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EFFECTS OF LEAD, IRON, AND BISMUTH ALLOY SHOT EMBEDDED IN THE BREAST MUSCLES OF GAME-FARM MALLARDS

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ABSTRACT: Effects of five lead (Pb), iron (Fe), or bismuth (Bi)/tin (Sn) alloy shot embedded in the breast muscles of game-farm mallards (*Anas platyrhynchos*) were studied from 28 March 1994 through 27 March 1995. We detected no differences in the mean survival times, mean hematocrits, or mean body weights among the three shot types. Connective tissue encapsulated Pb and Bi/Sn shot but only slight changes occurred in tissues surrounding the shot. Recovered Pb and Bi/Sn shot were essentially unchanged in appearance and weight. A thin zone of "oxide" surrounded Fe shot with a slight inflammatory response and a small amount of scarring adjacent to the embedded shot. Fe shot decreased slightly in weight while embedded. Bacterial infections were absent in all dosed ducks. Mean weights of kidneys, livers, and gonads did not vary by type of shot. Kidneys and livers of Bi-dosed ducks had higher concentrations of Bi than in Pb- and Fe-dosed ducks. Muscle and blood showed no differences in Bi concentrations among doses. We found no histological dose-related effects in kidneys, liver, and gonads from the embedded shot.

Key words: *Anas platyrhynchos*, bismuth alloy shot, breast muscle, embedded shot, iron shot, lead shot, waterfowl.

INTRODUCTION

Elder (1955) reported that after the close of the hunting season 26% of a pooled sample of mallards (*Anas platyrhynchos*) and black ducks (*Anas rubripes*) from the prairie provinces of Canada carried body shot. Havera et al. (1992) reported 15% of canvasbacks (*Aythya valisineria*) and 9% of lesser scaups (*Aythya affinis*) live trapped in spring at the Keokuk Pool on the Mississippi River (Illinois, USA) carried embedded shot.

Possible physiological effects of embedded shot in waterfowl, have caused concern, but few research data relate to this subject. Ducks Unlimited (1978) reported that little information was available on the fate of steel (Fe) shot embedded in flesh but that Fe shot in breast muscle of live ducks and chickens caused no ill effects after almost 1 yr. Also, Fe shot did not oxidize or discolor tissue after they were shot into chickens, which were then frozen for up to 1 yr. Ducks Unlimited (1978:20) concluded "the possible toxic effects of steel shot embedded in . . . waterfowl flesh remains small and . . . the need for more structured studies is evident." Bartels et al. (1991:856) reported that clinical problems seldom develop in dogs with chronically embedded Pb pellets. They stated that with Fe shot "the potential for corrosion (rusting) and related secondary tissue reaction is possible. If corrosion occurs in tissues, embedded steel shot could elicit inflammation that results in multiple fistulous tracts or abscesses." Bartels et al. (1991) found that Fe shot corroded and resulted in "a severe inflammatory response" in dogs. Kraabel et al. (1996) studied implanted Fe shot and tungsten-bismuth-tin shot in the pectoral muscles of 5-wk-old game-farm mallards for 1, 2, 4, and 8 wk. They reported corrosion of Fe shot but no adverse effects on health of ducks.

To understand the effects of embedded

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shot in waterfowl, we documented the effects of embedded lead (Pb), Fe, and bismuth (Bi/Sn) alloy shot in the breast muscles of game-farm mallards (*Anas platyrhynchos*). This study was part of a comprehensive investigation of the toxicity of Bi/Sn shot in waterfowl.

MATERIALS AND METHODS

General procedures

Fifteen male and 15 female wild-type, game-farm mallards 6- to 8-mo-old were purchased from Whistling Wings (Hanover, Illinois) and transported to Champaign (Illinois, USA; 40°05′N, 88°14′W) on 22 March 1994.

Assignments of ducks to doses and assignment of shot to ducks were randomized. We weighed the ducks and embedded the shot on 28 March 1994. Five No. 4 (3.3 mm diameter) pellets of either Pb, Fe, or Bi/Sn were surgically inserted 1.3 cm deep into the breast muscle of each of five females and five males. An aluminum foil template was fitted over the breast of each duck to standardize the locations of the shot. The five locations were marked on the breast feathers with a felt-tipped pen. The shot were not sterilized because the intent was to simulate shot fired by hunters.

