

CONTAMINANT BURDENS OF WILD TURKEYS INHABITING THE SAVANNAH
RIVER SITE AND THE POTENTIAL FOR CONSUMER EXPOSURE

by

CODY ALLEN TISDALE

(Under the Direction of James C. Beasley & James A. Martin)

ABSTRACT

Harvested wildlife present a potential vector of contaminant exposure to consumers, yet contaminant levels in many game species are not well understood. I compared contaminant concentrations in tissues of wild turkeys (*Meleagris gallopavo*) inhabiting the Savannah River Site (SRS), an area of known contamination, to those inhabiting uncontaminated areas. I also compared lead concentrations in tissues of birds harvested with two common shot types. Overall, turkeys had relatively low contaminant burdens, even on the SRS, although mercury concentrations in SRS birds were greater than those from uncontaminated areas. In addition, I found contaminant concentrations in muscle of turkeys harvested with lead shot did not differ from those harvested with copper-coated lead, and the majority of this contamination was confined to the wound channel. Collectively, these results suggest turkeys likely are not important vectors of human exposure to contaminants, but hunters should take care to remove muscle tissue surrounding pellet wound channels.

INDEX WORDS: contaminants, consumption risks, wild turkey, lead shot, lead toxicity, Savannah River Site, mercury, anthropogenic

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

An estimated one-third to one-half of earth's land surface has been transformed by anthropogenic activities (Vitousek et al. 1997). Habitat loss and fragmentation tend to be the most common and profound effects humans have on the environment. However, while often less perceptible than these anthropogenic alterations, environmental contamination can also have detrimental effects on local wildlife. Many anthropogenic contaminants are byproducts of industry and may introduce a wide variety of elements into the environment (Chertow 1998). These contaminants include trace elements and heavy metals, many of which are needed by wildlife, but become toxic in higher doses.

Among anthropogenic contaminants, arsenic, lead, mercury, and selenium are products of industrial waste that are of particular concern as they can negatively impact both wildlife and humans, even in low doses. Chronic exposure to high levels of selenium can negatively impact endocrine function, as well as cause nail and hair loss (Vinceti et al. 2001). Mercury and lead can cause weight loss and incoordination in wildlife, while arsenic, mercury, and lead can all affect the central nervous system and cause neuropathy in humans (Haley 2005, Zahir et al. 2005, Choong et al. 2007, Gidlow 2015). Lead can also be toxic at extremely low levels, especially in children, as it can impair their cognitive development (Lanphear et al. 2005). Elevated blood lead levels in young adults also has been associated with increased major depression and panic disorders (Bouchard et al. 2009), while in adults it can lead to increased blood pressure, decreased kidney

function, and reproductive problems (United States Environmental Protection Agency [EPA] 2019b). In addition, mercury can cause severe neurological damage and delay neurological development in children (Clarkson et al. 2003). Many of these trace elements are also of special concern to expecting mothers, as they can be transferred through the womb and negatively affect fetal development (Haley 2005, EPA 2019a).

Radionuclides, while not as common as other contaminants in industrial waste, can also have profound impacts on the surrounding environment, as well as the species living in them. The Chernobyl and Fukushima nuclear accidents are the most notable accidental releases of radionuclides in human history, releasing approximately 5,300 and 520 Petabecquerels of radioactivity in the form of various volatile elements into the surrounding environment, respectively (Dreicer et al. 1996, Steinhauser et al. 2014). However, smaller localized releases and nuclear weapons testing have released radionuclides worldwide, and thus radiological contamination is a global issue, and not restricted to these two disasters. Radionuclides released due to anthropogenic activities can then persist in the environment for decades, especially those with a relatively long half-life (Paller et al. 2014). In fact, wildlife living near areas of known radiological contamination have been found to contain elevated activity concentrations of these anthropogenic contaminants, even long after the releases have taken place (Kennamer et al. 2017, Oldenkamp et al. 2017).

Radiocesium (Cs^{137}) is a commonly studied radionuclide due to its relatively long half-life of 30.2 years, allowing it to persist in the environment for decades following releases. Radiocesium also acts as an analog for potassium, allowing it to accumulate in the soft tissues of exposed wildlife, which then may act as a vector of exposure to

predators and human consumers (Jannik 1999). Given that potassium has also been found to accumulate more in ‘red’ (e.g., leg) muscle compared to ‘white’ (e.g., breast) muscle in birds (Chutkow 1973), accumulation patterns of Cs¹³⁷ also may vary among tissue types. Indeed, Oldenkamp et al. (2017) found Cs¹³⁷ to accumulate in higher concentrations in the muscle tissue of wild boar compared to liver tissue. As humans may consume breast and leg muscles of harvested gamebirds at varying rates, understanding the relationship in Cs¹³⁷ concentrations between muscle types is paramount to understanding consumer risk.

Wildlife are exposed to anthropogenic contaminants through the ingestion of water, soil, and plants that contain elevated levels of the radiocesium or heavy metals (Beresford and Howard 1991). Soil, in particular, may accumulate many of these contaminants (Zhu and Shaw 2000, Agrawal et al. 2010), which may then be consumed by wildlife while foraging. Beyer et al. (1994) used fecal samples of various species to estimate the proportion of soil consumed in each species’ diet. For example, 9% of the diet of wild turkeys (*Meleagris gallopavo*) was comprised of soil, indicating birds inhabiting areas of soil contamination may be at increased risk of contaminant exposure. These turkeys may then act as vectors of contaminant exposure to predators and human consumers.

Among gamebirds, the wild turkey is one of the most commonly harvested and consumed species across North America. Over half a million birds were harvested in the lower 48 states during 2018 alone. Among hunters surveyed in South Carolina, >75% reported the harvest and consumption of wild turkeys, and >60% reported sharing wild turkey meat with people outside their household (Smith et al. 2017). Despite their

popularity as a game species, there are few data on contaminant levels carried by wild turkey or other gamebirds. Thus, information on contaminant burdens in consumable tissues of wild turkeys is important to elucidate potential risks to consumers, particularly in landscapes where environmental contaminants are known to be present.

Not only can consumers of game meat be exposed to contaminants naturally accumulated within tissues of harvested game species, they also may be exposed to toxic elements inadvertently introduced into consumable tissues during harvest. In particular, lead, despite its known toxicity, remains a popular ammunition used in the harvest of big game and upland game birds. Once fired, lead bullets and pellets have a tendency to fragment as they pass through game, leaving small particles and a fine powder of lead behind (Frank 1986, Hunt et al. 2009, Cruz-Martinez et al. 2015). These fragments have been shown to put avian scavengers at risk for lead poisoning, as they consume the carcasses and offal piles of harvested animals (Kelly and Johnson 2011, Bedrosian et al. 2012, Cruz-Martinez et al. 2012). Not only have these fragments been found in the carcasses and offal piles being scavenged, they have also been found in the muscle tissue of game meat commonly consumed by people (Hunt et al. 2009). Thus, game harvested with lead ammunition may also act as a vector of exposure to consumers.

While most consumers of game avoid ingesting lead pellets found in the muscle tissue of harvested birds, the small fragments and powder may escape detection and be unknowingly consumed (Frank 1986). Fragments of lead bullets have been shown to travel as far as 15 cm from the wound channel (Hunt et al. 2006, Cruz-Martinez et al. 2015). Fragmentation of lead ammunition is not limited to big game and rifle bullets, as studies have found shotgun pellet fragments in the carcasses of harvested gamebirds as

well (Johansen et al. 2001, Johansen et al. 2004, Pain et al. 2010). While no longer allowed in the hunting of waterfowl, lead-based shot is still commonly used for upland game species such as mourning dove (*Zenaida macroura*), ring-neck pheasant (*Phasianus colchicus*), and wild turkey, and many species of large game are harvested with lead bullets; thus, consumers of game meat may still be exposed to lead through these contamination pathways. In fact, Iqbal et al. (2009) found consumers of wild game have blood lead levels 0.30 $\mu\text{g}/\text{dl}$ higher than people who do not consume wild game.

Due to concerns over environmental and human exposure to lead, efforts have been made to limit the use of lead in ammunition through use of non-toxic shot types. Metals such as steel, copper, or various other non-toxic metals are commonly used in place of lead. In particular, the 1991 federal ban on the use of lead shot in the harvest of waterfowl made the use of non-toxic shot a requirement across the U.S. (Avery and Watson 2009). Since then, over half of the states in the U.S. have added additional non-lead requirements to the federal law, with California being the first state to ban the use of lead shot in the harvest of game entirely. However, widespread acceptance of these alternative shot types has been challenging due to hunter concerns over price and efficacy of lead-free ammunition (Schulz et al. 2007).

In addition to lead ammunition, there are other shot types where relatively non-toxic metals are used to coat lead, and while these coated shots are not legally deemed non-toxic, they may alter the amount of lead contamination in the tissues of harvested game. However, there have been no published studies investigating the effectiveness of non-toxic coatings of lead pellets at reducing lead contamination in the muscle of

harvested game. It should be noted however, that some of these metals may also be toxic to humans, although these toxic effects are only seen at much larger doses than lead.

My thesis aims to address the underlying gaps in our knowledge of the potential for wild turkeys to serve as vectors of contaminant exposure to humans and non-human predators and scavengers, as well as the effects of shot type on lead residues in the wound channels of harvested turkeys. In Chapter 2, I present a comparative study that quantifies differences in contaminant burdens in the tissues of wild turkeys vulnerable to annual harvest that inhabit an area of known contamination, the Savannah River Site, and nearby uncontaminated reference areas. In this study, I also compared concentrations of various contaminants between tissue types, in particular, between tissues and feathers in the case of Hg concentrations, to determine whether non-lethal sampling is a viable method for quantifying Hg burdens in turkeys. Finally, I related contaminant concentrations found in turkeys to consumption limits set by the Centers for Disease Control (CDC) and the Food and Drug Administration (FDA) to determine consumer risk. In Chapter 3, I present a study where I quantified lead and copper concentrations in turkeys harvested with copper-coated and traditional lead shot types. I sampled these concentrations at multiple locations in relation to the wound channel to determine the extent to which lead contamination may extend beyond wound channels. Finally, I used these concentrations to predict consumer risks for hunters harvesting turkeys using either shot type.

