Bull Environ Contam Toxicol (2009) 82:435–439
DOI 10.1007/s00128-009-9647-2

Determining Tissue-Lead Levels in Large Game Mammals Harvested with Lead Bullets: Human Health Concerns

L. J. S. Tsuji · B. C. Wainman · R. K. Jayasinghe · E. P. VanSpronsen · E. N. Liberda

Received: 30 January 2008 / Accepted: 7 January 2009 / Published online: 21 January 2009

Abstract Recently, the use of lead isotope ratios has definitively identified lead ammunition as a source of lead exposure for First Nations people, but the isotope ratios for lead pellets and bullets were indistinguishable. Thus, lead-contaminated meat from game harvested with lead bullets may also be contributing to the lead body burden; however, few studies have determined if lead bullet fragments are present in big game carcasses. We found elevated tissue-lead concentrations (up to 5,726.0 μg/g ww) in liver (5/9) and muscle (6/7) samples of big game harvested with lead bullets and radiographic evidence of lead fragments. Thus, we would advise that the tissue surrounding the wound channel be removed and discarded, as this tissue may be contaminated by lead bullet fragments.

Keywords Lead bullets · Lead-contaminated wild meat · Human consumption

Lead is a non-essential metal that has detrimental effects on wildlife and humans (Scheuhammer and Norris 1995; ATSDR 2005). Thus, substantial progress has been made over the last three decades in Canada and the US to decrease the amount of lead in the environment; major initiatives have drastically decreased or eliminated lead in paint, solder, and gasoline. Another environmental source of lead is lead ammunition. Indeed, Tsuji et al. (2008) have shown using stable lead isotope ratios that lead ammunition is a source of lead exposure in subsistence hunting people of northern Canada. Although lead shotshell was banned for the hunting of waterfowl in 1991, in the US, and for migratory bird hunting in Canada, in 1999 (Thomas 1997), the use of other types of lead ammunition, such as, lead-core bullets for the harvesting of large game is still allowed in North America, except for some areas in California (California Department of Fish and Game 2008). In Canada, there are no federal laws restricting the chemical composition of the ammunition that can be used to harvest these game animals because these organisms fall under the jurisdiction of the provincial governments (see for e.g., Ontario Provincial Offences 1999). Only migratory game birds are regulated by the federal "non-toxic" shot policy (Environment Canada 2000); a similar situation exists in the US between federal and state governments (Thomas 1997). Thus, there is a need to assess whether lead bullets used in the harvesting of large game mammals fragment to the extent where meat are not suitable for human consumption [>0.5 μg/g wet weight (ww) for fish protein, no guideline exists for wild game; Health Canada 1991].

Although Hunt et al. (2006) clearly show that deer (*Odocoileus* spp.) harvested with lead bullets become contaminated with lead bullet fragments, no toxicological tests were performed. The absence of toxicological tests was probably due to the fact that the authors were interested only in avian exposure through scavenging of carcasses and offal piles rather than human exposure through the consumption of lead-contaminated meat where lead-tissue levels are of importance. In this paper, we

L. J. S. Tsuji (✉) · E. P. VanSpronsen · E. N. Liberda
Department of Environment and Resource Studies,
University of Waterloo, 200 University Ave. West, Waterloo,
ON N2L 3G1, Canada
e-mail: ljtsuji@fes.uwaterloo.ca

B. C. Wainman
Department of Obstetrics and Gynecology,
McMaster University, Hamilton, ON L8N 4A6, Canada

R. K. Jayasinghe
Jacques Whitford Limited, Burlington, ON, Canada

determine tissue-lead levels in large mammals harvested with lead bullets; radiography is used to assess whether lead fragments contaminate the meats to an extent where there are human health concerns.

Materials and Methods

White-tailed deer (*Odocoileus virginianus*) livers (n = 9; identification codes: D1L–D9L) and striated muscle samples (n = 4; D1M–D4M) were collected from hunters in southern Ontario during the fall of 2000. Caribou (*Rangifer tarandus*) muscle samples (n = 3; note: two sub-samples were processed from C1 and C2; while, ten sub-samples (C3a–j) were processed for radiography from C3, but only C3a–c were analyzed for lead) were obtained from First Nation people of northern Ontario, in the winter of 2000. Caribou samples were excised near the wound channel.