William S. Montgomery, Jr. (Bismuth Cartridge Co., Dallas, Texas, USA) provided the Bi/Sn shot. Seven shot were chemically analyzed in the laboratories of the Illinois state Water Survey (Champaign, Illinois, USA). Bismuth in the Bi shot ranged from 97.27% to 100.05% (mean = 98.35%, SD = 0.86%) and Sn ranged from 1.69% to 1.98% (mean = 1.90%, SD = 0.10%). Other elements averaged <0.1% each; Pb ranged from 0.00% to 0.02% (mean = 0.10%, SD = 0.05%). Fe shot and Pb shot were removed from commercial 12 gauge shotgun shells.

The shot were embedded by staff of the College of Veterinary Medicine (University of Illinois, Urbana, Illinois, USA) and staff of the Illinois Natural History Survey (Champaign, Illinois, USA).

The ducks were anesthetized with Ketamine (Fort Dodge Laboratories, Fort Dodge, Iowa, USA), 100 mg/ml at a dosage of 50–80 mg/kg IP. An Ophthalmic Base Ointment (Merck, Sharpe & Dohme, Arlington, Texas, USA) was applied to the birds' eyes during anesthesia.

We used 70% alcohol to clear the site of feathers before embedding the shot. An 8G × 6″ stainless steel needle was used to puncture the skin and muscle. The beveled end of the needle was marked 1.3 cm from the end to determine the depth of insertion. Pellets were placed in the beveled end of the needle or were dropped through the needle if pellet size permitted. Although pellets were No. 4, some shot varied in diameter, especially the Bi/Sn pellets.

A metal trochar sanitized in Nolvasan (Fort Dodge Laboratories, Fort Dodge, Iowa, USA) was used to insert the pellet in place. The needles were dipped in sterile saline and dried with Sterile Nugauze (Johnson & Johnson, Arlington, Texas, USA) sponges between ducks as were the tips of the needles and trochar between pellets. Approximately 0.05 ml of Vetbond Tissue Adhesive (3-M Animal Care Products, St. Paul, Minnesota, USA) was used per insertion site, which was held closed by hand for 5 to 10 sec to ensure adhesion.

After surgery, the ducks were held in crates indoors (22 C) overnight. The birds were examined periodically, as they recovered from the anesthesia. The next day (29 March 1994), the fully recovered ducks were placed in crates and taken in an open truck some 160 km to the Natural History Survey's Forbes Biological Station (Havana, Illinois, USA; 40°17′N, 90°02′W).

The ducks were moved to the Forbes Biological Station where suitable facilities existed for holding ducks. Two elevated wire pens 5.8 × 1.8 × 0.8 m, each equipped with an enclosed feeding area and a tank with running water were used. Males and females were placed in separate pens. Duck pellets (Heinhold Feeds, Inc., Kouts, Indiana, USA), shelled corn, and commercial oyster shell (Southern Industries Corp., Mobile, Alabama, USA) were provided *ad libitum*. The station staff cared for the ducks and inspected them ≥5 days each week and weighed them each month.

One hundred eighty five days after the pellets were inserted into the breast muscle, we collected a blood sample from the wing vein of each of 28 surviving ducks with 20-gauge needles for determination of hematocrits and analysis for Pb, Fe, Bi, Sn, P, Ca, Mg, Zn, and Cu.

Blood was collected from the wing vein in hepranized microhematocrit capilets (Division American Hospital Supply Corp., Miami, Florida, USA) for hematocrit determinations. When 24 hematocrit samples (capacity of the centrifuge) were collected the samples were centrifuged at the site. The tubes were spun for 5 minutes at 11,500 RPM at 13,000 *g* force. The values were read with a Micro-Capillary Reader (Damon/IEC Division, Needham Heights, Massachusets, USA).

The whole blood was injected into 10-ml lithium-heparinized Vacutainer (Benton Dickson Vacutainer Systems, Rutherford, New Jersey, USA) tubes and frozen until analyzed. Eight females and seven males were randomly

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selected for euthanization by decapitation, after which the liver, kidneys, and gonads were removed and weighed.

We examined each euthanized duck for signs of toxic effects of the embedded shot and fixed representative samples of kidney, liver, gonads, muscle, lungs, and heart in formalin for histological study. Residual tissues, except of lungs and heart, were placed in individual numbered plastic bags, held temporarily on ice in a styrofoam cooler, and taken to the State Water Survey laboratories (Champaign, Illinois, USA) where they were stored in a freezer at −10 C until analysis for Pb, Fe, Bi, Sn, P, Ca, Mg, Zn, and Cu. The study continued until 27 March 1995 (day 365), when the remaining ducks were taken to Champaign, euthanized, and processed as described above.

