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Elevated lead concentrations in edible portions of game birds harvested with lead shot

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Abstract

Here, we report the results of a study to determine the frequency of elevated Pb concentrations in pectoral muscle tissue of hunter-killed game birds (mostly waterfowl), and to address the cause of occasionally observed high Pb values. Of 827 right pectoral muscle pools (1–12 individuals per pool), 92 had Pb concentrations greater than 0.5 μg/g wet weight, (~2 μg/g dry weight). The average Pb concentration for these 92 pools was 12 ± 38 μg/g wet weight (~40 ± 125 μg/g dry wt). When tissue from individuals making up some of these 'high Pb' pools were analysed, 40 of 190 individual birds had Pb concentrations > 5 μg/g dry weight in their right pectoral muscles. All tissue samples were examined visually prior to analysis, and none contained detectable Pb pellets. The average concentration of Pb in right pectoral muscle tissue of individual birds from high Pb pools with elevated muscle-Pb concentrations was 211 ± 634 μg/g ($n = 40$) and ranged from 5.5 to 3910 μg/g (dry wt). Large differences in Pb concentrations between right and left pectoral muscle of the same individuals, were often noted. The magnitude of the differences in Pb concentrations between left and right pectoral muscles of the same individual, and also between different samples taken from the same tissue, preclude both analytical error and biologically incorporated Pb as the cause of the elevated Pb concentrations in these animals. Radiography confirmed the presence of numerous small (< 1 mm diameter) metallic fragments in pectoral muscle samples from these birds. Embedded fragments of metallic Pb from shot disintegration are a potential source of dietary Pb exposure for predators, and for human consumers of wild game, especially in communities that rely on subsistence hunting and for whom hunter-killed wild game represents a major food source. This risk can be eliminated by the use of non-toxic shot for hunting.

Keywords: Lead; Muscle; Dietary exposure; Hunting; Game birds; Waterfowl

1. Introduction

Lead (Pb) is a toxic metal with no demonstrated biological function in living organisms. Ingestion of spent Pb shot is the primary source of Pb poisoning in Canadian waterfowl (Scheuhammer and Norris, 1995). In addition, embedded Pb shot pellets are commonly present in the flesh of otherwise healthy, free-flying waterfowl in Canada and elsewhere (Scheuhammer and Norris, 1995). Secondary poisoning from ingestion of Pb shot embedded in the flesh of game animals is responsible for 10–15% of recorded post-fledgling mortality in Bald and Golden Eagles in the USA and Canada (Scheuhammer and Norris, 1995). In an effort to reduce Pb exposure in wild birds, the USA and Canada have implemented bans on the use of Pb shot for the purpose of hunting most migratory game birds (Morehouse, 1992; Environment Canada, 1997). Pb shot is still permitted for hunting most upland game birds and small mammals, and for target shooting.

Ingestion of Pb shot pellets is also a potential source of Pb exposure for humans through the consumption of game animals that have Pb pellets or pellet fragments embedded in their tissues. The risk is increased for populations with traditional diets (Smith and Rea, 1995; Tsuji and Nieboer, 1997). An increase in blood Pb levels and negative health effects have occasionally been attributed to ingested Pb shot pellets retained in the appendices of humans (Lees et al., 1988; Madsen et al., 1988; Tsuji and Nieboer, 1997).

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For animals killed with Pb shot, Frank (1986) showed that small pieces of Pb may be present in kidney and liver tissue due to fragmentation of pellets upon collision with bone. These Pb fragments may be extremely small but are detectable in radiographs.

To address concerns raised by native and non-native hunters, a national survey of contaminants in game bird flesh was undertaken by the Canadian Wildlife Service (Braune et al., 1998). The objectives of the survey were (1) to obtain recent data on contaminants in game birds to assess the risk to human consumers, and (2) to identify any potential avian health issues related to contaminant exposure. Here, we summarize the findings of the survey that pertain to Pb. We then report the results of a further study to determine Pb concentrations in individual pectoral muscles from a large sample of hunter-killed game birds; and we present results of radiographic analyses to visualize small fragments of metallic Pb in avian muscle tissue.

2. Materials and methods

2.1. Specimen collection

Collections of birds were made from 125 sites across Canada (Fig. 1), mostly in the fall hunting seasons between 1988 and 1995. Close to 4000 individual birds were collected, almost all by shotgun using Pb shotshell ammunition. For contaminant analysis (including Pb), birds were pooled according to species and location. If there was an adequate number of birds available from each location, birds were divided further by age and sex. Pool sizes ranged from 1 to 12 individuals, and averaged about 5 individuals per pool. Most of the 44 avian species represented in the 827 pools analysed for Pb were waterfowl; however, other species such as ptarmigan, grouse, woodcock, and various seabirds were also collected.

