Published in final edited form as:

Sci Total Environ. 2019 January 10; 647: 639–644. doi:10.1016/j.scitotenv.2018.08.043.

Lead exposure biomarkers in the Common Loon

Aaron J. Specht^{1,*}, Kimberley E. Kirchner², Marc G. Weisskopf¹, and Mark A. Pokras³

¹Harvard T.H. Chan School of Public Health, Boston, MA

²Worcester Polytechnic Institute, Worcester, MA

³Cummings School of Veterinary Medicine, Tufts University, N. Grafton, MA

Abstract

Lead in fishing tackle is a significant source of exposure to the environment, wildlife, and potentially humans. Common Loons (*Gavia immer*) are exposed to lead by eating fish which have lead tackle, or ingesting fishing weights or spent ammunition when they ingest small stones to aid in digestion. Blood lead is traditionally used as a biomarker of exposure in loons, but it only reflects recent exposures. Cumulative exposure measured via bone lead may better reflect the overall health of loons and their aquatic habitat. This study compared a portable x-ray fluorescence (XRF) device for measurement of bone lead with and without tissue overlying the bone on loon cadavers with measurements made by inductively coupled plasma mass spectrometry (ICP-MS) of different tissues from the loons. For this study we had 75 bone samples, 19 body fluid samples, and 17 liver lead samples. We found significant correlations between portable XRF bone lead measurements made with overlying tissue and ICP-MS measures of bone lead ($R=0.88$), body fluid lead ($R=0.65$), and liver lead ($R=0.71$). Bone lead was found to be higher in loons collected from non-coastal regions. In assessing lead-related cause of death, bone lead proved more predictive than liver lead. Future studies should investigate the value of these biomarkers for both aquatic health and loon health to further validate our findings.

Graphical Abstract:

*Corresponding Author: Aaron J Specht, aspecth@hsph.harvard.edu, 655 Huntington Ave Building 1 Rm 1402, Boston, MA 02215.

6. Conflicts of Interest

The authors declare no conflicts of interest with this work.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

metals; environmental monitoring; avian; lead; aquatic health; loon

1. Introduction

Lead in fishing tackle is a significant source of exposure to the environment, wildlife, and potentially humans. Lead ingestion in Common Loons has been shown to be the leading cause of death in New Hampshire, accounting for 44% of adult loon deaths.(Committee, 2013) Loons ingest the lead by eating fish that have ingested lead tackle, or directly by consuming the tackle, mistaking it for a pebble when they ingest small stones to aid in digestion.(Committee, 2013) Additionally, loons may consume lead from spent ammunition, again, mistaking them as pebbles. Most loon mortality from lead fishing gear occurs during the peak fishing times of the summer, July through August, suggesting that it is lead deposition from current fishing activities that primarily affects loon health, rather than lost fishing gear from past years.(Committee, 2013)

Loons are often considered indicators of aquatic health, as they are extremely sensitive to changes in their habitat.(Evers, Paruk, W., & Barr, 2010; Evers et al., 2003) Contaminants that bioaccumulate, including PCBs, PBDEs, and heavy metals have been shown to be problems for loons.(Evers, 2006) Since loons are representative of the overall aquatic environment, it is likely that other species that are hunted or fished are also exposed, which in turn may expose humans. Similarly, it was shown that hunters displayed increased blood lead levels in comparison to the general population, which likely arises from the use of lead bullets and shot.(Hunt et al., 2009; Iqbal et al., 2009; Johansen, Pedersen, Asmund, & Riget, 2006) Thus, tracking lead exposure typical of aquatic species through loons will answer questions pertinent to preserving the species itself, but also for protecting the environment and human health.