All tissue samples were sealed in marked, plastic, Ziploc^{TM} bags and stored frozen until processed further. Samples were lyophilized to constant weight and radiographed (to detect lead pellets/fragments), prior to being ground in a spice mill with stainless steel blades. Dry weight was used as wet weight values are variable; conversion of dry weight (dw) to wet weight (ww) values was done according to Medvedev (1999), where average moisture content was reported as 72% in livers and 75% in muscle of large mammals.

Ultrapure water was used in the preparation of all solutions and digestions and is referred to as distilled double-deionized water (DDW). Working standards of 0, 2.5, 5, 10, 15, 25, 50 and 100 μg/L lead were made from a lead intermediate standard (2,000 μg/L). Working standards were constituted by adding 0–1,250 μL of intermediate lead standard plus 25 μL Triton solution then diluting to 50 mL with 0.1% HNO_3.

Individual tissues samples were weighed (0.10 g) and placed into microtubes (Sarstedt). One ml of HNO_3 (Ultrex, JT Baker) was placed into each microtube, sealed with lid locks (DiaMed), and left in a fumehood overnight. Tissue samples were then placed into microtube heating blocks and placed in a block heater (Multi-Blok, Lab-Line). Samples were digested initially at a temperature of 60°C for 1 h and then at 80°C for another hour. After cooling (usually 10 min at room temperature), samples were placed in a centrifuge for 10 min (11,000 rpm). The entire contents of each microtube was subsequently transferred into 15-mL test tubes (Pyrex) with DDW. Samples were then diluted to a volume of 4-mL using DDW, vortexed and placed into cylindrical heating blocks on hot plates; tissue samples were digested until dryness (5–6 h) at a hot plate temperature setting of 120°C. The residue was taken up in 5-mL of 0.1% HNO_3. Subsequently, samples were vortexed and placed in a test tube rack with lid locks until lead determination.

Tissue digests were analyzed using a Varian Electrothermal Atomic Absorption Spectrometer (EAAS; model Spectra AA 220; Zeeman correction is not an accessory of this spectrometer) with graphite furnace. All storage vials were tested to be free of lead. 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 ten samples. The assigned values for the reference standard were within 15% of the certified concentration. All blanks were below the detection limit of 0.3 μg/L (three times the standard deviation of the 0.1% HNO_3 or DDW blank). Duplicates were also run.

Results and Discussion

Elevated tissue-lead concentrations (>0.5 μg/g ww) were found in deer livers (5/9 samples) and muscle (3/4 samples; Table 1). Deer samples with radiographic evidence of lead fragments had elevated tissue-lead levels (range 23–1,243.6 μg/g ww; Table 1), but some samples without radiographic evidence of lead fragments were also elevated (e.g., D1L = 1.2 μg/g ww; D4 M = 1.2 μg/g ww; Table 1). All caribou muscle-lead concentrations (range: 1.0–5,726.0 μg/g ww; Table 2) were above the level for human consumption irrespective of whether or not there was radiographic evidence of lead fragments being present in the tissue. Sub-samples (i.e., duplicates etc.) taken from the same animal were found to vary considerable (e.g., C3a vs. C3b: 23 vs. 5,726.0 μg/g ww; Table 2).

Table 1 Tissue-lead levels in white-tailed deer harvested with lead bullets

ID #	Tissue type	Radiographic evidence of lead fragments in tissue	Lead concentration (μg/g wet weight)
D1L	Liver	No	1.2
D2L	Liver	Yes	23.0
D3L	Liver	No	0.4
D4L	Liver	No	0.7
D5L	Liver	No	0.2
D6L	Liver	No	0.1
D7L	Liver	No	0.1
D8L	Liver	Yes	1,243.6
D9L	Liver	Yes	42.5
D1M	Muscle	No	0.9
D2M	Muscle	No	0.3
D3M	Muscle	Yes	867.4
D4M	Muscle	No	1.2

Table 2 Tissue-lead levels in caribou harvested with lead bullets

ID #	Tissue type	Radiographic evidence of lead fragments in tissue	Lead concentration (μg/g wet weight)
C1a	Muscle	Yes	36.9
C1b	Muscle	No	1.0
C2a	Muscle	Yes	2.3
C2b	Muscle	No	1.0
C2a	Muscle	Yes	23.0
C2b	Muscle	Yes	5,726.0
C2c	Muscle	Yes	52.6

