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Chronic lead exposure is epidemic in obligate scavenger populations in eastern North America

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ABSTRACT

Lead is a prominent and highly toxic contaminant with important impacts to wildlife. To understand the degree to which wildlife populations are chronically exposed, we quantified lead levels within American black vultures (*Coragyps atratus*; BLVU) and turkey vultures (*Cathartes aura*; TUVU), two species that are useful as environmental sentinels in eastern North America. Every individual sampled (n = 108) had bone lead levels indicative of chronic exposure to anthropogenic lead (BLVU: $\bar{x} = 36.99 \pm 55.21$ mg Pb/kg tissue (±SD); TUVU: $\bar{x} = 23.02 \pm 18.77$ mg/kg). Only a few showed evidence of recent lead exposure (BLVU liver: $\bar{x} = 0.78 \pm 0.93$ mg/kg; TUVU liver: $\bar{x} = 0.55 \pm 0.34$ mg/kg). Isotopic ratios suggested multiple potential sources of lead including ammunition, gasoline, coal-fired power plants, and zinc smelting. Black and turkey vultures range across eastern North America, from Quebec to Florida and individuals may traverse thousands of kilometers annually. The extent to which vultures are exposed suggests that anthropogenic lead permeates eastern North American ecosystems to a previously unrecognized degree. Discovery of an epidemic of chronic lead exposure in such widespread and common species and the failure of soft-tissue sampling to diagnose this pattern has dramatic implications for understanding modern wildlife and human health concerns.

1. Introduction

Lead exposure increases behavioral problems and decreases the attention span and the IQ of children (Carpenter, 2001; Dietrich et al., 2001; Canfield et al., 2003). These effects are likely irreversible despite lowering blood lead levels through chelation (Carpenter, 2001). Within the U.S., lead-free paint and automobile gasoline now are mandated because of these dangers. Nevertheless, lead is still regularly used by humans in manufacturing and ammunition and is prevalent in human and natural ecosystems (Vucetich et al., 2009; Robbins et al., 2010). The quantity of spent lead shotgun shot may reach 400,000–2 million pellets per acre (Pain, 1991; Fry and Maurer, 2003), while estimations of annually discarded lead-tainted carcasses include 28,000 "varmint" carcasses and 36,000 big game gut piles in California (Fry and Maurer, 2003) and >1.05 million prairie dogs (*Cynomys ludovicianus*) killed with lead bullets per year in South Dakota (Huxoll, 2012). Obligate scavengers therefore are likely exposed to anthropogenic lead when foraging, and the degree to which they are exposed may be a strong indicator of the ecosystem-wide distribution of environmental lead. These processes have substantial indirect implications for human exposure, since one of the most common vulture food sources – hunter-killed game – is also common in human diet.

High concentrations of lead in blood of birds can have a suite of detrimental physiological effects (summarized in Haig et al., 2014), including reduction in hemoglobin synthesis, decreases in bone mineralization (Locke and Thomas, 1996; Gangoso et al., 2009), oxidative insult and demyelinization of nerve cells (Mateo et al., 2003) and increases in the mortality rate of the iconic and critically endangered California condor (*Gymnogyps californianus*; Church et al., 2006; Finkelstein et al., 2012). However, in spite of the frequency with which they are used in avian toxicology studies (i.e. liver, blood; Locke and Thomas, 1996; Snyder and Snyder, 2000; Carpenter et al., 2003; Fry and Maurer, 2003; Church et al., 2006; Finkelstein et al., 2012; Harmata and Restani, 2013), blood metrics are an imperfect indicator of lifetime total exposure to lead because they are responsive primarily to recent events. In fact, avian rehabilitators describe regular admission of birds with no neurological symptoms of lead poisoning (wing drooping, flightless, listlessness; Friend and Franson, 1999) but high blood lead levels, and admission of birds with neurological symptoms consistent with lead poisoning but low blood lead levels. This is because lead competes with and replaces calcium within the central nervous and skeletal systems (Finkelstein et al., 2008; Mason et al.,

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2014; Pain et al., 2005; Gangoso et al., 2009). Thus, animals chronically exposed to lead show high bone lead levels, regardless of the time since recent exposure. Likewise, blood lead levels could be indicative of either recent exposure or of long-stored lead leached from bone into blood (Ambrose et al., 2000; Mason et al., 2014). It is for this reason that bone lead metrics can be a better indicator than blood of long-term lead burden and exposure (Ambrose et al., 2000).

