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ACUTE TOXICITY OF LEAD, STEEL, AND AN IRON-TUNGSTEN-NICKEL SHOT TO MALLARD DUCKS (*ANAS PLATYRHYNCHOS*)

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ABSTRACT: Twenty mallards (*Anas platyrhynchos*) of both sexes were dosed by oral gavage with Heavi-Shot™ (H-S; Environ-Metal, Inc., Sweet Home, Oregon, USA) pellets, 20 with steel shot, and 10 with lead (Pb) pellets, all of equal size. All pellets were fired from a shotgun into an absorbent material, retrieved, and weighed prior to introduction into the ducks. Birds were fed whole kernel corn and grit and observed for signs of toxicity for 30 days following dosing. Hevi-Shot pellets lost an average of 6.2% of their mass and steel shot pellets lost 57% of their mass in the birds' gizzards. Almost all (90%) of the Pb shot dosed birds died before the end of the study, while no mortality was observed in the steel or H-S dosed groups. Even though total food consumption differed between the H-S and steel shot groups, mean bird weight change was not different. There were no significant morphologic or histopathologic abnormalities of the liver and kidney in the H-S and steel shot groups. Results indicated that mallards dosed orally with eight No. 4 H-S pellets were not adversely affected over a 30-day period, and that H-S provides another environmentally safe nontoxic shot for use in waterfowl hunting.

Key words: *Anas platyrhynchos,* lead shot, mallard, nontoxic shot.

INTRODUCTION

Lead (Pb) shot was banned for waterfowl hunting within the USA in 1991 (Ringleman et al., 1993; Kelley et al., 1998), and although it is declining in prevalence it can still be found in wetlands and other areas (Grasman and Scanlon, 1995). Lead poisoning continues to occur when waterfowl feed or obtain grit from the bottom of lakes, ponds, and marshes, although at a declining rate (Samuel and Frank, 2000). Birds ingest spent Pb shotgun pellets and fishing sinkers, which usually are retained in the gizzard where they are ground into small, easily absorbed particles (Kelley et al., 1998). Poisoning from Pb pellets has been implicated in many types of birds, from black ducks (*Anas rubripes*) and mallards (*A. platyrhynchos*) (Rattner et al., 1989), to Canada geese (*Branta canadensis*) and mute swans (*Cygnus olor*) (Simpson and Hunt, 1979; Windingstad and Hinds, 1987; O'Halloran et al., 1988), flamingos (*Phoenicopterus ruber*) (Mateo et al., 1997), bald eagles (*Haliaeetus leucocephalus*) (Jacobson et al., 1977; Hoffman et al., 1981) and spectacled and common eiders (*Somateria fischeri* and *S. mollissima,* respectively) (Franson et al., 1998).

Steel shot has been used as a substitute for Pb, but lacks the favorable ballistics of Pb shot. Various types of shot have been tested for toxicity to mallards (Bursian et al., 1997; Kelley et al., 1998). Several tungsten-containing shotgun shot have been tested for ballistics and toxicity. Tungsten-iron and tungsten-polymer shots did not adversely affect mallards during a 30 day acute effects trial (Kelley et al., 1998). Hevi-Shot™ (H-S; Environ-Metal, Inc., Sweet Home, Oregon, USA) is a commercially available shot that contains a mixture of tungsten (W), nickel (Ni), and iron (Fe). It is predicted that H-S will show no avian toxicity because studies with 100% W and steel shot and nickel-coated W shot have been shown to be nontoxic (USFWS, 1976; Bursian et al., 1997; Kelly et al., 1998).

This study was conducted to provide information to the US Fish and Wildlife Service on potential toxicity of H-S shot (US Fish and Wildlife Service, 2001) and gen-

erally followed approved test guidelines (US Fish and Wildlife Service, 1997). The objective of this study was to document corrosion and erosion of the candidate shot under worst-case conditions in vivo and to monitor any subsequent effects on mallard feed consumption, body weight, survival, and other health parameters as identified through blood hematology and biochemistry, as well as liver and kidney tissue pathology. The study was designed to determine whether shot can remain in a bird gizzard for up to 30 days without significant loss of pellet mass or deterioration of health of mallards. Potential for toxicity was compared to steel shot, the baseline for approval by the US Fish and Wildlife Service (1976, 2001). Lead shot-dosed birds were included as required by the US Fish and Wildlife Service (1997) for interlaboratory quality control purposes.

