Wildlife Society Bulletin 40(1):174–180; 2016; DOI: 10.1002/wsb.615

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Tools and Technology

An Evaluation of Excrement as an Alternative to Blood for Characterizing Lead Exposure in Bald Eagles

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ABSTRACT Lead (Pb) exposure in free-flying bald eagles (*Haliaeetus leucocephalus*) is an important conservation concern. One of the challenges to characterizing the magnitude of Pb exposure in free-flying bald eagles is obtaining samples. Although blood samples are the most commonly used biomarker to quantify Pb exposure in living animals, the potential for negative impacts resulting from handling bald eagles make blood sampling less than ideal in a large-scale field study. We partnered with wildlife rehabilitators, who simultaneously collected blood and excrement samples from bald eagles admitted for rehabilitation in Iowa, USA, 2012 and 2013, to determine whether excrement could provide information comparable to blood about Pb exposure. We used linear regression to examine the relationship between excrement and blood Pb levels from 45 bald eagles admitted for rehabilitation and found that excrement Pb levels were statistically significantly related to blood Pb levels ($P < 0.01$, $R^2 = 0.68$). Specifically, when excrement Pb levels were low, blood Pb levels were also low. We also found patterns in Pb levels as a function of sex, age, season admitted, and fate. We conclude that excrement has the potential to be a valuable tool for investigating Pb exposure in bald eagles. © 2015 The Wildlife Society.

KEY WORDS bald eagle, blood, excrement, *Haliaeetus leucocephalus*, heavy metal, Iowa, lead (Pb).

The impact of lead (Pb) on free-flying bald eagles (*Haliaeetus leucocephalus*) is a subject of considerable concern. Wildlife researchers and rehabilitators continue to report cases of Pb poisoning in bald eagles (Nam et al. 2011, Bedrosian et al. 2012, Warner et al. 2014). The ingestion of spent Pb ammunition, Pb fragments embedded in food items, and Pb fishing tackle have been linked to morbidity and mortality in several avian species including bald eagles (Locke et al. 1982, Clark and Scheuhammer 2003, Cade 2007, Craighead and Bedrosian 2008, Domenech and Langner 2009).

Lead is a substance that is poisonous to all animals, including humans (Eisler 1988, Scheuhammer and Norris 1995, Goddard et al. 2008). Bald eagles do not have a muscular gizzard, which can erode ingested Pb fragments through the grinding action of grit; but their stomach pH is low (pH 1–4), which makes Pb very soluble (Clemens et al. 1975, Dieter and Finley 1978, Roscoe et al. 1979, Pain et al. 1997). When ingested Pb is not successfully excreted through regurgitation or defecation, it is absorbed into the bloodstream through the gastrointestinal tract, where it can have a diverse array of toxicological effects (Pattee et al. 1981, Eisler 1988, Sanderson 2002, Nam et al. 2004, Mateo et al. 2006). Dissolved Pb is deposited in soft tissues, such as the liver and kidneys, and ultimately accumulates in the skeleton, especially in areas of active bone formation (Scheuhammer 1987, Eisler 1988, Ek et al. 2004, Martin et al. 2008, Martinez-Haro et al. 2010). As it accumulates, Pb replaces other metals, primarily calcium, normally used for metabolism, and increasingly disrupts the nervous, vascular, renal, reproductive, and hematopoietic systems (Eisler 1988, Franson and Pain 2011).

Lead exposure can lead to acute or chronic poisoning (Eisler 1988). Acute poisoning may severely damage internal organs, often rapidly leading to death, without the occurrence of obvious external signs such as emaciation or lack of coordination (Locke and Thomas 1996). Chronic Pb exposure is associated with lethargy, loss of coordination, drooping wings, diminished growth, stunted development, abnormal metabolism, difficulty learning, altered behavior, impaired reproduction, and reduced survival (Victery 1988, Carpenter et al. 2003, Mateo et al. 2003). There is significant inter- and intraspecific variation in the impacts of Pb exposure (Hoffman et al. 1981, Carpenter et al. 2003, Finkelstein et al. 2012). In eagles, some of the factors associated with variation in Pb absorption, retention, and impact include age, sex, and amount of Pb ingested (Pattee et al. 1981, Wayland and Bollinger 1999, Wayland et al. 1999).