Blood lead is a traditional marker of lead exposure in humans and many different animals.(Beyer & Meador, 2011; Finkelstein et al., 2012; Newth et al., 2016; Rabinowitz, 1998) However, as shown in humans and other species, blood lead is only indicative of recent exposure of one week to one month, whereas the biological half-life of bone lead in humans is on the order of decades.(Finkelstein et al., 2012; Rabinowitz, 1991) In human studies, it has been shown that cumulative lead exposure measured using bone lead levels is a better biomarker for studying such sublethal problems as cardiovascular disease and cognitive function.(Navas-Acien, Guallar, Silbergeld, & Rothenberg, 2007; Shih, Hu, Weisskopf, & Schwartz, 2007; Weisskopf et al., 2004) Cumulative lifetime exposure levels would be the optimal measure to best reflect the overall health of different species and the impact of lead on the environment. Bone lead has been measured in humans using a K-shell x-ray fluorescence (KXRF) device that used a cadmium-109 radioisotope source.(Chettle, Scott, & Somervaille, 1991; Hu, Milder, & Burger, 1989) Recently, a portable L-shell x-ray fluorescence (XRF) device has been used to measure bone lead in humans *in vivo* and condor samples.(A. J. Specht et al., 2016; A. J. Specht et al., 2018; Aaron James Specht, Weisskopf, & Nie, 2014) The portable XRF has a ten times lower measurement time than

KXRF measurements and can easily be transported for site visits. This handheld device would lend itself to in-field studies of environmental contamination and cumulative lead analyses of wildlife using bone lead as a biomarker.

In pursuit of a methodology to easily identify bone lead levels, this study examined measurements of bone lead using a novel portable XRF method.(A. J. Specht et al., 2016; A. J. Specht et al., 2018; Aaron James Specht et al., 2014) We tested the portable XRF methods in comparison to bone measurements made using inductively coupled plasma mass spectrometry (ICP-MS). Since we had many stored carcasses of loons, we were able to test the measurement capability along with the effect of soft tissue thickness overlying the bone, which can affect the uncertainty of measurements from the device.(Aaron James Specht et al., 2014) We also had liver and body fluid measurements on a small number of birds, which we used for comparison between exposure assessment methods. Finally, we were able to look at differences in exposure based on location and bone, liver, and body fluid lead level associated with a clinical veterinary diagnosis of lead poisoning.

2. Methods

2.1 Loon samples

As part of a long-term, regional loon mortality study, loon cadavers were collected from Massachusetts, New Hampshire, and Maine, and were stored at Tufts Cummings School of Veterinary Medicine. Cadavers were obtained from several different sources, including the Loon Preservation Committee, WildCare, Biodiversity Research Institute, and a variety of fish and wildlife agencies. Necropsies were performed and data were obtained on age, gender, body metrics, associated pathology, and any interesting findings. Leg samples were saved both from birds that had no known lead exposure (as assayed by body fluid lead levels), and from birds with elevated blood or body fluid lead concentrations. Some leg samples were saved from birds with lead exposure seen in radiographs that showed lead fishing gear or lead shot. In those cases, the metal fragments were confirmed to be lead by using LeadCheck® Swabs (3M St. Paul, MN, USA).(Buehler & Rhoda, 2012) The legs were then stored in a freezer at -40°C until analysis. Liver samples from some of the birds were also saved and stored in the same freezer until analysis. For this study, 75 tarsometatarsus bone samples were used in the validation portion of this study. Seventeen liver samples, relating to birds that had bone measurements were then analyzed to determine the relationship between chronic and acute lead exposure. Post mortem body fluids in 19 of the birds were collected by sampling mixed fluids at the time of autopsy on the loon cadavers. Only 19 loons had sufficient fluids for sampling at that time.

2.2 Sample Analysis

2.2.1 Portable X-ray Fluorescence Measurements—In this study, we used a portable XRF from Thermo Fisher (Niton XL3t GOLDD+, Thermo Fisher Scientific Billerica, MA). The same device was used in previous studies that made lead measurements of human and condor samples.(A. J. Specht et al., 2016; A. J. Specht et al., 2018; Aaron James Specht et al., 2014) We used x-ray tube settings of 50kV 40uA and filtration of silver and iron. The device was calibrated against known concentration doped plaster-of-Paris