MATERIALS AND METHODS

The study was conducted with 50 hatchling year (4 to 6 mo old) mallards, 25 male and 25 female, obtained from Whistling Wings (Hanover, Illinois, USA). Upon arrival at the testing facility (Snow Camp, North Carolina, USA, 35.9°N, 79.4°W), the birds were quarantined and acclimated from 24 February to 27 March 2000. Birds were identified individually by numbered leg bands and were caged as pairs. Cages were 76 cm deep, 83 cm wide, and 46 cm maximum height, with 8% floor slope. The floor, tops, and sides of cages were polycarbonate-coated wire mesh. All cage support structures and feeders were stainless steel. Birds were housed in indoor study rooms maintained at an ambient temperature of approximately 15±5 C. The light schedule was 8 hr light and 16 hr dark.

Prior to the start of acclimation, each individual cage was numbered and the order of cage assignment of the ducks was randomly determined. The first male bird banded was placed into the first cage designated in the randomization. The second male banded went into the second designated cage, etc. The females were banded and assigned to a cage having a male matching closest in body weight. Body weights were measured to the nearest gram at the start of acclimation. Birds were in normal health during the acclimation.

Purina Game Bird Flight Conditioner (Ralston Purina, Inc., St. Louis, Missouri, USA) was initially fed during quarantine. During the acclimation period, birds were fed cracked corn and finally, by initiation of the experimental phase, were placed on a whole kernel corn diet (Lindley Mill, Snow Camp, North Carolina, USA). This diet was designed to simulate a winter diet, when birds would most likely be exposed to shot, and to provide a maximum challenge for nutrition-induced toxicity. Water from a well and feed were provided ad libitum except for a 16-hr fasting period just prior to dosing. Number 2 and smaller quartzite grit (Farm Services, Graham, North Carolina) was provided ad libitum. This provided the ducks with a hard diet that required grit for maximum utilization of the corn, and presented them the opportunity to ingest as much grit as they required. By allowing the birds to self select grit intake rate, the harsh grinding environment of the gizzard was maintained, thereby achieving the desired maximum challenge for pellet corrosion/erosion rates.

Cages of birds were randomly sorted into three treatment groups (H-S, steel, or Pb shot). The steel and H-S groups had equal numbers of males and females (n=10 for each sex). The Pb group had 10 birds (n=5 for each sex). The experimental period was 27 March to 26 April 2000.

Test shot

Size 4 H-S pellets were fired from a standard shotgun into absorbent material, retrieved, and weighed to the nearest microgram prior to dosing. Commercial size No. 4 steel shot and Pb shot were purchased (Remington Arms Co., Inc., Madison, North Carolina) and fired and weighed in a similar fashion. Eight shot then were placed in a single gelatin capsule. Each bird received one capsule by oral intubation, to ensure that the shot were all placed into the lower portion of the crop.

During daily husbandry, fecal pans were cleaned of all material, then sieved, and searched for pellets visually and with magnets. The wastes were run through a hydraulic separator (Brewer, 1981) equipped with magnets to assure collection of iron-containing shot. Magnets placed around the floor drain increased the probability that any pellets that fell from the fecal pans were recovered. At the end of the test period, mallard gizzard contents also were run through the hydraulic separator to recover remaining shot.

Physiologic endpoints

Feed consumption within each cage of two birds was measured to the nearest gram at four

7-day intervals plus a final 2-day interval for a total of five time periods in the 30 day test. The weights were summed for total food consumption during the trial. Spillage of feed was not accounted for in this process because it was assumed to be independent of treatment.

Body weight of each bird was measured to the nearest gram at the beginning of the acclimation period, immediately prior to dosing, and at days 15 and 30 post-dosing.

Change in mass of the pellets before and after placement in the ducks was determined. Pellets were cleaned with water and examined under magnification to make sure that no food particles were adhering to the pellets. They were dried (approximately 38 C) for 24 hr prior to weighing.

Blood was collected 15 and 30 days postexposure by brachial venipuncture into heparinized glass evacuated tubes and stored at 4 C until submitted to the laboratory for testing. All tests were run within 48 hr of blood collection. Blood samples were submitted to Veterinary Laboratory Services of Rex Health Care (University of North Carolina, Chapel Hill, North Carolina) for hematology (packed cell volume [PCV], hemoglobin, total and differential white blood cell [WBC] counts), and plasma biochemistry (plasma protein, glucose, creatine kinase [CK], aspartate aminotransferase [AST], calcium, and uric acid) (Fudge, 2000).