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CHAPTER 2
TRACE ELEMENT AND RADIOCESIUM LEVELS IN WILD TURKEYS ON THE
SAVANNAH RIVER SITE¹

¹Tisdale, C.A., J.C. Leaphart, J.A. Martin, and J.C. Beasley. To be submitted to *Environmental Toxicology and Chemistry*

Abstract

Despite the popularity of the wild turkey (*Meleagris gallopavo*) as a game species across North America, little is known about contaminant burdens of turkeys inhabiting areas of environmental contamination. Wildlife inhabiting environments containing anthropogenic contaminants may incorporate these potential toxins into their tissues, which may act as a potential vector of exposure to human consumers. We tested heavy metal, trace element, and radionuclide concentrations in the tissues of turkeys inhabiting the Savannah River Site (SRS), an area of known contamination, and compared them to samples collected from turkeys inhabiting areas of relatively little to no contamination. We tested breast muscle, leg muscle, and liver for 11 trace elements (Hg, Cr, Ni, Cu, Zn, As, Se, Sr, Cd, Pb, U) as well as counted samples for radiocesium (Cs^{137}) to assess potential consumption risks. In addition, we sampled breast feathers for Hg to assess the viability of non-lethal sampling as a substitute for lethal breast/liver sampling to detect Hg concentrations. Finally, we sampled for As, Hg, and Se, commonly occurring contaminants in coal combustion residuals (CCR), in the blood of turkeys known to be inhabiting an ash basin on the SRS and in those in nearby reference areas. We found that turkeys inhabiting SRS contain elevated levels of Hg in both breast and liver tissues as compared to offsite birds, but we found Cr concentrations to be lower. We also found a positive correlation between breast muscle and feather Hg concentrations, indicating that non-lethal sampling may be a viable method of detecting Hg in turkeys. We were unable to detect differences in the remaining elements, including Cs^{137} . Finally, we found that the blood of turkeys inhabiting the ash basin likely contain higher Se than birds in the

surrounding area, but further research is required. All elements analyzed remained below reference limits set by the Centers for Disease Control for safe consumption.

Introduction

It is estimated between one-third and one-half of the earth's land surface has been transformed by anthropogenic activities (Vitousek et al. 1997). These changes to the landscape can negatively impact wildlife, both directly and indirectly. Most commonly, habitat fragmentation and loss can have negative population level effects by destroying or separating populations from key resources needed for persistence. While often not as perceptible as habitat destruction and fragmentation, the introduction of contaminants to the environment is a global issue that also can have negative effects on wildlife populations. Within the United States alone industrial processes create billions of tonnes of waste per year (Chertow 1998). The disposal of this effluent or industrial byproduct can inadvertently introduce contaminants to the environment at both local and global spatial scales. Many contaminants in industrial waste are trace elements or heavy metals, some of which are required by wildlife in trace amounts; however, these elements become toxic to wildlife in large doses.

Of the various contaminants produced by industrial activities, arsenic (As), mercury (Hg), selenium (Se), and lead (Pb) are of particular concern due to the negative impacts they can have on the health of both wildlife and humans. Chronic exposure to high levels of selenium can negatively impact endocrine function, as well as cause nail and hair loss (Vinceti et al. 2001). Mercury and lead can cause weight loss, and incoordination in wildlife, while arsenic, mercury, and lead can all affect the central nervous system and cause neuropathy in humans (Haley 2005, Zahir et al. 2005, Choong

et al. 2007, Gidlow 2015). In addition, mercury can cause severe neurological damage and delay neurological development in children (Clarkson et al. 2003). Many of these trace elements are also of special concern to pregnant women, as they can be transferred through the womb and negatively affect fetal development (Haley 2005, United States Environmental Protection Agency [EPA] 2019).

While not as common as industrial waste, the release of radionuclides via nuclear accidents can have a profound effect on the surrounding landscape and can linger in the environment for centuries. For example, the Chernobyl and Fukushima nuclear accidents released approximately 5300 and 520 Petabecquerels of radioactivity in the form of various volatile elements into the surrounding environment, respectively (Dreicer et al. 1996, Steinhauser et al. 2014). Far smaller, but numerous radionuclide releases have occurred around the world as a result of weapons testing and nuclear power generation (446 active nuclear reactors in 30 countries). Wildlife living near these areas are then exposed to radionuclides, and research has found that their tissues contain higher activity concentrations of these anthropogenic contaminants than wildlife living in other areas (Connelly and Markham 1983, Oldenkamp et al. 2017). Because of the long half-lives of some radionuclides, such as radiocesium (Cs^{137}), contamination effects can persist in the environment for decades (Paller et al. 2014).

Cs^{137} can accumulate in the tissues of wildlife and can pose health risks to exposed wildlife and human consumers. When consumed, Cs^{137} acts as an analog to potassium, allowing it to accumulate in muscle and other soft tissues of animals, which then act as a vector of exposure to predators and human consumers of game meat (Paller et al. 2014). Potassium has been found to accumulate in higher concentrations in ‘red’

(e.g., leg) muscle compared to ‘white’ (e.g., breast) muscle (Chutkow 1973), indicating Cs¹³⁷ activity levels also may vary among muscle types in birds. However, the relationship between leg and breast muscle Cs¹³⁷ activity levels is largely unknown for wild gamebirds. Given the potential that humans may consume leg muscles and breast muscle of harvested gamebirds at different frequencies, understanding the distribution of Cs¹³⁷ among animal tissues is important to understanding risk to consumers of game meat.

Wildlife are likely exposed to radionuclide and heavy metal contamination through ingestion of contaminated water, soil, or plants (Beresford and Howard 1991). Many contaminants accumulate in soil (Zhu and Shaw 2000, Agrawal et al. 2010), which may be indirectly consumed by wildlife while feeding. Beyer et al. (1994) used fecal samples of various species to estimate the proportion of soil consumed in each species’ diet. For example, 9% of the diet of wild turkeys (*Meleagris gallopavo*) was comprised of soil, indicating these birds may be at increased risk of contaminant exposure. As wild turkeys are amongst the most widely consumed game species, information on contaminant levels in wild turkeys is important to better understand potential risks to human consumers. In fact, over half a million birds were harvested in the lower 48 states during 2018 alone. In South Carolina, >75% of all hunters report the harvest and consumption of wild turkeys, and >60% of those hunters report sharing wild turkey meat with people outside their household (Smith et al. 2017). Despite this, there are currently few data available on accumulation of contaminants in free-ranging wild turkey populations. In particular, no data are currently available on trace element concentrations

in turkeys on the Department of Energy's (DOE) Savannah River Site (SRS), where known releases of contaminants have occurred.

We sampled muscle, liver, feathers, and blood of wild turkeys on a site with known contamination (SRS), in order to quantify Cs¹³⁷ and trace element levels at the site and to compare to samples taken from birds in nearby, relatively uncontaminated areas. We compared Hg, As, and Se concentrations in blood from turkeys on the SRS known to be inhabiting an ash basin, to blood from turkeys away from areas of known contamination. We also tested for correlations between feather, breast muscle, and liver tissue Hg concentrations, to determine whether non-lethal feather sampling could be an adequate substitute for lethal muscle/liver sampling to test for Hg concentrations. In addition, we analyzed any possible differences in Cs¹³⁷ concentrations between leg and breast muscle due to differences in 'red' and 'white' muscle fiber ratios. We tested the predictions that trace element and Cs¹³⁷ levels would be greater in birds inhabiting the SRS than those of the surrounding areas, element concentrations would be positively correlated between tissue types, and Cs¹³⁷ concentrations would differ between breast and leg muscle.

Methods

Study Area

The SRS is an ~78,000 ha tract of contiguous land owned by the United States Department of Energy (DOE) located in Upper Coastal Plain of South Carolina, USA and situated on the eastern bank of the Savannah River (Figure 2.1). The creation of the SRS in 1951 caused a dramatic change in the landscape, transforming agricultural fields into slash (*Pinus elliottii*), loblolly (*P. taeda*), and longleaf pines (*P. palustris*) (White and

Gaines 2000). These upland pine forests cover 64% of the site, interspersed by a mosaic of industrial complexes and bottomland hardwood forests (Workman and McLeod 1990). The industrial complexes include decommissioned nuclear reactors and coal combustion plants that were used for the creation of power. While on a smaller scale than the Chernobyl and Fukushima disasters, these reactors have a history of inadvertent radionuclide discharge via natural streams and reservoirs used to disperse heat (Cummins et al. 1991). Though many radionuclides were released on SRS, ^{137}Cs has been the subject of intensive study, due to its relatively long half-life of 30.2 years, and its ability to readily accumulate within soft tissues of wildlife, as the isotope acts as an analog to potassium (Jannik 1999).

Trace element contamination has also taken place on SRS via coal combustion plants. Metals such as aluminum (Al), As, cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), Pb, manganese (Mn), nickel (Ni), Se, and zinc (Zn) have been introduced into the environment at SRS via the release of fly ash, as well as coal combustion residuals (CCR) placed into impoundments on site (White and Gaines 2000). Mercury contamination is also present on the SRS, which was primarily introduced through the pumping of river water from the Savannah River, which contained elevated levels of mercury, to cool the nuclear reactors, inadvertently contaminating some natural streams and reservoirs on the SRS.

The SRS represents an ideal landscape to conduct this study as there are both uncontaminated areas and areas contaminated with radiocesium, mercury, and trace elements such as arsenic, selenium, and lead from historic nuclear production and coal power plants. Indeed, elevated levels of trace elements have been found in numerous

species on the SRS such as waterfowl (*Anas* spp., *Aythya* spp.), wild pigs, (*Sus scrofa*), bobcat (*Lynx rufus*), Virginia opossum (*Didelphis virginiana*), river otter (*Lontra canadensis*) and gray fox (*Urocyon cinereoargenteus* (Cumbie and Jenkins 1974, Oldenkamp et al. 2017, Borchert et al. 2019). Mercury concentrations in raccoons (*Procyon lotor*) and American alligators (*Alligator mississippiensis*) have also been found to be greater in animals frequenting known areas of contamination on the SRS (Yanochko et al. 1997, Lord et al. 2002).

Sample Collection

In 2016 and 2017, we collected breast muscle, leg muscle, and liver from turkeys harvested by hunters during a two-day hunt held on the SRS each spring. We collected breast feathers from harvested birds during the 2017 hunt as well. Offsite (i.e. reference) breast and liver samples were donated by hunters during the spring seasons of 2016-2019 from South Carolina and eastern Georgia, USA (Figure 2.1). We weighed all samples and placed them in individual Whirl-Paks (Nasco, Janesville, WI, USA) before freezing them until further analysis could be completed.

We also harvested 17 turkeys in the spring of 2018 within 1500 m of Cs¹³⁷ contamination areas and collected breast muscle samples. These birds were harvested due to their likelihood of inhabiting areas of known contamination. As 1500 m is within the average daily movement distance (3725 m) of wild turkeys and the average home range size of birds on the SRS has been found to be 766 ha (Kilgo and Blake 2005, Gross et al. 2015), it is likely that harvested birds had previously used these contaminated areas, and had potentially been exposed to elevated Cs¹³⁷ levels.

In addition to samples collected from harvested birds, we also collected blood samples from turkeys captured via Net Blaster (Wildlife Control Supplies, East Granby, CT, USA) on the SRS for a concurrent study (2018-2019). During capture, we affixed 80g VHF (Advanced Telemetry Systems, Isanti, MN, USA), or 90g GPS VHF (Lotek Wireless, Havelock North, New Zealand) backpack transmitters to adult male turkeys. We also collected 5ml of blood from the superficial plantar metatarsal vein, which we then froze at -80°C until further analysis. Turkeys were then released at the captured location after processing. These birds were then located via VHF and GPS and locations were overlaid with contaminant data layers to determine which individuals were inhabiting areas of known contamination. All collections were done under SCDNR collection permit SC-08-2019 and approved by the University of Georgia Institutional Animal Care and Use Committee protocol A2019 01-012.