2.2. Tissue preparation and analysis (pooled samples)

Pectoral muscle tissue was chosen for analysis because it best represents the edible portion of game animals. Collected specimens were processed at the National Wildlife Research Centre Specimen Bank (Hull, Quebec) according to in-house standard operating procedures. Each bird was weighed, aged and sexed. A median portion (~10 g) of the right pectoral muscle was excised, and visually detectable Pb fragments or pellets were removed. Pectoral muscle tissue samples from individual birds were pooled predominantly according to species and location, and homogenized. Homogenized pooled tissue samples were stored in a

Fig. 1. Locations of sampling sites across Canada for game birds analysed for Pb and other contaminants.

−20°C freezer. Remaining portions of right pectoral muscle, along with whole left pectoral muscles of individuals, were archived in the tissue bank in a −40°C freezer.

A portion of each frozen tissue pool was transferred into a pre-weighed acid-washed test tube and freeze-dried for 24–48 h. Tissue was then weighed, rehydrated with deionized H_2O, and digested in high-purity concentrated HNO_3.

Graphite furnace atomic absorption spectrophotometry (GFAAS) was used to measure Pb concentrations of the digests. The upper 10th percentile of Pb concentrations for the 827 pooled right pectoral muscle samples were designated as 'high'. This threshold corresponded to Pb concentrations >0.5 μg/g (wet wt), or >2 μg/g (dry wt) based on an estimated mean moisture content of 70% in muscle samples. The detection limit for Pb in pooled tissue samples by GFAAS was approximately 0.05 μg/g dry weight.

2.3. Tissue preparation and analysis (individual birds)

Of the muscle tissue pools that had high Pb concentrations, 36 were randomly selected for Pb analysis of the individuals making up the pools (190 individuals). Individuals from 6 pools that had pooled tissue-Pb concentrations below 2 μg/g dry weight were also analysed (19 individuals). Muscle tissue samples were freeze-dried and digested as described for pooled samples. Again, visually detected Pb fragments or pellets were removed from tissue samples during processing. For these samples, Pb was measured by standard flame atomic absorption spectrophotometry (AAS), with a practical detection limit for Pb of approximately 1.5 μg/g dry weight in muscle tissue. For individual muscle samples, Pb concentrations >5 μg/g dry weight were considered to be high.

When the Pb concentration in an individual right pectoral muscle sample was high (>5 μg/g), Pb was measured in the corresponding left pectoral muscle (if available) of the same individual for comparison. Left pectoral muscles for 32 individuals with non-detectable Pb concentrations in individual right pectoral muscle samples were also analysed.

2.4. Detecting Pb pellet fragments with radiography

Radiographs of selected muscle samples were taken to determine if embedded fragments of Pb shot pellets could be detected. Pectoral muscle tissue remaining after the initial AAS analyses was used for radiography. A cabinet model Faxitron Model 8050 X-ray machine was used (Field Emission Corporation, McMinnville, OR). This is an automatic machine with a voltage of 10–110 kV peak, and a current of 3 mA at 110 kV. Kodak Industrex AA 400 X-ray film was used. Radiography was performed by staff of the Department of Pathology, Ontario Veterinary College, Guelph University, Guelph, Ontario.

2.5. Quality assurance

Quality assurance for the analysis of Pb included the analysis of replicate samples, reagent blanks, and the following Standard Reference Materials (SRMs): International Atomic Energy Agency fish tissue (MA-B-3/TM); and National Research Council of Canada dogfish muscle (DORM-2) and dogfish liver (DOLT-2).

3. Results

3.1. Quality assurance results

Recovery of Pb from SRMs averaged 98(±17)% ($n=22$). Based on these excellent recoveries, no adjustments were made to Pb concentrations as measured in tissue digests.

3.2. Pooled muscle sample analyses

Pb concentrations averaged 1.4±13 μg/g wet weight (~4.7±43 μg/g dry wt) for all right pectoral muscle pools ($n=827$) analysed for the National Contaminants Survey. There were 735 of 827 pools with low Pb concentrations (≤2 μg/g dry wt), and 92 pools with high Pb concentrations (>2 μg/g). For the 92 pools with high Pb, concentrations averaged 40±125 μg Pb/g (dry wt). Out of the 92 pools with high Pb concentrations, 36 were selected for analysis of individuals ($n=190$ individuals). Pb concentrations in these 36 pools averaged 67±165 μg/g dry weight, with a maximum of 767 μg/g dry weight.