To evaluate the degree to which wildlife are exposed to lead and, therefore, to give insight into the extent to which anthropogenic lead pervades modern trophic systems, we evaluated chronic lead exposure of two species of obligate scavengers, the American black vulture (*Coragyps atratus*; hereafter black vulture) and the turkey vulture (*Cathartes aura*), collected in the U.S. state of Virginia. These species are two of the three avian obligate scavengers in North America (the other is the California condor whose modern range does not include eastern North America; Snyder and Snyder, 2000). East of the Mississippi River, turkey vultures are found south of ~51° in Quebec (Kirk and Mossman, 1998) and black vultures south of ~41° in Pennsylvania (Buckley, 1999). Both species aggregate in Virginia and other southern parts of their range when not breeding and birds marked at this location have been observed 900 km apart (A. Duerr unpublished observations). Although black vultures are generally assumed to scavenge larger carcasses than turkey vultures, both species eat a wide variety of prey, including remains (including gut-piles or offal) of domestic and wild ungulates (e.g., cows, *Bos taurus*; sheep, *Ovis aries*; white-tailed deer, *Odocoileus virginianus*) and small- and medium-sized mammals (e.g., gray squirrel, *Sciurus carolinensis*; groundhog, *Marmota monax*) that die of natural or anthropogenic causes (Kirk and Mossman, 1998; Buckley, 1999). Individuals of both vulture species are known to be more resistant to the effects of lead toxicity than are many other animals and thus are better as environmental sentinels than are species that die quickly when exposed to low concentrations of lead (i.e., these vultures can accumulate and harbor more lead for a longer period of time than more sensitive species; Bravo et al., 2005; Carpenter et al., 2003).

Chronic lead exposure was evaluated by quantifying lead concentrations in femurs of culled vultures. To understand differences in chronic and recent exposure, we also quantified lead concentration in liver tissue from the same individuals. While blood is the typical medium for assessing recent exposure for live birds, liver is the preferred tissue collected from bird carcasses to understand recent toxicological events (Friend and Franson, 1999). Once harvested, tissue samples were digested in nitric acid and lead concentration measured by inductively coupled plasma mass spectrometry (ICP-MS). We also measured lead isotope ratios (^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb) of these same black and turkey vulture femur samples to determine probable sources of lead.

2. Materials and methods

2.1. Sample collection

Vultures (n = 98 black vultures and 10 turkey vultures) were culled by the U.S. Department of Agriculture, Wildlife Services in Chesterfield County Virginia, on 26 and 27 July 2011 and between 24 April 2012 and 02 May 2012. Carcasses were frozen within 24 h of culling. Frozen carcasses were thawed and necropsied in October 2011 and in July and August 2012. At necropsy, we collected samples from liver, kidney, breast muscle, thigh muscle, and femur. All samples were immediately wrapped in aluminum foil, placed in a labeled plastic bag, and stored in a conventional freezer (~−18 °C) until sample preparation.

Foil-wrapped samples were thawed to room temperature for preparation. We cleaned the target tissue manually to remove traces of non-target tissue (e.g., muscle, connective tissue on bones or liver). We dissected target tissue and weighed 0.5000–0.5449 g (wet weight) on a digital balance (Sargent-Welch SWA 200DR). To allow comparison between samples with different masses, we used the measurement of mg of lead per kg of tissue (mg/kg). Each sample was handled with new scalpel blades and gloves and stored in a previously unused sample vial to prevent cross contamination between samples.