phantoms as done in previous works.(A. J. Specht et al., 2016; Aaron James Specht et al., 2014) The lead L-beta peak at 12.6 keV was used for quantification to avoid interference from peaks at the alpha region (10.55 keV). Matlab was used for spectral fitting using a Gaussian function with an exponential background fitting. The measurements were made for 3 minutes each at mid-length tarsometatarsus bone. Using a mid-length long bone in humans was shown to be the most consistent for comparison to cumulative lifetime exposure levels. (Castellino, Castellino, & Sannolo, 1995; Erkkila et al., 1992) Two sets of measurements were done on each sample; one with soft tissue intact, and one after tissue had been removed. The detection limit of the device changed based on the thickness of soft tissues covering the bone being measured.

Calibration of the portable XRF was performed using lead doped plaster-of-Paris phantoms and tissue phantoms made from poly-methyl methacrylate (Lucite®) as done in previous work.(A. J. Specht et al., 2016; A. J. Specht et al., 2018; Aaron James Specht et al., 2014) Lucite® was shown to be a good approximation to skin thickness in humans, and likely can be comparable to tissue thickness in loons as well.(Aaron James Specht et al., 2014)

2.2.2 Inductively Coupled Plasma Mass Spectrometry Measurements—The ICP-MS measurements were made using a standard ICP mass spectrometer (NexION 350X ICP-MS, PerkinElmer Inc., Waltham, MA). A 1 cm sample of the bone from the mid-diaphysis was taken, dried in an oven, weighed out to approximately 1.0 grams, and digested along with 0.04 mL of 10ppm rhodium standard in an aluminum block heater ramping up to 130 Celsius in 1.5 hours with continuous heat for 14–16 hours in 5 mL trace metal grade nitric acid (TraceMetal grade, Fisher A509–212). Then 1 mL of 30% hydrogen peroxide (Certified A.C.S. grade, Fisher H325– 500) was added to the samples. Standard quality control included initial and continuing calibration verification, reagent blanks, and a standard reference material. The ICP-MS measurements were validated with 10 subsamples from the same bones that were sent to the PADLS Toxicology Laboratory at the University of Pennsylvania School of Veterinary Medicine. Thirteen liver samples from loons for which we also had bone samples were sent to the PADLS Toxicology Laboratory for lead analysis and 4 liver samples were previously measured using ICP-MS.

At the PADLS Toxicology Laboratory the following procedures were used for ICP-MS analysis. The tissue and bone samples were weighed into Teflon PFA (Perfluoroalkoxy alkane) vials (Saville, Minnetonka, MN) and digested overnight in a Precision Oven™ (Thermo Scientific, Waltham, MA) set at 70±3°C with 12.5 times the quantity (weight/volume) of 70% nitric acid. A 0.1 mL portion of each digested tissue or bone sample was individually mixed with 0.05 mL of 2 ppm internal standard containing Ge (germanium), In (indium), Tb (terbium), and Y (yttrium) and each mixture was diluted with deionized water to a final volume of 5 mL for analysis. The concentration of lead in the submitted sample was measured using a calibration curve of aqueous standards prepared at four different concentrations of each metal. The accuracy of the results was monitored by analyzing reference material (NIST 1577C) with known values of metals of interest with each batch of samples.

2.2.3 Body Fluid Measurements—The 19 body fluid samples were analyzed for lead using a desk-top LeadCare® blood lead testing system (Magellan Diagnostics, North Billerica, Massachusetts USA). (Kornetsky, Rock, & Pokras, 2013) In using this system, there is an upper limit of 65 ug/dL for measurements. Since our analyses focus on correlations of these continuous variables, we use values of 65 ug/dL for the five measurements found to be >65 ug/dL.

