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Heavy Metal Concentrations of Duck Tissues in Relation to Ingestion of Spent Shot

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Lead poisoning of waterfowl from dissolution of ingested lead shot has been recognized as a major problem in wildlife management for almost a century. The accumulation of lead in liver, bones, and other tissues has been shown to correlate with the presence of lead shot in the gizzard (Longcore et al. 1974, Anderson 1975, White and Stendell 1977, Scanlon et al. 1980, Calle et al. 1982). And the toxic effects of lead on waterfowl have been extensively documented (Bellrose 1959). However, lead shot also contains considerable amounts of antimony as a hardening agent and arsenic which aids in forming spherical shot. In addition, the shot is sometimes plated with nickel and copper to increase resistance to deformation. Whether these additional elements accumulate in tissues upon dissolution of lead shot in the gizzard, or if possible toxic interactions affect waterfowl are unknown.

Aside from estimating delta-aminolevulinic acid dehydratase activity there is no way to immediately assess the lead burden of an individual without sacrificing it for tissue analysis. Even the detection of ingested shot by fluoroscopy indicates nothing about past exposure or whether the detected shot is steel or lead. Since arsenic exposure can be assessed by analysis of hair and fingernails, we anticipated that elemental analysis of feathers could be used to assess ingestion of shot without sacrificing the bird. While the concentration of lead in liver tissue appears to be an indicator of short term exposure (Longcore et al. 1974), the amount in bones is considered to be more representative of long term exposure (White and Stendell 1977). If any of the other elements present in lead shot are accumulated by waterfowl, they too might be detected in bones. The aims of this study were 1) to determine if any of the additional components of lead or steel shot were accumulated upon shot ingestion as indicated by their concentrations in bones and feathers, and 2) to determine the feasibility of heavy metal analysis of feathers to assess exposure to spent shot.

MATERIALS AND METHODS

Specimens were collected during the 1981-1982 and 1982-1983 hunting seasons from brackish marshes in Chambers County, Texas. Wings and gizzards were frozen until analysis. The presence of ingested shot in the gizzard was determined by x-ray analysis, (Torrex 150 x-ray inspection system, Torr X-ray Corp., 65-75 KV, 3 ma, 30 sec exposure) followed by visual inspection.

Tissues from specimens collected during the 1981-1982 season were prepared for inductively coupled plasma emission spectrometric analysis (ICP) following EPA guidelines (1979). Tissues were not ashed prior to acid digestion to avoid volatization and decreased recovery of any of the elements (Dahlquist and Knoll 1978). An ulna from each specimen was dissected from the wing, broken in half and the marrow was discarded. All tissue was scraped from the bone with a glass microscope slide. Wings showing evidence of shot damage were not used. Three to six primary feathers were washed extensively in glass distilled water to remove as much contaminating soil as possible and then dried at 50 C. Analyses were run in duplicate or triplicate.

All glassware was acid washed as prescribed by EPA methods. Ten ml of glass distilled water and 3 ml of concentrated nitric acid (Baker analyzed reagent grade) were added to weighed tissue samples in 100 ml beakers. These were placed on hot plates at 180 C and evaporated to near dryness. Another 3 ml of acid was added and the beaker was covered with a watch glass. The samples were refluxed until digestion was complete. After filtering the sample through a medium porosity Buchner fritted glass funnel, the sample volume was adjusted to 25 or 50 ml, depending on initial weight of the tissue. Samples of 15 sources of lead and steel shot were digested in a similar manner, except complete digestion required an additional 6 ml of acid and the final volume was adjusted to 500 or 1000 ml.

Concentrations of 17 elements were simultaneously determined in each digested sample using a Jarrell-Ash inductively coupled plasma emission spectrophotometer (atomic series 855). This technique is superior in sensitivity, speed and convenience to the more commonly employed atomic absorption spectroscopy. Data for bones are expressed as mg per kg wet weight while feather results are reported as mg per kg dry weight. The effect of ingested shot in the gizzard on the tissue consentration of each of the elements was assessed using one-way analysis of variance. In addition, Pearson correlation coefficiants were computed to test the relationship between the occurrence of the elements in bones and feathers.

