Lead and Mercury in Fall Migrant Golden Eagles from Western North America

Heiko W. Langner [1,*]

Email heiko.langner@kaust.edu.sa

Robert Domenech [2]

Vincent A. Slabe [2]

Sean P. Sullivan [3]

[1] King Abdullah University of Science and Technology, Thuwal, 23955-6900 Saudi Arabia

[2] Raptor View Research Institute, Missoula, MT, 59801 USA

[3] Rhithron Associates Inc., Missoula, MT, 59801 USA

Abstract

Lead exposure from ingestion of bullet fragments is a serious environmental hazard to eagles. We determined blood lead levels (BLL) in 178 golden eagles (*Aquila chrysaetos*) captured during fall migration along a major North American flyway. These eagles spent the breeding season distributed over a large range and are the best currently available representation of free flying golden eagles on the continent. We found 58 % of these eagles containing increased BLL > 0.1 mg/L; 10 % were clinically lead poisoned with BLL > 0.6 mg/L; and 4 % were lethally exposed with BLL > 1.2 mg/L. No statistical difference in BLL existed between golden and bald eagles (*Haliaeetus leucocephalu*s). Golden eagles captured on carrion had higher BLL than those captured using live bait suggesting differences in feeding habits among individuals. Median BLL increased with age class. We propose a conceptual model for the long-term

increase in BLL after ingestion of lead particles. The mean blood mercury level in golden eagles was 0.023 mg/L. We evaluate a field test for BLL that is based on anodic stripping voltammetry. This cost-effective and immediate method correlated well with results from inductively coupled plasma–mass spectrometry, although results needed to be corrected for each calibration of the test kit.

Lead poisoning may be a common cause of disease and death among scavenging birds including golden eagles (Cruz-Martinez et al. 2012; Gangoso et al. 2009; Kramer and Redig 1997; Pattee et al. 1990; Stauber et al. 2010; Watson et al. ~~2008~~2009). Causes of lead toxicity in these birds have been subject of numerous studies that collectively point to the ingestion of lead fragments from ammunition used in hunting as the major source rather than natural sources or other anthropogenic sources of lead (Craighead and Bedrosian 2008; Cruz-Martinez et al. 2012; Harmata and Restani 1995; Hunt et al. 2009; Kramer and Redig 1997; Rogers et al. 2012 ~~2011~~; Stauber et al. 2010). Between 25 and 60 % of golden and bald eagles that were admitted to rehabilitation centers in the United States had toxic lead levels depending on the location, season, and age of the birds tested (Cruz-Martinez et al. 2012; Kramer and Redig 1997; Stauber et al. 2010). Recent studies show a strong conformity in timing between increased lead levels in eagles and big-game hunting seasons (Bedrosian et al. 2012; Cruz-Martinez et al. 2012; Stauber et al. 2010). Moreover, Bedrosian et al. (2012) found a positive correlation between blood lead levels (BLL) of wild-caught bald eagles and the fraction of hunters using lead-containing ammunition near Jackson, Wyoming, USA. Harmata and Restani (1995) sampled spring migrant eagles in areas with extensive ground-squirrel hunting and found only 38 % of golden eagles and 5 % of bald eagles had lower than the presumed background BLL of 0.2 mg/L. These studies, along with physical evidence of often hundreds of lead fragments remaining in the field in game carcasses and offal (gut piles) (Hunt et al. 2006; Minnesota Department of Natural Resources 2008), provide strong arguments that lead poisoning among North American eagles is to a large extent caused by fragmenting lead bullets used for hunting. However, the population-wide extent of lead exposure is difficult to assess from these data because birds submitted to

rehabilitation centers are usually compromised and therefore may not be representative of the overall population. Similarly, sampling of wild-caught eagles in locations of extensive big-game and squirrel hunting may be biased toward birds that reflect temporary local conditions. Results from Bedrosian et al. (2012) suggest that the hunting activity itself may attract individuals that specialize in feeding on carrion and thus are not representative of the overall population. Sampling eagles during migration and away from hunting hot spots may provide more representative estimates of the population-wide extent of lead exposure in golden and bald eagles.

AQ1

AQ2

AQ3

Published information on mercury levels in golden eagles is sparse because concentrations in golden eagles are usually much lower and raise less concern than concentrations measured in bald eagles given that species' tendency to feed in mercury-rich aquatic food webs. Blood and feather samples of free-flying golden eagles in southwestern Montana were analyzed for total mercury by Harmata and Restani (2013), but the majority of blood samples were lower than their detection limit, and concentrations in feathers were mostly <1 mg/kg. Kochert et al. (2002) reported increased mercury concentrations in golden eagle nestlings that apparently ate contaminated ring-necked pheasants (*Phasanius colchicus*). Golden eagle eggs collected over several decades in Scotland contained higher mercury levels if the nests were located near the coast than if they were located farther inland (Walker et al. 2010) suggesting that coastal birds are more likely to feed in aquatic food webs. Eggs sampled in Canada and Idaho (Kochert et al. 2002) did not contain mercury levels of concern. However, atmospheric deposition rates of mercury in northwestern North America are expected to increase over the coming decades due to increasing emissions in Asia (Corbitt et al. 2011). Blood samples from predators, such as golden eagles, may provide valuable baseline data for the assessment and prediction of changes in mercury loads to the mountainous environments across the northern continent.

Blood lead levels have traditionally been determined in the laboratory using spectrophotometric (graphite furnace atomic absorption spectrometry, inductively coupled plasma (ICP)–optical emission spectroscopy), or mass

spectrometric (ICP–mass spectrometry) methods. Recently, a cost-efficient field method using anodic stripping voltammetry has emerged (Magellan Diagnostics 2012), which can be performed on site, sometimes within minutes after a bird was captured (Bedrosian et al. 2012; Craighead and Bedrosian 2008; Cruz-Martinez et al. 2012; Rogers et al.2012 ~~2011~~). Because the field method was specifically developed and calibrated for human blood tests performed by specifically trained personnel, its accuracy and precision for avian applications must be tested and adjusted, which will make results more comparable with traditional laboratory methods.