We divided the breast muscle along the breast bone to recover the embedded shot. Each shot was placed in the same numbered vial used to store the shot before embedding. One or two samples of breast muscle surrounding the embedded shot were removed and fixed in 10% neutral buffered formalin for histological examination. Muscle samples for chemical analyses were collected from each of the 25 ducks submitted for histological examination, with care to avoid tissue adjacent to embedded shot.

We cleaned the shot by rolling them between the thumb and forefinger and then carefully removing any bits of adhering tissue with narrow-tipped forceps. Pb shot, which sometimes had bits of tissue adhering to their surfaces, were placed in tap water, with a small amount of the detergent Biz (Proctor & Gamble, Cincinnati, Ohio, USA) added, for 7 days. The shot were then removed, wiped dry with a facial tissue, and air dried for 3 days. Tissue did not adhere to Bi/Sn or Fe shot. No other cleaning was attempted to ensure against accidental removal of metal from the shot. The recovered pellets were weighed to the nearest 0.1 mg and weights were compared with original weights.

The gonads (testis or ovary), liver, kidney, muscle, lungs, and heart were sectioned at 4μm and stained with hematoxylin and eosin. The samples were examined without knowledge of the dosed group assignment. Subsequently, group assignment and organ/body weight data were associated with histologic findings for interpretation.

In this report, day 0 is the day the shot were embedded in the breast muscles. Day 185, for example, is 185 days after the shot were embedded in the breast muscles, and the term "Bi-dosed ducks" refers to ducks with Bi shot embedded in their breast muscles.

The Method Detection Limit (MDL) (Glaser et al., 1981) used to establish the detection limits for levels of elements in tissues and other materials was based on the mean weight of the tissue samples. The MDL procedure should give a value that averages ≥ two times larger than the MDL to be considered a meaningful value (Glaser et al., 1981). For statistical analysis, values < MDL were entered as one-half the MDL value.

Sample concentrations of Bi and Pb were generally lower than the MDL for these elements when analyzed by inductively coupled argon emission plasma spectroscopy (ICP-Model 1100, Vacuum, Thermo Jarrel Ash, Franklin, Massachusetts, USA). We used graphite furnace atomic absorption (GFAA-Model 957, Atomic Absorption Spectrophotometer, Model 188, Furnace, Thermal Jarrel Ash, Franklin, Massachusetts, USA), a more sensitive analytical technique, for the measurement of Bi and PB at low concentrations in the blood, livers, kidney, and gonad samples. ICP was used to measure Ca, P, Mg, Zn, Cu, Sn, and Fe, along with beryllium (Be) as an internal standard, in all samples. All chemical analyses were made in the laboratories of the Illinois State Water Survey (Champaign, Illinois, USA).

The MDL were set at ≤0.3 μ/g for Pb, at ≤0.5 μ/g for Bi, and at ≥5.0 μ/g for Sn all on a wet weight basis. MDL considerably lower than these were achieved for all analyses.

Sample preparation

Blood and tissue samples were digested in acid before analysis for metals with ICP and GFAA. Because wet weight concentrations of the blood and organs were desired, these samples were not dried before digestion. The elements of interest were Bi, Sn, Fe, Pb, Ca, Mg, P, Zn, and Cu. The protocol for the study, as approved the Canadian Wildlife Service and the U.S. Fish and Wildlife Service, required analysis of all elements that comprised ≥0.1% by weight in the shot plus "nutritionally essential elements (Ca, Mg, P, Zn, and Cu)." Sanderson et al. (1997a:189) describe the digestions and analytical methods used for ICP and GFAA analyses.

Statistical analyses

When no differences existed between sexes they were combined for data analysis. Thus, when sexes are not identified in the text and tables, sample sizes include combined females and males.

Comparisons among doses for variables that were measured only once (typically after nec-

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TABLE 1. Mean days survived, mean hematocrit (Hct), and mean body weights for game-farm mallards by shot treatment (Pb, Fe, Bi).

Treatment	Mean ± SE (*n*)						
	Survival		Hct		Body weight		
	Day 185	Day 365	Day 185	Day 365	Day 0	Day 185	Day 365
Pb	185.0 ± 0.0 (10)	349.3 ± 14.5 (7)	42.6 ± 0.7 (10)	44.7 ± 2.3 (6)	1.0 ± 0.0 (10)	1.2 ± 0.1 (7)	1.2 ± 0.1 (6)
Fe	183.6 ± 0.9 (10)	331.0 ± 29.4 (4)	42.1 ± 1.3 (8)	45.3 ± 0.3 (3)	1.1 ± 0.0 (10)	1.2 ± 0.2 (4)	1.2 ± 0.0 (3)
Bi	185.0 ± 0.0 (10)	336.8 ± 24.5 (4)	42.2 ± 1.1 (10)	42.0 ± 4.0 (3)	1.1 ± 0.0 (10)	1.1 ± 0.1 (4)	1.2 ± 0.0 (3)

ropsy) were made by ANOVA. Equality of variances among groups was evaluated with Levene's test. If significant heteroscedasticity ($P < 0.05$) was detected, Brown-Forsythe statistics were used and degrees of freedom were approximated. Pairwise differences among groups were evaluated with Bonferroni comparisons.