Elemental Analysis

We analyzed concentrations of 11 trace elements (Hg, Cr, Ni, Cu, Zn, As, Se, Sr, Cd, Pb, U) as well as counted samples for Cs¹³⁷. Feathers were thoroughly washed with Milli-Q purified water (EMD, Millipore, Merck KGaA, Darmstadt, Germany) and metal free detergent before lyophilization. Muscle (breast and leg), liver, and blood samples were weighed before and after lyophilization, to determine percent moisture content, and subsequently homogenized. Feather samples were cut into small pieces with scissors and lyophilized blood samples were shaken within microcentrifuge tubes to ensure homogenization.

All samples were tested for total mercury, hereafter mercury (Hg), via thermal decomposition, amalgamation, and atomic spectrophotometry using a direct mercury

analyzer (DMA-80; Milestone, Shelton, CT, USA in accordance with EPA method 7473, United States Environmental Protection Agency; EPA 2007). For muscle and liver tissue, approximately 15 mg dry, homogenized tissue from each sample was tested. A duplicate, blank, and two reference materials (TORT-3 and PACS-2; National Research Council of Canada, Ottawa, ON) were tested after every 10 samples for quality control. If duplicate sample results were not within 20% of one another, a triplicate was tested. Replicate means were used for analysis. Percent recoveries of certified reference material ranged from 88.6-108.8%.

We analyzed the remaining elements (Cr, Ni, Cu, Zn, As, Se, Sr, Cd, Pb, U) via inductively coupled-mass spectroscopy (ICP-MS) in breast muscle and liver samples only. First, we microwave digested 250 mg of each dry, homogenized sample (MARSX Xpress, CEM, Corporation, Matthews, NC) in 10 ml of 70% nitric acid (HNO₃) according to EPA method 3052 (United States Environmental Protection Agency; EPA 1995). After digestion, we diluted samples with 5 ml Milli-Q purified water. In accordance to quality assurance protocols outlined by the EPA, we then diluted samples further to 1:10 ratio of digested sample to Milli-Q water, before analysis with ICP-MS (Nexlon 300X ICP-MS; Perkin Elmer, Norwalk, CT, USA)(United States Environmental Protection Agency; EPA 2004). Due to the small amount of blood sample available, we performed a micro-digestion using 50 mg dried sample, 1ml nitric acid, and adding 3ml Milli-Q water post-microwave digestion. We then diluted these blood samples to a final ratio of 3:10 nitric digested sample to Milli-Q water, before analysis on the ICP-MS. Acid digested blood samples were only analyzed for As and Se concentrations in this way. As a quality control for all digested samples, we included a blank, a duplicate, and

certified reference material (TORT-3) every 20 samples. If duplicate differences exceeded 20%, a triplicate was tested. Replicate averages were used for analysis. Certified reference material percent recoveries ranged from 77-107%.

All samples were also counted for Cs¹³⁷. We packed approximately 1g of dry homogenized sample into a vial and placed them on a Packard Cobra II Auto-Gamma Counter (Model Cobra II 5003; GMI Inc., Ramsey, MN, USA) for one hour to analyze for Cs¹³⁷ activity. The counter was auto calibrated each day before use using a traceable Cs¹³⁷ source. We also included an empty tube after every fourth sample to obtain background radiation levels. We then adjusted sample counts to account for background activity.

Statistical Analysis

We compared trace element levels from wild turkeys collected from SRS to those from birds collected in areas with no known trace element or Cs¹³⁷ inputs. We removed trace elements from analysis if >50% of the samples in a given group were below minimum detectable limits (MDL). These minimum detectable limits were unique for each element and each batch of samples ran (Table 2.1). For the remaining elements, we set any trace element levels below MDL, to 50% of the elements' respective MDL for analysis (Hall et al. 2009, Fletcher et al. 2014). We then tested the remaining concentrations of each element in each tissue type for normality using the Shapiro-Wilks test and log transformed data as necessary. The Levene test was used to ensure homogeneity of variance between locations for each element (Milliken and Johnson 2009). We then used multivariate analysis of variance (MANOVA) using Pillai-Bartlett's statistic to test for differences in element concentrations between the SRS and reference

samples (Hand and Taylor 1987). Finally, univariate analysis were used to follow up significant MANOVA results to determine where differences lie. Due to previous research indicating element concentrations can differ between tissue types, we analyzed element concentrations of breast, leg, and liver separately, (Szymczyk and Zalewski 2003, Havelková et al. 2008, Mansouri et al. 2015). We also tested the blood of captured turkeys for Hg, As, and Se concentrations, to test for differences between locations within the SRS. Finally, we tested for correlations in contaminant concentrations between breast muscle, liver, and in the case of mercury, feathers using Pearson correlations. In the case of breast muscle and liver correlation, linear regression was performed with and without an outlier that fell well outside the majority of data points (see results), to ensure it was not having an overwhelming influence on the results.

To compare trace element concentrations to consumption limits set by the EPA, we used an average adult weight of 70 kg and average meal size of 0.227 kg based on EPA fish consumption advisory calculations (United States Environmental Protection Agency; EPA 2000). Mercury can be found in multiple forms in the environment, the most toxic of which is methyl mercury (MeHg). We were unable to differentiate between the various forms of Hg within our samples with the equipment available. However, as most Hg in birds has been found to be in the form of MeHg (75-100%) (Gerrard and St. Louis 2001, Campbell et al. 2005), we assumed all THg found in a sample was in the MeHg form, which likely results in conservative consumption risk estimates.

Results

We collected samples from 49 male turkeys on the SRS, and 17 male turkeys harvested offsite in South Carolina and eastern Georgia to determine trace element and

Cs¹³⁷ levels. We collected breast muscle samples from 48 of the SRS birds (1 sample donated was mainly fatty tissue and was discarded), and leg muscle and liver samples from all 49 birds. Nineteen breast feather samples were also collected from the turkeys harvested during the 2017 season. We received breast muscle samples from all 17 offsite turkeys, but only received liver samples from 14. In addition, we collected 13 blood samples from male turkeys captured on SRS during the winters of 2018 and 2019. Breast muscle percent moisture values averaged 73.7% (range: 70.7-77.5%), leg muscle averaged 76.0% (range: 71.1-89.6%), liver samples averaged 75.7% (range: 56.4-81.0%) and blood samples averaged 81.2% (range: 75.2-83.9%).

We quantified trace elements (Hg, Cr, Ni, Cu, Zn, As, Se, Sr, Cd, and Pb) for all breast muscle, and liver samples. After removing elements with >50% of samples <MDL at either location, Hg, Cr, Ni, Cu, and Zn were included in further analyses for breast muscle, and Hg, Cr, Cu, Zn, Se, Cd for liver samples. We found differences in trace element concentrations in both breast muscle and liver tissue between turkeys from the SRS and the surrounding area (breast muscle: $F_{5, 57} = 4.95$ $P < 0.001$, liver: $F_{6, 29} = 6.22$ $P < 0.001$). Further analysis indicated differences in Hg concentrations in both tissue types and Cr concentrations in breast muscle between the SRS and offsite locations. Breast muscle and liver tissues of turkeys inhabiting the SRS contained higher levels of Hg than turkeys in the surrounding area, but breast muscle contained lower concentrations of Cr (Tables 2 and 3).

In addition, we quantified Hg concentrations in the breast feathers of 19 wild turkeys harvested during 2017 on the SRS. Average Hg concentration was 0.122 $\mu\text{g/g}$ dry weight (d.w.) (range: 0.052-0.214). Linear regression and Pearson's coefficients revealed

a correlation between Hg concentrations in feathers and breast muscle, but no significant correlation between feather Hg and liver Hg. The best fit regression line for the feather and breast muscle correlation was ($R^2 = 0.44$, $P = 0.003$): [Breast Hg = Intercept + $\beta_{\text{Feather Hg}}$ * (Feather Hg)], where the Intercept = 0.0088 (95% CI: -0.001-0.019) and $\beta_{\text{Feather Hg}} = 0.14$ (95% CI: 0.054-0.206, Figure 2.2). While the feather and liver correlation test was not significant, the best fit regression line was ($R^2 = 0.16$, $P = 0.09$): [Liver Hg = Intercept + $\beta_{\text{Feather Hg}}$ * (Feather Hg)], where $\beta_{\text{Feather Hg}} = 0.18$ (95% CI: -0.021-0.375) and Intercept = 0.042 (95% CI: 0.016-0.068, Figure 2.3). However, a correlation was found between breast muscle Hg and liver Hg concentrations both with an outlier ($R^2 = 0.82$, $P < 0.001$): [Breast Hg = Intercept + $\beta_{\text{Liver Hg}}$ * (Liver Hg)], where $\beta_{\text{Liver Hg}} = 0.52$ (95% CI: 0.454-0.582) and Intercept = -0.004 (95% CI: -0.008-0) and without ($R^2 = 0.49$, $P < 0.001$): [Breast Hg = Intercept + $\beta_{\text{Liver Hg}}$ * (Liver Hg)], where $\beta_{\text{Liver Hg}} = 0.30$ (95% CI: 0.219-0.379) and the Intercept = 0.008 (95% CI: 0.004-0.010, Figure 2.4). The remaining elements with the majority of samples >MDL for both breast muscle and liver were Cr, Cu, and Zn. The concentrations we found of these elements did not display any correlations between tissue types.

We also collected blood samples from 13 captured birds on the SRS, 4 of which were observed inhabiting a coal ash basin known to be contaminated with Hg, As, and Se. The majority of samples fell below the MDL for As and no further analysis was completed. The average Hg concentrations in the blood of wild turkeys inhabiting this basin was 0.037 $\mu\text{g/g}$ d.w. (SD: 0.005, range: 0.030-0.042), while the SRS turkeys inhabiting relatively uncontaminated areas on the SRS averaged 0.031 $\mu\text{g/g}$ d.w. (SD: 0.014, range: 0.020-0.055). Blood Se levels of the ash basin birds averaged 0.270 $\mu\text{g/g}$

d.w. (SD = 0.039, range: 0.233-0.316), while the 9 other SRS turkeys averaged only 0.090 µg/g d.w. (SD = 0.041, range: 0.034-0.138). Due to the low sample size, and as the birds inhabiting the ash basins were captured together, potentially violating the assumption of independence, we refrained from performing any tests for differences in Hg and Se levels between locations. However, as can be seen by the means and standard deviations, it seems likely that the ash basin birds contain significantly higher Se concentrations than that of turkeys inhabiting relatively uncontaminated areas of the SRS.

Minimum detectable counts (MDC) for Cs¹³⁷ averaged 0.25 Bq/g d.w. (range: 0.03-0.54) across all samples. Every sample from both SRS and offsite fell below their respective MDC, and thus, we were unable to analyze any potential differences between muscle types, or by geographic location.

Discussion

We compared various tissues from turkeys inhabiting an area of known contamination to tissues from turkeys inhabiting relatively uncontaminated areas to better understand environmental contaminants that wild turkeys may be acquiring from the environment. We found both liver and breast muscle samples taken from turkeys inhabiting the SRS contained greater concentrations of Hg, but lower concentrations of Cr in breast muscle than birds collected from offsite, and exhibited no difference for the remaining trace elements evaluated (i.e., Cu, Zn, Se, Cd, Ni). A positive correlation between feather Hg and breast Hg levels was discovered, but no correlation was found between feather Hg and liver Hg levels. We also did not find any evidence of Cs¹³⁷ contamination in any of the wild turkey samples collected, even among individuals harvested from areas nearby water sources with known Cs¹³⁷ contamination.