One objective of this study was to document the degree of lead exposure in fall migrant golden eagles along the Rocky Mountain Front and to compare different demographic groups to draw conclusions regarding the origin of the lead. Another objective was to determine total mercury levels in the blood of these migrant eagles. Because they spent the summer season widely distributed along northern latitudes of the North American continent, fall migrant golden eagles should be suitable indicators for mercury exposure from global atmospheric sources. Our final objective was to test the validity of and provide guidance for the adoption of a field test for BLL based on anodic stripping voltammetry compared with an established laboratory method.

Materials and Methods

Capture and Sampling

The Rocky Mountain Front Migratory Flyway extends from Alaska to Mexico and holds the world's largest known concentration of migrant golden eagles (Tilly 2008). These birds spend summers in northern North America and winters in southern North America. We chose capture locations along the flyway in the Helena National Forest in Montana where orographic features have a funneling effect, such that several hundred golden eagles can be observed on major migration days in the fall (Nora Ridge, 47.02°N, 112.40°W; Grassy Mountain, 46.31°N, 111.11°W).

Between 2006 and 2012, we captured golden eagles using traditional ridgeline trapping techniques using bow-nets with rock pigeons (*Columbia*

livia) as lures (Bloom 1987). In addition, eagles were captured at the base of Nora Ridge using carcasses of road-killed deer and mini-net launchers (Trapping Innovations LLC, Jackson Hole, Wyoming, USA). Each successfully trapped bird was banded with a United States Geological Survey (USGS) band. Small blood samples (4 mL) were drawn from the brachial vein using sterile syringes with 25-gauge needles. Whole blood samples were split in the field between one or two 1.5-mL microcentrifuge tubes and 2-mL glass blood collection tubes ("lavender top" with ethylene diamine tetra acetic acid [EDTA](K_3) anticoagulant). Blood samples were immediately cooled on ice, and the microcentrifuge tubes were frozen at −18 °C on the day of sampling until analysis. These activities were covered by the following permits: USGS Federal Banding and Sampling Permit 23353; Montana Fish, Wildlife, and Parks Wild Bird Banding and Possession Permits 573 and 029; and University of Montana–Missoula Institutional Animal Care and Use Committee Animal Use Protocol No. 013-07EG-DBS-060807.

Chemical Analyses

Laboratory analyses were performed by the University of Montana, Department of Geosciences Environmental Biogeochemistry Laboratory, as described elsewhere (Langner et al. 2012). Briefly, samples were digested with concentrated HNO_3 and H_2O_2 at increased temperature and analyzed for lead by ICP–MS and for mercury by cold vapor atomic fluorescence spectrometry. Method quantitation limits were 0.5 and 0.2 μg/L for lead and mercury in blood, respectively; thus all measured concentrations were well within the quantifiable range. Measured concentrations for standard reference samples (Bio-Rad Lypochek Whole Blood Metals Controls; levels 1, 2, 3; $N = 6$) were within the ranges listed by the manufacturer (Langner et al. 2012).

Analysis of BLL was also performed by way of anodic stripping voltammetry using an ESA LeadCare Blood Lead Analyzer System (LCS; ESA Biosciences Inc., Chelmsford, Massachusetts, USA Magellan Diagnostics 2012). Although the LCS is portable and designed for field use, we generally analyzed blood samples once daily after return from the trapping stations. This modification was optional according to the manufacturer and was preferred due to the highly variable and often adverse conditions at the

ridgetop sampling stations. Subsamples (50 μL) were pipetted from the blood collection tubes (with EDTA(K_3) anticoagulant) and transferred into LCS treatment reagent tubes before the treated blood was dripped onto an LCS sensor and inserted into the LCS instrument. A BLL read-out was available within 3 min. Measured concentrations for standard reference samples (Bio-Rad Lypochek Whole Blood Metals Controls; levels 1, 2, 3; $N = 3$) were within the ranges listed by the manufacturer.