2.2. Sample preparation, lead (Pb) concentration, and isotope ratio analysis

Tissue samples collected and weighed on a wet weight basis were digested in 10 ml of ~67–70% nitric acid (TraceMetal Grade, Fisher Scientific) and maintained at room temperature until analysis. Prior to analysis, samples were heated at 105 °C for 3 h and diluted to 50 ml with ultrapure water and then read by ICP-MS at REIC Laboratories, Beaver, WV. Method detection limits (MDL) averaged 0.0569 ± 0.0904 (mean ± SD) for black vulture samples and 0.0399 ± 0.0008 for turkey vulture samples.

To test the consistency of our results within tissue types, we collected new tissue samples from a small subset of the frozen tissue already analyzed. Two of the samples re-evaluated were samples whose lead concentration was within 1 standard deviation of the population mean found after the first run (mean = 36 mg/kg; samples chosen = 11.8 mg/kg and 15.3 mg/kg) and the third was one extreme outlier (629 mg/kg). In all cases, results were within 15% of the original measurements (11.0 mg/kg, 14.0 mg/kg, and 540 mg/kg, respectively). In statistical analysis of the three tissues sampled twice, we only included the one measurement for each tissue sample that was closer to the population mean for lead concentration.

In addition to standard methodological controls implemented by the analytical lab, we submitted for analysis two types of putative blanks as controls. We prepared three samples from the femur of store-bought organically raised domestic chicken (*Gallus gallus*; Pb = 0.766 ± 0.19 mg/kg; MDL = 0.039 ± 0.0006) and four samples of nitric acid (no tissue; Pb = 0.0079 ± 0.0028 mg/L; MDL = 0.0002 ± 0.00). These represent either, at worst, typical levels of potential contamination in our sampling, or at best, actual background measures of lead in these materials.

Finally, to evaluate both lead isotope ratios in femur samples and potential lab- and instrument-specific failure points, we sent for re-analysis, 114 of our original, pre-digested vulture and control samples to a second analytical lab (Michigan State University Diagnostic Center for Population and Animal Health; MSU — DCPAH, East Lansing, MI). At this lab, NIST SRM (Standard Reference Material) 2976 mussel (0.250 g) (NIST Office of Reference Materials, Gaithersburg, MD), NIST SRM 981 common lead isotopic standard (0.250 g) (NIST Office of Reference Materials, Gaithersburg, MD), and three blank tubes were processed as quality control (QC) on lead quantitation accuracy and on background lead levels. All samples, QC materials, and blanks were brought to a mass of 10 g with water on a top-loading laboratory balance capable of measuring to ±0.001 g. This was done by quantitatively transferring the digested contents to a separate tube tared on the balance; the original tube was rinsed several times with Milli-Q reverse osmosis deionized water (Millipore Corporation, Billerica, MA). Dilution factors for calculation of lead concentrations were combined with the weights provided for the original samples during calculation.

Lead concentrations were determined on an Agilent 7500ce ICP-MS (Agilent Technologies, Santa Clara, CA) equipped with a Cetac Auto Sampler (Cetac, Omaha, NE) and MicroMist Nebulizer (Agilent). The instrument was calibrated with appropriate dilutions of Specpure 1000 μg/ml stock solutions (Alfa Aesar, Ward Hill, MA) of each element of interest according to in-house generated standard operating procedures derived from the Agilent Operator's Manual (Agilent Technologies 2004). Each quantitative analysis used a matrix matched quality control NIST SRM. Accordingly, analyses performed on bone were matched to NIST SRM 1400 (bone ash) and analyses on tissue were matched to NIST SRM 1577c (bovine liver). Digested samples were diluted in an aqueous solution of 0.05% (w/v) ethylenediaminetetraacetic acid (EDTA), 1% (w/v) ammonium hydroxide, 0.05% (w/v) Triton-X 100, and 2% (w/v) butanol prior to analysis. Listed reagents were from