Mercury concentrations have been found to be pervasive in aquatic environments throughout the Southeastern United States (Cumbie and Jenkins 1974, Facemire et al. 1995). As Hg concentrations tend to be higher in aquatic environments, turkeys inhabiting areas in upland areas may be less susceptible to Hg exposure. A negative correlation has also been found between distance from the coast and Hg levels in wildlife inhabiting the Southeast, likely due to the poor drainage and higher acidity of Coastal Plain soils, making Hg more bioavailable to organisms (Harris 1971, Guentzel 2009, Borchert et al. 2019). However, as samples were harvested both closer and farther from the coast as the SRS, it is unlikely that distance to coast was a confounding factor.

Mercury levels in the breast muscle of wild turkeys harvested on the SRS could present a potential vector of exposure to human consumers. Using average meal sizes used for fish consumption advisories by the EPA, the average meal of wild turkey sampled in this study would contain 1.81 μg Hg, less than the daily maximum oral reference dose for the average person (7.00 $\mu\text{g}/\text{day}$). One turkey sampled on the SRS had an abnormally high concentration of Hg as compared to the majority of samples in both breast muscle (0.179 $\mu\text{g}/\text{g}$, d.w.) and liver tissue (0.299, d.w.). This one elevated Hg concentration out of 48 total breast samples would exceed the daily maximum oral reference dose at 11.89 μg Hg in one meal. However, while over the reference dose, unless particularly sensitive to Hg contamination, exceeding a single daily reference dose by this low amount is unlikely to have detrimental effects to health, especially given that on average turkey hunters in South Carolina consume only 12 meals of wild turkey each year (Smith et al. 2017). As not all Hg contamination is specific to the SRS, birds

inhabiting other contaminated areas of the Southeast, chiefly the Savannah River, may contain elevated Hg levels, and should be researched further.

Further, as there is no research on Hg levels in wild turkeys throughout the U.S., we could only compare the SRS samples to those we collected from western SC and eastern GA. Mercury concentrations can vary widely by region and we cannot be certain if the levels found in the present research are representative to concentrations throughout the range of the Eastern wild turkey (Bigler et al. 1975, Wren 1986). In addition, as Hg concentrations can differ by sex for some species (Robinson et al. 2012, Provencher et al. 2016), and many states allow the harvest of female birds (hens), there is a need for research to determine whether differences in Hg concentrations exist between male and female wild turkeys (Wren 1986). Given the paucity of data on trace element and heavy metal concentrations in wild turkeys and other game species, there is a need for more research on the trace element levels in tissues of game, especially for Hg due to human consumption risks.

Chromium was the only other trace element that differed between the two locations, with significantly lower concentrations in the breast muscle of turkeys inhabiting the SRS than those of the surrounding area. This is similar to chromium concentrations found in mourning doves and wild pigs on SRS, but opposite what was found for raccoons (Burger et al. 1997, Burger et al. 2002, Oldenkamp et al. 2017). This could potentially be due to the habitat used by the various species and the availability of Cr in their corresponding habitats. Acute chromium toxicity can lead to mouth sores and gastrointestinal issues, while chronic toxicity may be carcinogenic (Baruthio 1992, Smith

and Steinmaus 2009). However, levels found in turkeys both on and offsite are well below reference levels set by the EPA and do not pose a health risk to consumers.

We found a moderate correlation between Hg concentrations in feathers and muscle tissue, but no correlation between feather and liver Hg concentrations. Thus, collection of feather samples appears to be a reasonable non-lethal method for assessing Hg concentrations in wild turkey muscle tissue. However, given the variability in the relationship between feather and muscle Hg concentrations ($R^2 = 0.44$), additional testing of muscle tissue also may be needed to ensure wild turkeys are safe for hunter consumption in areas of known Hg contamination. Deviations between Hg concentrations in feather and muscle/liver tissues from this correlation could likely be due to temporal variations in Hg exposure, as Hg will only be deposited into feathers during feather growth (Goede and De Bruin 1986). We also found a correlation between Hg concentrations in muscle and liver ($R^2 = 0.49$ without outlier). This is somewhat surprising, as Oldenkamp et al. (2017) did not find any correlations in Hg concentrations between tissue types in wild pig, gray squirrel (*Scurius carolinensis*), waterfowl (*Anas platyrhynchos*, *Aix sponsa*), or waterbirds (*Fulica americana*, *Podilymbus podiceps*, *Phalacrocorax auratus*) on SRS. We were also unable to find correlations between breast muscle and liver for Cr, Cu, and Zn, similar to the findings of Oldenkamp et al. (2017).

While limited to a low sample size, we found that turkeys inhabiting an ash basin on site had elevated levels of Se in their blood as compared to birds sampled across the SRS. This could likely be caused by turkeys consuming soil while foraging in the contaminated area, or possibly eating plants that contain elevated concentrations of Se. However, there is no research determining what levels of Se are detrimental to the health

of turkeys, so further research is required to determine if the concentrations the turkeys are exposed to are high enough to cause any negative health effects. In addition, as there are currently no data on any potential correlations between Se levels in turkey blood and breast muscle, we cannot determine any potential exposure to consumers of turkeys inhabiting this particular area of the SRS.

The SRS contains areas of known Cs¹³⁷ contamination and many species inhabiting the site have been found to contain elevated Cs¹³⁷ in their tissues (Kennamer et al. 1998, Gaines et al. 2000, Burger et al. 2001, Oldenkamp et al. 2017). However, we found no evidence of Cs¹³⁷ contamination in any tissues of wild turkeys inhabiting the site. The majority of Cs¹³⁷ contamination is confined to a small percentage of the site and many turkeys harvested may never inhabit these areas, and therefore, may not be exposed to the environmental contamination. However, 17 turkeys were harvested within 1500m of known areas contaminated with Cs¹³⁷, and none of the samples exceeded their respective MDC. Most of the Cs¹³⁷ contamination on the SRS lies within the soil of wetlands and the sediment beneath reservoirs. While a turkey's diet consists of an estimated 9% soil, they may not forage enough in contaminated areas to be exposed to detectable amounts of Cs¹³⁷ (Beyer et al. 1994). Also, Cs¹³⁷ has a rapid excretion rate from living organisms, with a biological half-life of 10-11 days (Moss and Horrill 1996). This can lead to varying Cs¹³⁷ concentrations temporally, influenced by recent foraging habits. As turkey habitat use changes by season, they are likely exposed to different levels of contamination while foraging (Godwin et al. 1992, Miller et al. 1999). All of our turkeys were harvested during a 1-month period during the spring, but concentrations

have the potential to differ across seasons. As many states allow the harvest of turkeys during the fall, there is potential for concentrations to vary from the spring.

Our findings indicate that wild turkeys exposed to environmental contamination may incorporate some of these contaminants into their tissues. We found that turkeys inhabiting the SRS, contain elevated concentrations of Hg and decreased concentrations of Cr as compared to the surrounding area, while samples taken from wild turkeys inhabiting areas of known Cs¹³⁷ contamination were not found to have any increased Cs¹³⁷ activity. Our findings indicate that birds inhabiting the site do not pose a risk to human consumers. However, our findings indicate that wild turkeys are susceptible to Hg and Se exposure and have the potential to incorporate these elements into their tissues. More research is needed on potential detrimental effects of Se exposure in turkeys. Also, due to the popularity of the harvest and consumption of turkeys across the U.S., it may be warranted to conduct further research on birds inhabiting other areas of contamination across their range.

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Table 2.1. Average minimum detectable limits (MDL) and percentage of samples below these MDL by element for wild turkey tissues taken from the Savannah River Site and the surrounding area (offsite) and tested via ICP-MS.

Element	Tissue Type	SRS		Offsite	
		\bar{x} MDL (range)	%<MDL	\bar{x} MDL (range)	%<MDL
Cr	Liver	0.084 (0.032-0.135)	22.4	0.038	0
	Breast Muscle	0.029 (0.026-0.032)	25.5	0.035 (0.032-0.038)	0
Ni	Liver	0.107 (0.020-0.194)	63.3	0.017	0
	Breast Muscle	0.019 (0.019-0.020)	6.4	0.018 (0.017-0.020)	0
Cu	Liver	0.116 (0.027-0.205)	0	0.010	0
	Breast Muscle	0.022 (0.018-0.027)	0	0.018 (0.010-0.027)	0
Zn	Liver	0.686 (0.417-0.954)	0	0.157	0
	Breast Muscle	0.282 (0.146-0.417)	0	0.287 (0.157-0.417)	0
As	Liver	0.075 (0.048-0.102)	79.6	0.082	100
	Breast Muscle	0.051 (0.048-0.054)	76.6	0.065 (0.048-0.082)	100
Se	Liver	1.019 (0.565-1.472)	18.4	0.904	7.7
	Breast Muscle	1.546 (1.472-1.620)	95.6	1.188 (0.904-1.472)	88.2
Sr	Liver	0.263 (0.068-0.458)	73.5	0.024	0
	Breast Muscle	0.049 (0.029-0.068)	61.7	0.046 (0.024-0.068)	58.8
Cd	Liver	0.070 (0.030-0.109)	2.0	0.024	0
	Breast Muscle	0.028 (0.025-0.030)	66.0	0.027 (0.024-0.030)	64.7
Pb	Liver	0.147 (0.034-0.113)	59.2	0.017	15.4
	Breast Muscle	0.028 (0.021-0.034)	40.0	0.025 (0.017-0.034)	68.8
U	Liver	0.073 (0.021-0.124)	98.0	0.025	100
	Breast Muscle	0.029 (0.021-0.038)	100	0.023 (0.021-0.025)	94.1

Table 2.2. Descriptive statistics of trace element concentrations in the breast muscle of wild turkeys sampled both on and off the Savannah River Site. Only elements with >50% of samples >MDL are displayed.

Element	SRS (<i>n</i> = 47)		Offsite (<i>n</i> = 17)	
	Mean (range)	SE	Mean (range)	SE
Hg	0.030 (0.012-0.179)	0.004	0.018 (0.009-0.036)	0.002
Cr	0.083 (0.013-0.290)	0.011	0.168 (0.039-0.773)	0.045
Ni	0.116 (0.010-0.591)	0.019	0.086 (0.022-0.363)	0.021
Cu	4.007 (0.622-59.580)	1.307	2.668 (1.305-11.056)	0.582
Zn	27.920 (12.573-67.798)	1.766	31.284 (17.220-70.528)	3.108

Table 2.3. Descriptive statistics of trace element concentrations in the liver tissue of wild turkeys sampled both on and off the Savannah River Site. Only elements with >50% of samples >MDL are displayed.

