Bulletin of Environmental Contamination and Toxicology
https://doi.org/10.1007/s00128-020-02967-x

Lead Contamination in Ground Venison from Shotgun-Harvested White-Tailed Deer (*Odocoileus virginianus*) in Illinois

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Received: 17 July 2020 / Accepted: 13 August 2020

Abstract
Ground venison packets from shotgun- and archery-harvested White-tailed Deer in Illinois in 2013 and 2014 were analyzed for metal contamination. Radiographs indicated that 48% of 27 ground venison packets from 10 shotgun-harvested deer contained metal fragments, while none of the 15 packets from three archery-harvested deer contained fragments. ICP-MS analysis verified that all metal fragments from seven of the venison samples from shotgun-harvested deer were composed of lead, with average concentrations from 1.04 to 8.42 μg g^{-1}, dry weight. A single serving of ground venison containing one of these metal fragments embedded in it would be predicted to have a lead concentration ranging from 6.4 to 51.8 μg g^{-1}. Sixty percent of 20 commercial meat processing plants surveyed by phone in 2018 and 2019 indicated that they mixed venison from multiple deer when preparing ground venison products. However, our results do not show any cross-contamination in archery-harvested ground venison processed prior to the firearm hunting seasons.

Keywords White-tailed deer · Shotgun · Slugs · Lead contamination

In order to help manage the White-tailed Deer (*Odocoileus virginianus*) population in Illinois, the Illinois Department of Natural Resources (IDNR) coordinates a statewide hunting season. Between 89,577 and 135,455 deer were harvested each year 2005–2018 during the firearm hunting seasons (IDNR 2020a). Firearm deer hunters in Illinois cannot use rifles (IDNR 2020b), and more than 90% of the firearm-harvested deer in Illinois from the 2005 to 2018 seasons were harvested by shotgun (P McDonald, pers. comm.). Lead shotgun slugs (i.e., solid projectiles) are the most frequently used shotgun ammunition for hunting deer (Grund et al. 2010).

Lead slugs typically fragment upon impact with bones and muscle tissue of deer, and these fragments spread throughout the surrounding tissues (Grund et al. 2010; Cruz-Martinez et al. 2015). Prior studies of shotgun-harvested White-tailed Deer have found evidence of metal fragments in offal piles (Warner et al. 2014), and metal fragments in rifle-harvested deer carcasses (Hunt et al. 2009; Grund et al. 2010) and packages of ground venison (Hunt et al. 2009; Tsuji et al. 2009). The presence of lead fragments in venison has led states to recall or destroy venison donated by hunters to food pantries (Grund et al. 2010).

Lead toxicity in humans typically occurs via chronic environmental exposure, with children being both more likely to ingest and retain it, as well as being most susceptible to its neurological, immunotoxic, and developmental effects (Liu et al. 2008). No level of lead is currently considered safe for human consumption (World Health Organization 2011, National Institute of Environmental Health Sciences 2019). Therefore, even small fragments from ammunition could have a deleterious effect.

Little research has been conducted to track the prevalence and contribution to tissue concentrations of individual lead fragments from shotgun slugs. In the present study, metal fragments were first identified in packets of ground venison using radiography, verified with imaging software, and then, for a subset, chemical analysis. These results were compared with packets of ground venison from archery-killed deer to

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Published online: 25 August 2020

determine if metal fragments entered the venison during processing. We then used these data to estimate potential exposure to lead from servings of venison. In addition, we conducted phone surveys of commercial meat processing plants to determine the extent to which venison from more than one deer was intermixed. To the best of our knowledge, this is the first study to identify, isolate, and analyze fragments of lead shotgun slugs from packets of commercially prepared ground venison.

Materials and Methods

Forty-two ground venison packets were obtained from hunters during the 2013 and 2014 Illinois hunting seasons. The venison packets were processed by three different commercial meat-processing plants in western and central Illinois (Adams, Bureau, and McLean Counties). These plants typically mix meat from multiple animals when processing ground venison products; however, for purposes of this study, we will assume that the number of deer being sampled was equal to the number of deer submitted by the hunters for processing. The packets were identified as either harvested using lead slugs (21 packets from four deer) or stainless steel broadhead arrows (15 packets from three deer). Hereafter, we will refer to these as "shotgun-harvested" and "archery-harvested" packets. This resulted in a comparable sample size to previous studies of ground meat from large game mammals (Fachehoun et al. 2015). All archery-harvested deer were processed by a commercial processor prior to the firearm hunting seasons. In order to investigate whether the processing method influences fragment frequency, six (6) additional shotgun-harvested packets were obtained from a custom processor in LaSalle County, Illinois (hereafter referred to as "custom"). This facility specialized in processing venison only, and each of these packets contained meat from a single deer. All venison packets had an average mass of 453.59 g, and were stored in a − 20 °C freezer until analysis.

