

Lead Pellet Ingestion and Liver-Lead Concentrations in Upland Game Birds from Southern Ontario, Canada

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Abstract One-hundred twenty-three gizzards from upland game birds (chukar, *Alectoris chukar*; and common pheasant, *Phasianus colchicus*) harvested by hunters in southern Ontario, Canada, were examined for lead pellet ingestion by manual examination of gizzard contents and by radiography. Lead pellets were found to be ingested by chukars (6/76; 8%) and the common pheasant (16/47; 34%). Further, 13% (17/129) of the bird (wild turkey, *Meleagris gallopavo*; Hungarian partridge, *Perdix perdix*; chukar; and common pheasant) livers analyzed had elevated lead concentrations (≥ 6 $\mu\text{g/g}$ wet weight [ww]). Liver-lead concentrations above Health Canada's guideline for human consumption of fish protein (< 0.5 $\mu\text{g/g}$ ww) were found in 40% (51/129) of livers analyzed. Data indicate that the ingestion of lead pellets in upland game birds and the potential consumption of lead-contaminated meat by humans are concerns related to the continued use of lead shotshell for hunting.

Keywords Lead pellet ingestion · Liver-lead concentrations · Upland game birds · Canada · Lead shotshell

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Introduction

Lead is a toxic substance to wildlife and humans (Scheuhammer and Norris 1995; ATSDR 2005). On September 1, 1999, the use of lead shotshell for the hunting of most migratory game birds was banned in Canada by the federal government (Environment Canada 1997). As a result, “nontoxic” shotshell types are required for hunting of migratory game birds in accordance with the federal regulations; by contrast, the hunting of most upland game birds falls under provincial jurisdictions (Ontario Provincial Offences 1999), because only a few species are migratory (e.g., American woodcock, *Scolopax minor*) and fall under federal jurisdiction (Environment Canada 2000). Thus, upland game birds can become exposed to lead pellets by mistakenly ingesting them as grit, while feeding in areas where lead pellets are deposited. Although lead pellet ingestion rates and tissue-lead levels have been studied extensively in other parts of the world (e.g., Butler et al. 2005; see Rodrigue et al. 2005 for a brief review), few studies (Tsuji et al. 1998; Scheuhammer et al. 1999; 2003; Rodrigue et al. 2005; Stevenson et al. 2005) have examined the lead shotshell issue in upland game birds in Canada. Taking into account that criteria have been developed for biologically incorporated lead in livers (concentrations ≥ 6 $\mu\text{g/g}$ wet weight [ww] are diagnostic of lead poisoning in birds [Friend 1985]), caution must be advised in interpreting liver-lead levels of game birds harvested with lead shotshell, as lead pellets/fragments often become embedded in livers (Frank 1986). Ideally, policy makers require data to make an informed decision on the use of lead shotshell for the hunting of upland game birds in Canada.

There is also concern that the use of lead shotshell in all game hunting may be contaminating wild meat to the point where the harvested meat is unsafe for human consumption