All birds that survived until day 30 post dosing were examined for gross lesions at necropsy (n=20 for H-S and steel shot groups, and n=1 for Pb shot group). Histopathologic evaluations were conducted on liver and kidney sections from each bird and were evaluated without knowledge of the test group to which the sample belonged. Samples of blood, kidney, and liver from five male and five female birds from the H-S shot and the steel shot group were submitted to Frontier Geosciences, Inc. (Seattle, Washington, USA) for metals analysis by inductively coupled plasma–mass spectroscopy (US Environmental Protection Agency, 1996; Method 1638). Tissues from the Pb shot group were not chemically analyzed because it is well documented that lead is quickly passed via the blood to the liver and kidney (Pain, 1996). Minimum detection limits were the same for all tissues and reported as parts per million (ppm): Fe=3.0; Ni=0.01; Pb=0.01; W=0.03.

Statistical analyses

Feed consumption by the H-S and steel shot treatment groups was analyzed for mean differences by time period using a repeated-measures analysis of variance (ANOVA) blocked by cage. Post hoc comparisons between treatment groups within days were done by ANOVA. Total feed consumption over the 30-day period and percentage change in body weight of birds in the H-S and steel shot groups were tested for mean differences using a two-tailed Student's t-test. For each treatment group, total mass of pellets recovered from the ducks after 30 days was compared to the total mass gavaged into the ducks using a one-tailed Student's t-test to determine if significant loss of weight had occurred. These data also were examined to determine the average loss of mass per pellet.

Tissue residues of W, Ni, and Fe were compared between the H-S test group and the steel shot test group, using a one-tailed Student's t-test, to investigate whether H-S dosed birds had higher tissue residues for each metal than did steel dosed birds. Comparison of hematologic and plasma biochemistry values between H-S dosed birds and steel dosed birds were made using a two-tailed Student's t-test. Steel shot is considered nontoxic (US Fish and Wildlife Service, 1976); therefore, the steel-dosed birds were used as the baseline for comparisons. Parameters in the Pb dosed birds were measured as a laboratory quality control measure, to ensure that our results were similar to those conducted by other laboratories running similar studies. All statistics were conducted using Systat for Windows, version 5 (Systat, Evanston, Illinois).

RESULTS

Clinical signs

There were no clinical signs of toxicity or mortality in the H-S or steel shot groups. The Pb shot group had 90% mortality with the first death occurring 6 days post dosing. The Pb-dosed mallards, including the single male bird that survived, showed typical clinical signs of Pb poisoning, including loss of weight, bile-stained cloacal area, lethargy, and instability. By the end of the 30-day study the surviving duck had recovered substantially. Because of this high mortality, day 30 comparisons between the Pb group and other groups were not made.

Mean food consumption differed between the H-S and steel shot treatment groups over the duration of the study. Post hoc, one-way ANOVA indicated this was due to differences in food consumption days 21–28 post dosing (Table 1). Food

TABLE 1. Feed consumption (g/cage) by mallards throughout a 30-day trial with shot.[a]

Treatment group[b]	Days 0–7	Days 7–14	Days 14–21	Days 21–28	Days 28–30	Days 0–30
H-S	551 ± 42.6 (525–577)	450 ± 116 (378–522)	772 ± 117 (700–844)	831 ± 123[c] (755–907)	239 ± 66.3 (198–280)	2843 ± 273[c] (2,674–3,012)
Steel	605 ± 130 (525–685)	468 ± 227 (327–609)	783 ± 120 (708–858)	1,009 ± 170[c] (904–1,808)	292 ± 79.6 (243-341)	3,137 ± 262[c] (2,975–3,299)

[a] Mean ± standard deviation with 95% confidence limits in parenthesis. Sample size is 20 birds.
[b] H-S = Hevi-Shot pellets, Steel = steel pellets.
[c] Statistically significant difference between treatment groups ($P < 0.05$).

consumption increased in both groups of birds during this interval, although at a lesser rate of increase for the H-S birds than for the steel-dosed birds.

Mean body weight and variance within each treatment group (by sex) was equal (P=0.05) at the start of the study. The percentage change in mean male and female body weight in the H-S group during the trial was not different from birds in the steel shot group (Table 2). Lead-dosed birds lost weight and died prior to the end of the study period.