2.3 Statistical Analysis

We used linear regressions for comparisons between different biomarkers of exposure and logistic regressions to estimate how biomarkers predict a lead related death. With logistic regression analyses, we calculated odds ratio estimates for a standard deviation change in biomarker exposure level. Statistical analysis was done using R statistical software, version 3.0.3 (Foundation for Statistical Computing, Vienna, Austria). The bone lead results were not normally distributed, so Wilcoxon rank sum tests were done rather than standard t tests for comparisons of lead level to location. Negative bone lead values were left as such to prevent artificial variance reduction, since the values themselves are a point estimate of bone lead with associated uncertainty. The uncertainty (σ) of each portable XRF measurement was calculated using the following equation,

$$\sigma = \frac{c \times \sqrt{\frac{BKG + Gross}{t}}}{Net} \quad (1)$$

where c is the concentration, BKG is the background counts as estimated by our fitting, $Gross$ is gross counts over the area of the fitted peak, t is measurement time, and Net is the net lead counts from the Gaussian function in our fitting of each individual loon bone measurement spectra in this work. (20)

3. Results

3.1 Summary of Lead Results from Bone, Body fluids, and Liver

In Table 1, we present the summary of results from ICP-MS and portable XRF measurements of bone lead, body fluid, and liver analyses. The average uncertainty for the portable XRF measurements of bone with overlying tissue was 1.65 ± 0.51 ug/g. The average uncertainty for the portable XRF measurements of bone without overlying tissue was 0.78 ± 0.06 ug/g. When considering only those that also had liver measurements, the average of the bone lead values remained similar at 10.8, 3.8, and 2.8 ug/g for portable XRF with tissue, portable XRF without tissue, and ICP-MS respectively. When considering bone lead for only those that also had body fluid measurements, the bone lead averages were 8.0, 4.0, and 2.6 ug/g for portable XRF with tissue, portable XRF without tissue, and ICP-MS, respectively.

3.2 Correlation between ICP-MS and Portable XRF with Tissue

Figure 1 shows the significant correlation between measurements of portable XRF bone lead with intact overlying tissue and ICP-MS bone lead measurements ($R=0.877$, 95% CI (0.813, 0.921), $Beta= 3.75$ (3.5, 3.98), $p\text{-value}<0.001$).

3.3 Correlation between ICP-MS and Portable XRF of Bone without Overlying Tissue

Figure 2 is the significant correlation between portable XRF bone lead measurements without tissue and ICP-MS bone lead measurements ($R=0.911$, 95% CI (0.863, 0.942), $Beta= 1.59$ (1.51, 1.67), $p\text{-value}<0.001$).

3.4 Correlation between Portable XRF of Bone with and without Overlying Tissue

Figure 3 shows the significant correlation between portable XRF measurements made with and without tissue ($R=0.822$, 95% CI (0.732, 0.884), $Beta= 2.01$ (1.85, 2.17), $p\text{-value}<0.001$).

3.5 Detection Limit and Tissue Thickness

Table 2 shows the distribution of tissue thickness, the associated detection limit of the device, and the spread of values observed in this study. This table helps to demonstrate the validity of this method for field measurements indicating that there is a low bone lead detection limit for loon measurements even when considering tissue thickness and the typical lead level observed in loons is higher than the detection limit.

3.6 Correlation between liver, fluids, and bone lead measurements

Table 3 shows a correlation matrix with rho values comparing the lead results from different biomarkers. The bone markers are similar, although they vary slightly in comparison to liver and body fluid measures.

3.6 Relationships Between Bone Lead, Location, and Anticipated Exposure Level

For 32 of the samples collected, we had an origin location along with suspected cause of death, which included many with documented ingestion of lead fishing gear. We looked at the differences between loons collected from Maine, New Hampshire, and Massachusetts. We found that bone lead measured via XRF values from specimens collected in Massachusetts (bone lead 1.3 ± 3.2 ug/g) were significantly lower compared to either New Hampshire (bone lead 4.9 ± 7.2 ug/g, $p\text{-value}=0.019$) or Maine (bone lead 4.0 ± 4.0 ug/g, $p\text{-value}=0.019$) collected specimens. Using bone lead measured by ICP-MS did not change these results.