Sigma-Aldrich (St. Louis, MO). Lead isotopes were determined as counts per second (cps) at m/z 204, 206, 207, and 208, and m/z 204 values were adjusted for the presence of trace mercury values determined at m/z 202 assuming a normal distribution for mercury of m/z 196 (0.153%), 198 (9.968%), 199 (16.873%), 200 (23.096%), 201 (13.181%), 202 (29.863%), and 204 (6.865%; Rosman and Taylor, 1997). M/z 207 has an explicit requirement for adjustment likely owing to an instrument-specific mass discrimination, and it is an accepted technique to correct for such bias by using a suitable isotope standard reference material (Becker, 2005). Therefore cps values for lead were adjusted according to responses obtained from a standard of NIST SRM 981 for lead isotopes certified at m/z 204 (1.4255%), m/z 206 (24.1442%), m/z 207 (22.0833%), and m/z 208 (52.347%). This reference material was analyzed in separate nitric acid digests twenty times over a three week period to gather an average response, and application of these average responses back to the SRM provided average responses of m/z 204 (1.3968% ± 0.0372), m/z 206 (24.2802% ± 0.1812), m/z 207 (21.9149% ± 0.2259), and m/z 208 (52.4801% ± 0.0861) to one standard deviation, with resultant precision in terms of %RSD of 2.66%, 0.746%, 1.03%, and 0.164%, respectively, and accuracies of 98.0%, 100.6%, 99.2%, and 100.1%, respectively.

Lead concentration measurements from the two labs were well correlated (R = 0.95). These same 114 samples included the 108 evaluated for lead isotope ratios.

2.3. Data analysis

Data analysis of lead concentrations in femur and liver were conducted on the original REIC results. To compare femur and liver concentrations among black and turkey vultures, we used a *t*-test. Statistical comparisons were performed without the femur outlier (540 mg/kg). We used a similar test to compare our lead isotope data with those from California condors (Finkelstein et al., 2012). Where appropriate, we log-transformed data to meet assumptions of the statistical tests. We correlated lead levels in femur and in liver with a Pearson correlation coefficient on the 105 birds from which both femur and liver samples were collected.

3. Results and discussion

All 108 vultures had bone lead levels indicative of long-term lead exposure. Femur lead levels averaged 36.99 ± 55.21 mg/kg (±SD; range: 4.5–540; n = 98 black vultures) and 23.02 ± 18.77 mg/kg (±SD; range: 6.17–70; n = 10 turkey vultures). There was no difference between the femur lead concentrations of the two vulture species ($t_{10.5} = 1.51$, $p = 0.16$; Fig. 1a). The range of bone lead levels we found here were, with the exception of our one outlier, broadly similar to those reported for red kites (*Milvus milvus*; 0–187 μg/g; n = 86; Pain et al., 2007) and Spanish imperial eagles (*Aquila adalberti*; 0–155 μg/g; n = 34; Pain et al., 2005). However, our measurements were higher than those of Egyptian vultures (*Neophron percnopterus*; 0–30 μg/g; n = 39; Gangoso et al., 2009) and California condors (4.13–14.6 μg/g; n = 2; Finkelstein et al., 2010), the second of which are regularly chelated to remove lead from their body.

Only a few vulture samples had liver lead levels consistent with recent exposure or leaching from bone. Liver lead levels of black vultures averaged 0.78 mg/kg ± 0.93 (±SD; range: 0.12–6.17 mg/kg; n = 96) and 0.55 ± 0.34 mg/kg (±SD; range: 0.23–1.3 mg/kg; n = 9) in turkey vultures. There was no difference between the liver lead concentrations of the two species ($t_{10.5} = 0.82$, $p = 0.43$; Fig. 1b). Finally, lead levels in femur and liver were not correlated (r = 0.09, without outlier; Fig. 2).