(e.g., Tsuji et al. 1999; Rodrigue et al. 2005). In Canada, it has been shown through radiography and spectrometry that lead pellets/fragments commonly contaminate waterfowl harvested with lead shotshell (Scheuhammer et al. 1998; Tsuji et al. 1999) and can cause elevated lead concentrations in the edible portions of game birds. Health Canada guidelines for human consumption of fish protein is a lead concentration of $<0.5 \mu\text{g/g}$ ww (Health Canada 1991; no guidelines exist for game birds). In addition, for humans who subsist on wild game in Canada, it has been shown that the ingestion of lead pellets/fragments is common, with pellets being found in the gastrointestinal tract including the appendix (Carey 1977; Reddy 1985; Tsuji and Nieboer 1997). More than 500 pellets have been found in a surgically removed appendix (Carey 1977). Taking into account that the presence of lead pellets/fragments in the gastrointestinal system has been shown to increase a person's lead body burden (Durlach et al. 1986; Madsen et al. 1998) and that lead has no known function in the human body and acts detrimentally on almost all bodily systems (Health Canada 2002; ATSDR 2005), the human health concerns are warranted. Indeed, elevated tissue-lead levels have been reported in subsistence harvesting groups in Canada where no other source of lead exposure has been identified (Tsuji et al. 1997, 2001; Hanning et al. 2003; Levesque et al. 2003). In this study, we examine lead pellet ingestion rates for upland game birds (chukar, *Alectoris chukar*; and common pheasant, *Phasianus colchicus*) harvested from a heavily hunted area in southern Ontario, Canada, to evaluate bird health. In addition, the potential for lead contamination of upland game bird livers via the embedding of lead pellets/fragments will also be examined through radiography and electrothermal atomic absorption spectrometry (EAAS), as offal is consumed by Native Canadians (Tsuji and Nieboer 1999) and some Europeans (Guitart et al. 2002) as a delicacy. Indeed, in a study by Guitart et al. (2002) where 411 bird livers were analyzed from waterfowl carcasses obtained randomly from Spanish hunters, it was reported that approximately 40% of livers had lead concentrations higher than $0.5 \mu\text{g/g}$ ww; the European Union's current regulation permits a maximum lead concentration of $0.5 \mu\text{g/g}$ ww in poultry offal. As livers of waterfowl are often consumed in Spain, alone or as a sauce mixture, Guitart et al. (2002) established a risk to human health from the consumption of game bird livers.

Materials and Methods

Gizzards ($n = 123$) from chukars and common pheasants, and livers ($n = 129$) from four species of upland game birds (wild turkey, *Meleagris gallopavo*; Hungarian partridge, *Perdix perdix*; chukar; and common pheasant), were

collected whole from upland habitat located on a private island (approximately 1950 acres) in southern Ontario, Canada. The island was characterized by mixed woodland with monospecific stands of grass (*Gramineae*), while some fields also contained wheat (*Agropyron* spp.), burdock (*Arctium* spp.), and thistle (*Sonchus* spp.) (Holdner et al. 2004). Different areas of the island experienced different hunting pressure: shooting stations were used primarily for clay target shooting; fields were generally heavily hunted over, with some fields being tilled annually, while others were never tilled; and the fly pen enclosure area housed the upland game birds prior to release (Holdner et al. 2004). Farm-raised birds were released the day of the hunt, though whatever birds that survived earlier hunts were still in the fields. There is not a large overwinter population of birds. The study area has been heavily hunted with respect to indigenous wild turkeys and imported farm-raised chukars, Hungarian partridges and common pheasants for released bird hunting, since the 1930s.

Sample collection was conducted in the fall of 2000. Gizzard and liver samples were not always matched; that is, gizzard and liver were not always collected for each bird. Only lead shotshell has been used to hunt the upland game birds at this location and hunters are restricted to the use of lead shotshells provided by the hunting club. Lead pellet density ranged from 14 pellets per 0.25 m^2 in the hunting fields to 2051 pellets per 0.25 m^2 in clay target shooting areas (Holdner et al. 2004). Bird movement was not restricted by any physical barriers between target shooting and hunting areas; however, birds were collected from the hunting fields.

Gizzards were examined to identify any pellets that had been shot in; these pellets were not considered in the count of ingested pellets. Pellets were removed from the grit following a method described by Tsuji et al. (1998). After the removal of pellets from the grit manually, the grit was placed in a petri dish and radiographed; radiography identifies any lead pellets missed through manual examination (Tsuji et al. 1998).

Upland game bird livers ($n = 129$) were collected whole, sealed in marked, plastic, Ziploc bags, and stored frozen until further processing. Briefly, tissue samples were lyophilized to constant weight, radiographed to detect lead pellets/fragments, and then ground in a spice mill with stainless-steel blades. As most lead fragments were too small to be felt through manual manipulation, fragments were not removed prior to processing. Liver dry weight was used, as ww values are variable; conversion of dry weight (dw) to ww values was done according to Tsuji et al. (1999), where average moisture content was 66% in bird liver tissues.