One of the challenges to characterizing the magnitude of Pb exposure in living, free-flying bald eagles is obtaining

Received: 28 January 2015; Accepted: 11 September 2015
Published: 24 December 2015

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samples. Blood is the biomarker most commonly used to quantify Pb exposure in living birds (Bergdahl and Skerfving 2008). Blood Pb levels can be indicative of exposure over the previous few weeks, and can also reflect past exposure when it contains Pb that had previously been stored in bone (Ambrose et al. 2000, Fry et al. 2009, Mason et al. 2014). Obtaining blood samples from free-flying eagles, however, is challenging. Capturing free-flying birds, which is necessary in order to draw blood, is logistically complicated; this makes obtaining sufficient sample sizes over spatial areas or over time difficult. Additionally, capturing and drawing blood from bald eagles can be stressful for both the bird and the human. Although samples can be obtained from dead eagles or eagles admitted to rehabilitation centers, it is unclear how representative these animals are of the entire population because of individual variation in Pb sensitivity.

The difficulty and potential for negative impacts resulting from handling bald eagles makes blood sampling to monitor Pb exposure less than ideal in a large-scale field study (Richardson and Miller 1997, Rosenfield et al. 2007). A potential alternative to using blood to quantify Pb exposure is to test excrement for Pb. Excrement is an attractive alternative because it is noninvasive and can be collected from the ground below nest and roost trees. In birds, excrement is a combination of fecal and urinary excretions that exit the cloaca. Lead found in the fecal component of excrement is Pb that is moving through the digestive system, and, therefore, is indicative of recent dietary exposure to Pb. In birds, urine contains uric acid particles called urate spheres, which bind heavy metals (Bal et al. 1998). Lead in the urine component of excrement is derived from Pb that has been taken up by the bloodstream and is excreted by blood as it passes through the kidneys (Furness 1993).

Excrement has been used to measure Pb exposure in other bird species, including domestic chickens (*Gallus gallus domesticus*; Clapp et al. 2012), great (*Parus major*) and blue (*Cyanistes caeruleus*) tits (Dauwe et al. 2000), great blue herons (*Ardea herodias*; Fitzner et al. 1995), peregrine falcons (*Falco peregrinus*; Ek et al. 2004), pied flycatchers (*Ficedula hypoleuca*; Berglund et al. 2010), little blue herons (*Egretta caerulea*; Spahn and Sherry 1999), and mallards (*Anas platyrhynchos*; Martinez-Haro et al. 2010). Excrement Pb levels have also been positively correlated with Pb levels in the blood and liver in greylag geese (*Anser anser*; Mateo et al. 2006) and in humans (Bergdahl and Skerfving 2008). We partnered with professional wildlife rehabilitators in Iowa, USA, to simultaneously collect blood and excrement samples from bald eagles as they were admitted for rehabilitation, and we had them tested for Pb. We used these samples to evaluate whether excrement could provide comparable information to blood about Pb exposure in bald eagles in order to identify individuals at risk or monitor spatial or temporal patterns of Pb exposure.

STUDY AREA AND SPECIES

The bald eagle is a year-round resident in Iowa, although eagle numbers vary seasonally. There are an estimated 200–300 active bald eagle nests in the state (Shepherd 2013) and the number of wintering eagles is in the thousands. Bald eagle numbers peak in Iowa between November and February, when migrants arrive from their breeding grounds in more northern states and Canada (Jackson et al. 1996). Bald eagles nest in Iowa from late January through July, with incubation typically starting in late February. Eagles occur in the greatest concentrations wherever there is open water and an abundant food source (Dyar 2010). Bald eagle nests are most concentrated in the northeastern part of Iowa along the Mississippi River. Bald eagles also nest along river corridors throughout the remainder of Iowa, but at lower densities, with the fewest nests occurring in western Iowa (Shepherd 2013).