In order to identify potential metal fragments and effectively sample the venison packets, radiographs of entire frozen or dried ground venison packets were made in 2014, 2015, and 2017 at the Prairie Oak Veterinary Center in Normal, Illinois. All radiographs were made using an Innovet Select Veterinary X-ray System (Summit Industries LLC, Niles, IL). Steel pellets that were 4.5 mm in diameter were included in the radiographs by placement adjacent to the venison, in order to serve as size and color intensity comparisons. Areas within the radiograph with color intensity similar to the pellets were considered possible metal fragments.

To verify the presence of metal fragments, ImageJ (version 1.51m9) was used to calculate the mean grayscale values (MGV) of metal fragments within a packet, and of 10 randomly selected areas (via a grid, and random numbers from www.random.org) per packet that were not adjacent to fragments. The areas of the randomly-selected portions were the same as the mean area of the suspected metal fragments in the radiographs. Values for MGVs ranged from 0 (black) to 255 (white), and were calculated for each sampled area as the sum of the grayscale values of all measured pixels, divided by the number of pixels sampled. ImageJ was also used to measure the two-dimensional length and width of each fragment. A one-way ANOVA indicated a highly significant difference in the MGV's between pixels representing metal fragments, random locations within packets, and steel pellets ($F_{2,337} = 103.92$, $\eta^2_p = 0.38$, $p < 0.001$). Hochberg's GT2 post-hoc tests indicated that the mean MGV's for metal fragments (51.75 ± 14.74 MGV) were significantly lower (i.e., darker) than that for random locations within the packets (122.97 ± 35.41 MGV; $p < 0.001$), while steel pellets (20.67 ± 7.01 MGV) had significantly lower mean MGV's than metal fragments ($p = 0.02$) and random locations ($p < 0.001$).

After metal fragments were identified using radiography, samples of venison containing fragments were analyzed using inductively coupled plasma-mass spectrometry (ICP-MS), by the Washington Animal Disease Diagnostic Lab at Washington State University (Pullman, WA). To establish background metal concentrations, seven randomly-selected samples (not in the vicinity of suspected particles) of venison from shotgun-harvested deer, and three samples from archery-harvested deer were also analyzed in 2014. All background samples demonstrated concentrations of lead and copper that were below detection limits. Therefore, detectable concentrations could reasonably be assumed to come from fragments of ammunition.

Due to budgetary limitations, not all suspected particles could be analyzed. Seven suspected metal fragments from six packets were chosen for chemical analysis because they originated from a single deer via a single commercial processor. Radiographic images were used to isolate the suspected fragments. The ground venison packets were partially thawed, and a ca. 0.2 cm thick slice of ground venison containing the suspected particle was removed using a stainless steel knife. The venison slices were dried in a Nesco American Harvester Dehydrator (600 W) for 10 h to eliminate water weight. A second round of radiographs were then taken of the dried venison slices to identify the locations of the suspected metal fragments. Dried venison that contained suspected metal fragments was then sampled (mass range 0.57–2.40 g). These samples were sent to the Washington State lab for analysis in 2017.

At the Washington State University lab, all analytical samples of dried tissue were divided into subsamples

of ~ 0.25 g or less, and all were analyzed (2–8 analyses per sample, depending on sample mass). This approach ensured that any fragment present in a sample would be analyzed. These analytical subsamples were digested in 3 mL of trace metal grade concentrated nitric acid in test tubes for 6 h at 30 °C, followed by 1 h at 70 °C, and 8 h at 120 °C. Following digestion, all digests were diluted in Type I (ultrapure) water. All analytical subsamples were digested until completion. Reagent blanks and standard reference materials (SRMs; TORT, lobster hepatopancreas; National Resource Council Canada) were included in each digestion batch. Trace element (arsenic, cadmium, cobalt, copper, iron, manganese, molybdenum, lead, and zinc) analyses were performed using ICP-MS (Agilent 7800 ICP-MScx, Santa Clara, CA). Reporting limits for dry samples were based on an estimated 0.25 g of samples. They ranged from 0.20 μg g^{-1} for manganese to 40 μg g^{-1} for arsenic. The reporting limit for lead was 10 μg g^{-1} and the limit for copper was 4.0 μg g^{-1}. Masses of lead in each subsample were calculated using analytical concentrations, then summed for all subsamples taken from a particular sample and divided by the mass of that sample. In this way, the concentration of lead in the entire sample was calculated.