RESULTS AND DISCUSSION

Although lead generally made up over 95% of the weight of the shot, 9 other elements were present at average concentrations greater than 30 ppm. Antimony ranged from 0.41% to 1.47% of the weight, while arsenic made up 0.23% to 0.84% of the shot analyzed. Tin, selenium, manganese, cadmium, chromium, copper and nickel were also present in all the samples of lead shot at relatively high levels.

Of the 6 species analyzed, 3 are considered "indicator" species or those which can be expected to have high incidence of shot ingestion (Texas Parks and Wildlife 1983). Thus data from lesser scaup, pintails, and mottled ducks can be compared to the concentrations of metals in "nonindicator" species, green-winged teal, shovelers, and gadwalls. Table 1 summarizes tissue concentrations of lead and arsenic, and ingestion rates.

Table 1. Summary of 1981-1982 mean tissue concentrations of lead and arsenic (mg per kg) and occurrence of shot in the gizzard. A = 1981-1982 season, B = 1982-1983 season, C = from Bellrose (1959).

Species	% With Injested Shot			Bone		Feathers	
	A	B	C	Pb (SE)	As (SE)	Pb (SE)	As (SE)
Teal	8.3	2.6	1.4	9.1	14.6	3.8	8.1
n =	34	313		(0.8)	(1.1)	(0.3)	(0.6)
Shoveler	23.1	7.1	1.6	9.7	11.8	0.8	5.2
n =	13	42		(0.4)	(0.7)	(0.5)	(2.3)
Gadwall	16.7	0.0	1.8	9.7	5.9	0.3	44.2
n =	13	50		(0.9)	(0.7)	(0.2)	(2.8)
Scaup	56.3	24.2	13.1	7.5	11.4	29.1	62.8
n =	16	33		(1.7)	(5.8)	(10.2)	(21.1)
Pintail	75.0	7.1	8.9	21.6	12.2	2.9	12.0
n =	4	14		(3.2)	(1.0)	(0.4)	(1.3)
Mottled	50.0	17.6	49.6*	57.1	10.3	15.7	13.0
n =	4	17		(7.9)	(1.2)	(2.5)	(1.1)

* Data for 248 mottled ducks collected in 1978 provided by C.D. Stutzenbaker, Murphree Wildlife Management Area, Port Arthur, Texas.

Bones and feathers of indicator species had higher concentrations of lead than did the tissues of nonindicator species. Bones of the three indicator species averaged 28.8 ppm lead as compared to only 9.5 ppm lead in bones of nonindicator species. Likewise, feathers of indicator species contained an average of 15.9 ppm lead while nonindicator species averaged only 1.6 ppm lead in their feathers. There was no apparent difference in arsenic concentrations in feathers or bones of the two groups of ducks.

The tissue concentrations of 9 elements (those present in shot at >30 ppm) in relation to presence of ingested shot in the gizzard are presented in Tables 2 & 3. Calcium interfered with accurate determination of antimony, thus antimony concentrations were omitted from further analyses.

Of the 108 ANOVAs that were performed (9 elements, 6 species, 2 tissues), 24 (22.2%) showed significance at the level of $p < 0.05$. In 18 (16.7%) of these cases, the concentration of the element was greater in the tissue of birds with shot in the gizzard than in the tissue of specimens without any ingested shot. Only 5.6% of the significant analyses showed a greater concentration of the element in tissues of birds without shot present. Of the 18 analyses which indicated a significantly greater concentration of an element in birds with shot, 12 (11.1%) were in nonindicator species and only 6 (5.6%) occurred in analyses of indicator species. In addition, 50% of these 18 significant analyses involved feathers, and 50% were with bone tissue.