Results and Discussion

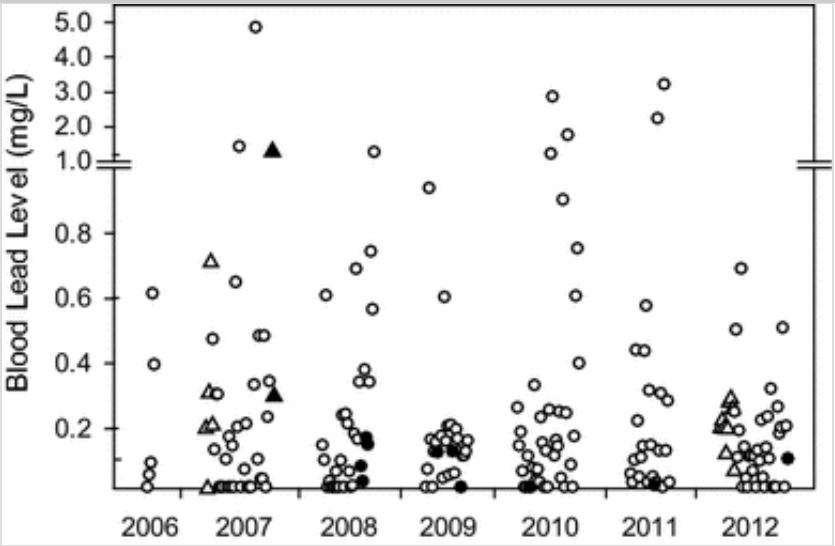
Lead Levels in Eagles

We measured BLL in 192 golden eagles and 12 bald eagles between 2006 and 2012 (Fig. 1). For 17 eagles only ICP–MS results were obtained, and for 27 eagles only LCS results were obtained, including 11 eagles in 2007, 5 in 2008, 2 in 2010, and 9 in 2012. For 160 eagles, both ICP–MS and LCS analyses were performed. Statistical analyses for this study were based on BLL obtained from ICP–MS analysis, if available, unless stated otherwise. For the 16 eagles for which only LCS results were available, we calculated corrected BLL using conversion factors for the specific year as described later in the text. Altogether, 133 golden and 2 bald eagles were captured using the bow-net ridgetop technique. A total of 59 golden and 10 bald eagles were trapped on carcasses. The large majority of blood samples (178 golden and 10 bald eagles) was collected during fall migration, which we arbitrarily defined to last from September 18 to October 25 of each year, thus bracketing the time period when migrating eagles were captured on the Nora Ridge site. Outside of this time period, a small number of birds was captured with carcass traps, including five golden eagles in March 2007, two bald eagles in November and December 2007, and nine golden eagles between December and March 2012. BLL results from these different groups of eagles were pooled only for the comparison of lead analytical methods, whereas we focus other discussion on fall migrant golden eagles only ($N = 178$) using data from other seasons and bald eagles for reference where explicitly stated.

Fig. 1

BLL of golden eagles (*open symbols*) and bald eagles (*filled symbols*). *Circles* and *triangles* represent samples obtained during and outside of fall migration,

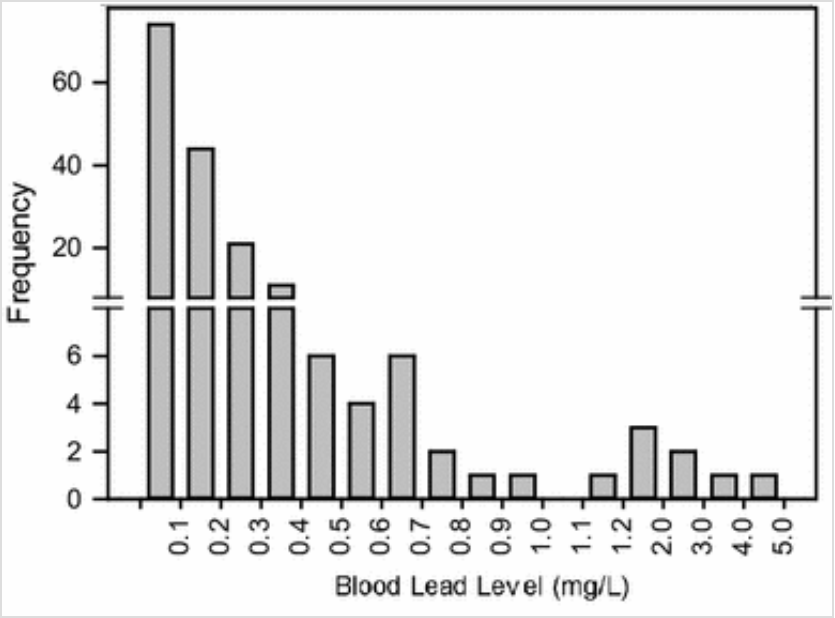
respectively. *Dashed lines* Dashed Lines are missing in Fig. 1. They are present as desired in the submitted version. They are present but TOO THIN in the pdf version that you sent to me on 13 Feb. designate background threshold (0.1 mg/L), Kramer and Redig's (1997) historic detection level (0.2 mg/L), and transitions from subclinical to clinical levels (0.6 mg/L) and to acute levels invariably associated with death (1.2 mg/L). Note the change of scale on the y-axis at BLL = 1 mg/L



Blood lead levels ranged from lower than detection (<0.03 mg/L) to 4.8 mg/L (Fig. 1) with 74 fall migrant golden eagles having BLL up to 0.1 mg/L, 44 from >0.1 to 0.2 mg/L, 42 from >0.2 to 0.6 mg/L, and 11 from >0.6 to 1.2 mg/L, whereas seven of these birds had BLL > 1.2 mg/L (Fig. 2).

Fig. 2

Frequency of BLL ranges measured in fall migrant golden eagles (*N* = 178). Bin size increases above BLL = 1.2 mg/L



Comparison of Methods for Lead Analysis

LCS is an economical method of testing BLL optimized for the analysis of human blood samples, although it is increasingly being used for wildlife (Craighead and Bedrosian 2008; Cruz-Martinez et al. 2012; Rogers et al. 2011). The near-immediate results of the LCS may potentially provide real-time information regarding the need for immediate action in the field. However, limits of LCS for testing BLL in wildlife are its limited reportable range of 0.033–0.65 mg/L in whole blood (Magellan Diagnostics 2012) and the tendency to underestimate BLL in birds compared with other methods (Bedrosian et al. 2009; Craighead and Bedrosian 2008).This may be compounded by bias due to variable instrument calibration (Craighead and Bedrosian 2008). To evaluate the results of LCS compared with those from ICP–MS analysis, we fitted a simple linear regression model to concentrations obtained with each method: [ICP–MS] = a[LCS] + b, where brackets represent concentrations in mg/L, and a and b are model estimated parameters. Concentrations outside the reporting limits of the LCS (<0.033 and ≥0.65 mg/L) were eliminated resulting in N = 108 usable data pairs. Fitting of data including all years yielded a conversion factor a = 1.27 (SE = 0.04, r^2 = 0.88, P < 0.0001), with insignificant b, to convert LCS to ICP–MS (Table 1; Fig. 3). Inspection of the residuals associated with this model suggests that all assumptions for a simple linear regression model are not fully met. Specifically, model results exhibit slight heteroscedasticity and slight deviations from normality in the residuals. Common methods of

variable transformation, including log and square root transformations, resulted in little to no increase in meeting the assumptions. Despite these violations, which are likely the result of large deviations of LCS measurements near its upper detection limit (Fig. 3), fitting the model using simple linear regression substantially improves the accuracy of the LCS readings.