METHODS

Blood and Excrement Sampling of Bald Eagles Admitted for Rehabilitation

In 2012 and 2013, professional wildlife rehabilitators in Iowa were recruited to collect blood and excrement samples from bald eagles as they were admitted. We worked with 3 raptor rehabilitation centers in Iowa: Saving Our Avian Resources in Dedham, the Macbride Raptor Project in Solon, and the Wildlife Care Clinic in Ames. Excrement and blood were collected from eagles regardless of the reason for admittance and prior to treatment, including chelation therapy. Rehabilitators collected blood samples into Capiject T-MQK 500-uL tubes with 0.78-mg K2-EDTA. Two vials of blood were collected, when possible, from the ulnar vein of each eagle. Wax paper was placed on the floor of an eagle's cage in order to collect excrement when an eagle defecated. After an eagle defecated, the wax paper was removed from the cage, the excrement was allowed to air-dry, and then the excrement was removed from the wax paper using a sterile disposable plastic spatula and placed in a sterile plastic collection tube. Blood and excrement samples were stored frozen until transferred to the Iowa State Hygienic Laboratory for testing. The timeframe from sample collection to transfer to the State Hygienic Laboratory for testing was 1–4 months. Along with sample collection, rehabilitators collected information about each eagle, including date of admission, reason for admission, age, sex, and final disposition. The sampling procedures described above were conducted under the permissions of the rehabilitators' licenses and permission from the U.S. Fish and Wildlife Service (permit MB52842A-0) and the State of Iowa (permit SC-871 and SC-872).

Lead Testing

For testing blood for Pb, approximately 0.5 mL of whole blood was combined with 10 mL of deionized water and 2.5 mL of concentrated nitric acid in a 50-mL polypropylene digestion tube and heated in a hot block at approximately 95° C until the volume was reduced to 5 mL. The samples were removed from the hot block and allowed to cool to room temperature before adding 0.5 mL of hydrogen peroxide (35%). Samples were reheated until the volume was reduced to 2 mL. After cooling, the samples were diluted to 25 mL using deionized water. Inductively Coupled Plasma Mass Spectrometry (Perkin–Elmer ELAN DRC II;

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Waltham, MA) was used to analyze the blood samples. Calibration of instrument response was accomplished using external standards. Check standards, reference materials, and reagent blanks were used to evaluate accuracy. Lead concentrations for blood were reported in μg/dL with a quantitation limit that ranged from 0.5 μg/dL to 20 μg/dL, depending on the run. For analysis and comparison with excrement, blood Pb levels were converted to parts per million (ppm), with an adjustment for the density of blood.

For testing excrement for Pb, approximately 0.5 g was weighed out and placed in a 50-mL polypropylene digestion tube. To the tube, 5 mL of concentrated nitric acid was added, and the tube was heated in a hot block at approximately 95° C for 15 min. After cooling to room temperature, an additional 2.5 mL of nitric acid was added and the tube was reheated until the volume was reduced to 5 mL. After cooling to room temperature, 5 mL of double-distilled water and 2.5 mL of concentrated hydrochloric acid were added. Finally, the sample was diluted to 50 mL with deionized water. Inductively Coupled Plasma Mass Spectrometry (Agilent 7500ce) was used to analyze the excrement samples. As with blood, calibration of instrument response was accomplished using external standards. Check standards, reference materials, and reagent blanks were used to evaluate accuracy. Calibrations for excrement included 0.0 μg/L, 1.0 μg/L, 5.0 μg/L, 50.0 μg/L, 100.0 μg/L, and 500.0 μg/L. Lead concentrations in excrement were reported in mg/kg, with a quantitation limit of 1.0 mg/kg (ppm).

