kidney, and liver samples from the control, tungsten-iron-, and tungsten-polymer-dosed groups. In general, the concentration of iron was highest in the tissue samples from the tungsten-iron-dosed and control ducks and higher in females than males. The high concentration of iron in liver samples from control and tungsten-iron-dosed ducks agreed with the histological documentation of hemosiderosis (Mitchell et al., 2001a). Locke et al. (1967) dosed mallards with eight pellets of iron shot, which resulted in hemosiderosis of the liver and elevated hepatic iron concentrations. 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 five-fold increase in iron in the serum of ducks during the egg-laying season.

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). 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 comprised 55% and 95.5% of the tungsten-iron and tungsten-polymer shot, respectively. Tungsten was also detected in the kidneys of two control ducks. It was thought this was due to the normal variance expected 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, Ludington, Michigan, USA). In the tungsten-iron-dosed ducks, the number of tissue samples that tungsten was detected in and the concentration of tungsten in these samples were substantially greater compared to samples from tungsten-polymer-dosed ducks.

In summary, male and female mallards administered 40 #4 tungsten-iron and tungsten-polymer shot and maintained for 150 days were not adversely affected based on the variables measured. Of the two shot types tested, tungsten concentrations in selected tissues were lower in tungsten-polymer-dosed mallards.

ACKNOWLEDGMENTS

This research was funded by Federal Cartridge Company. Appreciation is expressed to A. Napolitano, A. Mitchell, M. Preisler, D. Mashek, and R. Sendow for their help with the study.

LITERATURE CITED

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

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. Comparative Biochemistry and Physiology 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.). E. and S. Livingstone, London, UK, pp. 70–72.

CAMPBELL, T. W., AND E. H. COLES. 1986. Avian clinical pathology. *In* Veterinary clinical pathology, E. H. Coles (ed.). W. B. Saunders, Philadelphia, Pennsylvania, pp. 279–301.

FEDERAL REGISTER. 1986. Nontoxic shot approval procedures for shot. Federal Register 51: 42098–42102.

GILL, J. L. 1990. Repeated measurements on animals: Why not assume autocorrelation? Journal of Animal Breeding and Genetics 107: 89–95.

GOYER, R. A. 1996. Toxic effects of metals. *In* Casarett and Doull's toxicology, C. D. Klaassen (ed.). McGraw-Hill, New York, New York, pp. 691–736.

KAYE, S. V. 1968. Distribution and retention of orally administered radiotungsten in the rat. Health Physics 15: 398–417.

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. Journal of Wildlife Diseases 34: 673–687.

KINARD, F. W., AND J. C. AULL. 1945. Distribution of tungsten in the rat following ingestion of tungsten compounds. Journal of Pharmacology and Experimental Therapeutics 72: 53–55.

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 Association 3: 143–147.

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. Journal of Wildlife Diseases 12: 14–19.

MITCHELL, R. R., S. D. FITZGERALD, R. J. AULERICH, R. J. BALANDER, D. C. POWELL, R. J. TEMPELMAN, R. L. STICKLE, W. STEVENS, AND S. J. BURSIAN. 2001a. Health effects following chronic dosing with tungsten-iron and tungsten-polymer shot in adult game-farm mallards. Journal of Wildlife Diseases 37: 451–458.

———, ———, ———, ———, ———, ———, ———, AND ———. 2001b. Reproductive effects and duckling survivability following chronic dosing with tungsten-iron and tungsten-polymer shot in adult game-farm mallards. Journal of Wildlife Diseases 37: 468–474.

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*. Bulletin of Environmental Contamination and Toxicology 40: 159–164.

RINGELMAN, J. K., M. W. MILLER, AND W. F. ANDELT. 1993. Effects of ingested tungsten-bismuth-tin shot on captive mallards. The Journal of Wildlife Management 57: 725–732.

ROZMAN, R. S., L. N. LOCKE, AND S. F. MCCLURE, III. 1974. Enzyme changes in mallard ducks fed iron or lead shot. Avian Diseases 18: 435–444.

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.

SCHEUHAMMER, A. M., AND S. L. NORRIS. 1995. A review of the environmental impacts of lead shotshell ammunition and lead fishing weights in Canada. Canadian Wildlife Service, Ottawa, Occasional Paper Number 88, Ontario, Canda, 54 pp.

STURKIE, P. D. 1976. Avian physiology. Springer-Verlag, New York, New York, 400 pp.

UNDERWOOD, E. J. 1971. Trace elements in human and animal nutrition. Academic Press, New York, New York, 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 Wildlife Service Office of Migratory Bird Management, Washington, D.C., 549 pp.

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.

———. 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. Archives of Biochemistry and Biophysics 61: 272–277.

Received for publication 21 February 2000.