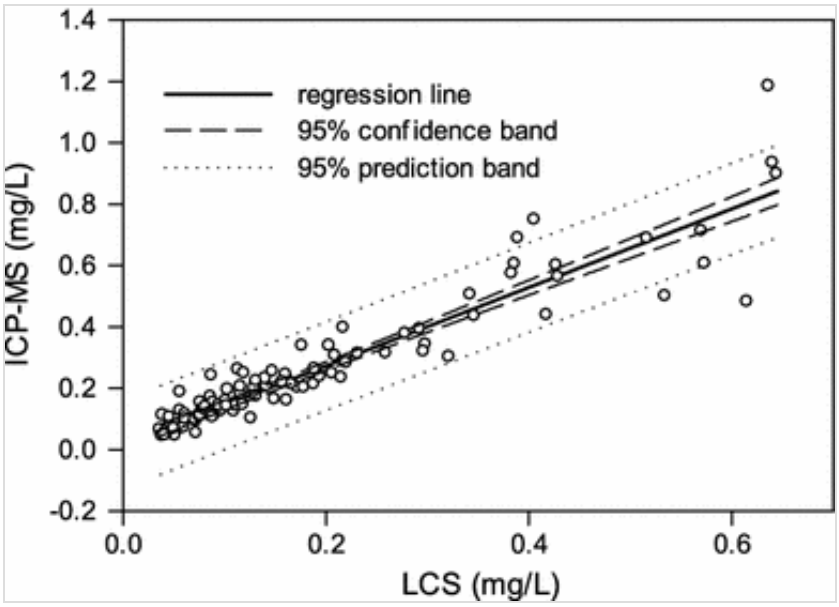
Table 1

Results of regressions between LCS and ICP–MS based BLL for individual years

Year (*N*)	***A***			***B***			**Adjusted r^2**
	Estimate	**SE**	***P***	**Estimate**	**SE**	***P***	
2006 (3)	1.34	0.03	0.02	0.00	0.01	0.758	0.9985
2007 (10)	0.93	0.15	<0.0001	0.04	0.05	0.456	0.8156
2008 (16)	1.13	0.08	<0.0001	0.04	0.02	0.0598	0.9216
2009 (19)	1.45	0.08	<0.0001	0.00	0.01	0.735	0.9834
2010 (21)	1.60	0.08	<0.0001	0.02	0.02	0.448	0.9539
2011 (15)	1.24	0.07	<0.0001	0.01	0.01	0.654	0.9454
2012 (24)	1.16	0.10	<0.0001	0.02	0.02	0.19	0.844
All years (108)	1.27	0.04	<0.0001	0.01	0.01	0.11	0.8814

Fig. 3

Regression between BLL generated by ICP–MS laboratory analysis and the LCS field method



We tested whether differences existed between individual years that would suggest differences between calibrations of the LCS because only one calibration was performed each year at the beginning of the fall migration season. Single-factor ANCOVA showed that the covariate of year has a significant effect on the fit of the regression model ($P < 0.001$; Table 1). Based on these data, we conclude that the LCS can produce quantitative results for BLL in eagles; however, for each calibration of the LCS, a subset of samples should be analyzed by a laboratory method to allow for adjustment of the LCS readings through simple linear regression. If no laboratory analysis is possible, the conversion factor $a = 1.27$ may be used to remove some of the bias of the LCS.

Levels of Lead Exposure

Among the 178 fall migrant golden eagles, 60 (34 %) exceeded a BLL of 0.2 mg/L, and 104 (58 %) exceeded 0.1 mg/L (Fig. 2). This is remarkably similar to the fraction reported recently by Harmata and Restani (2013) for free-flying golden eagles in southwestern Montana (35 % > 0.2 mg/L). Previous studies had reported A higher incidence of increased BLLS in hot spots of lead exposure. Harmata and Restani (1995) found BLLS > 0.2 mg/L in 65 % of vernal migrant eagles in an area of intensive ground-squirrel hunting. In a study area near Jackson, Wyoming (USA), 93 % of captured bald eagles had BLL > 0.1 mg/L during the big-game hunting season (Bedrosian et al. 2012). Higher fractions of eagles with BLL > 0.2 mg/L

were reported by Stauber et al. (2010) from the Inland Pacific Northwest (55 % for golden and bald eagles combined). Those studies were designed to determine lead exposure levels in eagles under conditions of increased lead supply and would be expected to yield above-average levels compared with the continental average. Studies performed among free-flying eagles away from known sources of lead that included migrants from multiple breeding areas, including Harmata and Restani (2013) and this study, recovered very similar rates of lead-exposed eagles. We believe these are the closest currently available estimates of exposure levels to lead within North American eagle populations.

We captured 18 golden eagles, or 10 %, that were clinically lead poisoned according to Kramer and Redig (1997) with BLL > 0.6 mg/L including seven eagles (4 %) with BLL > 1.2 mg/L, which was categorized as lethally exposed according to the same investigators. These percentages may be biased because highly exposed birds may be compromised and be more or less prone to being captured. Nevertheless, our results support the argument that human-caused lead toxicity is likely a common occurrence among these iconic predators even in areas of ordinary hunting pressure. Interestingly, all of the lethally exposed eagles were captured during the second half of fall migration (after October 10) when the supply of hunter-killed game carcasses was presumably increasing due to the start of legal big-game hunting in several North American regions.