Recovery of shot

Shot recovery rate for the H-S and steel shot groups was 100%. However, a single steel pellet was accidentally lost, so only 159 of 160 steel pellets were available for analysis. Only seven (9%) of 80 lead pellets were recovered. Of the H-S pellets recovered, 26 had passed through the gastrointestinal tract and were found on magnets surrounding the floor drain or in feces under individual cages. The remaining H-S pellets (134) were removed from gizzards during postmortem examinations at the end of the study. Of 159 steel shot pellets recovered, 22 had passed through the gastrointestinal tract. The remaining pellets (137) were recovered at necropsy. Only seven expelled Pb pellets were found. Because Pb is not magnetic and often is ground down to very small fragments in the gizzard, some likely were flushed past the drain magnets.

The 134 H-S pellets recovered from gizzards had an average weight loss of 6.2% (Table 3). The mean weight loss of 137 steel pellets removed from duck gizzards

TABLE 2. Male and female mallard body weights (g) by treatment plus change (g) and percent change in body weight.[a,b]

Treatment group	Acclimation period	Day 0	Day 30	Change	Percent change
Male					
H-S	1,025 ± 64.4 (985–1,065)	1,090 ± 98.4 (1,029–1,151)	1,024 ± 103 (960–1,88)	−65.6 ± 62.8 (−104)–(9–27)	−5.9 ± 2.7 (−9.0)–(−3.0)
Steel	1,034 ± 35.5 (998–1,056)	1,121 ± 85.1 (1,084–1,158)	1,050 ± 78.5 (1,001–1,099)	−70.1 ± 52.8 (−103)–(−37)	−6.1 ± 0.3 (−9.0)–(−3.0)
Female					
H-S	978 ± 55.3 (944–1,012)	999 ± 108 (932–1,066)	974 ± 120 (900–1,048)	−45.8 ± 79.4 (−72)–(−16)	−2.6 ± 2.7 (−5.3–0.1)
Steel	988 ± 37.4 (965–1,011)	1,013 ± 122 (938–1,088)	997 ± 122 (922–1,994)	−15.8 ± 49.8 (−46–16)	−1.5 ± 3.1 (−4.6–1.6)

[a] Mean ± standard deviation with 95% confidence limits in parenthesis. Sample size is 10 birds for each group. H-S = Hevi-Shot pellets, Steel = steel pellets.
[b] None of the mean body weights or mean percent change in body weights of the H-S group were statistically different from those of the steel shot group.

TABLE 3. Count and weights (g) of treatment pellets placed into gizzards of mallards during a 30-day dosing trial.[a]

Treatment group[a]	Day 0		Day 30[b]		Change (%)	Pellets expelled during study	
	Mean weight	Count	Mean weight	Count		Mean weight	Count
H-S	0.2227 ± 0.0001	160	0.2088 ± 0.0077	134	6.2	0.2010 ± 0.0278	26
Steel	0.1504 ± 0.0008	160	0.0654 ± 0.0222	137	57	0.0746 ± 0.0266	22[c]
Pb	0.2019 ± 0.0010	80		0[d]		0.1584 ± 0.0193	7

[a] Results are reported as mean ± standard deviation. H-S = Hevi-Steel pellets, steel = steel pellets, Pb = led pellets.
[b] Removed from gizzard.
[c] One pellet was lost during the study.
[d] Only one bird was alive at day 30, no pellets were found.

was 56.5% (Table 3). Thus, steel shot pellets lost about seven times more weight than did the H-S pellets that were in the gizzard for the same period. No Pb shot pellets were recovered 30 days post-dosing because only one bird in this treatment group was still alive and no shot were found in its gizzard.

The 26 H-S pellets expelled by ducks during the study period (found between days 3 and 27) lost 9.7% of their original weight (Table 3). The 22 steel shot pellets expelled (days 6 through 27) averaged a weight loss of 50% (Table 3). Thus, expelled steel shot pellets lost approximately five times more weight than did the expelled H-S pellets. The mean weight of seven expelled lead pellets (recovered between days 4 to 22) averaged a weight loss of 21.5% (Table 3). However, this weight loss value is biased because it is likely that only a portion of the total Pb shot expelled were recovered.