When variables were measured over ≥2 time periods, dose groups were compared and tested for variation over time with ANOVA with repeated-measures. When necessary, significance levels based on the Huynh-Feldt adjustment were used. Owing to loss of animals during the experiment, these ANOVA sometimes became unbalanced; for these analyses a restricted maximum likelihood model was used to estimate parameters and test for differences among doses with Wald statistics.

Survival rates of birds were assessed by estimating survival functions with the Kaplan-Meier product-limit method. Differences among groups were evaluated with the Mantel-Cox test. All statistical analyses were conducted with the BMDP statistical software package, version 7.0 (BMDP, 1992).

When two values are reported as "different" or that they "differ," it means that they differ statistically ($P \leq 0.05$).

RESULTS AND DISCUSSION

Survival

The study plan specified that one-half of the ducks were to be necropsied on day 185 and one-half on day 365. Two Fe-embedded male ducks died on day 177 and 179, respectively. These two ducks, not originally selected for sacrifice on day 185, were exchanged for two ducks of the same sex and dose that were scheduled for sacrifice. Fe-dosed ducks survived a mean of 183.6 days to day 185. All Pb- and Bi-dosed ducks survived to day 185 (Table 1). The mean number of days survived to day 185 did not differ among doses ($P = 0.17$).

One Pb-dosed female died on day 255, one Fe-dosed female died on day 229, and one Bi-dosed male died on day 252. No detectable relationship existed between the embedded shot and number of days to death for any of the ducks. For ducks not sacrificed on day 185, the mean numbers of days survived to day 365 were 331.0 for Fe-dosed, 336.8 for Bi-dosed, and 349.3 for Pb-dosed ducks, which were not different among doses. The mean number of days survived for all ducks not necropsied on day 185 was 341.1 and for all ducks was 265.5. In the text and tables of this report, day 185 and day 365 refer to ducks scheduled for necropsy on these days, including the ducks that died prior to their scheduled necropsy.

Hematocrit

No difference was detected in mean hematocrits among doses on either day 185 or day 365 (Table 1).

Body weight

Mean body weights of ducks did not differ among doses either 185 or 365 days after the shot were embedded. However, with sexes and doses combined, body weights differed ($F_{5,45} = 2.80$; $P = 0.05$) from day 0 to day 365 (Table 1). Except for minor declines on days 122 and 154, the ducks gained weight from the date

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TABLE 2. Mean weight (mg) and mean percent change in weight of five No. 4 Pb, Fe, or Bi shot when embedded in and after removal from the breast muscle of game-farm mallards.

Treatment	Weight of shot groups ± SE (*n*)		
	When embedded	At necropsy	% change Day 0 to necropsy[a]
Pb	1,049.3 ± 7.0 (10)	1,052.0 ± 6.3 (10)	−0.2 ± 0.2 (10)
Fe	743.7 ± 2.7 (10)	726.2 ± 2.8 (10)	−2.4 ± 0.2 (10)
Bi	987.9 ± 10.6 (10)	985.0 ± 10.2 (10)	−0.2 ± 0.2 (10)

[a] There was no difference in the percentage weight change of Pb and Bi shot on day 185 versus day 365, but Fe shot lost more weight (−3.14%) after 365 days than after 185 days (−2.01%) ($P = 0.02$).

shot were embedded (28 March 1994) through day 305 (27 January 1995) and then lost weight through day 365 (27 March 1995). The early weight gains were probably associated with continued growth of the 6- to 8-mo-old ducks.

Embedded shot

At necropsy, 129 of 130 embedded shot were recovered. Pb shot were encapsulated by connective tissue and appeared unchanged from the date they were embedded. Bi shot were sometimes completely or partially encapsulated, and a few of them showed slight indications of dissolution on the surface. Fe shot showed little or no signs of encapsulation, and each shot was surrounded by a thin zone of "oxidation." This zone crumbled easily into tiny bits when the shot were rolled between the thumb and forefinger.