Distilled double-deionized water (DDW) was used in the preparation of solutions and digestions. All liver

Table 1 Liver-lead levels of upland game birds

Species	Sample size (<i>n</i>)	Number of individuals with elevated lead levels ^a	Percentage of individuals with elevated lead levels	Liver-lead level range ($\mu\text{g/g}$ wet weight) ^b
Chukar	74	4	5%	<DL–7766 ^c
Hungarian partridge	5	0	0%	<DL–6.1
Wild turkey	1	1	100%	7.0
Common pheasant	49	12	24%	<DL–25.1
All species	129	17	13%	

^a Biologically incorporated liver-lead levels diagnostic of lead poisoning are $\geq 6 \mu\text{g/g}$ wet weight (Friend 1985); however, in this study we cannot discount the contribution of embedded lead fragments to the elevated concentration of lead seen in the livers

^b Data do not include duplicate sample measurements, only original values of analyzed samples

^c DL = 0.3 $\mu\text{g/L}$

Table 2 Original analysis versus duplicate analysis (μg lead/g wet weight) in upland game bird liver samples

Species	Sample ID	Original lead concentration	Duplicate lead concentration
Chukar	C51L	0.461	0.007
	C80L	<DL	<DL
Wild turkey	T85L	7.0	13.9

samples were weighed (0.10 g) and placed in individual 1.5-ml microtubes (Sarstedt) where 1 ml of HNO_3 (Ultrex, JT Baker) was added, prior to being sealed with lid locks (DiaMed) and left overnight in a fumehood to allow digestion. Liver samples were placed in two microtube heating blocks and placed in a block heater (Multi-Blok; Lab-Line). Samples were digested initially at 60°C for 1 h, followed by another hour at 80°C. Samples were allowed to cool and were then centrifuged (Eppendorf centrifuge model 5415). The contents of each microtube were then transferred into 15-ml test tubes (Pyrex) using DDW. Samples were then diluted to a volume of 4 ml using DDW, vortexed, and placed in heating blocks on hot plates (Corning model PC 100); samples were further digested for another 5–6 h until dryness. The residue was taken up in 5 ml of 0.1% HNO_3 , vortexed, and placed in a test tube rack with lid locks until lead determination using EAAS. Working standards were made following the protocol detailed by Jayasinghe et al. (2004). One blank and certified reference standards (water, SRM 1640; mussel tissue, 1974b; National Institute of Standards and Technology, Gaithersburg, MD, USA) were run with every 10 samples. Some duplicates were also run. The reference standards were within 15% of the certified concentration. All blanks were below the detection limit of 0.3 $\mu\text{g/L}$.

Only original values found for liver samples that had duplicate measurements are used in results. Duplicate values were not included, as duplicates were only used to examine if heterogeneity existed in the samples.

Differences between original measurements and duplicates have been reported in lead shotshell-harvested game birds (Scheuhammer et al. 1998; Rodrigue et al. 2005).

Results

An 8% ingestion rate of lead pellets by chukars (6/76) was found, with one or two pellets/fragments being identified through manual examination and radiography of the grit. The percentage of common pheasants ingesting lead pellets was much higher, at 34% (16/47; range of number of pellets/fragments in gizzard contents, 1–66). It should also be noted that approximately 5% of gizzard content samples for common pheasants had >10 pellets, which suggests that these birds had acute lead poisoning (Scheuhammer and Norris 1995).

All species of upland game birds, except the Hungarian partridge, had at least 5% of the birds sampled showing liver-lead concentrations indicative of lead poisoning (Table 1; liver-lead levels $\geq 6 \mu\text{g/g}$ ww are diagnostic of lead poisoning in birds [Friend 1985]); however, any elevation in liver lead could conceivably be due to small particles of metallic lead being embedded in the tissue (Frank 1986). To the point, one liver-lead concentration is too high (7766 $\mu\text{g/g}$ ww; chukar, Table 1) to be the result of biologically incorporated lead; the high liver-lead concentration is attributed to pellets/fragments of lead embedded in the tissue (Table 3), as it has been suggested that the upper limit for lead concentrations in livers of wild or lead-pellet-dosed waterfowl is approximately 283 $\mu\text{g/g}$ dw (Tsuji et al. 2002).