The “True” Background BLL

Despite being frequently quoted as a background limit in bald and golden eagles, a threshold of 0.2 mg/L was chosen by Kramer and Redig (1997) based on their instrumental method detection level and was founded in neither environmental nor toxicological considerations. Anecdotal evidence suggests a substantially lower background BLL for eagles: For example, Bedrosian and Craighead (2009) tested nine wild bald and golden eagle nestlings that had not lived through a big-game hunting season. They all had BLL ≤ 0.008 mg/L. We sampled two golden eagles that had been living in captivity for 18 and 23 years [Raptors of the Rockies, Florence, Montana (courtesy of Kate Davis)]. Their BLL were 0.05 and 0.06 mg/L, respectively. According to these data and based on numerous studies involving other

raptors (e.g., California condors (Cade 2007) and humans (see Centers for Disease Control 2012), the BLL of 0.1 mg/L as suggested by Pokras and Kneeland (2009) and by Neumann (2009) appears to be a more realistic background threshold for free-flying eagles. BLL between 0.1 and 0.2 mg/L are likely the result of lead exposure in the past with a possible mechanism for arriving at this level offered in the next section. If we assume a BLL of 0.1 mg/L as the background level for our data set, the number of eagles with increased lead levels increases to 104 eagles or 58 % of fall migrant golden eagles (Fig. 2).

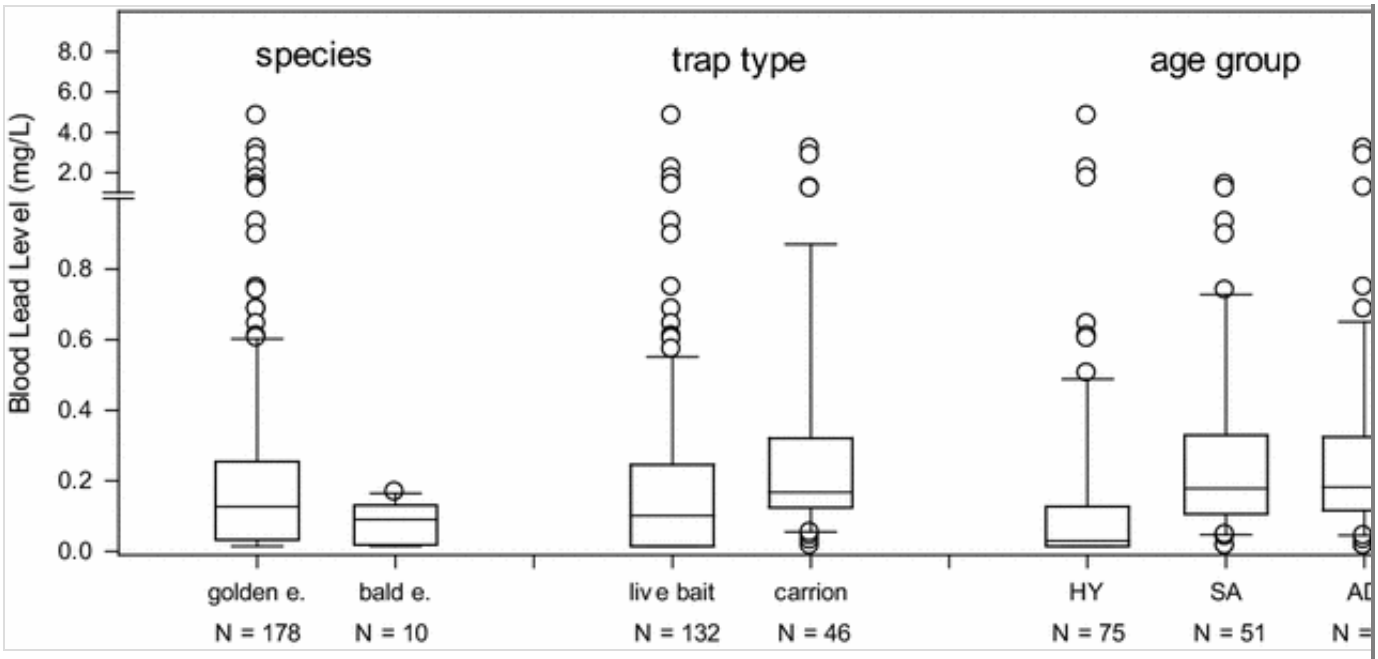
AQ4

Effect of Species, Trap Type, and Age on Blood Lead Levels

Due to lack of normality in the distribution of BLL, we used nonparametric analysis of variance [ANOVA; Kruskal–Wallis test (Kruskal and Wallis 1952; R Development Core Team 2012)] to evaluate differences between subpopulations. No statistical difference was found in BLL between golden eagles and bald eagles that were captured during fall migration ($P = 0.133$; Fig. 4). The similarity in lead exposure implies that both eagle species would be similarly affected by increases or decreases in the use of lead-containing ammunition by the hunting community.

Fig. 4

BLL for eagles captured during fall migration by species and, for golden eagles only, by trap type and age group. *Boxplots* show median, first, and third quartiles (*box*) and the 10th and 90th percentiles (*whiskersTechniques for the collection*), and data above and below these ranges are indicated by *circles*



AQ5

A significant statistical difference in BLL among fall migrant golden eagles was found as a function of trap type ($P < 0.001$; Fig. 4). This finding suggests that golden eagles captured on carrion tend to specialize in this type of feeding and therefore are more prone to ingesting lead fragments that cause lead toxicity than individuals that avoid carrion. This finding further supports the presumption that the use of lead-free ammunition could decrease the occurrence of lead toxicity in eagles.