We also looked at a logistic regression of lead in the different tissue predicting a dichotomized lead-related cause of death variable of either 1) definitely exposed through ingestion of lead fishing gear or known lead poisoning, or 2) non-lead related cause of death (i.e. trauma or fatal gunshot). We had these data on 29 of the specimens collected. Bone lead as measured by XRF with tissue was shown to be a predictor of lead-related cause of death with a standard deviation increase in bone lead increasing odds of lead-related cause of death by 1.37 (95% CI: 1.27, 1.47). Using the portable XRF measurement of lead in bone

without overlying tissue slightly reduced the odds ratio with an odds ratio of 1.24 (95% CI: 1.16, 1.33) for lead-related cause of death per standard deviation increase in XRF bone lead without tissue. The odds ratio for a lead-related cause of death per standard deviation increase in ICP-MS bone lead measurement was 1.32 (95% CI: 1.23, 1.42). A standard deviation increase in body fluid lead level had an odds ratio of 1.49 (95% CI: 1.38, 1.61) for lead-related cause of death. Finally, the odds ratio per standard deviation increase in liver lead levels for lead-related cause of death was 1.15 (95% CI: 1.03, 1.29). When only using the data for specimens with every measurement (N=10), the results for liver (OR=1.36) increased to a similar level as body fluids (OR=1.34) with the other trends remaining the same.

4. Discussion

The results clearly demonstrate that portable XRF measurements of lead in bone have good comparability to ICP-MS measures of lead in bone. Both correlations with and without overlying tissue demonstrated an adequate quantification from the portable XRF measurements. The skin thickness values gave detection limits that were lower than those in human population studies of bone lead, which, again, demonstrate the ability of the portable XRF to determine bone lead for the majority of the samples in this study. The biomarker of bone lead in loons was also shown to have positive correlations with body fluid and liver lead levels.

The correlations between ICP-MS and portable XRF of lead in bone with overlying tissue demonstrated how tissue could affect the relation between the two measurements. The detection limits even with skin were low enough to quantify the bone lead for the specimens in our study. Although the correlation coefficient was very high for this relationship ($R=0.88$), the beta estimate in our correlation was 3.75, which indicates that the XRF measurement was reading higher than the ICP-MS measurement. We believe this was either a result of lead being stored in the overlying tissue during a process of lead toxicosis, a difference in the composition of tissues in loons compared to our calibration, or residual contamination on the skin of the loons in the portable XRF measurements. It is unlikely residual contamination would produce correlations so reflective of those inside the body, but this could be the case, as loons should reflect their environment well. Calibration differences could exist with loons, although human populations show beta estimates closer to 1 for this comparison. If these results are from the tissue itself, not residual contamination, that may indicate some mechanism of storage in tissues during the lead toxicosis process for this species. This would not necessarily be contradictory to studies of human body burden, as a majority of these birds were suspected to have died due to lead toxicosis. It is possible that during this process the lead in their bodies did not have sufficient time to be incorporated into bone prior to death, which would help to explain the high readings with tissue. This is supported by the lower, closer to 1, beta estimate (1.59) for the correlation between portable XRF without overlying tissue and ICP-MS measurements of bone lead, and the closer concentration readings (Table 1). The correlation between body fluids or liver lead and bone lead was higher for the XRF measurements with tissue than without, which also may be because lead in the tissue itself affects the XRF measurement. More study on this point is

needed to clearly delineate where this excess lead arises and identify how this may impact future *in vivo* measurements.

The beta value for the relation between bone lead measured with ICP-MS and XRF without overlying tissue was not one, which might have been expected. However, the two measurement approaches sampled different portions of the bone, which could have influenced the measurements depending on the biological accumulation differences of those regions of the bones. ICP-MS measurements averaged over a cross-section of the bone including the trabecular bone at the bone core and the cortical bone on the surface. (Rabinowitz, 1991) The portable XRF only samples to a depth of around 0.2–0.5mm below the surface, which would be predominantly cortical bone. (Todd, Parsons, Tang, & Moshier, 2001) In a previous study comparing XRF and ICP-MS measurements, similar slight differences were found. (A. J. Specht et al., 2018) Finally, in a study of human cadaver bones identifying differences between surface and core bone lead levels, significant differences were found, indicating that an averaging of the bone as done by ICP-MS may not reflect the surface bone measured via portable XRF. (Todd et al., 2001)