Data Analyses

For analysis, we assigned samples with Pb levels below the quantitation limit a value equal to one-half the quantitation limit, following Wiemeyer et al. (1989). The distributions of both the excrement and blood Pb data were highly skewed. As a result, we calculated geometric rather than arithmetic means. Also, prior to regression analysis, we $\log_{10}$ transformed excrement and blood Pb levels. We chose to conduct a $\log_{10}$ transformation to be consistent with our use of a geometric mean, which is the antilog of the $\log_{10}$ values of a data set (Sokal and Rohlf 1995). We calculated geometric means, 95% confidence limits, and ranges for blood and excrement Pb levels. We compared the geometric mean, 95% confidence limit, and range of blood Pb level for birds whose excrement Pb levels were below the quantitation limit and for birds whose excrement Pb levels were above the quantitation limit. We used linear regression in project R version 3.2.2 (R Core Team 2015) to examine the relationship between paired blood and excrement Pb levels. The relationship was considered significant if $P < 0.01$. Finally, we compared geometric means and 95% confidence limits of blood and excrement Pb levels by sex, age, season admitted (defined by solstice), and fate of the eagle. Means whose 95% confidence limits did not overlap were considered significantly different.

RESULTS

We collected paired blood and excrement samples from 45 bald eagles admitted for rehabilitation in Iowa in 2012 and 2013. Initial diagnoses made by rehabilitators included injury (generally a broken bone, $n = 18$), suspected Pb poisoning ($n = 11$), acute organophosphate poisoning ($n = 2$), starvation ($n = 2$), depression ($n = 6$), and unknown ($n = 6$).

Blood Pb levels were above the quantitation limit in all samples, but 57.8% (26/45) of the excrement samples had Pb levels below the quantitation limit of 1.0 ppm. Mean Pb levels were higher in excrement samples than in blood samples (Table 1). The mean blood Pb level for birds with excrement Pb levels above the quantitation limit was higher than the mean blood Pb level for birds with excrement Pb levels below the quantitation limit (Table 2).

Excrement and blood Pb levels had a significant positive relationship ($\beta = 0.78$, $SE = 0.08$, $P < 0.01$, $R^2 = 0.68$; Fig. 1). Reanalysis of the data after removing the samples with excrement Pb levels below the quantitation limit still

Table 1. Summary of blood and excrement Pb statistics for 45 bald eagles admitted for rehabilitation in Iowa, USA, 2012 and 2013.

Statistic	Blood	Excrement
No. of eagles	45	45
Pb geometric mean (ppm)	0.43	1.95
95% CL	0.25–0.75	1.11–3.41
Pb range (ppm)	0.03–34.91	<1.00–520.00

Pb, lead; ppm, parts per million.

Table 2. Summary of blood Pb statistics as a function of whether Pb levels in excrement samples were above or below the quantitation limit (1.0 ppm) for 45 bald eagles admitted for rehabilitation in Iowa, USA, 2012 and 2013.

Statistic	Excrement Pb <1.0 ppm	Excrement Pb >1.0 ppm
No. of eagles	26	19
Pb geometric mean (ppm)	0.15	1.83
95% CL	0.11–0.20	0.81–4.11
Pb range (ppm)	0.03–0.63	0.04–34.91

Pb, lead; ppm, parts per million.