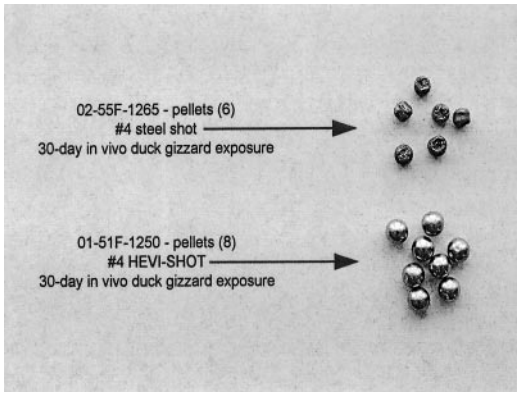

FIGURE 1. Photograph showing the physical condition of No. 4 steel shot and No. 4 H-S after 30 days in mallard gizzards when mallards were fed 100% whole kernal corn diet with quartzite grit available *ad libitum*. This photo is representative of the final condition of the two types of shot across all ducks examined.

The marked difference in the surface condition of the two types of shot after 30 days in the gizzard was visually apparent under a dissecting microscope (Fig. 1). The surface condition of the pellets shows the severity of the metal erosion in the steel shot pellets as compared to the H-S Viewed in cross-section, the size of the eroded pits in the steel shot pellets was even more apparent (Fig. 2).

Histopathology

Only one duck was alive in the lead-shot treatment group by the end of the study period. This bird had no significant lesions in the kidney, and had moderate, diffuse hepatocellular swelling and vacuolation.

No significant metal-related lesions were observed in the kidney of any duck. Periductular interstitial nephritis in the kidney of one duck from the steel shot group was considered an insignificant lesion unrelated to treatment. Lesions in the livers were similar in type and severity in all groups; hepatocellular swelling and vacuolation were not related to treatment.

Clinical pathology

Results of hematology are provided in Table 4. Mean hemoglobin levels for the Pb dosed birds were lower than in birds

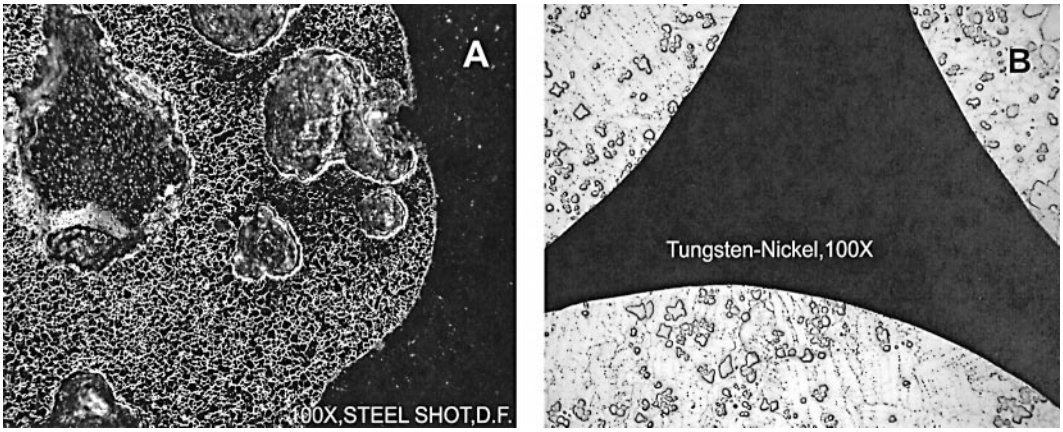

FIGURE 2. A photograph of the surface condition of a steel shot pellet (A) and parts of three H-S pellets (B) taken after they had resided in a mallard gizzard for 30 days. The magnification is 100×. The craters on the steel shot are the result of erosion. The H-S shot lack the large erosion craters seen on the steel pellets.

which received either H-S or steel shot on Day 15 post-dosing. Mean WBC and PCV of H-S ducks day 15 post-dosing were not significantly different from those of the steel shot treatment group, but the Pb group had elevated WBC as compared to the steel group (Table 4). At day 30 post-dosing, mean PCV and hemoglobin concentration differed between H-S and steel shot groups, but mean WBC for the two groups was not different.

None of the day 15 post-dosing mean plasma chemistry values differed between the H-S and steel shot groups (Table 5). However, all day 15 values for the Pb shot group were different from the steel shot group. The 30-day post-dosing mean plasma protein value for H-S birds was higher than that of the steel shot group. The H-S group's 30-day mean values for all other tests were not different from the steel shot group's mean values (Table 5).