3.3. Analyses of muscle samples from individual birds

Of the 190 individual right pectoral muscles analysed from the 36 high Pb pools, 40 individuals (21%) had >5 μg Pb/g (dry wt), an average concentration of 211±634 μg/g; ($n=40$), and a range of 5.5 to 3910 μg/g. Of these 40 individuals, 11 also had high Pb concentrations in their corresponding left pectoral muscles, while 27 had non-detectable or low (<5 μg/g dry wt) Pb concentrations in their left pectoral muscles. (Left pectoral muscles from the remaining 2 individuals were lost and so were not analysed). In addition, for 5 of the 36 high Pb right pectoral muscle pools, all individual muscle tissues ($n=24$) making up the pools had non-detectable (<1 μg/g dry wt) Pb concentrations.

For individual left muscles that were analysed when right muscle Pb concentrations were low, 3 out of 32

had high Pb concentrations, ranging from 7.2 to 44.5 μg/g dry weight. Table 1 lists the 10 highest Pb concentrations found in right pectoral muscle samples, with corresponding values for left pectoral muscle from the same birds.

For the 6 low Pb pools selected for analysis of individual right muscle tissue, 2 of 19 individuals had a high Pb concentration in the right pectoral muscle (24.7 and 5.58 μg/g dry wt).

3.4. Differences between tissue samples from the same individual

For individuals having high Pb concentrations in right or left pectoral muscle tissue, the average difference in Pb concentration between right and left pectoral muscles was 196 ± 623 μg/g ($n = 41$). The average difference in Pb concentration between the right and left pectoral muscle for individuals having high Pb concentrations in both muscles was still substantial (132 ± 220 μg/g; $n = 11$). Analyses of different subsamples from the same tissue (e.g. same right pectoral muscle) also indicated highly variable Pb concentrations in some cases (Table 2).

Table 1
Pb concentrations in left and right pectoral muscle tissue from specimens with >100 μg Pb/g dry weight in right pectoral muscle

	Species	Pb in left pectoral muscle	Pb in right pectoral muscle
Waterfowl	Snow goose	ND	3909.6
	Snow goose	ND	728.5
	Snow goose	ND	112.3
	Merganser	ND	689.1
	Mallard	ND	237.2
	Mallard	ND	124.7
	Bufflehead	ND	176.7
Upland species	Woodcock	183.0	844.0
	Spruce grouse	22.4	418.5
	Spruce grouse	ND	103.8

ND, not detected (<1 μg/g).

Table 2
Original analysis versus duplicate analysis of Pb (μg/g dry wt) in right pectoral muscle samples

ID # (species)	Original	Duplicate
52146 (willow ptarmigan)	35.8	20.8
55747 (surf scoter)	43.4	ND
52114 (American woodcock)	14.2	ND
52115 (American woodcock)	844.0	64.5

ND, not detected (<1 μg/g).

3.5. Atomic absorption spectrophotometry versus radiography

Radiography indicated the presence of Pb fragments in some muscle tissue samples (Fig. 2). Table 3 summarizes the presence or absence of radiodense fragments from radiographic evaluation of right and left pectoral muscles, compared with Pb concentrations as determined by AAS analysis of samples from the same muscles.