Element	SRS (<i>n</i> = 48)		Offsite (<i>n</i> = 14)	
	Mean (range)	SE	Mean (range)	SE
Hg	0.068 (0.022-0.299)	0.006	0.035 (0.015-0.058)	0.004
Cr	0.142 (0.016-0.635)	0.017	0.145 (0.069-0.390)	0.024
Cu	12.178 (3.511-25.788)	0.564	11.142 (7.340-19.083)	0.914
Zn	84.731 (36.614-125.143)	2.346	87.687 (65.600-119.079)	3.911
Se	1.624 (0.627-3.510)	0.102	1.917 (0.452-3.086)	0.195
Cd	1.632 (0.015-3.089)	0.105	1.233 (0.597-1.798)	0.107

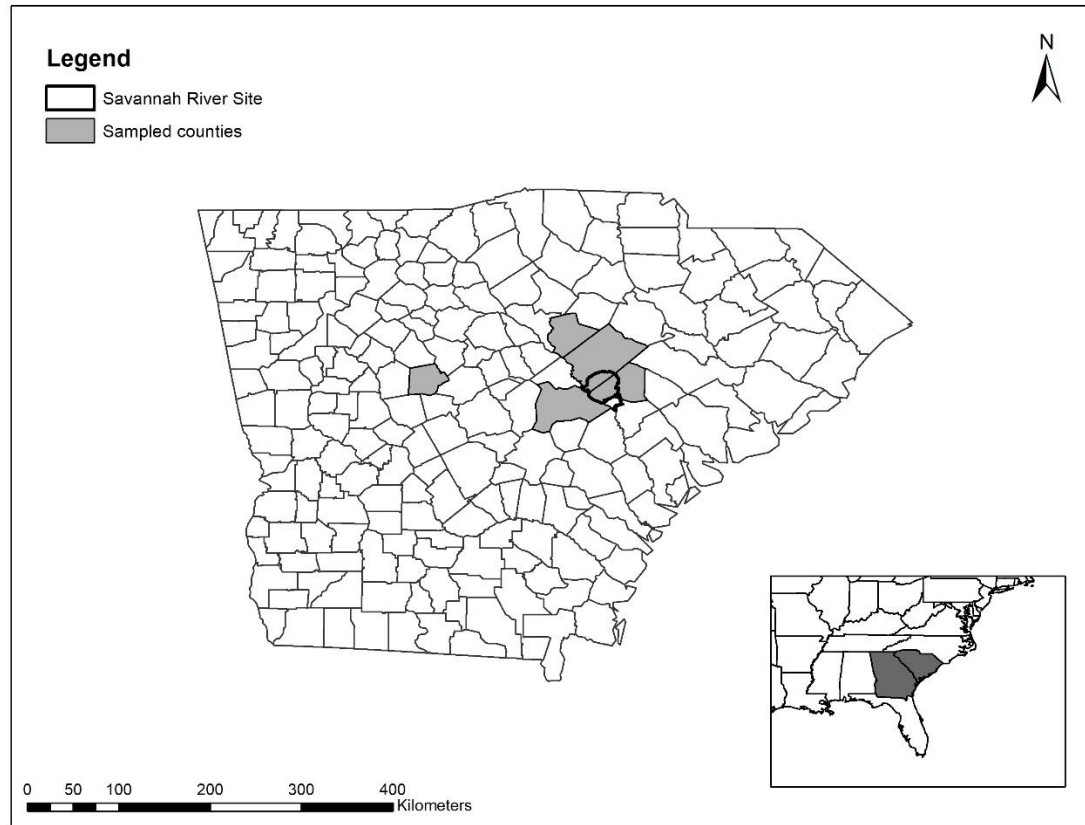


Figure 2.1. Counties wild turkey muscle samples were harvested in, in relation to the Savannah River Site.

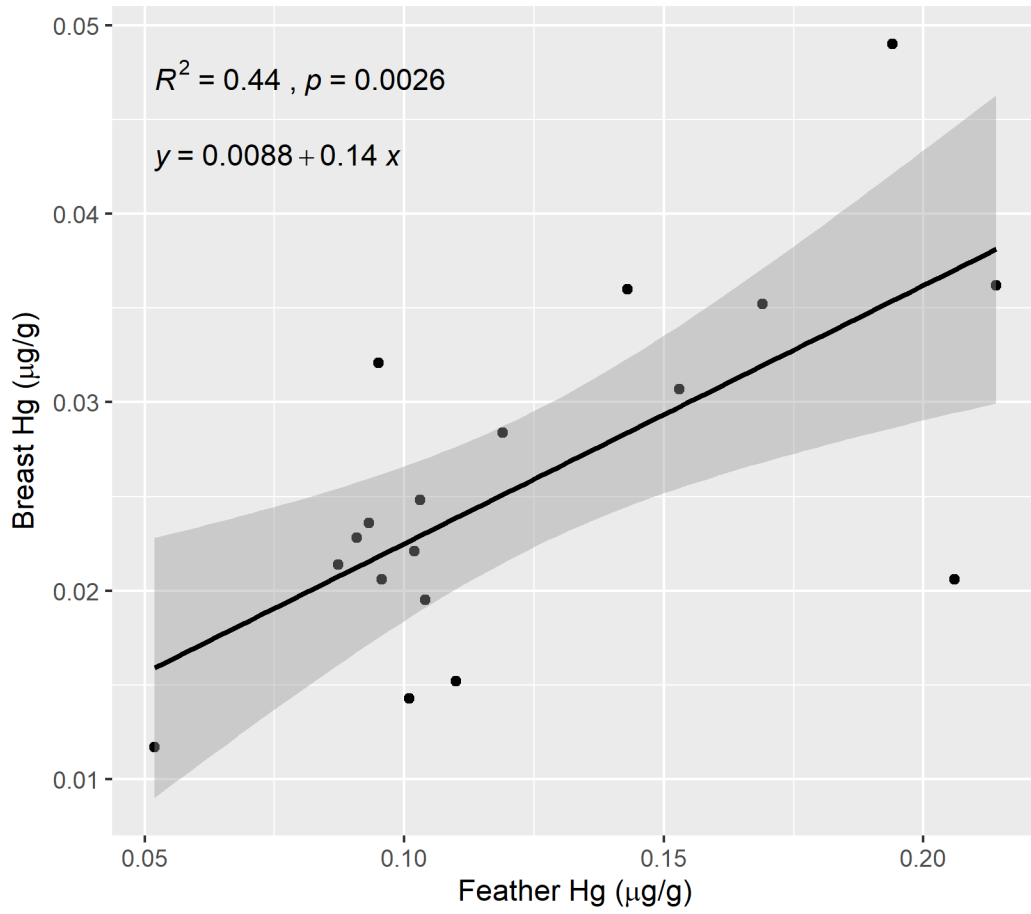


Figure 2.2. Wild turkey breast feather and breast muscle mercury (Hg) concentrations ($n = 19$) from birds inhabiting the Savannah River Site plotted with linear regression line.

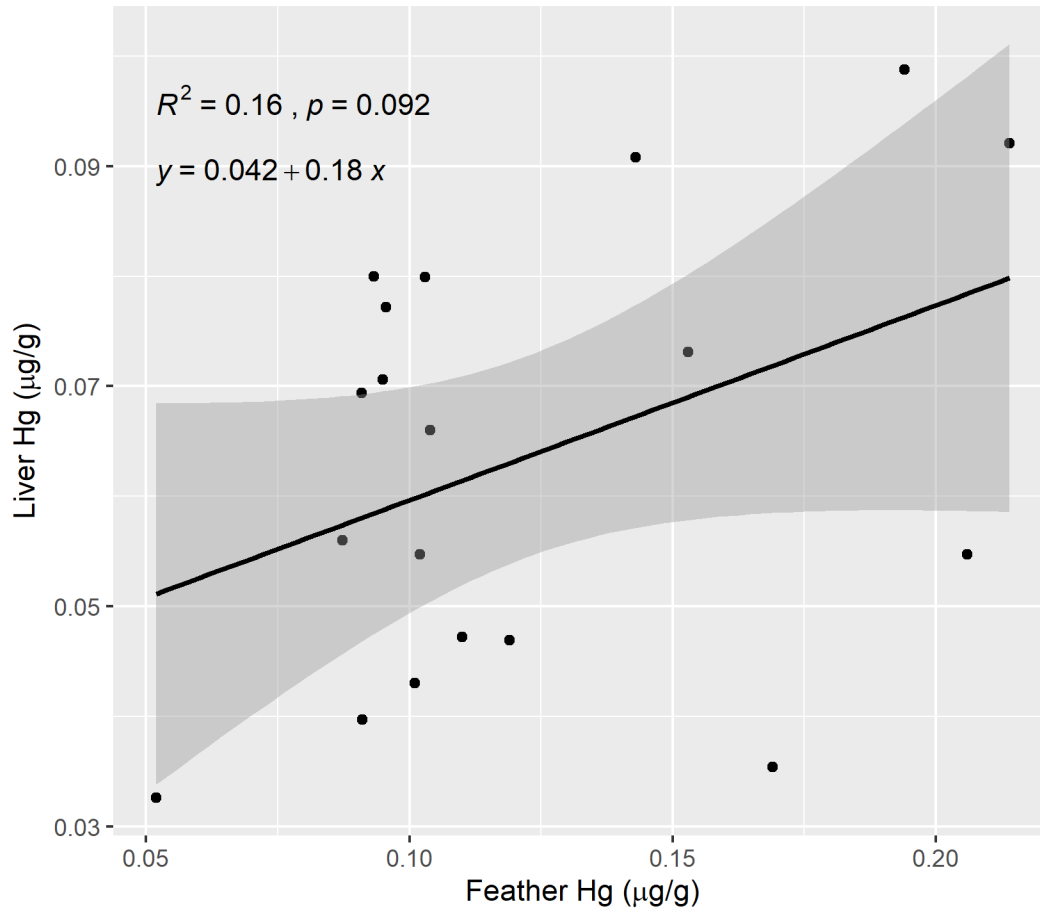


Figure 2.3. Wild turkey breast feather and liver mercury (Hg) concentrations ($n = 19$) from birds inhabiting the Savannah River Site plotted with linear regression line.