Analytical chemistry

Mean Fe residues were significantly lower in blood and liver but not in kidney of the H-S group compared to the steel shot group (Table 6). Mean Ni residues in the blood, liver, and kidney of the H-S group were higher than in those tissues of the steel shot group because, as expected, no Ni residues were observed in the tissues from the steel shot group (Table 6).

Mean Pb level in the blood of the H-S group was less than the steel shot group (Table 6). There were no differences in

TABLE 4. Results of hematology by treatment group at days 15 and 30 post-dosing.[a]

Treatment group	PCV (%)		Hemoglobin (g/dl)		WBC ($\times 10^3/\mu l$)	
	Day 15	Day 30	Day 15	Day 30	Day 15	Day 30
H-S	50.5 ± 4.0 (48.6–53.4)	47.5 ± 2.0[b] (46.2–48.8)	15.8 ± 1.6 (15.0–16.6)	13.8 ± 1.1[b] (13.3–14.2)	4.7 ± 2.1 (3.0–5.8)	5.1 ± 0.6 (4.8–5.4)
Steel	51.9 ± 4.0 (50.5–53.3)	49.2 ± 2.9 (47.8–50.6)	16.7 ± 1.0 (16.2–17.2)	14.5 ± 0.9 (14.1–14.9)	3.2 ± 1.4 (1.7–4.6)	6.1 ± 2.5 (4.9–7.3)
Pb	28.4 ± 6.1[b] (23.6–33.2)		5.3 ± 1.2[b] (4.26–6.26)		11.5 ± 8.1[b] (5.0–18)	

[a] Mean ± standard deviation with 95% confidence limits in parenthesis. H-S = Hevi-Shot pellets, steel = steel pellets, Pb = lead pellets. Sample size is 20 for the H-S and steel groups, and is nine for the lead shot group for Day 15.
[b] Statistically significant difference compared to the steel shot mean ($P < 0.05$).

TABLE 5. Results of serum chemistry by treatment group at 15 and 30 days post-dosing.[a]

Treatment group	Plasma protein (g/dl)		Glucose (mg/dl)		Calcium (mg/dl)	
	Day 15	Day 30	Day 15	Day 30	Day 15	Day 30
H-S	6.4 ± 1.2 (5.7–7.1)	6.7 ± 0.7[b] (6.3–7.1)	178 ± 21 (165–191)	189 ± 37 (169–209)	13.1 ± 6.4 (9.6–16.6)	10.9 ± 2.1 (9.8–12.0)
Steel	6.4 ± 0.9 (6.0–6.8)	6.3 ± 0.7 (5.8–6.6)	186 ± 16 (178–194)	188 ± 13 (182–194)	11.2 ± 2.2 (9.7–11.9)	11.7 ± 3.9 (9.8–13.6)
Pb	4.2 ± 0.7[b] (3.7–4.7)		164 ± 16.5[b] (151–177)		7.9 ± 0.3[b] (7.6–8.2)	

Treatment group	Uric acid (mg/dl)		AST (U/l)		CK (U/l)	
	Day 15	Day 30	Day 15	Day 30	Day 15	Day 30
H-S	2.5 ± 0.9 (2.0–3.0)	2.7 ± 1.9 (2.2–3.2)	48 ± 43 (24.3–71.1)	37.5 ± 19.0 (27.2–47.8)	916 ± 1,200 (290–1,568)	714 ± 405 (493–934)
Steel	2.6 ± 1.2 (2.0–3.2)	3.1 ± 1.0 (2.6–3.6)	40.4 ± 33.8 (23.8–57.0)	37.5 ± 25.6 (35.3–49.7)	749 ± 563 (473–1,024)	627 ± 350 (461–793)
Pb	4.6 ± 3.1[b] (2.1–7.1)		101 ± 58[b] (53–149)		1,753 ± 988[b] (963–2,543)	

[a] Mean ± standard deviation with 95% confidence limits in parenthesis. H-S = Hevi-Shot pellets, Steel = steel pellets, Pb = lead pellets. Sample size is 20 for the H-S and Steel groups, and is nine for the lead shot group for Day 15. AST = aspartate aminotransferase, CK = creatine kinase.
[b] Statistically significant difference compared to the steel shot mean ($P < 0.05$).