Little difference existed in the physical characteristics of Pb and Bi shot, or surrounding tissues, on day 185 versus day 365. No difference existed in the percent weight change of Pb and Bi shot on day 185 versus day 365. Fe shot lost small amounts of weight, which was caused by the oxidization; the weight loss was larger by day 365 (−3.14%) than by day 185 (−2.01%) ($P = 0.02$) (Table 2). Pb and Bi shot were essentially unchanged in weight on days 185 and 365. Fe shot lost a larger percentage of its weight than either Pb or Bi shot (Table 2).

Organ weights

Some of the weights of organs differed substantially between sexes and between ducks necropsied on days 185 and 365. These differences were sex and season related. Because only 30 ducks were used in the study, one-half males and one-half females, separated among three doses, most of the differences in organ weights were not statistically significant. No differences in organ weights were detected that were related to the type of embedded shot.

Livers of ducks necropsied on day 185 had mean weights that ranged from 22.7 g for Pb-dosed to 32.4 g for Bi-dosed ducks. Livers of females had mean weights that ranged from 20.7 g for Fe-dosed to 34.0 g for Bi-dosed ducks. On day 185, mean weights of livers of males and females did not differ, but by day 365, livers of females weighed approximately twice as much as livers of males (Table 3). Because of small

TABLE 3. Mean weights (g) of liver, kidneys, and gonads of game-farm mallards at 185 and 365 days combined by shot treatment (Pb, Fe, Bi).

Treatment	Mean ± SE (*n*)		
	Liver	Kidneys	Gonads
Pb	22.7 ± 2.9 (10)	7.0 ± 0.6 (10)	29.0 ± 7.2 (10)
Fe	28.6 ± 6.9 (9)	6.6 ± 0.5 (9)	16.0 ± 8.0 (9)
Bi	32.4 ± 3.8 (10)	6.6 ± 0.4 (10)	13.3 ± 6.5 (10)

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TABLE 4. Mean concentrations (μ/g, wet wt) of elements in kidneys of game-farm mallards at days 185 and 365 combined by shot treatment (Pb, Fe, Bi). n = 10 for all samples.

Treatment	Mean ± SE								
	Pb[a]	Fe	Bi[a,b,c]	Sn[c]	P	Ca	Mg	Zn	Cu
Pb	0.3 ±0.06	131 ±14.8	0.04 ±0.00	1.3 ±0.21	3,437 ±105	111 ±12.4	221 ±5.4	33.7 ±1.0	8.5 ±0.6
Fe	0.2 ±0.04	164 ±18.3	0.05 ±0.01	1.12 ±0.00	3,523 ±92	163 ±32.0	227 ±7.8	32.2 ±1.7	8.1 ±0.8
Bi	0.2 ±0.03	130 ±10.1	1.8 ±0.32	1.29 ±0.17	3,650 ±110	188 ±53.0	240 ±8.4	39.6 ±3.3	10.8 ±1.4

[a] MDL: Pb = 0.11 μ/g, Bi = 0.07 μ/g, Sn = 2.25 μ/g.
[b] Difference among doses in the mean amounts of Bi in kidneys: $F_{2,9}$ = 27.86; P = < 0.01.
[c] Mean amount of Bi in kidneys: Pb versus Bi; $P < 0.01$, Fe versus Bi; $P < 0.01$.

sample sizes, when analyzed separately for sex and time of necropsy (days 185 and 365), the differences were not statistically significant.

Mean weights of kidneys ranged from 6.6 g for Fe- and Bi-dosed ducks to 7.0 g for kidneys of Pb-dosed ducks (Table 3). No differences were detected in mean kidney weights between sexes, among doses, or between ducks necropsied on days 185 and 365.

Gonads of males on day 185 had mean weights that ranged from 6.3 g for Fe-dosed ducks to 10.9 g for Bi-dosed ducks. Gonads of females on day 185 had mean weights ranging from 1.4 g for Pb-dosed ducks to 2.6 g for Bi-dosed ducks. On day 365, gonads of all females had mean weights that varied from 6.5 g for Fe-dosed females to 31.4 g for Pb-dosed females. On day 365 gonads of all males had mean weights of 45.3 g compared with 19.6 g for gonads of all females. Gonads of all ducks necropsied on day 365 were heavier ($P < 0.01$) than gonads from all ducks necropsied on day 185. With days and sexes combined, no variation existed among doses in the mean weights of gonads.