Quality assurance indicated highly accurate analyses**,** with all SRM samples within their certified ranges for all elements. House reference materials (bovine liver) were also within the acceptance range for all elements tested. Duplicate samples were within 10% of one another, indicating good agreement and instrumental precision. None of the reagent blanks exceeded the reporting limit for any element. Tissue concentrations of the elements arsenic, cadmium, cobalt and molybdenum were below detection for all samples, while concentrations of iron and zinc were above detection limits for all samples. The elements copper, manganese and lead were below detection in 93.9%, 10.2% and 79.6% of the analytical subsamples, respectively. At least one subsample from each sample had a detectable quantity of lead.

In 2018 and 2019, phone surveys were conducted with 20 commercial deer-processing plants in the following Illinois counties: Clinton, Edgar, Franklin, Iroquois, Johnson, Kane, LaSalle, Livingston, McDonough, McLean ($n=2$), Peoria ($n=2$), Rock Island, Sangamon, Schuyler, Stephenson ($n=2$), Vermilion, and Woodford. During the survey, a plant employee was queried if ground venison products given to hunters would originate only from individual deer that the hunter brought in for processing.

Binomial logistic models on the effects of deer and processor on fragment data failed to produce any significant models. Likewise, comparative tests (equivalence test for proportions z-test and Wilcoxon Rank Sums test) indicated no significant difference between packets grouped by deer. Therefore, samples from all deer were pooled together for shotgun-harvested and archery-harvested deer, and equivalence test for proportions z-test and Wilcoxon Rank Sum tests were used for the likelihood of finding a fragment and number of fragments, respectively.

Results and Discussion

Thirteen of the 27 ground venison packets (48%) from shotgun-harvested deer contained at least one metal fragment (Table 1), while none (0%) of the 15 packets from archery-harvested deer contained any metal fragments. This constitutes a statistically significant difference ($z=3.234$; $\alpha=0.05$). In packets from shotgun-killed deer that contained metal fragments, the number of fragments ranged from 1 to 3, with an average of 1.46 ± 0.18 fragments per packet (mean ± standard error). The two-dimensional length of the fragments ranged from 0.18 to 4.92 mm (1.5 ± 0.64 mm; $\bar{x} \pm$ SD; $n=23$ fragments), while the two-dimensional width of the fragments ranged from 0.11 to 1.40 mm (1.09 ± 0.41 mm).

Samples of shotgun-harvested venison from the "custom" processor did not significantly differ from either shotgun-harvested or archery-harvested venison obtained from a commercial processor with respect to likelihood ($z=1.75$; $p=0.080$) or number ($W=112$; $p=0.102$) of fragments. However, shotgun-harvested venison packets from a commercial processor were more likely ($z=3.59$; $p<0.001$) to have fragments and had significantly more ($W=298.5$; $p=0.004$) fragments than archery-harvested packets from a commercial processor.

The results of the ICP-MS analysis indicated very high concentrations of lead (286–3518 μg g^{-1}, dw) in all seven

Table 1 Data from ground venison packets from White-tailed Deer harvested in Illinois by two different methods

Packet source	Processor	*n*	% with fragments	Number of fragments
Archery-harvested	Commercial	15	0.0 ± 0.0^{a}	0.00 ± 0.00^{a}
Shotgun-harvested	Commercial	21	57.1 ± 10.8^{b}	0.86 ± 0.19^{b}
	Custom	6	16.7 ± 29.8^{ab}	0.16 ± 0.15^{ab}

Number of packets, percent of packets with identified metal fragments (±95% confidence interval), and mean number of fragments in packets (± standard error) are all reported. Superscripts represent values that are not significantly different within each column

samples with suspected metal fragments (Table 2). These concentrations are from the venison immediately surrounding the metal fragments, and reflect the small mass of the sample relative to the entire packet. To better reflect potential human exposure to lead, we calculated the amount of lead in an average serving of venison taken from each shotgun-harvested packet that was analyzed chemically, based on: (1) a mean packet size of 450 g, (2) the mean number of 1.46 fragments per packet with fragments (for a mean of 0.86 fragments for all shotgun-harvested packets), and (3) a serving size of 85 g of venison. We also calculated the concentration of lead in a single serving with a single fragment with measured mass.