No statistical difference in BLL was shown in our data set between age classes of fall migrant golden eagles (Tukey's honest significant difference test $P > 0.05$). However, we did observe a striking difference in the distribution of BLL with hatch-year birds having much lower first, second, and third quartiles than subadults (2–4 years old) and adults [≥5 years old (Fig. 4)]. For example, the median BLL for hatch-year birds is 0.043, whereas it is 0.16 and 0.18 mg/L for subadults and adults, respectively. This implies that despite multiple individuals having extremely high BLL, a relatively large percentage of hatch-year eagles have background or low lead exposure. A similar relationship between age and level of lead exposure was reported by Cruz-Martinez et al. (2012) suggesting that BLL is a function of cumulative lead exposure over longer time periods. These observations cannot be explained simply by assuming an exponential decrease of BLL after a rapid increase due to lead ingestion, which is implied by reporting

half-lives (Bedrosian et al. 2012; Finkelstein et al. 2012; Harmata and Restani 2013). Instead, lead-exposure events appear to cause long-term increases in baseline BLL that are superimposed over any short-term changes. This hysteresis effect is likely due to the chemical sequestration of lead into bone minerals followed by its slow release into the bloodstream (Rabinowitz et al. 1976). Thermodynamically controlled release of lead, combined with the ongoing dissolution and rebuilding of bone tissue in live birds, may provide a long-term lead source to the bloodstream in all eagles that were exposed to lead fragments in food at some point throughout their lives. Half-lives of lead atoms in blood were estimated for humans by Rabinowitz et al. (1976) to be 36 ± 5 days, which may be similar for raptors. However, this does not imply a halving of BLL within approximately 36 days as previously interpreted in the raptor literature (Bedrosian et al. 2012; Harmata and Restani 2013) because this would ignore the rate of the reverse process, namely, lead release from bones. The combined sequestering and release of lead from bone tissue may cause changes of BLL in any direction, even increases in BLL, whereas the half-life concept of Rabinowitz et al. (1976) still holds.

AQ6

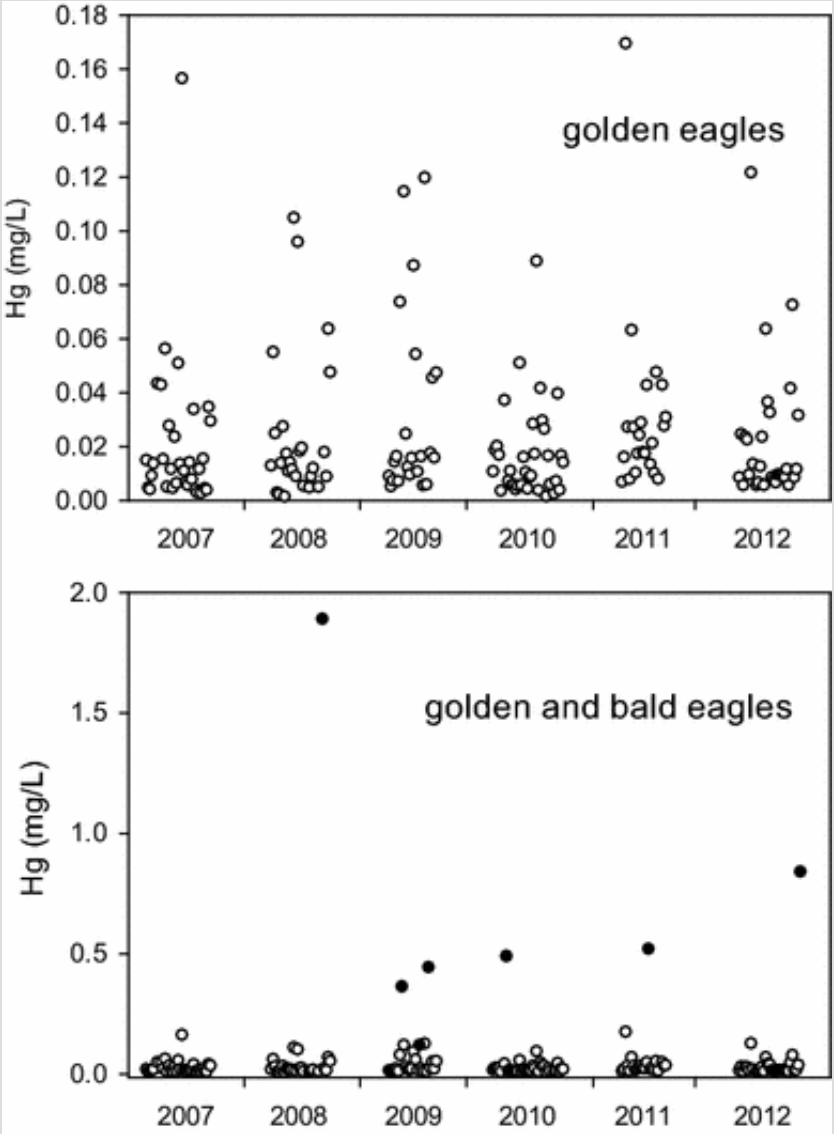
Mercury in Eagles

Analysis of total mercury in eagle blood was initiated in 2007 and was performed only on samples collected during fall migration. A total of 170 golden and 7 bald eagle samples was analyzed, all of which exceeded our method quantitation limit of 0.0002 mg/L in whole blood. Concentrations differed widely between golden and bald eagles (Fig. 5) with mean concentrations of 0.023 mg/L (SD = 0.028) in golden eagles and 0.66 mg/L (SD = 0.58) in bald eagles. The mean mercury concentration in blood was approximately 29 times greater in bald eagles compared with golden eagles, thus highlighting the fundamental differences in mercury load between aquatic and terrestrial food webs and the diverging feeding preferences of these two species. This was compared with the insignificant difference in blood lead levels (BLL) providing further evidence that lead exposure occurs while scavenging, an activity in which both species engage. Blood mercury levels in bald eagles bracketed mean concentrations reported by Harmata (2011) for bald eagles in southwestern Montana. Five of the seven bald

eagles tested exceeded 0.4 mg/L, which was adopted by several investigators as the background level for piscivorous birds (Burgess et al. 2005; Eisler 1987; Harmata 2011). Blood mercury levels among golden eagles were generally higher in this study than those reported by Harmata and Restani (2013).Although they found only 21 % of golden eagles exceeding their detection limit of 0.005 mg/L total mercury in blood [Table 1 in Harmata and Restani (2013)], we determined that 142, or 84 %, of our golden eagle blood samples exceeded this concentration. Mercury analysis in tissues at the low levels found in terrestrial species may require special precautions to avoid artificial loss of mercury by diffusion into plastic vial walls during storage (Lewis and Brigham 2004; Olson and DeWild 1999). We froze unpreserved blood samples until analysis, or we used glass blood vials for storage (United States Environmetnal Protection Agency 2001).