Elements in tissues

The only differences in the concentrations of the various elements in kidneys on day 185 versus day 365 were for Pb-embedded ducks. These ducks had more Mg (241 versus 212 μ/g [ppm]) and P (3,801 versus 3,281 μ/g) on day 185 than on day 365. The only variations among doses were for concentrations of Bi in kidneys of Pb-dosed ducks (0.04 μ/g) and Fe-dosed ducks (0.05 μ/g) versus Bi-dosed ducks (1.8 μ/g) (Table 4). Sanderson et al. (1997b:232) found 1.5 μg/g of Bi in the kidneys of game-farm mallards dosed with 8 No. 4 Bi shot on days 0, 30, 60, and 90. Although the concentration of Bi was higher than in the kidneys of controls, Fe-dosed, and Pb-dosed ducks, no toxic effects existed from this concentration of Bi in the kidneys. There was no difference in the mean concentrations of Bi in kidneys of Pb-dosed versus Fe-dosed ducks; both had Bi concentrations lower than the MDL (0.07 μ/g).

No differences existed in the mean concentrations of each of the various elements in the livers on day 185 versus day 365. Bi-dosed ducks had more Bi (0.19 μ/g) in their livers than either Pb-dosed (0.05 μ/g) or Fe-dosed (0.05 μ/g) ducks; both of the latter groups had lower concentrations of Bi than the MDL (0.07 μ/g) (Table 5). Sanderson et al. (1997b:233) found 0.6 μg/g of Bi in the livers of game-farm mallards dosed with 8 No. 4 Bi shot on days 0, 30, 60, and 90. This concentration of Bi in the liver was higher in Bi-dosed ducks than in controls, Fe-dosed, or Pb-dosed ducks, but no toxic effects existed as a result of this concentration of Bi in the liver. No difference existed in the mean concentrations of Bi in Pb-dosed versus Fe-dosed ducks and

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TABLE 5. Mean concentrations (μ/g, wet wt) of elements in livers of game-farm mallards at days 185 and 365 combined by shot treatment (Pb, Fe, Bi). For all samples $n = 10$.

Treatment	Mean ± SE								
	Pb[a]	Fe	Bi[a,b]	Sn[a]	P	Ca	Mg	Zn	Cu
Pb	0.12 ±0.02	972 ±229	0.05 ±0.01	1.2 ±0.13	3,611 ±246	65.2 ±5.7	255 ±16.7	60.2 ±5.8	73.0 ±17.0
Fe	0.12 ±0.04	877 ±161	0.05 ±0.01	1.5 ±0.28	3,232 ±254	64.1 ±6.0	220 ±19.3	49.8 ±6.9	55.5 ±14.9
Bi	0.16 ±0.04	598 ±116	0.19 ±0.06	1.1 ±0.00	2,889 ±299	49.5 ±4.6	196 ±19.0	49.4 ±7.0	74.8 ±27.0

[a] MDL: Pb = 0.11 μ/g, Bi = 0.07 μ/g, Sn = 2.23 μ/g.
[b] Difference among doses in the mean amount of Bi in the liver: $F_{2,11} = 2.28$; $P = < 0.04$.
[c] Mean amount of Bi in the liver: Pb versus Bi; $P < 0.05$, Fe versus Bi; $P < 0.05$.

no other differences existed among doses in the mean concentrations of any element in the livers.

Locke et al. (1987) found a marked hemosiderosis in the livers of ducks that had been dosed with Fe shot. In our study, no hemosiderosis was detected in the livers of Fe-dosed ducks on either days 185 or 365.

No difference in the mean concentrations in the gonads for any element was detected among doses. Mean concentrations of Pb in the gonads ranged from 0.17 μ/g for Pb-dosed ducks to 0.20 μ/g for Fe-dosed ducks. All concentrations were between the MDL and 2× the MDL (Table 6). Two Bi-dosed ducks had 4.0 and 5.7 μ/g Sn, respectively, in their gonads; however, all other ducks had lower concentrations of Sn than the MDL (3.72 μ/g) in their gonads.

Four of 10 Bi-dosed ducks had more Bi than the MDL (0.07 μ/g) in their gonads, and all four concentrations were >2× the MDL. Only 1 of 10 Pb-dosed (0.20 μ/g) and 1 of 10 Fe-dosed (0.24 μ/g) ducks had more than the MDL of Bi in their gonads: both were >2× the MDL. Three male Bi-dosed ducks had, respectively, 0.60 μ/g, 1.60 μ/g, and 1.94 μ/g Bi in their gonads.