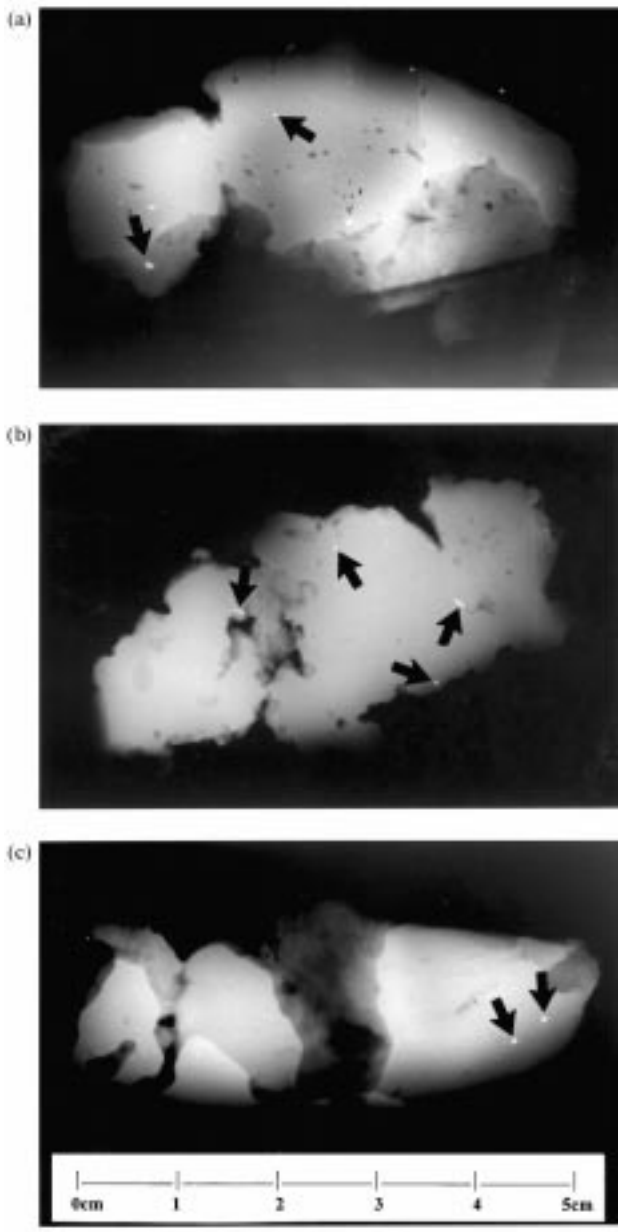

Fig. 2. Radiographs of pectoral muscle tissues of game birds showing X-ray dense particles. Arrows indicate some of the embedded Pb fragments. (a) ID #64896; (b) ID #64906; (c) ID #65546.

Table 3
Presence (P) or absence (A) of radiodense fragments in right and left pectoral muscles, with corresponding Pb concentrations

	ID # (species)	X-ray dense particles (right muscle)	Pb in right muscle (μg/g dry wt)	X-ray dense particles (left muscle)	Pb in left muscle (μg/g dry wt)
Waterfowl	66024 (Barrow's goldeneye)	P	24.7	P	ND
	64906 (common goldeneye)	P[a]	65.4	P	341
	65546 (northern shoveler)	P	7.8	P[a]	5.1
	63141 (northern shoveler)	nm	54.4	P	10.7
	59727 (bufflehead)	nm	13.5	P	9.0
	68429 (American wigeon)	P	41.7	A	ND
Upland species	64896 (spruce grouse)	P	419	P[a]	22.4
	65538 (rock ptarmigan)	P	14.4	P	32.3
	69120 (rock ptarmigan)	nm	97.8	P	104
	64897 (spruce grouse	A	104	A	ND

ND, not detected (< 1 μg/g); nm, not measured.
[a] Radiographs for these individuals depicted in Fig. 2.

4. Discussion

The main objectives of our study were (1) to determine if elevated Pb concentrations occur in edible (muscle) tissues of game birds killed with Pb shot, and (2) to determine whether elevated Pb levels in muscle tissue of game birds could be attributed to Pb shot fragments embedded in the tissue. Our initial analyses demonstrated that a significant number of pectoral muscle samples pooled from tissues taken from individual game birds killed with Pb shot had elevated (> 2 μg/g estimated dry wt) Pb concentrations. Further analyses frequently revealed large differences in apparent Pb concentration between samples of the original pooled tissues, and samples of the individual tissues making up the pool; between left and right pectoral muscles of the same individuals (Table 1); and even between different sub-samples excised from the same muscle (Table 2). Variability of the magnitude we observed indicates that the Pb present in the tissues was not biologically incorporated, as biologically incorporated Pb would be homogeneously distributed throughout the tissue. Although tissue samples of individual muscles with high Pb concentrations did not contain visually apparent Pb shot pellets, the presence of small metallic Pb pieces from the partial fragmentation of pellets passing through the muscle, as reported by Frank (1986) for other tissues (liver, kidney), might explain the large variations in Pb concentrations that we observed within and between individual birds.

Radiography has been used to determine the presence of very small Pb fragments in tissues of animals killed with Pb shot (Frank, 1986). Frank (1986) extracted the solid fragments from radiographed livers and kidneys of ducks and determined that the X-ray dense particles observed radiographically consisted of Pb fragments of varying size (from fine dust to irregular particles ~1–2 mm in diameter) that were readily distinguishable from bone splinters. In the present study, we also detected electron dense, metallic particles embedded in some samples of pectoral muscle of game birds shot with Pb ammunition (Fig. 2). Particles were frequently small (1 mm or less), and were scattered throughout the tissue.