The correlation between bone lead, body fluid lead, and liver lead demonstrate the feasibility of bone lead as a biomarker for lead exposure. The concentration of lead in bone was higher (~2 parts per million level) than in body fluid measurements (~0.3 parts per million), which is similar to what we would expect from previous studies of human and animal lead retention. (Rabinowitz, 1998; A. J. Specht et al., 2016; A. J. Specht et al., 2018) The body fluid lead correlations likely would have had stronger relationships, but the upper limit of detection for 5 data points possibly biased these results lower. An expanded study with more precise equipment would better elucidate these relationships. The liver lead levels were on average higher than the bone lead. This is likely because many of the liver tests were on loons suspected of dying due to lead poisoning, which would have meant very high more recent acute exposures that likely were not yet fully incorporated into bone, which has a slower uptake time. The odds ratio for body fluid lead level as a predictor of lead-related cause of death was the largest with a 49% increased odds for a standard deviation change in body fluid lead level. Long-term cumulative exposure from bone lead likely increases mortality from other causes, however more work on how this relates to health outcomes in general and lead poisoning in loons needs to be completed.

Specimens collected in Massachusetts had significantly lower lead levels than those from New Hampshire and Maine. This is likely reflective of the collection locations in the different states. Many of the New Hampshire and Maine collection locations were from lakes or inland areas that would be associated with fishing and hunting, whereas all of the specimens collected from Massachusetts were found on coastal beaches. It is less likely that coastal locations would experience as severe environmental contamination from lead-based hunting or fishing gear. Loons living in those areas would likely have lower overall lead body burden, which is reflected in our results. It is also possible that there are subtle, sublethal effects of lead, which would limit the ability of the bird to migrate, and, thus, limit them to certain locations inland with more easily accessible food sources. More studies on these differences need to be undertaken to properly understand the differences observed in this study.

In summary, portable XRF measurements can be used to measure cumulative lead exposure in loons, even when considering the tissue thickness present during in vivo measurements. Our high correlations between liver and body fluid lead and portable XRF bone lead measurements with intact overlying tissue demonstrate that even in cases of severe acute exposures, the portable XRF measurement may read a different concentration due to lead deposition in overlying tissue. We show that there are significant differences in lead levels by the locations of recovery of the loon. Lead levels were lower for loons recovered in coastal regions. Bone lead was shown to be a better predictor of veterinary diagnosed lead poisoning than both liver and post-mortem body fluid lead levels. More studies on health outcomes in loons and measures of lead exposure need to be completed to determine the best biomarkers for acute lead poisoning.

Acknowledgements

This work was supported by the Summer Star Foundation, Maine Outdoor Heritage Trust, the Edward Gorey Charitable Trust, the Dr. Scholl Foundation, and the U.S. Fish & Wildlife Service. This work was also supported by the Harvard T.H. Chan NIEHS Center for Environmental Health (P30 ES000002). We greatly appreciate the contributions from our collaborators who obtained and stored loon cadavers, including the Loon Preservation Committee, WildCare, Avian Haven, the Biodiversity Research Institute, Tufts Wildlife Clinic, and the fish and wildlife agencies of all New England states. The authors report no conflicts of interest with this work.