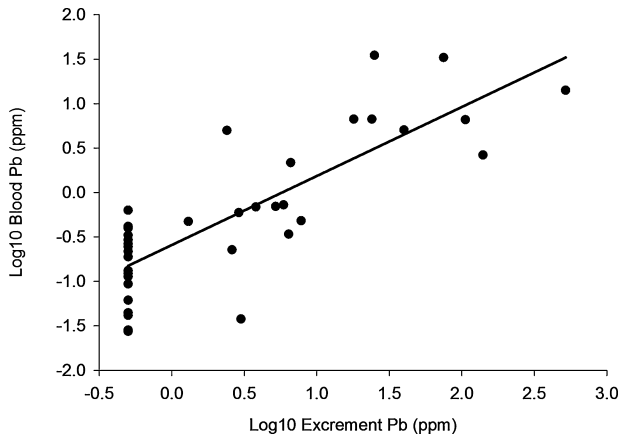

Figure 1. Log_{10} transformed Pb levels in paired blood and excrement samples collected from 45 bald eagles admitted for rehabilitation in Iowa, USA, in 2012 and 2013. The black line is the regression fit line ($P < 0.01$). Pb, lead; ppm, parts per million.

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resulted in a significant positive relationship between blood and excrement Pb levels ($\beta = 0.77$, SE = 0.19, $P < 0.01$, $R^2 = 0.49$).

When comparing Pb levels among birds, we found that Pb levels in blood were higher in adult birds compared with hatch-year birds, while there was no evidence for differences in excrement Pb levels among age classes (Table 3). There was no evidence that Pb levels in blood and excrement were different between females and males (Table 4). When comparing Pb levels in eagles as a function of season, blood Pb levels were higher in birds admitted in the winter compared with spring, while there was no evidence for differences in excrement Pb levels among seasons (Table 5). Although our sample size of birds for which we identified sex was small ($n = 23/45$, 51.1%), females sampled in the winter had higher Pb levels than females sampled in the spring, but there was not a difference for males (Table 6). Blood and excrement Pb levels were higher for eagles that died on their own than for eagles that were released (Table 7). Blood and excrement Pb levels of birds that were euthanized overlapped the levels of birds that survived and those that died on their own (Table 7).

Table 3. Geometric mean (95% CL) level of Pb in blood and excrement by age for 45 bald eagles admitted for rehabilitation in Iowa, USA, 2012 and 2013.

		Blood		Excrement	
Eagle age	No. of eagles	$\bar{x}$	95% CL	$\bar{x}$	95% CL
Hatch-year	5	0.08	0.03–0.20	0.72	0.35–1.44
Juv	7	0.41	0.12–1.44	2.43	0.36–16.28
Ad	28	0.55	0.27–1.12	2.36	1.12–4.95
Unknown	5	0.62	0.20–1.88	1.32	0.54–3.22

Pb, lead; ppm, parts per million.

Table 4. Geometric mean (95% CL) level of Pb in blood and excrement by sex for 45 bald eagles admitted for rehabilitation in Iowa, USA, 2012 and 2013.

		Blood		Excrement	
Eagle gender	No. of eagles	$\bar{x}$	95% CL	$\bar{x}$	95% CL
F	12	0.80	0.25–2.54	2.41	0.70–8.28
M	11	0.41	0.14–1.16	1.19	0.46–3.10
Unknown	22	0.31	0.16–0.64	2.21	0.98–5.00

Pb, lead; ppm, parts per million.

Table 5. Geometric mean (95% CL) level of Pb in blood and excrement by season for 45 bald eagles admitted for rehabilitation in Iowa, USA, 2012 and 2013.

		Blood		Excrement	
Season	No. of eagles	$\bar{x}$	%95 CL	$\bar{x}$	%95 CL
Winter	25	0.71	0.37–1.37	2.48	1.17–5.25
Spring	13	0.18	0.09–0.37	0.84	0.41–1.73
Summer	1	0.04	n/a	3.00	n/a
Autumn	6	0.54	0.07–4.00	4.06	0.43–37.81

Pb, lead; ppm, parts per million.

DISCUSSION

Our study suggests that excrement sampling could be a useful approach for examining Pb exposure in bald eagles. The positive linear correlation between blood and excrement Pb levels in the bald eagles means that excrement Pb levels mirror blood Pb levels. This is also important because collection of excrement samples is less stressful for the birds.