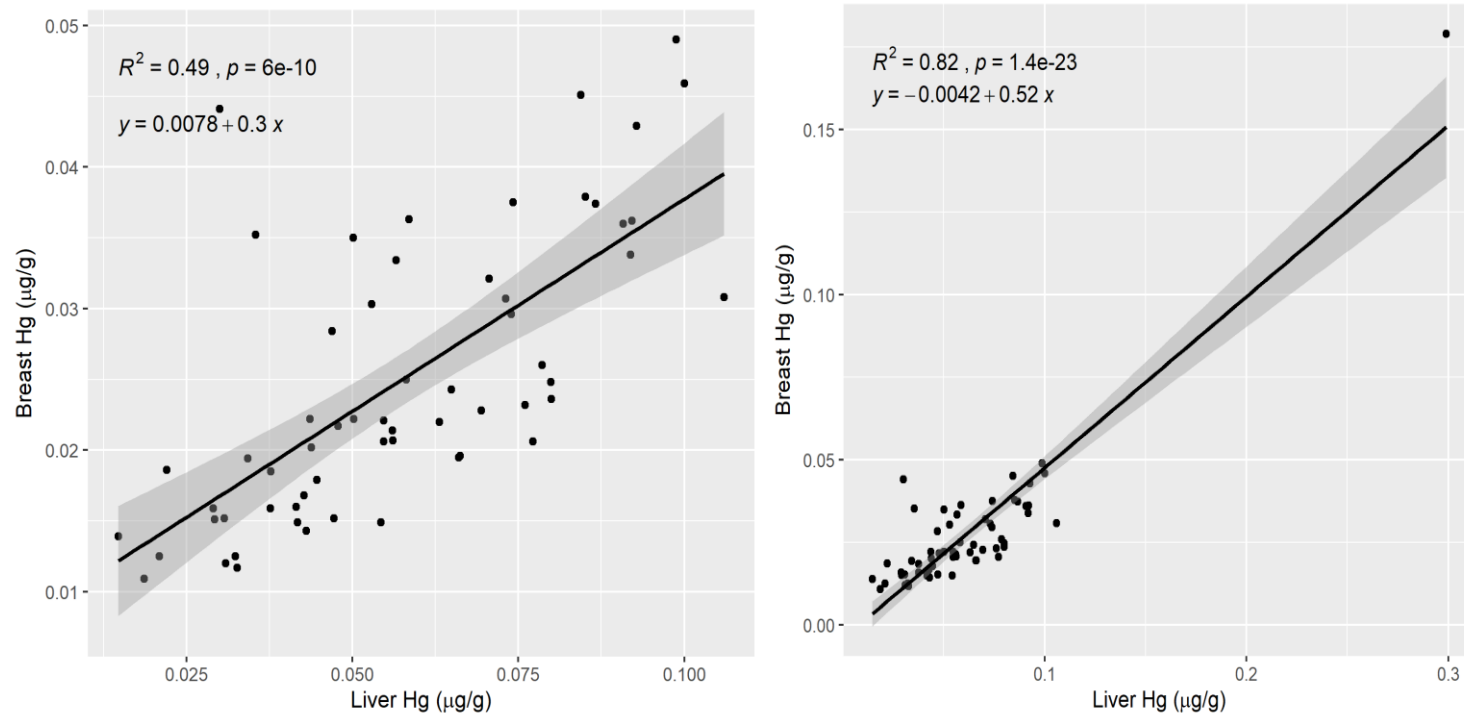


Figure 2.4. Wild turkey liver and breast muscle mercury (Hg) concentrations ($n = 48$) from birds inhabiting the Savannah River Site plotted with linear regression line with (right panel) and without (left panel) outlier.

CHAPTER 3

LEAD CONTAMINATION DIFFERENCES BETWEEN LEAD AND COPPER- COATED LEAD SHOT

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Abstract

Despite the known deleterious effects lead (Pb) exposure can have on the human body, it remains the most common type of ammunition used to harvest big game and upland game birds. We sampled wild turkey (*Meleagris gallopavo*) breast muscle shot with standard lead and copper-plated lead pellets to quantify lead residue concentrations within the wound channel and multiple adjacent locations to quantify how far lead contamination spreads through tissue. Birds harvested with standard lead ammunition did not differ in lead concentrations from those shot with copper-plated lead, suggesting copper-plating does not aid in reducing lead exposure. Additionally, we found samples taken from the wound channel contained more lead ($\bar{x} = 3.76 \mu\text{g/g}$, dry weight) than both the adjacent samples ($\bar{x} = 0.20 \mu\text{g/g}$, dry weight) and samples taken from >5 cm away ($\bar{x} = 0.15 \mu\text{g/g}$, dry weight). Our findings suggest that just one pellet passing through muscle tissue may expose consumers, especially children, to amounts of lead exceeding consumption limits set by the Food and Drug Administration (FDA) and Centers for Disease Control (CDC).

Introduction

Exposure to lead (Pb) can pose a substantial risk to human and wildlife health. Lead poisoning in adults can lead to increased blood pressure, decreased kidney function, and reproductive problems (United States Environmental Protection Agency; EPA 2019). Elevated blood lead levels in young adults also has been associated with increased odds of major depression and panic disorders (Bouchard et al. 2009). While lead is toxic to all age groups, children and pregnant women are especially susceptible to the detrimental effects of lead exposure, as blood lead levels as low as 10 $\mu\text{g/dL}$ have been found to

negatively affect IQ and cognitive development of children (Lanphear et al. 2005). Because of these deleterious effects, over the last 35 years the Center for Disease Control (CDC) has decreased the maximum acceptable blood lead levels for children from 25 $\mu\text{g}/\text{dL}$ to 5 $\mu\text{g}/\text{dL}$ (Roper et al. 1991, Baghurst et al. 1992). In the context of hunting, hunters can be exposed to lead by consuming meat that has been contaminated with fragments of ammunition (Hunt et al. 2009, Tsuji et al. 2009, Knott et al. 2010, Lindboe et al. 2012). However, more studies are needed to understand the amount of lead contamination found in hunter harvested animals, especially birds.

Despite the known toxicity of lead, its use in ammunition has been widespread in the harvest of game. Bullets and slugs are the predominant ammunition for large game, whereas shotgun pellets are used for upland small game. Once in the environment, spent lead ammunition can have detrimental effects on wildlife that directly or indirectly consume lead fragments or pellets (Cade 2007, Craighead and Bedrosian 2008, Neumann 2009, Cruz-Martinez et al. 2012). Avian scavengers such as eagles (*Accipitridae* spp.) and vultures (*Cathartidae* spp.) are particularly susceptible to lead exposure due to consumption of offal piles containing lead bullet fragments or unrecovered carcasses (Kelly and Johnson 2011, Bedrosian et al. 2012, Cruz-Martinez et al. 2012). Waterfowl and other aquatic species are also susceptible to lead toxicosis from the ingestion of spent shot while foraging in the sediment of hunted wetlands, which led to waterfowl die-offs as early as the 1940's (Jordan and Bellrose 1951, Olney 1960). An estimated 1.6-2.4 million ducks died annually in the U.S. before use of lead shot was prohibited for waterfowl harvest in 1991 (Degernes 2008, Avery and Watson 2009).

Not only is spent shot a risk to wildlife, animals shot with lead ammunition may also act as vectors of exposure to human consumers. While most hunters avoid consuming lead pellets found in the muscle tissue of harvested birds, the pellets can fragment into smaller pieces and also leave behind a fine powder that may escape detection (Frank 1986). Fragments of lead bullets have been shown to travel as far as 15 cm from the wound channel (Hunt et al. 2006, Cruz-Martinez et al. 2015). Fragmentation of lead ammunition is not limited to just big game and rifle bullets, as Pain et al. (2010) found shotgun pellet fragments in the carcasses of harvested gamebirds as well. Their study, along with others, have found elevated lead levels in the breasts of birds harvested with lead based ammunition (Johansen et al. 2001, Johansen et al. 2004, Pain et al. 2010). While no longer allowed in the hunting of waterfowl, lead-based shot is still commonly used for upland game species, such as mourning dove (*Zenaida macroura*), ring-neck pheasant (*Phasianus colchicus*), and wild turkey (*Meleagris gallopavo*), and many species of large game are harvested with lead bullets; thus, hunters may still be exposed to lead through consumption of these species. In fact, Iqbal et al. (2009) found consumers of wild game have blood lead levels 0.30 $\mu\text{g}/\text{dl}$ higher than people who do not consume wild game.

Efforts have been made to limit hunter exposure to lead via the production of 'non-toxic' ammunition. Metals such as steel, nickel, and copper have been used in place of lead or to coat lead pellets, but widespread acceptance of these alternatives has been challenging due to hunter concerns over price and efficacy of lead-free ammunition (Schulz et al. 2007). However, there have been no published studies investigating the effectiveness of non-toxic coatings of lead pellets at reducing lead contamination in the

muscle of harvested game. While coated lead pellets are often marketed for increased performance, they also have the potential to reduce lead exposure in consumers of harvested game.

Our objective was to test the efficacy of copper-plated shot at reducing lead contamination in muscle tissue. Using wild turkeys as a model species, we tested the hypothesis that lead contamination would vary by shot type and predicted higher levels of lead residues would be detected in individuals shot with standard lead shot as compared to those harvested with copper-coated lead ammunition. However, we predicted elevated lead levels would be limited to the wound channel and not to the adjacent muscle tissue. We also compared observed lead contamination levels to EPA standards to understand risks with using both types of ammunition. As copper can also be toxic when consumed at high doses, we also tested copper concentrations in the muscle samples of birds harvested with copper-coated lead shot to determine if copper levels exceeded EPA thresholds.

Study Area

All turkeys were harvested on the Savannah River Site (SRS), a ~78,000 ha tract of land owned by the United States Department of Energy (DOE). The SRS is located in the Upper Atlantic Coastal Plain in South Carolina with the Savannah River forming the western border. The site was created in 1951, transforming the ~40,000 ha of agricultural fields into slash (*Pinus elliottii*), loblolly (*P. taeda*), and longleaf pine (*P. palustris*) forests (White and Gaines 2000). In addition to these planted pines, oak (*Quercus* spp.) forests border most riparian areas, and cypress-tupelo (*Taxodium distichum*-*Nyssa aquatica*) swamps are scattered throughout the site. The SRS lies within a sub-tropical

climate, with temperatures frequently exceeding 32 °C between the months of May and September (Hunter 1990). Climate during the remainder of the year was mild, with temperatures infrequently dropping below freezing.

The SRS has a history of radionuclide and trace element contamination release into the surrounding environment due to anthropogenic activities. While wildlife inhabiting the site have been found to contain elevated concentrations of many of these contaminants, elevated lead concentrations have not been found in any of the biota inhabiting the site (Burger et al. 2002, Oldenkamp et al. 2017, Borchert et al. 2019).

Like much of the Southeast, turkeys were nearly extirpated and rare during the creation of SRS, with only 8 turkeys observed on site from 1951 to 1961 (Kilgo and Blake 2005). In 1973 and 1974, the South Carolina Department of Natural Resources (SCDNR) captured 48 turkeys in the Piedmont of South Carolina and released them onto the SRS. By 1977, the population had grown large enough that the SCDNR deemed the reintroduction a success and began using turkeys on the SRS as a source population for restocking birds across the remainder of the state. With the absence of hunting and active land use practices on site, the turkey population has flourished, with an estimated 2,000-2,200 individuals inhabiting the SRS in 2005 (Kilgo and Blake 2005).

Methods

Sample Collection

We harvested male wild turkeys on the SRS during the spring of 2018, with both standard lead and copper-coated lead pellets. We used size 5 shot for both ammunition types as a control against variation in lead concentration due to pellet size. We shot all birds twice, first in the head/neck area to ensure rapid euthanasia, and the second time

into the middle of the body from ~20 m away within 30 seconds of the first shot to ensure multiple pellets impacted the breast muscle. After harvesting each bird, we placed it in a freezer until samples could be taken. To facilitate sample collection, we partially thawed each bird for 1-2 days at 5°C, and partially skinned it to reveal the breast muscle. Once a pellet wound was located, we extracted a muscle plug centered on the wound channel using a 1.25-cm diameter piece of stainless-steel conduit, resulting in a ~5-cm long cylinder of muscle tissue. We extracted a second sample immediately adjacent and parallel to the wound channel sample, and a reference sample, from at least 5 cm from any sign of a pellet wound. This process was repeated for each of 2-3 pellet wounds per turkey. We removed all pellets detected in each sample while processing. We thoroughly cleaned conduit pieces between uses and stored samples in separate Whirl Paks (Nasco, Janesville, WI, USA) to prevent cross contamination. All collections were done under SCDNR collection permit SC-08-2019 and approved by University of Georgia Institutional Animal Care and Use Committee protocol A2019 01-012.