7. References

- Beyer WN, & Meador JP (2011). Environmental Contaminants in Biota: CRC Press.
- Buehler S, & Rhoda D (2012). 3M LeadCheck Swabs: Qualitative Spot Test Kit for Lead in Paint Environmental Protection Agency Report. Battelle.
- Castellino N, Castellino P, & Sannolo N (1995). Inorganic Lead Exposure: Metabolism and Intoxication. Lewis Publishers, 156–162, 446, 168–180.
- Chettle DR, Scott MC, & Somervaille LJ (1991). Lead in bone: sampling and quantitation using K X-rays excited by 109Cd. Environ Health Perspect, 91, 49–55. [PubMed: 2040251]
- Committee LP (2013). Effects if Lead Fishing Tackle on Loons in New Hampshire, 1989–2011. Retrieved from Loon Preservation Committee: <http://www.loon.org/ingested-leadtackle.php>
- Erkkila J, Armstrong R, Riihimaki V, Chettle DR, Paakkari A, Scott M, Aitio A (1992). In vivo measurements of lead in bone at four anatomical sites: long term occupational and consequent endogenous exposure. Br J Ind med, 49(9), 631–644. [PubMed: 1390269]
- Evers DC (2006). Loons as Biosentinels of Aquatic Integrity. Environmental Bioindicators, 1, 18–21.
- Evers DC, Paruk JDWMJ, & Barr JF (2010). Common Loon (*Gavia immer*), version 2.0 In The Birds of North America (Poole AF, Editor). Cornell Lab of Ornithology, Ithaca, NY, USA 10.2173/bna.313
- Evers DC, Taylor KM, Major A, Taylor RJ, Poppenga RH, & Scheuhammer AM (2003). Common loon eggs as indicators of methylmercury availability in North America. Ecotoxicology, 12(1–4), 69–81. [PubMed: 12739858]
- Finkelstein ME, Doak DF, George D, Burnett J, Brandt J, Church M, Smith DR (2012). Lead poisoning and the deceptive recovery of the critically endangered California condor. Proc Natl Acad Sci U S A, 109(28), 11449–11454. doi:10.1073/pnas.1203141109 [PubMed: 22733770]
- Hu H, Milder FL, & Burger DE (1989). X-ray fluorescence: issues surrounding the application of a new tool for measuring burden of lead. Environ Res, 49(2), 295–317. [PubMed: 2753011]
- Hunt WG, Watson RT, Oaks JL, Parish CN, Burnham KK, Tucker RL, Hart G (2009). Lead bullet fragments in venison from rifle-killed deer: potential for human dietary exposure. PLoS One, 4(4), e5330. doi:10.1371/journal.pone.0005330 [PubMed: 19390698]

- Iqbal S, Blumenthal W, Kennedy C, Yip FY, Pickard S, Flanders WD, Jean Brown M (2009). Hunting with lead: association between blood lead levels and wild game consumption. *Environ Res*, 109(8), 952–959. doi:10.1016/j.envres.2009.08.007 [PubMed: 19747676]
- Johansen P, Pedersen HS, Asmund G, & Riget F (2006). Lead shot from hunting as a source of lead in human blood. *Environ Pollut*, 142(1), 93–97. doi:10.1016/j.envpol.2005.09.015 [PubMed: 16280190]
- Kornetsky R, Rock M, & Pokras M (2013). A rapid postmortem screening test for lead toxicosis in common loons (*Gavia immer*) and bald eagles (*Haliaeetus leucocephalus*). *J Wildl Dis*, 49(3), 723–727. doi:10.7589/2011-11-336 [PubMed: 23778630]
- Navas-Acien A, Guallar E, Silbergeld EK, & Rothenberg SJ (2007). Lead exposure and cardiovascular disease--a systematic review. *Environ Health Perspect*, 115(3), 472–482. doi:10.1289/ehp.9785 [PubMed: 17431501]
- Newth JL, Rees EC, Cromie RL, McDonald RA, Bearhop S, Pain DJ, Hilton GM (2016). Widespread exposure to lead affects the body condition of free-living whooper swans *Cygnus cygnus* wintering in Britain. *Environ Pollut*, 209, 60–67. doi:10.1016/j.envpol.2015.11.007 [PubMed: 26629647]
- Rabinowitz MB (1991). Toxicokinetics of bone lead. *Environ Health Perspect*, 91, 4.
- Rabinowitz MB (1998). Bone lead as a biological marker in epidemiologic studies of chronic toxicity: conceptual paradigms. *Environ Health Perspect*, 106, 8.
- Shih RA, Hu H, Weisskopf MG, & Schwartz BS (2007). Cumulative lead dose and cognitive function in adults: a review of studies that measured both blood lead and bone lead. *Environ Health Perspect*, 115(3), 9. doi:10.1289/ehp.9786
- Specht AJ, Lin Y, Weisskopf M, Yan C, Hu H, Xu J, & Nie LH (2016). XRF-measured bone lead (Pb) as a biomarker for Pb exposure and toxicity among children diagnosed with Pb poisoning. *Biomarkers*, 21(4), 347–352. doi:10.3109/1354750X.2016.1139183 [PubMed: 26856822]
- Specht AJ, Parish CN, Wallens EK, Watson RT, Nie LH, & Weisskopf MG (2018). Feasibility of a portable X-ray fluorescence device for bone lead measurements of condor bones. *Sci Total Environ*, 615, 398–403. doi:10.1016/j.scitotenv.2017.09.123 [PubMed: 28988075]
- Specht AJ, Weisskopf M, & Nie LH (2014). Portable XRF technology to quantify Pb in bone in vivo. *Journal of Biomarkers*, 2014, 9.
- Todd AC, Parsons PJ, Tang S, & Moshier EL (2001). Individual variability in human tibia lead concentration. *Environ Health Perspect*, 109(11), 1139–1143. [PubMed: 11712999]
- Weisskopf MG, Wright RO, Schwartz J, Spiro A, Sparrow D, Aro A, & Hu H (2004). Cumulative lead exposure and prospective change in cognition among elderly men: the VA Normative Aging Study. *Am J Epidemiol*, 160(12), 1184–1193. doi:10.1093/aje/ [PubMed: 15583371]