Fig. 5

Total mercury concentrations in whole blood of golden (*open symbols*) and bald eagles (*filled symbols*) captured during fall migration. Golden eagle data are shown in the lower panel to illustrate the difference in scale



AQ7

AQ8

The primary source for mercury in most golden eagle habitats is aerial deposition of regionally or globally circulating atmospheric mercury. This source is expected to increase in the western United States over the coming decades due to an increase in coal burning, especially in eastern Asia. This is due to its position directly upstream of common atmospheric currents (Corbitt et al. 2011). It is important to document and predict future changes to create meaningful models for the environmental effect of these changes.

Conclusions

Our study involving 192 free-flying golden eagles in North America suggests that more than half of these eagles may be affected by increased BLL. This is

likely due to their opportunistic feeding habits targeting large-game carcasses and offal left in the field by human hunters. Lead particles from fragmenting ammunition have been shown to be common in those remains; and single incidents of lead ingestion by eagles may have life-long effects on their BLL.

We found that 10 % of the captured golden eagles had clinically high BLL suggesting recent ingestion of lead fragments. Although this percentage may be somewhat biased, it emphasizes that human-caused lead toxicity is likely a common occurrence among these iconic predators and deserves close attention by the conservation community.

We detected a significant difference of BLL as a function of trap type wherein birds captured on carcasses had higher lead levels than those captured with live bait. This suggests that golden eagles captured on carrion tend to specialize in this type of feeding and therefore are more prone to ingesting lead fragments that cause lead toxicity than individuals that avoid carrion.

Although the mean mercury level in whole blood of golden eagles was 29-fold lower than the level determined in bald eagles, careful measurement and documentation of mercury concentrations will be useful to track and predict future changes in environmental mercury levels.

Results from an anode stripping voltammetry-based field method for quantification of BLL correlated well with a traditional laboratory method. However, we found that an experimentally determined correction factor should be applied to adjust results of the field method that was designed specifically for humans. Furthermore, the confidence in values determined with the field method decreases near its stated upper detection limit.

Acknowledgments

We thank the many people who helped in the field, notably A. Shreading, B. Bedrosian, T. Pitz, R. Crandall, S. Wilson, S. Norton, and T. Veto. Kate Davis kindly allowed us to collect blood samples from captive eagles at Raptors of the Rockies. Logistical support was provided by Montana Fish, Wildlife and Parks, United States Forest Service and Helena National Forest

(P. Shanley). This work was funded by the Montana Bureau of Land Management, LCAO Foundation, M. J. Murdock Foundation, Maki Foundation, Mountaineers Foundation, Patagonia Foundation, Cinnabar Foundation, and private individuals. K. McKalip provided helpful comments on this manuscript. This work would not have been possible without support from the University of Montana Geosciences Department–Environmental Biogeochemistry Laboratory where all laboratory analyses were performed. Special thanks go to M. Young for help in the laboratory. We thank the two anonymous reviewers for their constructive criticism and suggestions.

References

Bedrosian B, Craighead D (2009) Blood lead levels of bald and golden eagles sampled during and after hunting seasons in the Greater Yellowstone Ecosystem. In: Watson RT, Fuller M, Pokras M, Hunt WG (eds) Ingestion of lead from spent ammunition: implications for wildlife and humans. The Peregrine Fund, Boise, pp 219–220

Bedrosian B, Parish CN, Craighead D (2009) Differences between blood lead level detection techniques: Analysis within and among three techniques and four avian species. In: Watson RT, Fuller M, Pokras M, Hunt WG (eds) Ingestion of lead from spent ammunition: Implications for wildlife and humans. The Peregrine Fund, Boise, pp 287–288

Bedrosian B, Craighead D, Crandall R (2012) Lead exposure in bald eagles from big game hunting, the continental implications and successful mitigation efforts. PLoS ONE 7(12):e51978

Bloom PH (1987) Capturing and handling raptors. In: Pendleton BG, Millsap BA, Cline KW, Bird DM (eds) Raptor management techniques manual. National Wildlife Federation, Washington, pp 99–123

Burgess NM, Evers DC, Kaplan JD (2005) Mercury and other contaminants in common loons breeding in Atlantic Canada. Ecotoxicology 14(1–2):241–252

Cade TJ (2007) Exposure of California condors to lead from spent

ammunition. J Wildl Manag 71(7):2125–2133

Centers for Disease Control (2012) Low level lead exposure harms children: A renewed call for primary prevention. Advisory Committee on Childhood Lead Poisoning Prevention, United States Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/nceh/lead/ACCLPP/Final_Document_030712.pdf. Accessed 16 Dec 2013. In: http://www.cdc.gov/nceh/lead/ACCLPP/Final_Document_030712.pdf

Corbitt ES, Jacob DJ, Holmes CD, Streets DG, Sunderland EM (2011) Global source-receptor relationships for mercury deposition under present-day and 2050 emissions scenarios. Environ Sci Technol 45(24):10477–10484

Craighead D, Bedrosian B (2008) Blood lead levels of Common Ravens with access to big-game offal. J Wildl Manag 72(1):240–245