In comparing the number of fragments and/or pellets found in the liver through radiography with the concentration of lead quantified, we can determine if lead pellets/fragments embedded in the tissue were influencing lead levels. However, some lead fragments may be too small to be discernable in a radiograph. In two chukar samples (C1L, C7L) there were lead-embedded fragments in the

liver tissue; however, lead concentrations were below the DL of 0.3 $\mu\text{g/L}$. By contrast, C54L was shown to have two lead fragments embedded in it and had a correspondingly high liver-lead concentration (7766 $\mu\text{g/g ww}$). A wide variation in the concentration of lead found in the livers occurred when only one radiopaque object was identified ($n = 5$; range, 0.107–5.767 $\mu\text{g/g ww}$). Also, it was evident that duplicate liver samples could be variable with respect to lead concentration (Table 2). Finally, livers of 40% of the upland game birds analyzed showed lead concentrations $>0.5 \mu\text{g/g ww}$ (Table 3). Caution is advised when interpreting this table, as there are no standards for the allowable levels of metallic lead in food; and only a small subsample of the liver was analyzed, which is of concern due to the heterogeneous nature of lead fragmentation. Ideally, further lead analyses using whole livers are required, but this approach has logistical and economic constraints.

Discussion

Hunting upland game birds with lead shotshell is a cause of concern on this southern Ontario island in that approximately 5% of the common pheasants sampled in the present study had ingested >10 pellets; the ingestion of ≥ 10 lead pellets causes birds to die within a few days (Scheuhammer and Norris 1995). The lead pellet ingestion rates found in the present study for upland game birds were much higher than what has been reported for other upland game bird studies in Canada (for a review see Rodrigue et al. 2005); these findings were most likely due to the fact that lead pellets were readily available in large numbers on the island and most species of upland game bird sampled were raised off island on farms. However, the fact remains that upland game birds will ingest lead pellets in dangerous quantities if given the chance.

Although the present study site was atypical in that it was a private shooting range/hunting club, there are at least

211 active public shooting ranges in Ontario where spent lead pellets are contributing to high lead loadings (tons/year) in the environment (Darling and Thomas 2003). Thus, the pellet densities found on the island (Holdner et al. 2004) are not atypical for outdoor shooting ranges in Ontario. The majority of these active shooting ranges are located in southern and central Ontario where luvisolic soils predominates (Darling and Thomas 2003). Since there is no provincial requirement with respect to site remediation of lead pellets at active public shooting ranges, as well as defunct public shooting ranges and private shooting ranges (Darling and Thomas 2003), lead pellets remain in the environment and a potential threat to upland game birds in Ontario.

Similar concern is also warranted when examining liver-lead levels in the present study in that 13% (17/129) of the upland game birds exhibited liver-lead concentrations consistent with lead poisoning, and only one of these livers had radiographic evidence of tissue embedded lead pellet/fragments. However, liver-lead levels must be interpreted with caution because even though there is not radiographic evidence of lead fragment contamination, lead fragments could be so fine (Frank 1986) as not to show up on a radiograph or appear as an artifact.

Three potential sources of lead exposure for humans from the consumption of upland game birds killed with lead pellets include (1) ingestion of tissues from lead-exposed birds that have biologically accumulated higher than normal concentrations of lead; (2) ingestion of minute fragments of metallic lead embedded in tissue; and (3) ingestion of lead shot pellets embedded in bird tissues (Scheuhammer and Norris 1995). In the present study, 40% (51/129) of upland game bird livers analyzed were above the level for human consumption; of these livers, four had radiographic evidence of lead pellets/fragments being embedded in the tissue. Thus, it appears that human concerns with the consumption of upland game bird livers relates to both biologically incorporated lead (this may be overestimated, as fine lead particles could have contaminated samples but

Table 3 Upland game bird liver-lead levels compared to human consumption guidelines

Species	Sample size (n)	Number of birds with liver-lead levels above human consumption guidelines ^a	Percentage of individuals with lead levels above human consumption guidelines
Chukar	74	21	28%
Hungarian partridge	5	2	40%
Wild turkey	1	1	100%
Common pheasant	49	27	55%
All species	129	51	40%

^a The Health Canada guideline for human consumption of fish protein is a lead (biologically incorporated) concentration of $<0.5 \mu\text{g/g}$ wet weight; no guidelines exist for game birds (Health Canada 1991)

not shown up radiographically) and embedded lead pellets/fragments. Other studies of upland game harvested with lead shotshell (Hubbard et al. 1965; Scheuhammer et al. 1998; Tsuji et al. 1999) or .22 caliber lead bullets (Rodrigue et al. 2005) have also shown elevation of edible tissue above the level for human consumption.