Although data are limited, it is clear from the results of the present study that large game harvested with lead bullets can be contaminated with lead to the extent whereby some portions of the tissues are no longer suitable for human consumption. Other researchers have reported lead-tissue contamination in large game mammals shot with lead bullets. However, studies are not consistent in their findings of lead-tissue contamination in large game shot with lead bullets (study: species, n, tissue type, range μg/g ww; Medvedev 1999: moose, 57, liver, 0.38–8.23; moose, 28, muscle, 0.35–3.75; caribou, 19, muscle, 0.03–6.88; Lewis et al. 2001: deer, 5, liver 0.08–0.67; Falandysz et al. 2005: deer, 82, liver, 0.047–1.0; deer, 82, muscle, 0.010–1.5). These results can be explained by taking into account that the distribution of lead ammunition fragments is not homogeneous within the animal shot (Fig. 1), the amount of lead-tissue contamination is dependent on the distance from where the animal was shot, where the lead pellet or bullet penetrated the tissue, and whether the lead projectile hit anything hard (e.g., bone, Frank 1986) that would cause further fragmentation of the lead projectile. Thus, it is not possible to accurately assess whether humans consuming big game shot with lead bullets 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 tissue-lead concentrations would vary greatly within tissue type (e.g., striated muscle) due to bullet fragmentation.

The heterogeneous distribution of lead in tissue through the use of lead bullets has been noted by several researchers (e.g., Fackler et al. 1984). Indeed, Rodrigue et al. (2005) report that in northern Quebec (Nunavik) where most hunters use a .22 caliber rifle to hunt ptarmigans (willow ptarmigan, *Lagopus lagopus*; rock ptarmigan, *L. mutus*), the tissue surrounding the entry wound site is the most lead-contaminated (26–650 μg/g ww), with tissue sampled farther away from the entry wound being less contaminated (0.001–0.07 μg/g ww). Similarly, Tsuji et al. (1999) report that none of the samples from 23 moose (*Alces alces*) and caribou shot with lead bullets in northern Canada had tissue-lead concentrations above the detection limit (0.3 μg/g ww); presumably, since none of these samples were collected near the wound channel. If <0.3 μg/g ww is adopted as a baseline of lead contamination (i.e., physiologically incorporated lead) in these ungulates, the present study clearly shows through EAAS (Table 2) and radiography that caribou samples excised from near the wound channel are highly lead-contaminated with associated lead signatures (i.e., embedded lead fragments) in the radiographs (Fig. 1). It should be noted that game tissue surrounding the wound channel is not typically thrown away; this damaged meat is made into stew and/or sausages by First Nation Canadians (Chief Billy Katapatuk, personal communication). Falandysz et al. (2005) reports that in Europe, lead-contaminated deer meat (embedded with fine dust particles from lead bullets) is also used in processed food, such as, pies. We would recommend that the tissue surrounding the wound channel be removed and