Average isotope ratios of $^{207}Pb/^{206}Pb$ in femur samples were 0.8272 ± 0.0121 (range: 0.8055–0.8813; n = 98) for black vultures and 0.8268 ± 0.0123 (range: 0.8121–0.8513; n = 10; Fig. 3) for turkey vultures. Isotope ratios were similar for femur samples with the highest (540 mg/kg; $^{207}Pb/^{206}Pb$ 0.8557) and lowest (4.5 mg/kg; $^{207}Pb/^{206}Pb$ = 0.8341) lead concentrations. Isotope ratios of lead from black vultures and turkey vultures were statistically different from those of pre-release California condors (condors: $\bar{x} = 0.8362 \pm 0.0056$ (±SD); range: 0.8296–0.8483; n = 22; BLVU: $t_{98,22} < 0.0001$; TUVU: $t_{10,22} = 0.0409$; Finkelstein et al., 2012). Isotope signatures were similar to post-release (lead-ammunition exposed) California condors ($\bar{x} = 0.8284 \pm 0.0230$; range: 0.7602–0.9164; n = 110; BLVU: $t_{98,110} = 0.6086$; TUVU: $t_{10,110} = 0.7217$; Finkelstein et al., 2012). Lead isotope ratios for black vultures overlapped with the upper range of published lead isotope ratios from ammunition from several different manufacturers sold in California ($\bar{x} = 0.8179 \pm 0.0115$; n = 76 bullets, range: 0.7858–0.8706; Finkelstein et al., 2012). Isotope ratios also overlapped to varying degrees with those reported for leaded gasoline in eastern North America ($\bar{x} = 0.845 \pm 0.103$; range: 0.719–0.962; year = 1987; Komárek et al., 2008), coal emissions ($\bar{x} = 0.833 \pm 0.023$; range: 0.799–0.888; Komárek et al., 2008), and zinc smelting (Pennsylvania; $\bar{x} = 0.824 \pm 0.055$; range: 0.817–0.829; Komárek et al., 2008). Lead isotope ratios from lead smelting (Missouri; $\bar{x} = 0.752 \pm 0.014$; range: 0.746–0.763; Komárek et al., 2008) and deteriorating lead-based paint from a fire tower in California ($\bar{x}$ =