Arsenic was significantly higher in feathers from shovelers with ingested shot than in those without shot (13.9 ppm vs 2.6 ppm). There was also an indication of arsenic accumulation in scaup and gadwall bones, but due to large variability, this difference was not significant. The amount of arsenic in teal and gadwall bones was highly correlated with the lead concentration ($r = 0.721$, $p < 0.001$; $r = 0.684$, $p < 0.001$). This relationship was also evident in lesser scaup bones and feathers ($r = 0.968$, $p < 0.001$; $r = 0.932$, $p < 0.001$) and in shoveler feathers ($r = 0.970$, $p < 0.001$). Surpisingly, the concentration of arsenic in teal and gadwall feathers was negatively correlated with the concentration of lead ($r = -0.283$, $p < 0.02$; $r = -0.717$, $p < 0.001$).

From our analysis of various kinds of lead shot, it is clear that upon ingestion of spent shot, waterfowl are exposed to several potentially toxic elements. According to Sittig (1976) As, Sb, Sn, Se, Mn, Cd, Cr, Cu, and Ni are all considered toxic by either the EPA

Table 2. Mean concentrations (standard errors) of heavy metals in feathers in relation to the presence of ingested shot.

	TEAL		SHOVELER		GADWALL		SCAUP		PINTAIL		MOTTLED	
	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH
Pb	3.9 (0.4)	3.4 (0.4)	0.2 (0.1)	2.5* (2.0)	0.3 (0.2)	0.0 (0.0)	21.5 (12.0)	35.1 (15.7)	3.4 (0.5)	2.7 (0.5)	9.9 (2.3)	21.4*** (1.2)
As	8.7 (0.6)	4.9** (1.4)	2.6 (0.5)	13.9* (9.7)	45.1 (2.7)	39.5 (12.4)	71.7 (37.9)	55.9 (24.1)	11.0 (1.4)	12.3 (1.7)	12.7 (1.7)	13.3 (1.7)
Sn	8.8 (0.5)	5.3** (0.8)	1.4 (0.3)	6.8* (3.9)	32.9 (2.5)	27.9 (9.6)	12.2 (1.1)	10.5 (1.0)	7.4 (1.5)	9.2 (1.4)	10.3 (2.1)	10.6 (1.5)
Se	6.5 (0.3)	7.7 (1.0)	1.1 (0.3)	3.0** (0.7)	10.5 (1.5)	6.8 (3.6)	8.9 (0.9)	7.1 (0.8)	5.4 (1.3)	7.5 (0.8)	7.7 (1.6)	7.6 (1.3)
Mn	4.5 (0.4)	4.2 (0.7)	5.2 (0.7)	10.5* (3.9)	26.7 (6.1)	58.1* (50.4)	2.5 (0.3)	1.9 (0.2)	6.4 (0.1)	3.3 (0.9)	6.2 (0.4)	2.5*** (0.4)
Cd	0.21 (0.05)	0.43 (0.16)	0.09 (0.02)	0.07 (0.03)	0.06 (0.01)	0.12 (0.02)	0.58 (0.29)	0.37 (0.16)	0.17 (0.01)	0.09* (0.02)	0.22 (0.03)	0.60*** (0.08)
Cr	0.4 (0.8)	0.4 (0.1)	0.2 (0.1)	0.4 (0.2)	5.5 (1.6)	3.1 (1.1)	0.5 (0.1)	1.1 (0.7)	0.4 (0.1)	0.0*** (0.0)	0.9 (0.5)	0.0 (0.0)
Cu	16.0 (1.3)	13.0 (1.1)	8.1 (0.6)	8.8 (0.8)	48.4 (4.5)	53.4 (16.6)	11.2 (0.9)	13.5 (1.6)	10.4 (0.2)	11.7 (0.4)	9.3 (0.6)	11.1 (1.3)
Ni	3.8 (0.6)	1.6 (0.5)	0.7 (0.2)	5.4*** (2.7)	17.2 (5.0)	9.5 (3.0)	3.5 (0.8)	2.8 (0.4)	0.3 (0.3)	0.4 (0.2)	0.3 (0.2)	0.3 (0.1)

* = p 0.05, ** = p 0.01, *** = p 0.001

Table 3. Mean concentrations (standard errors) of heavy metal in wing bones in relation to presence of ingested shot.