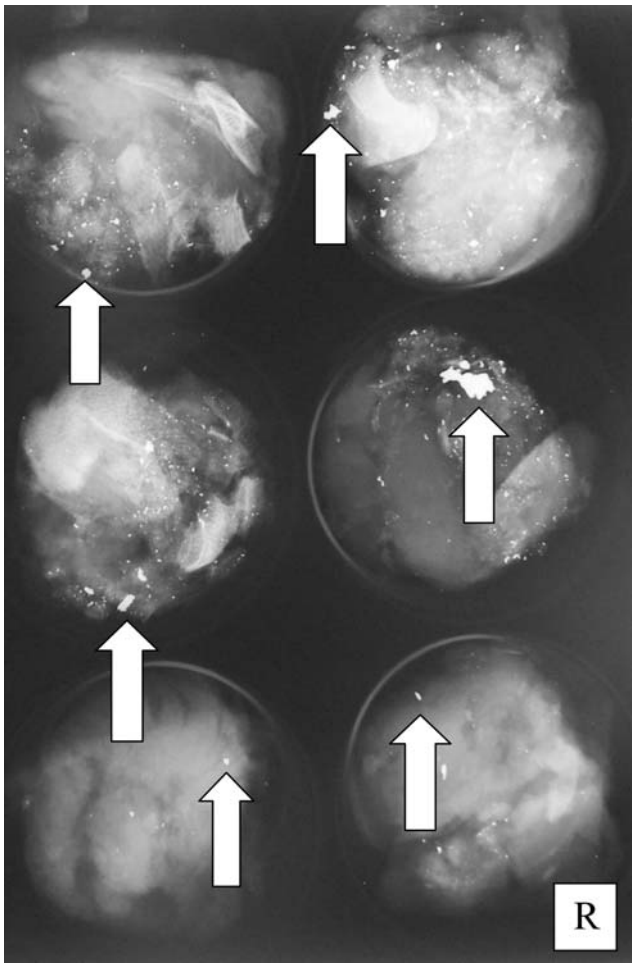

Fig. 1 Radiograph illustrating lead bullet fragmentation in caribou striated muscle. *Block arrows* point to some of the diffuse lead fragments. Sub-sample C3c (lead concentration = 52.6 μg/g ww) is located in the lower right position of the radiograph

discarded, as this tissue may be contaminated by lead bullet fragments. Indeed, Hubbard et al. (1965) report that even when game bird tissue is carefully teased apart and searched for lead pellets/fragments, tissue-lead concentrations were still in the range of 0.5–22.7 μg/g ww (n = 12), even after the removal of lead pellets/fragments from the tissue prior to analysis. Apparently, it is not possible to remove all lead pellets/fragments from game tissue harvested with lead ammunition due to the location, depth of penetration, and minuscule size of some of the fragments (Hubbard et al. 1965; Frank 1986).

Clearly, large game harvested with lead bullets may be contaminated to the point where the meat is unsuitable for human consumption, as studies have reported elevated blood-lead levels in individuals with lead pellets or lead bullet fragments determined radiographically and/or surgically as residing in the gastrointestinal system. Although most cases are typically, low-level chronic exposure (Madsen et al. 1988) there are cases of acute lead poisoning requiring medical intervention (e.g., Gustavsson and Gerhardson 2005). The only cases we are aware of concerning ingestion of lead bullet fragments are those reported by McQuirter et al. (2003). They studied gunshot injuries to the maxillofacial region and found that three patients ingested lead bullet fragments (shown radiographically to be located in the gastro-intestinal tract). In these patients, rapid elevation of blood-lead was noted, 1–2 months post-injury. Blood-lead levels were found to have decreased at the 3 year follow-up, but levels were still elevated (Patient 1: 540 to 369 μg/L; patient 2: 438 to 105 μg/L; patient 3: 330 to 182 μg/L). Evidently, ingestion of metallic lead in the form of lead pellets/fragments or lead bullet fragments can contribute to a rise in blood-lead level.

It should be mentioned that nonlead (<1% lead content by weight) ammunition, as of July 1, 2008, is required when hunting big game (e.g., deer) and non-game species in areas designated as within the California condor (*Gymnogyps californianus*) range in California, USA (California Department of Fish and Game 2008). This ban came about because raptor studies in North America (e.g., Kramer and Redig 1998; Miller et al. 1998) have suggested that big game (e.g., deer) harvested with lead ammunition may be a source of lead exposure for raptors through the ingestion of ammunition fragments embedded in carcasses, as shown radiographically by Hunt et al. (2006) and by isotope ratios (Church et al. 2006). A certified list of nonlead ammunition (e.g., "pure copper", polycarbonate tip) for hunting big game and links to specific manufacturer's information is given on the California Department of Fish and Game (2008) website. Thus, if the banning of lead bullets for all game hunting is contemplated (to eliminate a potential source of environmental lead, especially for subsistence hunting groups), viable nonlead bullet alternatives already exist.

Acknowledgments We thank Dr. E. Nieboer for use of his EAAS, all hunters who kindly donated large game mammal tissues and comments from Dr. H. N. Nigg.

References

ATSDR (2005) Toxicological profile for lead (draft) US department of health and human services, agency for toxic substances and disease registry, US. http://www.atsdr.cdc.gov/toxprofiles/tp2.html. Accessed February 27, 2007

California Department of Fish and Game (2008) Attention hunters: "it's time to get the lead out". http://www.dfg.ca.gov/wildlife/hunting/condor/. Accessed December 8, 2008

Church ME, Gwiazda R, Risebrough RW, Sorenson K, Chamberlain CP, Farry S, Heinreich W, Rideout BA, Smith DR (2006) Ammunition is the principal source of lead accumulated by California condors re-introduced to the wild. Environ Sci Tech 40:6143–6150