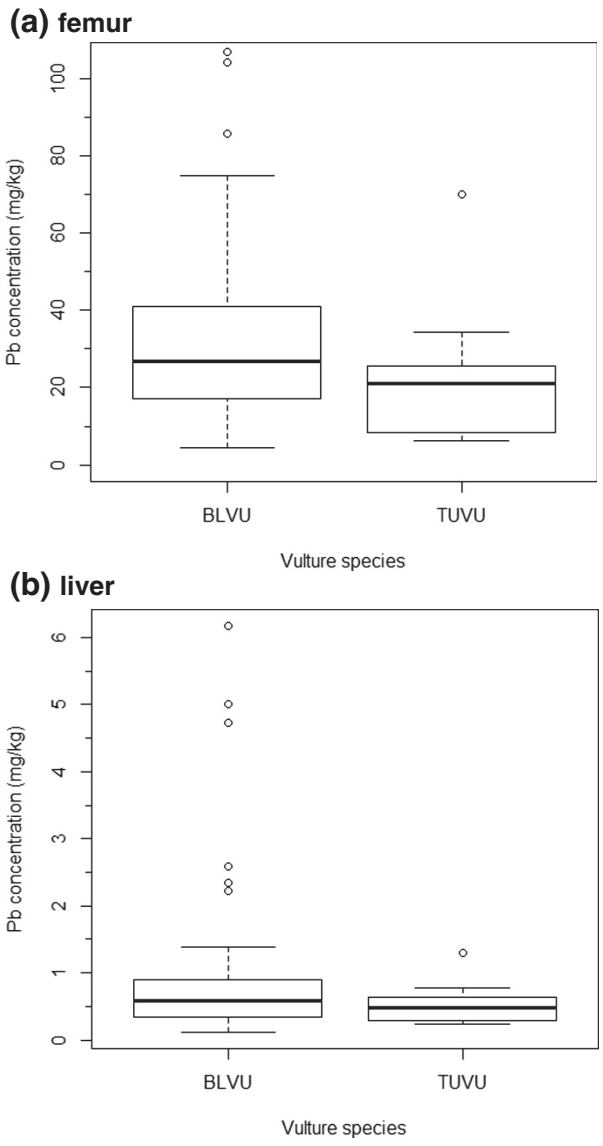

Fig. 1. Anthropogenic lead concentrations (mg/kg) in samples from (a) femurs of black (BLVU; n = 97, outlier at 540 mg/kg excluded) and turkey (TUVU; n = 10) vultures, and (b) livers of black (n = 96) and turkey (n = 9) vultures.

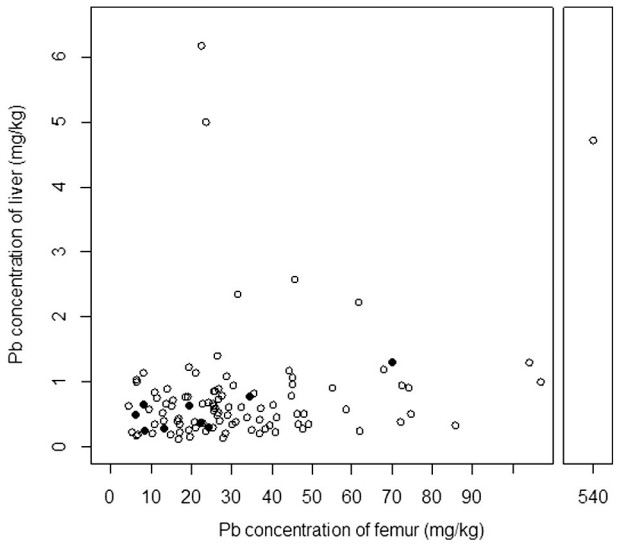

Fig. 2. Relationship between anthropogenic lead levels of liver and femur in 105 vultures. Turkey vultures (n = 9) are indicated by closed circles, while black vultures (n = 96) are indicated by open circles.

0.8925 ± 0.0161; range: 0.8734–0.9148; Finkelstein et al., 2012) were largely dissimilar to those found in eastern North American vultures.

The extent to which chronic lead exposure is epidemic in obligate scavengers in eastern North America provides important ecological insights. First, because sources of anthropogenic lead in the environment have changed dramatically with reduced use of lead in gasoline and paint (Komárek et al., 2008), the pervasiveness and chronic nature of anthropogenic lead exposure in vulture populations therefore is surprising. Furthermore, this trend would not have been evident with a sampling strategy based on standard blood or liver sampling. The fact that bone and liver lead levels were not correlated corroborates past evidence (Pain et al., 2007) that soft tissue and fluid sampling is of limited effectiveness in understanding long-term lead exposure of birds. Other studies of lead in birds have included feather sample analysis (Finkelstein et al., 2012; Harmata and Restani, 2013). Although a better measure than blood and liver of long-term exposure, even the largest birds grow their feathers over the course of <10 weeks, and thus lead levels in feathers are indicative only of a relatively short period