An average serving selected randomly from any one of our packets (shotgun-harvested, commercial processor) would be predicted to have lead concentrations within the range from BD to 8.42 µg g^{-1}, or a dose from ~ 0.00 to 4.4 mg of lead in one serving (Table 2). However, a single serving containing one of these metal fragments embedded in it would be predicted to have a lead concentration ranging from 6.4 to 51.8 µg g^{-1}. According to our data, approximately 15.0% of all venison servings from these packets would have a metal fragment in them, resulting in these higher exposure values. This analysis better estimates actual potential exposure (i.e., ingesting one or more fragments) than the overall average lead concentration of each packet.

The frequency of slug fragments in ground venison from all processors in our study (48%) was lower than that reported in ground venison donated to food pantries in North Dakota (59%, from rifle bullets; Cornatzer et al. 2009), but higher than that reported in Minnesota (32%, harvest type not specified; Grund et al. 2010) and Wyoming (32%, from rifle-bullets; Hunt et al. 2009). A similar rate (35%, harvest type not specified) to these other studies has been reported in ground Moose (*Alces alces*) meat from Sweden (Swedish National Food Agency 2012). Fragmentation of rifle bullets has been shown to vary based on ammunition type (Grund et al. 2010), therefore more research should be conducted on the effects of shotgun ammunition type on fragmentation and metal concentrations in ground venison.

Our phone survey revealed that venison from more than a single deer was mixed to produce at least one venison product (e.g., ground venison or sausage) at 60% (12/20) of the commercial deer processing plants (no such directory listings exist for custom processors). Even without intentional mixing of meat, cross-contamination from processing equipment is also a possibility. Thus, practices at commercial meat processing plants in Illinois may contribute to lead contamination of venison for hunters who used non-lead ammunition. However, no fragments were found in the archery-harvested venison packets that we examined, which were processed prior to the firearm hunting seasons. More research should be done to determine lead exposure via cross-contamination from processing equipment.

The concentrations of lead found in our venison samples were comparable to levels described in previous studies of firearm-harvested game from North America. Our estimated concentrations of lead within a serving of processed venison are also comparable to those obtained by Fachehoun et al. (2015), who found 0.005–4.2 µg g^{-1} in ground venison from Quebec, Canada. Mean lead concentration in ground Moose meat has been found to be 5.6 µg g^{-1} (Norway; Lindboe et al. 2012), 0.90 µg g^{-1} (Sweden; Swedish National Food Agency 2012), and 0.17 µg g^{-1} (Canada; Fachehoun et al. 2015).

Other work on lead levels in large game mammals have directly measured lead from tissue near the wound, comparable to our analytical samples, as opposed to mean tissue values. As in our study, Tsuji et al. (2009) confirmed the presence of metal fragments in their tissue samples from North American big game species prior to chemical analysis. Their samples of Caribou (*Rangifer tarandus*) and White-tailed Deer from northern Ontario with radiographic evidence of fragments contained lead concentrations 2.3–5726.0 µg g^{-1}, which is similar to that for our analytical samples. Likewise, Gerofke et al. (2018) found a maximum of 4728 µg g^{-1} in

Table 2 Lead concentrations of meat adjacent to ammunition fragments in seven samples of ground venison from shotgun-harvested (lead slug) deer provided by a commercial processor in Illinois, as determined by ICP-MS

Sample	Sample lead (µg g^{-1} dw)	Fragment mass (mg)	Mean serving lead (µg g^{-1} ww)[a]	Serving with fragment (µg g^{-1} ww)[a]
1	1303	2.58	4.93	30.3
2	1875	4.41	8.42	51.8
3	286	0.54	1.04	6.39
4	1800	2.43	4.64	28.6
5	314	0.60	1.15	7.10
6	3518	2.01	3.83	23.6
7	2754	3.25	6.21	38.2