	TEAL		SHOVELER		GADWALL		SCAUP		PINTAIL		MOTTLED	
	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH
Pb	8.4	11.1	9.8	9.2	8.8	14.0*	5.8	8.8	31.4	19.2	59.8	54.5
	(1.0)	(0.5)	(0.4)	(1.3)	(0.9)	(0.5)	(0.7)	(3.0)	(-)	(2.6)	(2.5)	(16.8)
As	13.9	16.6	12.0	11.2	5.3	8.7	4.1	17.0	9.7	12.8	11.0	9.6
	(1.5)	(0.5)	(0.9)	(1.1)	(0.6)	(3.0)	(1.0)	(10.3)	(-)	(1.0)	(2.1)	(1.5)
Sn	14.8	17.0	11.5	10.3	7.5	7.0	5.0	6.7	11.5	16.6	13.7	12.1
	(1.2)	(0.5)	(0.4)	(0.7)	(0.4)	(0.5)	(0.8)	(0.8)	(-)	(1.0)	(2.3)	(1.3)
Se	10.7	10.0	7.2	6.3)	5.5	6.2	3.3	4.9*	10.4	14.0	12.1	10.5
	(1.0)	(0.6)	(0.3)	(0.7)	(0.6)	(0.5)	(0.4)	(0.5)	(-)	(0.6)	(1.0)	(0.9)
Mn	10.3	8.0	19.2	16.1	16.5	25.5***	9.5	9.8	11.5	12.8	6.6	10.1*
	(0.9)	(0.3)	(1.5)	(1.1)	(1.3)	(1.3)	(0.5)	(0.7)	(-)	(1.4)	(0.6)	(0.9)
Cd	0.74	0.39	0.21	0.16	0.27	0.44*	0.25	0.31	0.52	0.25*	0.22	0.38***
	(0.15)	(0.03)	(0.04)	(0.06)	(0.03)	(0.04)*	(0.05)	(0.06)	(-)	(0.04)	(0.01)	(0.01)
Cr	1.3	3.6*	0.6	0.6	0.7	0.6	1.5	1.1	1.2	0.8	0.9	1.0
	(0.2)	(2.0)	(0.1)	(0.1)	(0.1)	(0.0)	(0.1)	(0.1)	(-)	(0.1)	(0.1)	(0.1)
Cu	0.9	0.4	2.8	2.6	0.9	1.1	2.6	13.3	1.0	1.0	0.7	1.4*
	(0.2)	(0.2)	(1.0)	(1.2)	(0.3)	(0.2)	(0.7)	(6.6)	(-)	(0.1)	(0.1)	(0.2)
Ni	0.6	2.3***	0.2	1.7	0.7	0.7	0.9	0.8	2.1	4.4	2.5	2.8
	(0.2)	(0.2)	(0.1)	(1.7)	(0.1)	(0.2)	(0.2)	(0.1)	(-)	(0.2)	(0.3)	(0.3)

* = p 0.05, ** = p 0.01, *** = p 0.001

or the Council on Environmental Quality. While none of these elements appeared in the tissues analyzed at toxic levels, very little is known about potentially detrimental interactions between them. Underwood (1979) cites numerous examples of heavy metal interactions both beneficial and negative. For example, arsenic can alleviate selenium poisoning while cobalt can enhance selenium toxicity. Tucker (1972) mentioned the possibility of toxic synergism between lead and arsenic. In addition, many more interactions and their mechanisms of action have been documented. Often those interactions which are most powerful concern heavy metals that are serious environmental contaminants (Underwood 1979).

Since the presence of ingested shot indicates nothing concerning past exposure, it is perhaps more meaningful to compare tissue concentrations on a species basis rather than on an individual basis. Species with high ingestion rates (scaup, pintail, and mottled) had higher lead levels in their bones and feathers. This was especially evident in the mottled ducks with an average 57.1 ppm lead in their bones, almost 3 times the next highest concentration (pintails). The lack of significant differences in lead concentration in bones of indicator species may be due to their high ingestion rates. Even though certain individuals did not have shot in their gizzard, there is a higher probability that they had some at one time, thus resulting in overall high levels in their bones. It is surprising that scaup bone levels were so low, for as Bellrose (1959) pointed out not only do they have high incidences of shot in the gizzard, but they are also more prone to have numerous pellets in their gizzards.