Although no differences existed among doses in the mean concentrations of Bi in the gonads, the three males with detectable Bi in their gonads suggest that some ducks may accumulate Bi in their gonads from Bi shot embedded in the breast muscles. Both kidneys and livers of Bi-dosed ducks had higher concentrations of Bi than kidneys and livers of Pb- and Fe-dosed ducks.

On day 365, no differences existed among doses for the concentrations of each of the nine elements in muscles (Table 7). Mean concentrations of Pb in the muscles ranged from 0.25 μ/g in Pb-dosed ducks to 1.12 μ/g in the Fe-dosed ducks. Muscle samples were not taken at the sites where the shot were embedded.

All muscle samples had lower concentrations of Sn than the MDL (2.26 μ/g). Only two ducks (one Fe-dosed and one Bi-

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TABLE 6. Mean concentrations (μ/g, wet weight) of elements in gonads of game-farm mallards at days 185 and 365 combined by shot treatment (Pb, Fe, Bi). For all samples $n = 10$.

Treatment	Mean ± SE								
	Pb[a]	Fe	Bi[a]	Sn[a]	P	Ca	Mg	Zn	Cu
Pb	0.17 ±0.05	59.3 ±17.8	0.05 ±0.02	2.6 ±0.5	3,480 ±441	431 ±240.1	206 ±14.4	26.0 ±5.4	2.1 ±0.6
Fe	0.20 ±0.05	125 ±57.4	0.06 ±0.02	1.9 ±0.0	2,901 ±250	204 ±108.6	195 ±16.5	20.9 ±2.5	2.1 ±0.5
Bi	0.17 ±0.06	75.5 ±18.4	0.46 ±0.23	1.9 ±0.0	3,245 ±257	288 ±135.2	189 ±17.8	25.3 ±3.6	2.3 ±0.4

[a] MDLs: Bi = 0.07 μ/g, Sn = 3.72 μ/g, Pb = 0.11 μ/g.

dosed) had more Bi than the MDL (0.11 μ/g). The mean value for Bi was <MDL.

No differences were detected in the mean concentrations of each of the nine elements in blood on day 185 versus day 365 (Table 8). The only differences among doses in the concentrations of the elements in the blood on either day 185 or day 365 were as follows: more Cu in Fe-dosed ducks (0.33 μ/g) than in Pb-dosed ducks (0.19 μ/g) on day 185, and more Mg in Pb-dosed ducks (80.7 μ/g) than in Bi-dosed (67.5 μ/g) ducks on day 365.

Histopathology

When the histopathology results were reviewed, no significant differences existed between Bi-embedded and Pb-embedded ducks. Typically, in muscles of both dosed groups, a minimal inflammatory response was evident, consisting primarily of macrophages with a small amount of fibrosis (scarring) immediately adjacent to the location of the embedded shot. For Fe-dosed ducks, slightly more fibrosis and inflammation was evident because of the "rust" formation on the shot and the resulting tissue reaction to the oxidized metal. This reaction also was evident in the tissues histologically because oxidized tissue had been phagocytized by nearby macrophages. No dose-related differences were observed in the kidneys, liver, gonads, hearts, and lungs.

The fact of no change in Hcts, organ weights, and histopathology does not preclude subtle biochemical changes. However, Sanderson et al. (1997b:223, 229) dosed game-farm mallards with 0 or 8 No. 4 Bi, Fe, or Pb shot on days 0, 30, 60, and 90. On day 120, Hcts, weights of livers, and kidneys of controls, Bi-, and Fe-dosed ducks not were not significantly different. These parameters were also similar to the same parameters found in our study. For example, Sanderson et al. (1997b:229) reported mean livers weights in Fe-dosed ducks of 33 g compared with 29 g in the present study and 31 g in Bi-dosed ducks compared with 32 g in the present study.

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TABLE 7. Mean concentrations (μ/g, wet wt) of elements in breast muscle of game-farm mallards at day 365 by shot treatment (Pb, Fe, Bi). For Pb-dosed $n = 7$ and $n = 4$ each for Fe- and Bi-dosed ducks.

Treatment	Mean ± SE								
	Pb[a]	Fe	Bi[a]	Sn[a]	P	Ca	Mg	Zn	Cu
Pb	0.25 ±0.08	66.0 ±10.6	0.07 ±0.01	1.1 ±0.0	3,064 ±68	64.9 ±12.6	352 ±8.5	12.9 ±1.3	3.8 ±0.5
Fe	1.12 ±0.90	79.1 ±24.5	0.10 ±0.06	1.1 ±0.0	3,006 ±105	63.5 ±12.6	346 ±13.8	12.3 ±1.4	3.4 ±0.6
Bi	0.49 ±0.37	67.9 ±21.3	0.06 ±0.00	1.1 ±0.0	2,764 ±210	65.5 ±12.1	308 ±23.2	14.5 ±4.2	3.7 ±0.9

[a] MDL: Pb = 0.11 μ/g, wet wt, Bi = 0.11 μ/g, wet wt, Sn = 2.26 μ/g wet wt.