Analytical sample concentrations were calculated using the concentrations of 2–8 subsamples

[a]These values are estimated based on mean packet size, percentage of packets with fragments, number of fragments per packet with fragments, and serving size

Roe Deer (*Capreolus capreolus*) tissue near the wound, while Martin et al. (2019) found a maximum of 3442 µg g^{-1} in Red Deer (*Cervus elaphus*). Other studies have reported that some of the meat near a wound channel is often used by butchers and hunters (Tsuji et al. 2009; Fachehoun et al. 2015). Our data suggest that meat processers in Illinois frequently use meat that includes metal fragments. However, packets from deer recorded as hunted by archery had no fragments in them and no detectable concentrations of lead. This supports previous research demonstrating lower lead concentrations in tissues from animals harvested with either crossbows (Fachehoun et al. 2015), or non-lead ammunition (Gerofke et al. 2018).

Martin et al. (2019) noted that measuring lead in game meat from randomly sampled tissue results in a heavily censored data set, with a small number of high-concentration values (where fragments are present) and a large number of below-detection samples. They found an overall mean lead concentration of 19.42 µg g^{-1}; however, to account for below detection values, the researchers estimated median values of 0.0123 µg g^{-1}, a difference of three orders of magnitude. While averaging lead concentrations across the entire animal may be a statistically more rigorous measurement of lead concentrations, this estimate does not consider the fact that at least some lead exposure in game mammal meat would likely come in fragment form. Exposure to fragments would result in a more concentrated dose that is more representative of 95th percentile risk assessments (Fachehoun et al. 2015; Gerofke et al. 2018). Our study identified fragments representing 0.54–4.4 mg of lead in a single serving from lead shotgun slugs, while past studies have found metal fragments from rifle bullets representing 0.2–168 mg (Hunt et al. 2009). This suggests that more research should be done that combines fragment prevalence with lead concentration in game meat meant for human consumption, especially ground meat that could result in dispersing fragments during processing.

The levels of lead detected in venison packets in our study could have negative human health effects, as there is no safe amount of lead exposure (World Health Organization 2011, National Institute of Environmental Health Sciences 2019). Lead exposure to humans from large game sources has been confirmed using isotopic analysis (Tsuji et al. 2008; Hunt et al. 2009), epidemiological studies (Iqbal et al. 2009; Liberda et al. 2018), and bioavailability experiments (Hunt et al. 2009). To assess blood exposure in human populations, blood lead levels (BLL) known to be associated with adverse effects are used as benchmarks. All dietary sources of lead can contribute to BLLs. The U.S. EPA currently considers 10 µg DL^{-1} to be the benchmark for small children (USEPA 2016), although some researchers have found no threshold for developmental effects in children (Lanphear et al. 2005). Recent epidemiological studies in First Nations hunting communities in northern Quebec have found lower BLLs in children than adults, although higher consumption of large game was positively correlated to BLL (Liberda et al. 2018). A similar relationship with age was also found by Iqbal et al. (2009) among North Dakota communities; however, children were under-sampled in that study.

Slug fragments in venison are also a route of lead exposure for scavengers that consume unrecovered deer carcasses and offal piles (Hunt et al. 2009; Iqbal et al. 2009; Bedrosian et al. 2012). Species at risk from fragments in unrecovered carcasses and offal include Bald Eagles (*Haliaeetus leucocephalus*; Harper et al. 1988), with recent studies showing a high prevalence of elevated levels of lead in blood and tissue, some within the lethal range for lead poisoning (Warner et al. 2014; Yaw et al. 2017).

The results of this study indicate that both humans and wildlife may ingest lead fragments from deer hunters who use lead shotgun slugs to harvest deer. We found no evidence that the practices of commercial meat processing plants in mixing ground venison from more than a single deer contributes to lead contamination in venison from archery hunters, which were processed prior to the firearm hunting seasons. Our analysis provides a better estimate of the actual potential exposure (i.e., ingesting one or more fragments) than the overall average lead concentration of individual packets.

Acknowledgements This research was supported by an endowment from the Beach Lewis fund from Illinois Wesleyan University. AP Capparella, W Jaeckle and two anonymous reviewers provided useful comments on drafts of the manuscript, and D. Oehler provided the archery-harvested venison packets.

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