With the exception of pintails, indicator species also had higher levels of lead in their feathers. The analysis of feathers is complicated by the molting cycle of each particular species. Except for mottled ducks, which generally complete their molt by August or September, the other species reach full winter plumage between October and January (Kortright 1967).

Arsenic concentrations in bones of these 6 species were remarkably uniform. However, feather concentrations varied considerably. Pintails and mottled ducks had almost twice the arsenic concentration in their feathers as did the nonindicator species, green-winged teal and shovelers. Again, scaup had the highest concentration, but gadwalls also had high arsenic levels in their feathers. Only shovelers had a significantly greater arsenic concentration in feathers of those individuals with gizzard shot.

The concentration of lead in a sample correlated most often with its cadmium, arsenic, and antimony concentration. Cadmium is often a by-product of zinc, lead, and copper smelting, perhaps explaining its relationship with lead. Of the comparisons of lead and arsenic concentrations 38.5% (5 of 13) were postively correlated, while only 2 indicated a negative relationship. These two were gadwall and teal feathers. In the case of gadwall feathers, the concentration of lead was very low while the average arsenic level was very high. This could account for the negative relationship. Teal feathers showed an unexpected effect of presence of gizzard shot on the concentration of arsenic. Individuals without ingested shot had a significantly higher arsenic concentration in their feathers than did specimens with shot in their gizzard. This could account for the negative correlation of lead and arsenic in their feathers.

The concentrations of lead that we measured compare favorably with bone-lead levels reported in the literature. For example, Stendell et al. (1979) determined lead concentrations in the wingbones of several species of ducks from throughout the country. They found an average of 54.4 ppm lead in mottled ducks from Texas as compared to our value of 57.1 ppm. The average lead concentration in wingbones of scaup in their study was 2.8 while ours was 7.5 ppm. In contrast, our pintail bone average was 21.6 ppm, much greater than the 9.2 ppm reported for Texas birds in their study. Our high values for pintail and scaup samples may be due to the high ingestion rates of the 1981-1982 season as compared to the ingestion levels of 1982-1983. 1981 was a very dry year, and waterfowl were concentrated in areas with very high shot density. In addition, our 1981-1982 sample for pintails contained only 4 individuals and may not be representative of the entire population. White and Stendel (1977) reported bone-lead concentrations for pintails up to 214 ppm, with the average being 9.8 ppm. Perhaps our value of 21.6 ppm is not that extreme. As for lesser scaup, Anderson (1975) reported an average of 40 ppm lead in the birds he analyzed. However, these had died from lead poisoning and would be expected to a much higher concentration of lead.

There is little data in the literature to which we can compare the concentrations of the other elements. The cadmium concentration of bones of various coastal birds ranges from 0.01 to 0.09 ppm (Hulse et al. 1980, Cheney et al. 1981, Maedgen et al. 1982). We measured cadmium bone levels ranging from an average of 0.16 to 0.74 ppm depending on the species. This compares favorably with the levels reported by Reid and Hacker (1982) in laughing gulls (0.61-0.71 ppm).

White and Stendell (1977) demonstrated a relationship between the frequency of lead shot in the gizzard and lead residues in wingbones on a population basis, but Stendell et al. (1979) expressed doubt that any relationship could be established on an individual basis due to the effect of retention time of shot in the gizzard. We were able to show a significant relationship in several cases, and the tendency for greater lead concentration in the particular tissue of individuals with ingested shot was apparent in many more. We had expected the analyses of feathers to be useful in predicting lead ingestion. However, it appears that stage of molt must be taken into consideration before this analysis can be reliable. The presence of other metals at higher concentrations in individuals with ingested shot is intriguing. This is especially true of arsenic, antimony, and cadmium. ICP analysis allows more rapid determinations of these on a large number of samples and it is possible that with additional work on background levels and effect of age, the analysis of these additional elements may prove to be useful in assessing environmental contamination from sources other than shot ingestion. Possible changes in tissue levels of these elements as the use of non-toxic shot replaces lead should be monitored.

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