TABLE 8. Mean concentrations (μ/g, wet wt) of elements in blood of game-farm mallards at 185 and 365 days by shot treatment (Pb, Fe, Bi).

Treatment	Mean ± SE (n)								
	Pb[a]	Fe	Bi[a]	Sn[a]	P	Ca	Mg	Zn	Cu[a]
					Day 185				
Pb	0.11 ± 0.02 (10)	438 ± 21.0 (10)	0.05 ± 0.01 (10)	1.1 ± 0.0 (10)	1,434 ± 73 (10)	76.8 ± 8.4 (10)	72.1 ± 3.0 (10)	5.8 ± 0.4 (10)	0.19 ± 0.03 (10)
Fe	0.08 ± 0.01 (8)	453 ± 23.9 (8)	0.07 ± 0.03 (8)	1.1 ± 0.0 (8)	1,472 ± 63 (8)	73.1 ± 8.8 (8)	76.1 ± 2.0 (8)	5.7 ± 0.3 (8)	0.33 ± 0.06 (8)
Bi	0.08 ± 0.01 (10)	445 ± 16.4 (10)	0.07 ± 0.02 (10)	1.1 ± 0.0 (10)	1,533 ± 39 (10)	84.1 ± 21.3 (10)	75.8 ± 1.2 (10)	6.2 ± 0.2 (10)	0.27 ± 0.04 (10)
					Day 365				
Pb	0.18 ± 0.09 (6)	425 ± 39.7 (6)	0.04 ± 0.00 (6)	1.4 ± 0.3 (6)	1,378 ± 119 (6)	81.1 ± 13.9 (6)	80.7 ± 3.1 (6)	6.0 ± 0.4 (6)	0.44 ± 0.06 (6)
Fe	0.15 ± 0.04 (3)	478 ± 34.6 (3)	0.06 ± 0.02 (3)	1.6 ± 0.5 (3)	1,314 ± 240 (3)	94.7 ± 40.4 (3)	78.3 ± 7.8 (3)	6.0 ± 1.0 (3)	0.48 ± 0.06 (3)
Bi	0.19 ± 0.12 (3)	450 ± 26.0 (3)	0.04 ± 0.00 (3)	1.1 ± 0.0 (3)	1,098 ± 269 (3)	100 ± 29.6 (3)	67.5 ± 4.5 (3)	6.6 ± 0.8 (3)	0.47 ± 0.03 (3)

[a] MDL: Bi = 0.08 μ/g, Sn = 2.14 μ/g, Pb = 0.13 μ/g, Cu = 0.18 μ/g.

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They found kidney weights of 8 g in Fe-dosed and Bi-dosed ducks compared with 7 g in the present study for Fe- and Bi-dosed ducks. Sanderson et al. (1997b:223) reported mean Hcts of 37 for female and 48 for male game-farm mallards 120 days after dosing with eight No. 4 Bi shot compared with 42 for sexes combined in the present study 185 and 365 days after dosing (Table 1). They found no significant differences in the mean Hcts of control, Fe-, and Bi-dosed ducks. They concluded (p. 250) "eight No. 4 Bi shot, repeatedly dosed in game-farm mallards, resulted in no demonstrable effects on adult ducks or on the eggs and ducklings they produced." Sanderson et al. (1992:538) concluded "Two, four or eight Number Two Bi shot, or four Number two Bi shot, when ducks had access to soil, had no adverse effects when administered to ducks."

CONCLUSIONS

Essentially no change was detected in Pb and Bi shot embedded in the breast muscle of ducks for 1 yr. Fe shot embedded in the breast muscle of ducks decreased slightly in weight as a thin zone of "rust" formed surrounding the shot. A slight increase in the concentration of Bi in the kidneys and liver was noted, and perhaps in the testes, of Bi-dosed ducks versus Fe- and Pb-dosed ducks, but no dose-related changes were detected in these organs. Pb, Fe, or Bi shot embedded in the breast muscles of ducks for up to 365 days resulted in no significant changes in the concentrations of Pb, Fe, Bi, Sn, P, Ca, Mg, Zn, or Cu in the breast muscles; the primary portion of wild ducks consumed by humans.

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