Sample Analysis

We conducted trace element analysis on muscle samples to determine lead and copper concentrations. Samples were weighed, freeze-dried, and then weighed again to calculate moisture content and subsequently ground and homogenized into a powder. We then microwave digested 250 mg of the dry, homogenized tissue (MARSX Xpress, CEM Corporation, Matthews, NC, USA) with 10 ml trace metal grade nitric acid (70% HNO₃). Following digestion, samples were diluted with 5 ml Milli-Q water (18MΩ; Millipore, Billerica, MA, USA). As a final step, the sample was diluted further, to 1:10, with 1 ml of the digested sample diluted in 9 ml Milli-Q water before analysis with inductively

coupled plasma mass spectroscopy (Nexlon 300X ICP-MS; Perkin Elmer, Norwalk, CT, USA). Every 20 samples, we included at least one sample of certified reference material (TORT-3 lobster hepatopancreas; National Research Council, Ottawa, ON, Canada) and a sample duplicate as a quality check. Percent recoveries ranged from 96-116%. For analysis, we used values of estimated lead concentration ($\mu\text{g/g}$) provided by these procedures. However, for values not exceeding the minimum detectable limit for lead (MDL; $0.065 \mu\text{g/g}$), we set the concentration value to 50% of the MDL ($\sim 0.033 \mu\text{g/g}$). All copper concentrations were above the MDL ($0.057 \mu\text{g/g}$).

Statistical Analysis

We compared lead concentrations (dry weight) taken from the wound channel of turkeys shot with standard lead shot to those shot with copper-coated lead shot. We also compared copper concentrations (dry weight) between breast sample locations for birds shot with the copper-coated lead pellets. Lead concentrations were tested for normality using the Shapiro-Wilks test, and were subsequently log transformed to approach normality. We conducted an analysis of variance using linear mixed effects models with the 'lme' function in package nlme in R to explore differences in lead levels between turkeys shot with each shot type (R Core Team 2019). To calculate degrees of freedom for each test, we used the Welch-Satterthwaite method. In addition, we used the 'multcomp' package to determine where any differences existed between sample locations. We used mixed effect models to allow for the random effect of bird identity because multiple tissue samples at each location were taken from each bird.

Results

We harvested 17 male wild turkeys from the SRS, 9 of which were shot with copper-coated lead pellets and the remaining 8 were shot with standard lead pellets. We sampled 2-3 wound channels, including an adjacent sample for every wound channel, as well as 2 reference samples from each bird. Lead concentrations were below MDL for $\geq 50\%$ of reference and adjacent samples for both shot types, whereas 29% of wound channel samples were below MDL for copper-coated shot and only 8% of wound channel samples were below MDL for those shot with standard lead pellets. Given the large proportion of samples in the adjacent and reference locations with concentrations below the MDL, we tested for differences between the wound channel and the pooled reference and adjacent locations, but not between the adjacent and reference locations.

Lead concentrations in the wound channels of turkeys harvested with copper-coated lead shot did not differ from lead concentrations in wound channels caused by standard lead shot ($F_{1,122} = 1.46$, $P = 0.26$, Figure 3.1). Hence, we pooled the 2 shot types together for further analyses. Using these combined data, we found a difference in lead concentration between locations ($F_{2,105} = 27.24$, $P < 0.001$), with tissue within the wound channel containing greater lead concentrations than both the adjacent and reference locations.

All samples taken from birds shot with copper-coated lead pellets had copper concentrations $> \text{MDL}$ ($0.05 \mu\text{g/g}$). Our linear mixed effects model analysis revealed a difference in copper concentration between breast locations ($F_{2,54} = 17.79$, $P < 0.001$, Figure 3.2). Similar to our lead analysis, *post hoc* analysis revealed the wound channel location contained greater copper concentrations than both the adjacent and reference

locations, but that copper concentrations did not differ between the adjacent and reference sample locations.

As can be seen by the standard deviations, lead and copper levels tended to be highly variable, even within locations. Also, due to the skewed nature and outliers within the dataset the means were skewed towards larger averages. This can be easily noticed when compared to the medians (Table 3.1 & 3.2). As such, we report the proportion of wound channel samples that exceed consumption limits set by The Food and Drug Administration (FDA) and Centers for Disease Control (CDC).

Discussion

We tested lead levels in multiple locations in the breast muscle of wild turkeys shot with two types of lead ammunition. Lead concentrations did not differ between shot types and were highest at the wound channel location. The Food and Drug Administration (FDA) and Centers for Disease Control (CDC) set a maximum consumption limit to avoid possible deleterious effects for lead at 3 $\mu\text{g}/\text{day}$ for children and 12.5 $\mu\text{g}/\text{day}$ for adults. Pooling shot types together, a single wound channel contained enough lead to put a child over this limit in 28.3% (13 of 46) wound channel samples. For adults, a single wound channel exceeded their consumption limit in 8.7% (4 of 46) of samples. At the adjacent location, only 2.2% (1 of 46) of samples exceed the child consumption limit, while none would exceed the daily limit set for adults. None of the samples at the reference location would exceed consumption limits set for children or adults. The reference dose set for copper is 10 $\mu\text{g}/\text{kg}/\text{day}$, or 700 $\mu\text{g}/\text{day}$ for the average adult. We did not find any samples at any location that would approach this consumption limit.

Copper-coated lead shot surprisingly did not reduce lead concentrations in the wound channel. This indicates the copper coating on pellets is likely not robust enough to contain the lead after being fired. Interestingly, the wound channel samples harvested with copper-coated lead shot had much higher variability in lead concentration than that of the lead shot harvested samples, including a few outliers (71.29, 22.27 $\mu\text{g/g}$). These high values suggest small lead fragments likely were present in the sample that escaped detection during initial laboratory dissection. Due to these fragments escaping detection during a thorough dissection, it is likely they would not have been found by hunters while preparing and cooking meat and thus potentially be ingested by consumers. Previous studies have radiographed birds harvested with various types of shot and found fragments of pellets throughout the body (Pain et al. 2010). However, in these studies, fragments were generally clustered around bone, likely due to pellets fragmenting when impacting the bone. As we were sampling the wound channel through breast muscle, we were less likely to sample the areas of increased pellet fragmentation near bones as observed in previous studies.

We did not make an attempt to clean or cook the muscle tissue, both of which have the potential to alter lead concentrations before consumption. However, Grund et al. (2010) found that rinsing carcasses of domestic sheep (*Ovis aries*) shot with lead bullets only spread the lead to other areas, making the contamination harder to avoid. Also, multiple studies have found elevated levels of lead in the tissue of cooked game meat harvested with lead shot (Johansen et al. 2001, Johansen et al. 2004, Pain et al. 2010). However, these studies did not test lead levels before preparation, so it is unknown what effect cooking may have on contamination levels and requires further study.

We shot turkeys an additional time in the breast during harvest, which is uncommon among normal turkey hunting practices. However, while turkey hunters typically only shoot a turkey in the head/neck area and do not fire a second round (Keck and Langston 1992), due to the spread of a shotgun pattern (Arslan et al. 2011) multiple pellets may often impact the breast muscle tissue. Also, most other species of birds are harvested while flying, making it very common for many pellets to impact the breast muscle (Burger et al. 1998, Johansen et al. 2001). Our results should be interpreted in the context of the risk of consuming a single wound channel in the breast. Nonetheless, game meat consumers across the United States are at risk of consuming lead particles and fragments being left behind by pellets in amounts that our results suggest may be higher than previously found. However, the likelihood of consuming a pellet wound channel is highly influenced by the amount of pellets impacting the breast upon harvest. It should be taken into consideration, however, that hunters use varying shot sizes to harvest game, more research is needed on the influence shot size may have on lead concentrations.

Our results suggest that the lead left behind via harvest with either lead or copper-coated lead shot may expose consumers to risk of lead ingestion at levels detrimental to their health. Our findings also indicate that lead contamination does not spread past the wound channel, indicating that if proper precautions are taken, such as removing the muscle tissue surrounding the wound channel, the majority of the lead contamination can be avoided. The lead concentrations we found suggest it may only take one pellet passing through muscle tissue to expose consumers, especially children, to amounts of lead

exceeding consumption limits. Our results suggest hunters should avoid eating muscle immediately surrounding the wound channel or use non-toxic shot types to minimize potential lead exposure from consuming harvested upland game birds.

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Table 3.1. Observed mean lead concentrations (dry weight) in wild turkey breast tissue harvested with two different types of shot on the Savannah River Site in 2018.

		Standard lead ($\mu\text{g/g}$)	Copper-plated lead ($\mu\text{g/g}$)
Wound Channel	\bar{x}	1.361 (0.033-11.046)	5.965 (0.033-71.291)
	Median	0.58	0.31
	SD	2.46	14.84
	n	22	24
Adjacent	\bar{x}	0.113 (0.033-0.619)	0.284 (0.033-2.756)
	Median	0.03	0.06
	SD	0.14	0.57
	n	22	23
Reference (>5cm)	\bar{x}	0.163 (0.033-1.727)	0.141 (0.033-0.401)
	Median	0.03	0.09
	SD	0.44	0.13
	n	15	18

Table 3.2. Observed mean copper concentrations (dry weight) in wild turkey breast tissue harvested with copper-coated lead shot on the Savannah River Site in 2018. Range is displayed in parentheses.

	\bar{x} ($\mu\text{g/g}$)	Median ($\mu\text{g/g}$)	SD	<i>n</i>
Wound Channel	10.75 (2.20-54.67)	3.90	12.89	24
Adjacent	2.63 (1.71-4.84)	2.42	0.82	23
Reference (>5cm)	2.64 (1.43-5.52)	2.38	0.83	18

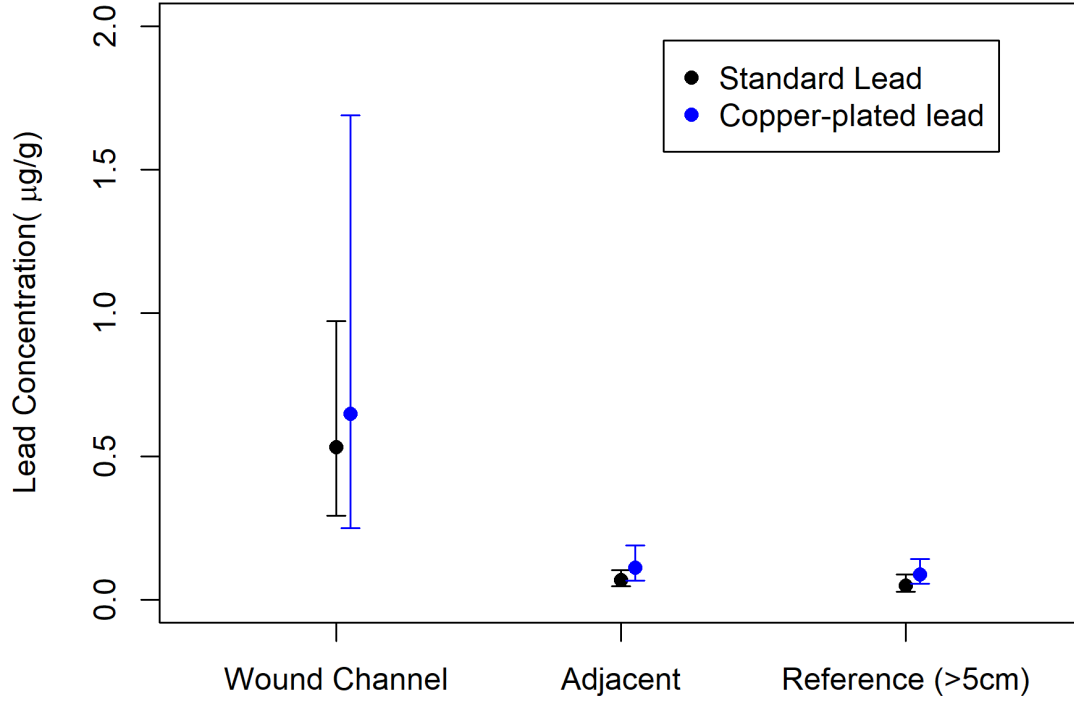


Figure 3.1. Model predicted lead concentrations of wild turkey tissue harvested with two types of shot on the Savannah River Site during the spring of 2018 shown with 95% confidence intervals.