TABLE 6. Effect of treatment on residue levels of iron, nickel, lead, and tungsten in mallards after a 30-day dosing.[a]

Treatment group	Metal			
	Fe	Ni	Pb	W
		Blood (mg/l)		
H-S	492 ± 73[c] (546–528)	0.03 ± 0.02[c] (0.02–0.04)	0.03 ± 0.01[c] (0.027–0.033)	0.24 ± 0.09[c] (0.20–0.28)
Steel	573 ± 145 (483–662)	<0.01[b]	0.04 ± 0.012 (0.033–0.047)	<0.03[b]
		Liver (mg/kg)		
H-S	541 ± 178[c] (453–628)	0.09 ± 0.05[c] (0.06–0.12)	0.15 ± 0.51 (0.0–0.40)	1.65 ± 0.84[c] (1.25–1.40)
Steel	1,134 ± 352 (916–1,352)	<0.01	0.03 ± 0.016 (0.14–0.16)	<0.03
		Kidney (mg/kg)		
H-S	148 ± 29.8 (133–162)	0.44 ± 0.28[c] (0.30–0.59)	0.05 ± 0.02 (0.04–0.06)	0.64 ± 0.29[c] (0.50–0.78)
Steel	143 ± 28.5 (123–160)	0.01 ± 0.006 (0.006–0.014)	0.04 ± 0.02 (0.03–0.05)	<0.03

[a] Mean ± standard deviation with 95% confidence limits in parenthesis. H-S = Hevi-Shot pellets, Steel = steel pellets, Pb = lead pellets. Sample size is 20 for each treatment and endpoint.
[b] Minimum detection limit: Fe = 3.0; Ni = 0.01; Pb = 0.01; W = 0.03.
[c] Statistically significant differences between means ($P < 0.05$).

mean Pb levels in liver or kidney between the H-S and steel shot treatment groups (Table 6). Because no W was detected in any tissues of the steel shot group, the levels in the tissues of the H-S group were greater than in the tissues of the steel shot group.

DISCUSSION

Mallards dosed with eight No. 4 H-S pellets did not show any signs of clinical toxicity from exposure to the W, Fe, or Ni contained in the shot. There was no significant difference in mean body weight of male or female mallards between the H-S dosed birds and those treated with similar-sized steel pellets, a known nontoxic shot. Although the H-S treatment group appeared to ingest less food over the 30-day post-dosing period, this was due to differential intake only for a single week (days 21–28) when both treatment groups increased food consumption. All birds, whether dosed with H-S or the steel shot, lost the same amount of weight during the 30-day treatment period due to the nutritional inadequacy of a corn-only diet.

Lack of toxic effects to mallards dosed with H-S was confirmed by absence of histopathologic lesions in liver or kidney, known target organs for these metals (Ennever, 1994). Minor metabolic insults to hepatic cells that were unrelated to metals treatment were observed in nearly all the birds in the study. Hematologic and plasma chemistry analyses indicated that the birds responded to H-S exposure similarly to ducks exposed to other commercially available shot, including tungsten-iron, tungsten-polymer, and nontoxic steel shot (Kelly et al., 1998). Mean PCV values for birds exposed to steel shot in this study were similar to those of Kelly et al. (1998) at all times post-dosing (current study: 51.9±1.4 day 15; 49.2±1.4 day 30; Kelly et al. [1998]: 49.3±0.65 day 7; 50.8±0.45 day 30), verifying that the control groups from both studies were similar. Furthermore, PCVs from birds treated with either H-S shot (this study) or tungsten-iron (Kelly et al., 1998) also did not differ. Values from all birds in both studies were within the normal range for ducks (Fairbrother and O'Loughlin, 1990). Likewise, mean hemoglobin values for both the steel shot and H-S groups were similar to the hemoglobin values reported by Kelly et al. (1998) for steel and tungsten-iron dosed mallards, respectively, and are within the normal range (Fairbrother and O'Loughlin, 1990). Kelly et al. (1998) did not report hemoglobin levels after day 7 post-dosing so further comparisons between studies could not be made. In the current study, the mean hemoglobin values for both the steel shot group and the H-S group declined from the 15-day values by approximately 2 g/dl (Table 4). This may reflect the declining nutritional state of the ducks. As expected, the mean hemoglobin value for the Pb shot group measured on day 15 post exposure was substantially lower than the mean value for both the steel-shot and H-S groups.