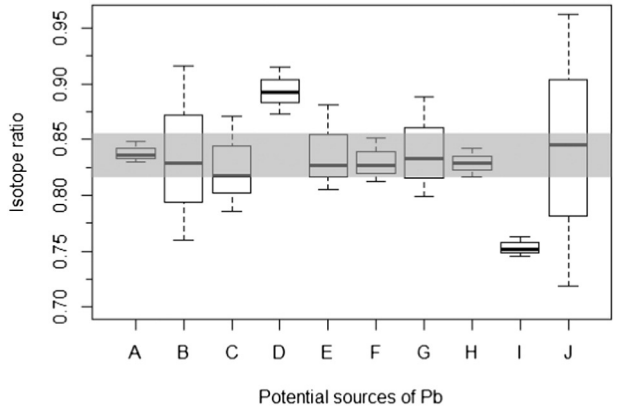

Fig. 3. Isotope ratios ($^{207}Pb/^{206}Pb$) of lead from vultures (this study), from California condors (Finkelstein et al., 2012), and from potential anthropogenic sources (Komárek et al., 2008). Ratios are from: (A) background lead in pre-release California condors, (B) post-release California condors, (C) lead ammunition, (D) lead-based paint from a fire tower in CA, (E) black vultures in this study, (F) turkey vultures in this study, (G) coal emissions, (H) zinc smelting, (I) lead smelting, and (J) leaded gasoline. The gray box shows the area of overlap between the two quantiles of the range of lead isotopes of black vultures and of potential lead sources.

of the long lifespan (>20 years) of these birds. Considering the kinetics of lead in an animal's body, measuring concentration in bone is likely the most effective manner to sample the long-term burden animals face from repeated lead exposure (Ambrose et al., 2000).

Second, our analysis provides key information on the potential sources of environmental lead that vultures encounter. The lead isotope ratios we measured are largely inconsistent with exposure to lead-based paint used at a fire tower in California (only one of 98 black vulture samples and zero of 10 turkey vulture samples fall in the range of paint chips that impacted condors in one study; Finkelstein et al., 2012) or with exposure from lead smelting operations. However, isotope ratios were nearly identical to those of post-release condors and therefore consistent with lead used in ammunition and emitted from coal-fired power plants and zinc smelting operations. In fact, coal and zinc are produced and heavily used, and recreational shooting and hunting are common in eastern North America. Future work with these and related species should consider not only the typical potential sources of lead (ammunition, paint), but also potential sources associated with their economically damaging behavior (vultures damage and ingest vehicle rubber, household waste, roofing material; Lowney, 1999).

Third, the thorough extent of exposure in these environmental sentinels suggests that anthropogenic lead permeates natural eastern North American ecosystems in a previously unrecognized manner. Because lead exposure is completely pervasive in this population, because it appears to come from multiple sources, and because it is ubiquitous in birds that range across eastern North America, it is likely that other species occupying the same space, including humans, also are exposed to this toxin, lethally and sublethally.

In the western U.S., lead poisoning is the exclusive factor holding back the recovery of the critically endangered California condor (Finkelstein et al., 2012). Although black and turkey vultures are more robust to lead poisoning than are condors (Carpenter et al., 2003) and their populations are not declining, these species play an important role as environmental sentinels in eastern North American ecosystems. Our analysis therefore suggests two important consequences for understanding human and wildlife health concerns. First, although human exposure to lead is still monitored (Cave et al., 2010; Amato et al., 2013), eastern U.S. humans at high risk (those consuming hunter-killed game or living or working near coal-fired power plants) may acquire lead with similar frequency as do these vultures. Second, the comparison between lead in liver and bone suggests that blood, feather, or soft-tissue measurements typical for use in avian toxicology studies (i.e. liver, blood; Locke and Thomas, 1996; Snyder and Snyder, 2000; Carpenter et al., 2003; Fry and Maurer, 2003; Church et al., 2006; Finkelstein et al., 2012; Harmata and Restani, 2013), may be reliable indicators of only relatively short-term lead exposure. Therefore, studies that rely solely on liver, feather, or blood samples will underestimate the true pervasiveness of lead within organisms and modern ecosystems.

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Author contributions:

SB and TK designed the study. SB, TK, JF, and AD collected and statistically analyzed data. AL and JB performed laboratory analyses. All authors wrote the manuscript.

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