Highlights:

- Loon bone lead can be used as a predictor of lead poisoning.
- Portable XRF can be used to assess loon bone lead in vivo in field measurements.
- Loon bone lead is a good marker of long-term aquatic health.
- There were significant differences of loon lead level by location.

Figure 1.
Correlation between portable XRF bone lead with tissue and ICP-MS bone lead concentrations.

Figure 2.
Correlation between portable XRF bone lead without tissue and ICP-MS bone lead concentrations.

Figure 3.
Correlation between the portable XRF measurements with and without tissue.

Table 1.

Summary of portable XRF bone lead, ICP-MS bone lead and liver, and LeadCare body fluid measurements in the loon samples.

	Portable XRF with Tissue (ug/g)	Portable XRF without Tissue (ug/g)	ICP- MS (ug/g)	Body Fluid Lead (ug/dL)	ICP-MS Liver Lead (ug/g wet weight)
<i>N</i>	75	75	75	19	17
<i>Average</i>	7.41	3.17	2.10	31.61	15.52
<i>Min</i>	-4.01	-1.34	0.03	0.90	0.10
<i>Max</i>	49.63	25.38	12.18	>65	95.30
<i>Standard Deviation</i>	11.70	4.79	2.74	28.06	28.55
<i>Detection Limit</i>	3.31	1.58			

Table 2.

Average, range, and standard deviation of tissue thickness, portable XRF detection limit for measurements with and without tissue, and the XRF bone lead estimate with tissue (N=75).

	Tissue Thickness (mm)	Detection Limit with Tissue (ug/g)	Detection Limit without Tissue (ug/g)	XRF Bone Lead without Tissue (ug/g)
Average	1.15	3.31	1.58	3.17
Min	0.37	2.20	1.33	-1.34
Max	3.02	7.63	1.91	25.38
Standard Deviation	0.55	1.02	0.11	4.79

Table 3.

Correlation matrix between lead measured in different tissues.

	Bone ICP- MS	Bone XRF without Tissue	Bone XRF with Tissue	Lead Care Body Fluids	ICP- MS Liver
<i>Bone ICP-MS</i>	1	0.91	0.88	0.57	0.45
<i>Bone XRF without Tissue</i>		1	0.82	0.5	0.27
<i>Bone XRF with Tissue</i>			1	0.62	0.56
<i>Body Fluids</i>				1	0.58
<i>Liver</i>					1