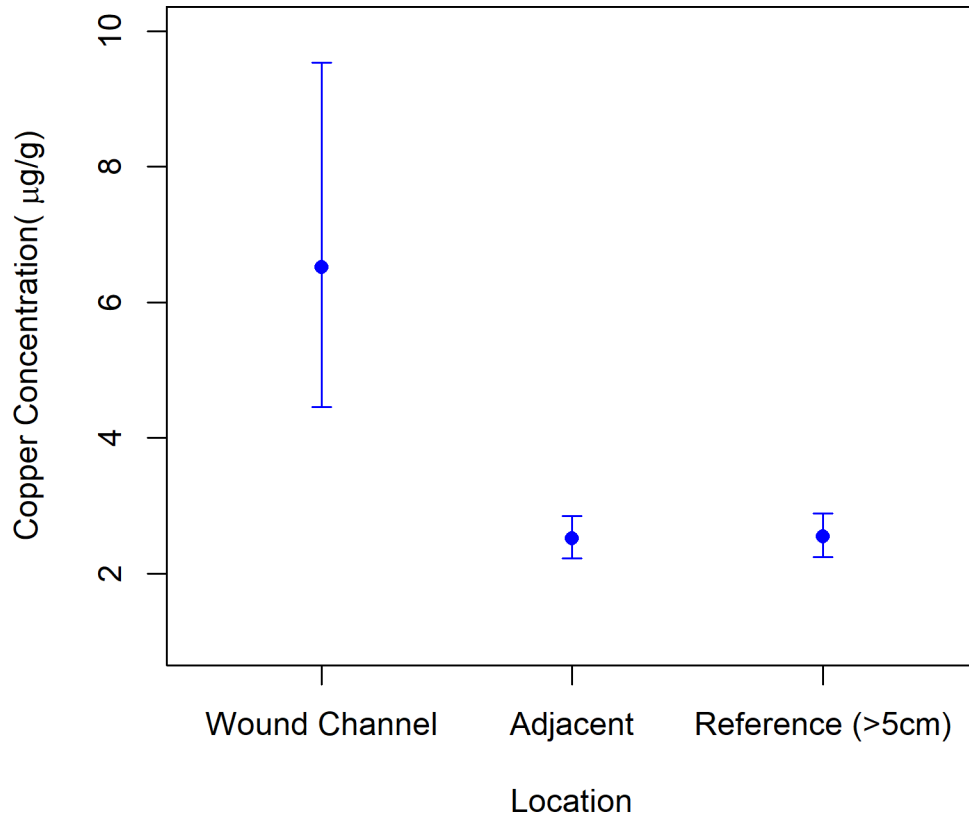


Figure 3.2. Model predicted copper concentrations of wild turkey tissue harvested with copper-coated lead shot on the Savannah River Site during the spring of 2018 shown with 95% confidence intervals. Samples sizes of n=24, 23, 18 for wound channel, adjacent, and references locations respectively.

CHAPTER 4

CONCLUSION

Despite the popularity of the wild turkey as a game bird, there has been little research on the potential for turkeys to serve as vectors of contaminant exposure to humans, either through natural accumulation of pollutants, or through contaminants (e.g., lead) that may be introduced during harvest. To better understand the concentrations of various contaminants in the tissues of this widely popular game bird, the objectives of my thesis were to 1) compare the concentrations of heavy metal, trace element, and radionuclide contaminants in a hunted population of wild turkeys inhabiting an area of known contamination relative to those inhabiting nearby reference areas and 2) compare lead concentrations in the muscle tissues of turkeys harvested with lead and copper coated lead shot. An overarching theme between both chapters was to discover if any of the contaminant concentrations found in the tissues of turkeys would pose a risk to consumers.

In Chapter 2, I sampled tissues of wild turkeys and compared the contaminant burdens to those of birds inhabiting nearby uncontaminated areas. I found that turkeys inhabiting the SRS contained elevated levels of Hg compared to birds sampled offsite, but were still below reference doses set by the CDC and FDA. This is similar to observations for other species inhabiting the SRS (Oldenkamp et al. 2017, Borchert et al. 2019). Conversely, I found Chromium (Cr) concentrations to be higher in turkeys offsite than in birds inhabiting SRS, which is consistent with previous studies on mourning

doves and wild pigs inhabiting the site, but inconsistent with concentrations found in raccoon tissues (Burger et al. 1997, Burger et al. 2002, Oldenkamp et al. 2017).

Variations in Cr accumulation patterns among species may reflect differences in diet and habitat, as Cr has been found to accumulate in plant tissues at different concentrations, with higher concentrations found in roots than in seeds (Cary et al. 1977). Concentrations of the remaining trace elements did not differ between SRS and offsite turkeys, suggesting turkeys are not an important vector of contamination exposure on the SRS for most elements. Although many species on the SRS have been found to contain elevated Cs¹³⁷ activity concentrations (Kennamer et al. 1998, Gaines et al. 2000, Burger et al. 2001, Oldenkamp et al. 2017), turkeys did not show any evidence of elevated Cs¹³⁷, even among 17 birds harvested near areas of known Cs¹³⁷ contamination.

I also analyzed blood samples from turkeys inhabiting an area adjacent to an ash basin on the SRS for Hg, As, and Se, common contaminants found in coal ash. While I did not find elevated levels of Hg or As, Se concentrations in the blood of the turkeys inhabiting the basin were greater than those in uncontaminated areas. However, low sample sizes restricted any further analysis of the data. There is no research into the potential effects of Se on the health of wild turkeys, so it is unknown if the levels we found are detrimental to the birds. Also, there is no research correlating Se levels in blood to those found in the tissues of the same individual, so it is unknown whether birds inhabiting the area are a consumer risk and requires further research.

Also in Chapter 2, I compared contaminant concentrations between multiple tissue types of turkeys, including both lethal (muscle and liver) and non-lethal (feather) samples. I found a moderate correlation between Hg concentrations in feathers and

muscle tissue, but no correlation between feather and liver Hg concentrations. Thus, collection of feather samples appears to be a reasonable non-lethal method for assessing Hg concentrations in wild turkey muscle tissue. However, given the variability in the relationship between feather and muscle Hg concentrations ($R^2 = 0.44$), additional testing of muscle tissue also may be needed to ensure wild turkeys are safe for hunter consumption in areas of known Hg contamination. The remaining elements with the majority of samples >MDL for both breast muscle and liver did not display a correlation between tissue types, although these contaminants were generally found in low concentrations.

Not only can hunters be exposed to contaminants already present in turkey tissues at harvest, they may introduce additional contaminants when harvesting birds with ammunition containing lead. While fragmentation of lead rifle bullets has been extensively studied (Hunt et al. 2006, Hunt et al. 2009, Tsuji et al. 2009, Grund et al. 2010, Knott et al. 2010), shotgun pellets have largely been overlooked. Previous studies have found shotgun pellets can fragment and introduce elevated lead concentrations into muscle tissue (Johansen et al. 2001, Johansen et al. 2004, Pain et al. 2010). In Chapter 3, I expanded on this research by comparing lead concentrations in the muscle tissue of turkeys harvested with two popular shot types among turkey hunters, including standard lead and copper-coated lead shot. I also compared lead concentrations between the wound channel, adjacent, and reference locations.

Surprisingly, I found that copper-coated lead shot did not reduce lead residues in the wound channel of harvested turkeys. Upon inspection of pellets post-harvest, the copper-coating had been scraped off in many areas, likely due to impacting the walls of

the gun barrel when fired. Also, the wound channel samples from individuals harvested with copper-coated lead shot had much higher variability in lead concentration than that of the lead shot harvested samples, including a few high outliers (71.29, 22.27 $\mu\text{g/g}$). These high values suggest small lead fragments were likely present in the sample that escaped detection during initial laboratory dissection. Due to these fragments escaping detection during a thorough dissection, it is likely they would not have been found by hunters while preparing and cooking meat and thus could potentially be ingested by consumers.

In addition, I found that the majority of lead contamination was constrained to the wound channel. This indicates that any potential fragments left behind as the pellet passes through the muscle tissue do not travel far through the carcass. However, previous studies have radiographed birds harvested with various types of shot and found fragments of pellets throughout the body (Pain et al. 2010). In these studies, fragments were generally clustered around bone, likely due to pellets fragmenting when impacting the bone. As I sampled just the wound channel through breast muscle, I may have been less likely to sample the areas of increased pellet fragmentation near bones as observed in previous studies. While there are studies that have found elevated lead concentrations in the muscle tissue of game birds after cooking (Johansen et al. 2001, Johansen et al. 2004, Pain et al. 2010), none have measured before and after effects on concentrations. Thus I was unable to predict any changes cooking may have on the lead concentrations found.

Finally, I compared lead and copper concentrations found in harvested turkeys to limits set by the CDC and FDA. For lead, these agencies set a maximum consumption limit to avoid possible deleterious effects for lead at 3 $\mu\text{g/day}$ for children and 12.5

$\mu\text{g}/\text{day}$ for adults. Just one wound channel contained enough lead to put a child over this limit in 28.3% (13 of 46) of wound channel samples. For adults, a single wound channel exceeded their consumption limit in 8.7% (4 of 46) of samples. At the adjacent sample location, only 2.2% (1 of 46) of samples exceed the child consumption limit, while none would exceed the daily limit set for adults. None of the samples at the reference location would exceed consumption limits set for children or adults.

The findings in my thesis expand our knowledge of the potential for environmental contaminants to be taken up by wild turkeys. Sampling turkeys at a site of known contamination, I found that while turkeys may incorporate environmental contaminants into their tissues, the birds inhabiting the SRS likely do not pose a risk to consumers. However, in Chapter 3, I found that harvesting birds with lead and copper-coated lead ammunition may be contaminating muscle with lead. However, if care is taken to remove the tissue around the wound channel, much of this contamination can be avoided. Or, to ensure all lead contamination is avoided full non-toxic shot may be used. These conclusions drawn from my thesis further contribute to our understanding of both wildlife exposure to environmental contamination, as well as human consumer exposure risk in relation to a popular game species.

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