It is important to point out that although we found a significant correlation between blood and excrement Pb levels, the relationship is not perfect. Excrement Pb levels were, on average, higher than blood Pb levels in the eagles we sampled. Other studies have also found Pb levels to be higher in excrement than other sample types (Spahn and Sherry 1999, Dauwe et al. 2000, Ek et al. 2004, Morrissey et al. 2005). For instance, Ek et al. (2004) found higher lead levels in excrement than in blood, liver, kidney, eggs, and feathers of peregrine falcons. One explanation for differences in Pb levels between blood and excrement and the imperfect correlation between them is that blood and excrement provide somewhat different histories of an individual's exposure to Pb. Blood Pb levels are often indicative of recent exposure over the past few weeks. For example, in a study in the California condor (*Gymnogyps californianus*), the half-life of Pb in blood was approximately 2 weeks (Fry et al. 2009). Blood Pb levels, however, can also reflect previously stored Pb that has leached from bone into blood (Ambrose et al. 2000, Mason et al. 2014). In our study, excrement samples were a combination of both feces and urate. Lead present in feces has moved through the gastrointestinal tract, thereby providing information about very recent dietary exposure to Pb. Lead in the urate component of excrement comes from the bloodstream via the kidneys and may be more comparable in terms of history to a blood sample (Furness 1993). Because of these differing histories, we cannot expect Pb levels in blood and excrement to be perfectly reflective of one another. As such, it is not possible to precisely predict blood lead levels from excrement Pb levels, and therefore it is not advisable to try to predict clinical outcomes using excrement data as has been done with blood (e.g., Kramer and Redig 1997).

Another source of variation in our results is individual variation. Lead absorption, retention, and impact can vary greatly among individual eagles (Pattee et al. 1981, Wayland et al. 1999, Pattee and Pain 2003). Female eagles that we sampled had higher blood and excrement Pb levels in winter than in the spring, while no such pattern was found for males. Part of the seasonal difference in Pb levels in females may be related to reproduction. During egg-laying (late Feb to early Mar in IA) females may have higher circulating blood Pb levels because calcium, along with any stored Pb, is mobilized from bone for eggshell formation (Burger 1994). We also found age differences in Pb levels. Hatch-year birds in our study had lower blood Pb levels than adult birds. Lower Pb exposure in young eagles compared with adult eagles has also been reported in other studies (Harmata and Restani 2013, Franson and Russell 2014). This may be due to adult birds actively foraging, whereas hatch-year birds are more frequently fed by their parents, perhaps making them less

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Table 6. Geometric mean (sample size) and 95% confidence limit level of Pb in blood and excrement as a function of sex and season for bald eagles admitted for rehabilitation in Iowa, USA, 2012 and 2013.

	Blood				Excrement			
	F		M		F		M	
Season	$\bar{x}$ (n)	95% CI	$\bar{x}$ (n)	95% CI	$\bar{x}$ (n)	95% CI	$\bar{x}$ (n)	95% CI
Winter	1.78 (8)	0.43–7.44	0.33 (5)	0.08–1.36	5.31 (8)	1.07–26.26	0.68 (5)	0.37–1.27
Spring	0.16 (4)	0.10–0.26	0.31 (4)	0.04–2.22	0.50 (4)	0.50–0.50	1.50 (4)	0.17–12.80

Pb, lead; ppm, parts per million.

Table 7. Geometric mean (95% CL) level of Pb in blood and excrement by fate for 45 bald eagles admitted for rehabilitation in Iowa, USA, 2012 and 2013.