Cruz-Martinez L, Redig PT, Deen J (2012) Lead from spent ammunition: a source of exposure and poisoning in bald eagles. Human Wildl Interact 6(1):94–104

Eisler R (1987) Mercury hazards to fish, wildlife, and invertebrates: A synoptic review. United States Department of the Interior, Fish and Wildlife Service, Laurel

Finkelstein ME, Doak DF, George D et al (2012) Lead poisoning and the deceptive recovery of the critically endangered California condor. Proc Natl Acad Sci USA 109(28):11449–11454

Gangoso L, Alvarez-Lloret P, Rodriguez-Navarro A, Mateo R, Hiraldo F, Donazar JA (2009) Long-term effects of lead poisoning on bone mineralization in vultures exposed to ammunition sources. Environ Pollut 157(2):569–574

Harmata AR (2011) Environmental contaminants in tissues of bald eagles sampled in southwestern Montana, 2006–2008. J Raptor Res 45(2):119–

135

Harmata AR, Restani M (1995) Environmental contaminants and cholinesterase in blood of vernal migrant bald and golden eagles in Montana. Intermt J Sci 1(1):1–15

Harmata AR, Restani M (2013) Lead, mercury, selenium, and other trace elements in tissues of golden eagles from southwestern Montana, USA. J Wildl Dis 49(1):114–124

Hunt WG, Burnham WILL, Parish CN, Burnham KK, Mutch BRIA, Oaks JL (2006) Bullet fragments in deer remains: implications for lead exposure in avian scavengers. Wildl Soc Bull 34(1):167–170

Hunt WG, Parish CN, Orr K, Aguilar RF (2009) Lead poisoning and the reintroduction of the California Condor in northern Arizona. J Avian Med Surg 23(2):145–150

Kochert M, Steenhof K, McIntyre C, Craig E, Poole A, Gill F (2002) The birds of North America, No. 684. The Academy of Natural Sciences Philadelphia, Phialdelphia, PA, and the American Ornithologists' Union, Washington, DC

Kramer JL, Redig PT (1997) Sixteen years of lead poisoning in eagles, 1980–95: an epizootiologic view. J Raptor Res 31(4):327–332

Kruskal WH, Wallis WA (1952) Use of ranks in one-criterion analysis of variance. J Am Stat Assoc 47:583–621

Langner HW, Greene E, Domenech R, Staats MF (2012) Mercury and other mining-related contaminants in ospreys along the Upper Clark Fork River, Montana, USA. Arch Environ Contam Toxicol 62(4):681–695

Lewis ME, Brigham ME (2004) 5.6.4.B Low-level mercury. In: Wilde FD, Radtke DB, Gibs J, Iwatsubo RT (eds) National field manual for the collection of water-quality data (TWRI Book 9): Chapter A5: Processing of water samples

Magellan Diagnostics (2012) LeadCare II: Instant diagnosis + early intervention = healthier kids. http://www.leadcare2.com. Accessed 4 Apr 2012

Minnesota Department of Natural Resources (2008) Bullet fragmentation. http://files.dnr.state.mn.us/fish_wildlife/lead/. In: Minnesota Department of Natural Resources, Fish & Wildlife Division. Accessed: 12 Dec 2012

Neumann K (2009) Bald eagle lead poisoning in winter. In: Watson RT, Fuller M, Pokras M, Hunt WG (eds) Ingestion of lead from spent ammunition: Implications for wildlife and humans. The Peregrine Fund, Boise, pp 210–218

Olson ML, DeWild JF (1999) Techniques for the coldigestion and BrCl oxidation. USEPA,ÿÿÿÿlection and species-specific analysis of low levels of mercury in water, sediments, and biota. p 1–11

Pattee OH, Bloom PH, Scott JM, Milton RS (1990) Lead hazards within the range of the California Condor. Condor 92(4):931–937

Pokras MA, Kneeland MR (2009) Understanding lead uptake and effects across species lines: A conservation medicine based approach. In: Watson RT, Fuller M, Pokras M, Hunt WG (eds) Ingestion of lead from spent ammunition: Implications for wildlife and humans. The Peregrine Fund, Boise, pp 7–22

Rabinowitz MB, Wetherill GW, Kopple JD (1976) Kinetic analysis of lead metabolism in healthy humans. J Clin Invest 58(2):260–270

Rogers TA, Bedrosian B, Graham J, Foresman KR (2012) ~~(2011)~~ Lead exposure in large carnivores in the greater Yellowstone ecosystem. J Wildl Manag 76:575-582

Stauber E, Finch N, Talcott PA, Gay JM (2010) Lead poisoning of Bald (*Haliaeetus leucocephalus*) and Golden (*Aquila chrysaetos*) Eagles in the US Inland Pacific Northwest Region—an 18-year retrospective study: 1991–2008. J Avian Med Surg 24(4):279–287

R Development Core Team (2012) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Tilly F (2008) HMANA western mountain flyway report. Hawk Migr Stud 24:47–56

United States Environmental Protection Agency (2001) Appendix to method 1631: total mercury in tissue, sludge, sediment, and soil by acid digestion and BrCl oxidation. USEPA, report EPA-821-R-01-13, pp 1–13

Walker LA, Lawlor AJ, Llewellyn N, et al. (2010) The predatory bird monitoring scheme (PBMS) report 2006–7. Project Number C03310 Centre for Ecology & Hydrology to Natural England, UK

Watson RT, Fuller M, Pokras M, Hunt WG (2009 This refers to the proceedings of a conference as a whole. Please format as needed.) Ingestion of lead from spent ammunition: Implications for wildlife and humans.Conference proceedings, 12/5/2008 in Boise, Idaho ~~In: Watson RT, Fuller M, Pokras M, Hunt WG (eds) 12/5/2008 2008.~~ The Peregrine Fund, Boise