Fackler ML, Surinchak JS, Malinowski JA, Bowen RE (1984) Bullet fragmentation: a major cause of tissue disruption. J Trauma 24:35–39

Falandysz J, Szymczyk-Kobrzynska K, Brzostowski A, Zalewski K, Zasadowski A (2005) Concentrations of heavy metals in the tissues of red deer (*Cervus elaphus*) from the region of Warmia and Mazuruy, Poland. Food Add Contam 22:141–149. doi:10.1080/02652030500047273

Frank A (1986) Lead fragments in tissues from wild birds: a cause for misleading analytical results. Sci Total Environ 54:275–281. doi:10.1016/0048-9697(86)90272-X

Gustavsson P, Gerhardson L (2005) Intoxication from an accidently ingested lead shot retained in the gastro-intestinal tract. Environ Health Perspect 113:491–493

Health Canada Food and drug regulations (1991) Food and drug regulations. Table 1: division 15 (Revised 14 February 1991). Health Canada, Ottawa

Hubbard AW, Pocklington WD, Wood EC (1965) The lead content of game. J Assoc Public Analy 3:29–32

Hunt WG, Burnham W, Paris CN, Burnham KK, Mutch B, Oaks JL (2006) Bullet fragments in deer remains: implications for lead exposure in avian scavengers. Wild Soc Bull 34:167–170. doi:10.2193/0091-7648(2006)34[167:BFIDRI]2.0.CO;2

Kramer JL, Redig PT (1998) Sixteen years of lead poisoning in eagles, 1980–1995: an epizooiologic view In: Money S (ed) Hunting with lead shot–wildlife and human health concerns. Canadian Wildlife Service, Hull, pp 1–15

Lewis LA, Poppenga RJ, Davidson WR, Fischer JR, Morgan KA (2001) Lead toxicosis and trace element levels in wild birds and mammals at a firearmes training facility. Arch Environ Contam Toxicol 41:208–214

Madsen HHT, Kkjom T, Jorgensen PJ, Grandjean P (1988) Blood levels in patients with lead shot retained in the appendix. Acta Radiol 29:745–746

McQuirter JL, Rothenberg SJ, Dinkins GA, Norris K, Kondrashov V, Manalo M, Todd AC (2003) Elevated blood lead resulting from maxillofacial gunshot injuries with lead ingestion. J Oral Maxillofac Surg 61:593–603. doi:10.1053/joms.2003.50117

Medvedev N (1999) Levels of heavy metals in Karelian wildlife, 1989–91. Environ Monit Assess 56:177–193. doi:10.1023/A:1005988511058

Miller MJR, Restani M, Harmata AR, Bortolotti GR, Wayland ME (1998) A comparison of blood lead levels in bald eagles from two regions on the Great Plains of North America. J Wildl Dis 34:704–714

Ontario Provincial Offences (1999) Justice of the Peace/1999 Edition. Ontario Government, Toronto, Ontario

Rodrigue J, McNicoll R, Leclair D, Duchesne J-F (2005) Lead concentrations in ruffed grouse, rock ptarmigan, and willow ptarmigan in Quebec. Arch Environ Contam Toxicol 49:97–104. doi:10.1007/s00244-003-0265-4

Scheuhammer AM, Norris SL (1995) A review of the environmental impacts of lead shotshell ammunition and lead fishing sinker products in Canada. Canadian Wildlife Service Occasional Paper Number 88, Ontario

Thomas VG (1997) The environmental and ethical implications of lead shot contamination of rural lands in North America. J Agric Environ Ethics 10:41–54. doi:10.1023/A:1007757607468

Tsuji LJS, Nieboer E, Karagatzides JD, Hanning RM, Katapatuk B (1999) Lead shot contamination in edible portions of game birds and its dietary implications. Ecosyst Health 5:183–192. doi:10.1046/j.1526-0992.1999.09929.x

Tsuji LJS, Wainman BC, Martin ID, Sutherland C, Weber J-P, Dumas P, Nieboer E (2008) The identification of lead ammunition as a source of lead exposure in first nations: the use of lead isotope ratios. Sci Total Environ 393:291–298. doi:10.1016/j.scitotenv.2008.01.022

WHO/FAO (1999) Joint FAO/WHO 53 Meeting: summary and conclusions. Committee on food additives, World Health Organization/Food and Agricultural Organization of the United Nations, Rome, pp 21