		Blood		Excrement	
Fate	No. of eagles	$\bar{x}$	95% CL	$\bar{x}$	95% CL
Died	14	1.64	0.56–4.78	6.54	2.41–17.77
Euthanized	16	0.31	0.14–0.68	1.62	0.56–4.63
Released	13	0.18	0.10–0.30	0.82	0.49–1.36
Nonreleasable and retained for education	2	0.18	0.07–0.46	0.50	0.50–0.50

Pb, lead; ppm, parts per million.

likely to be directly exposed to Pb. We also found higher blood Pb levels in winter (coincident with the deer-harvest season in IA) compared with spring. This finding is consistent with several studies that have documented temporal correlations between the incidence of Pb exposure and the timing of upland and big-game hunting seasons (Strom et al. 2009, Stauber et al. 2010, Cruz-Martinez et al. 2012). For example, from 2005 to 2010, free-flying bald eagles captured in Wyoming, USA, during the big-game hunting season had higher blood Pb levels than did birds captured before or after it (Bedrosian et al. 2012).

We also observed a relationship between the ultimate fate of an eagle in our study and its Pb level. We found that blood and excrement Pb levels were higher in birds that died on their own than birds that survived, whereas levels in euthanized birds overlapped these 2 groups. This overlap is likely due to variation in the reasons that the birds were brought to the clinics. Some of the birds that were euthanized were suffering from Pb poisoning, but most of the eagles that ended up being euthanized were admitted because of broken bones and had relatively low Pb levels ($n = 11/16$ [68.8%]; geometric mean blood Pb level = 0.14, 95% CI = 0.08–0.22; 10/11 [90.9%] had an excrement Pb level below the quantitation limit of 1.0 ppm).

Understanding of the relationship between Pb levels in blood and excrement can probably be improved with additional paired samples from bald eagles admitted for rehabilitation and from free-flying bald eagles. Unfortunately, individual variation not related to age or sex, along with time since Pb exposure relative to when sampling takes place, are sources of variation that will be difficult to resolve with observational data alone. There are, however, modifications to our approach that can be made in future studies to improve the utility of excrement for quantifying Pb exposure in bald eagles. We did not standardize the ratio of urate to feces in our samples. There was likely variation among our excrement samples in the proportions of each component present, which may have contributed to some of the variation we observed in the relationship between excrement and blood Pb levels. Studies that use excrement to examine spatial and/or temporal patterns of Pb exposure should, if possible, standardize the ratio of urate to feces in samples. Additionally, 58% of our excrement samples had Pb levels below the quantitation limit. Future studies should, if possible, use a lower quantitation limit to more precisely quantify Pb levels in excrement.

Future studies of Pb exposure in bald eagles could also employ techniques that enable the source of Pb to be identified and the negative impacts of Pb exposure to be quantified. For example, Martinez-Haro et al. (2011) analyzed aluminum concentrations together with Pb levels in excrement to assess soil ingestion as a source of Pb exposure in mallards. These authors, along with others, have characterized Pb isotope ratios to discriminate among different sources of Pb exposure (Church et al. 2006; Redig et al. 2009; Martinez-Haro et al. 2011, 2013; Finkelstein et al. 2012). Finally, porphyrin and biliverdin concentrations in excrement samples could be used as biomarkers of adverse or toxicological effects related to Pb exposure (Mateo et al. 2006, Martinez-Haro et al. 2013).

ACKNOWLEDGMENTS

The authors thank J. Coats and S. Shepherd for their guidance and expertise, Iowa State University's Wildlife Care Clinic (B. Zaffarano), the Macbride Raptor Project (J. Cancilla, L. Hart), Saving Our Avian Resources (K. Neumann), the Iowa Department of Natural Resources, the U.S. Fish and Wildlife Service, and personnel at the University of Iowa State Hygienic Laboratory (S. Bernholtz, B. Wels). Comments and suggestions from the Associate Editor and 3 anonymous reviewers contributed to the improvement of this manuscript. This research was funded by a State Wildlife Grant from the Iowa Department of Natural Resources (T-54-R-1) and a grant from the American Eagle Foundation.

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Associate Editor: Gervais.

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