The only statistically significant difference between the H-S and steel shot groups in plasma chemistry values was the mean plasma protein level 30 days post-dosing. The H-S group's mean protein level (6.7 g/dl) was higher than the steel-shot group's mean protein level (6.3 g/dl). Both of these values are higher than reported by Kelly et al. (1998) (4.4 g/dl for steel dosed birds and 4.2 for tungsten-iron dosed mallards). Fairbrother et al. (1990) reported mean plasma protein levels for nonreproductive adult mallards as 3.8 g/dl for males and 4.2 g/dl for females. The difference between the current study and the previous reports may be due to methodologic differences or to the nutritional state of the birds. However, because H-S and steel dosed birds had similar protein levels, it is unlikely that the H-S shot was having a negative effect plasma protein. Furthermore, H-S treatment did not affect glucose, calcium, uric acid, AST, or CK levels relative to those observed in the steel-shot group. Values in both the groups were at or below normal (Fairbrother et

al., 1990). Reduced levels in these plasma chemistry values are likely nutritionally related (Levengood et al., 2000).

Lead dosed birds demonstrated expected signs of toxicity. Only one duck survived until day 30, so plasma chemistry measurements could be taken only on day 15 post-dosing. At this time, 40% (2/5) of the birds had below normal glucose levels. Uric acid and AST were within the normal range, indicating normal kidney function. Unlike mammals AST is found in skeletal muscle, heart, kidney, brain, and liver of mallards (Fairbrother et al., 1990). All five of the Pb group ducks had CK levels elevated well above the normal range. Creatine kinase is released from the muscles during periods of tissue injury, heat stress, or reduced oxygenation (Mitchell and Sandercock, 1995). Lead-induced toxemia inhibits delta-aminolevulinic acid dehydratase and heme synthesis, with subsequent reductions in hemoglobin production and oxygen carrying capacity of the blood (Pain, 1996).

The substantial difference in erosion of individual shot pellets between H-S and steel shot when in the mallard gizzard is an important factor in reducing the potential for health-impairing exposure to the metals in H-S. The degree of metal erosion directly correlates to the amount of metal that is available for uptake and circulation. H-S pellets extracted from gizzards or expelled lost little of their initial weight in contrast to significant weight loss by the steel pellets. H-S is much harder than steel mainly due to the incorporation of Ni and the use of an alloying process during manufacture. Thus, due to the small amount of erosion of H-S shot as compared to steel shot, the amount of Fe available for uptake in the duck gastrointestinal tract is much less. Similarly, the potential for significant amounts of W and Ni to be present and available for gut uptake is likewise very low.

The low bioavailability of the metals in H-S, in combination with the reduced total amount of Fe or W in each pellet, results in significantly lower levels of circulating Fe in the blood and liver of the H-S group as compared to the steel shot birds (Table 6), or of either Fe or W when compared to those exposed to commercially available tungsten-iron shot (Kelly et al., 1998). Thirty days after dosing mallards with eight No. 4 tungsten-iron shot, Kelly et al. (1998) found 8.5 times as much W in liver and 10.5 times as much W in kidney as was measured in birds dosed with H-S. Furthermore, Eastin and O'Shea (1981) measured Ni concentrations in mallards fed a diet containing 800 ppm Ni for 90 days and found approximately five times as much Ni in their tissues as was measured in this study with H-S dosed birds.

Finally, verification of receipt of a nontoxic dose of Ni from ingestion of H-S shot is found by comparing exposure to ducklings fed diets containing highly soluble nickel sulfate (Cain and Pafford, 1981). These authors found no effects on duckling survival or growth when birds were fed 176 ppm Ni in the diet (determined to be the no observable adverse effects level [NOAEL]). The lowest observable adverse effects level (LOAEL) determined in this same study was 774 ppm (equivalent to 77.4 mg/day). The NOAEL dose is approximately 10 times greater than the dose to mallards given H-S pellets and the LOAEL dose is close to 700 times greater than the H-S dose. Furthermore, all birds that showed effects of the Ni exposure had liver and kidney residues at least 10 times higher (Cain and Pafford, 1981) than those detected in H-S dosed birds.

Ingestion of up to eight No. 4 H-S pellets by mallards had no effect on body weight nor does it cause any pathologic effects detected in this study. Because of the hardness imparted to the alloy by Ni, this shot shows very little erosion and corrosion within the mallard gizzard. Therefore, exposure to target organs is very low, as relatively little of the material is absorbed from the gastrointestinal tract. Consequently, H-S provides even less risk to wild

birds that ingest spent shot than do other commercially available nontoxic shot including steel, tungsten-iron or tungsten-polymer. The data produced in this study indicate that H-S is an environmentally safe alternative shot for use in waterfowl and upland bird hunting.

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