We did not radiographically observe Pb fragments in all muscle samples for which AAS analysis indicated elevated Pb concentrations (e.g. individual 64897; Table 3). Similarly, X-ray analysis of intact gizzards of waterfowl failed to detect 28% of seeded Pb shot fragments of varying sizes (Sanderson and Bellrose, 1986). The reverse situation can also occur. In one of our samples (#66024—left pectoral muscle), Pb was below the detection limit by AAS, yet metallic fragments were clearly visible in radiographs. Radiographs of our tissue samples were taken after an initial aliquot was removed for AAS Pb analysis, thus it is possible that tissue samples devoid of Pb fragments were used for Pb analysis, even though fragments were present in other parts of the same tissue.

Before widespread prohibitions on the use of Pb shot for hunting, it was estimated that 2–3% of North American waterfowl died of Pb poisoning from the ingestion of spent Pb shot pellets (Bellrose, 1959). Scheuhammer and Norris (1995) calculated that the Canadian contribution to this mortality was probably about 250 000 birds annually. In order to reduce the poisoning of wild birds from ingestion of spent Pb shot, several countries have restricted the use of Pb shot for hunting. The USA banned the use of Pb shot for waterfowl hunting nationwide in 1991 (Anderson, 1992). A similar ban on the use of Pb shot for most migratory game bird hunting near wetlands went into effect in Canada on 1 September 1997, and will apply throughout Canada in 1999 (Environment

Canada, 1997). Other countries that have taken action include the Scandinavian nations, the Netherlands, Great Britain, and Australia (Scheuhammer and Norris, 1995).

Regulations against the use of Pb shot for hunting have been established primarily to protect waterfowl and other birds from Pb toxicity due to ingestion of spent shot pellets deposited onto wetlands and fields. However, another important issue is the potential for elevated Pb exposure in predatory wildlife, and in humans, from consumption of the flesh of animals killed with Pb shot. Game animals often have shot pellets or pellet fragments embedded in their tissues. Ingestion of Pb shot embedded in dead or wounded game animals eaten by eagles and other raptorial birds accounts for about 10% of the recorded post-fledging mortality of Bald Eagles in the USA, and about 15% of adult Bald and Golden Eagle mortality in some locations on the Canadian Prairies and British Columbia (Scheuhammer and Norris, 1995). Healthy game birds also carry embedded Pb shot. Approximately 20% of free-living waterfowl of a number of species carry embedded Pb shot in their tissues from non-lethal, non-crippling shootings (Scheuhammer and Norris, 1995). Thus, in addition to the game birds that die annually of Pb poisoning from shot ingestion, millions of other birds carry one or more Pb shot pellets, and fragments of pellets, embedded in their flesh.

For human consumers of wild game, ingestion of whole Pb shot pellets may occur, adding to an individual's normal Pb exposure (Madsen et al., 1988). Most undigested foreign bodies are rapidly expelled from the mammalian gastrointestinal system; however, the density and small size of a Pb shot pellet may cause it to be retained intraluminally or in the appendix (Reddy, 1985; Tsuji and Nieboer, 1997). Madsen et al. (1988) reported that blood Pb levels of individuals with a few (1–2) Pb shot in their appendices were almost twice that of controls without shot retention. Occasionally, overt Pb poisoning in humans from Pb shot retained in the appendix has been reported (Hillman, 1967; Durlach et al., 1986).

In addition to the ingestion of whole Pb shot pellets, consumers of game harvested with Pb shot also risk dietary Pb exposure from the presence of small Pb fragments heterogeneously scattered throughout the edible tissues. As shown in the present study, small fragments of metallic Pb can be present in the muscle tissue of game birds even though whole pellets are not in evidence. Pb concentrations within a pectoral muscle of an individual game bird can vary greatly, sometimes rising as high as many hundreds of parts per million.

In the present study, of the 40 birds identified with elevated Pb levels in right pectoral muscle tissue, the average Pb concentration was 211 μg/g and values ranged from as low as 5.5 μg/g to almost 4000 μg/g. No tissue guidelines for Pb have been established for the flesh of poultry or other avian species; however, 0.5 μg/g wet weight (~2 μg/g dry wt) has been set as an acceptable Pb limit for formulated fish protein (Health Canada, 1991). The presence of Pb shot fragments in edible tissues of game animals poses a risk for increased human dietary Pb exposure, especially in communities where subsistence hunting, and the consumption of wild game, is common (Tsuji and Nieboer, 1997). This form of dietary Pb exposure in people is completely unnecessary, and can be avoided by the use of non-toxic shot for hunting.

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