Heterogeneity within a sample was also found with some duplicate samples differing from original samples in orders of magnitude. Heterogeneity of tissue-lead concentrations for duplicate samples with respect to birds harvested with lead shotshell has also been shown by other researchers (Frank 1986; Scheuhammer et al. 1998; Johansen et al. 2001, 2004). Similarly, heterogeneity within individuals harvested with lead shotshell has also been reported when left and right breast tissues were analyzed for lead (Scheuhammer et al. 1998; Johansen et al. 2001). These findings are due to the fact that there is heterogeneous scattering of lead fragments within the tissue generated from the impacting of lead pellets with soft and hard tissues (Tsuji et al. 1999). Depending on what part of the subsample of tissue (or side of breast) was analyzed for lead concentrations, there would be a different value obtained. The risk to human health is difficult to assess when eating hunted game birds harvested with lead shotshell because the concentration of lead is heterogeneous due to lead pellet fragmentation even when tissues have been inspected for lead pellets prior to consumption (Hubbard et al. 1965). Thus, it is difficult to accurately assess whether humans consuming upland game birds are at risk of exceeding the Provisional Tolerable Weekly Intake for lead (25 µg/kg body weight/week for adults and children [WHO/FAO 1999]).

As stated by Thomas (1997, p. 48), “In 1995, the Canadian federal Standing Committee on the Environment and Sustainable Development recommended that the Canadian Environmental Protection Act be used to stop the manufacture, sale and use of all lead shot by 1997,” but instead the Minister of the Environment chose to ban only lead shot use in migratory bird hunting. Although the use of lead pellets for migratory game bird hunting is now prohibited in Canada, hunters can still legally use lead shotshell for upland game bird hunting. As shown in the present study, upland game birds are ingesting lead pellets. The Canadian ban on hunting migratory birds with lead pellets has caused a 70% decline in the mean bone-lead levels in hatch-year ducks in Ontario since the nontoxic shot regulations were put into place; this corresponds to a >80% hunters compliance rate with the regulations (Stevenson et al. 2005). The nontoxic shot regulations do not apply to upland game species such as the American woodcock, which has shown no decrease in mean bone-lead concentrations since the regulations came into effect (Stevenson et al. 2005). Moreover, Scheuhammer et al.

(2003) have shown, using stable lead isotopes ratios, that the elevated lead levels in American woodcock were consistent with lead shot ingestion. These studies suggest that lead pellets need to also be banned for upland game bird hunting in order to remove a source of environmental lead. The Canadian government has cited lack of evidence of problems as the reason for not extending the ban of lead pellets to all uses (Thomas 1997). Denmark and the Netherlands in 1993 banned all uses of lead shotshell regardless of lack of scientific evidence, invoking the Precautionary Principle (Thomas 1997). Denmark first banned lead shotshell for hunting in designated areas, but noncompliance and enforcement difficulties resulted in a complete ban on the use of lead shotshell for all hunting to ensure reduced lead poisoning in waterfowl (Clausen 1992). Canada may face the same challenges as Denmark, as Canada’s partial ban on lead shotshell use is difficult to enforce effectively and ensure 100% compliance since lead shotshell is still available and can be legally purchased by upland game bird hunters (Scheuhammer and Norris 1995). Although approximately 25% of upland game bird hunters in Ontario have voluntarily begun to use nontoxic shot (Stevenson et al. 2005), a complete ban on the use of lead shotshell is needed in Canada to halt importation and availability. The result would be a significant decrease in lead pellet deposition (and resulting poisoning of upland game birds) and a decrease risk of human consumption of lead-contaminated game-tissue (where lead is biologically incorporated and/or embedded as fragments).

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