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ENVIRONMENTAL CONTAMINANTS IN TISSUES OF BALD EAGLES SAMPLED IN SOUTHWESTERN MONTANA, 2006–2008

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ABSTRACT.—Blood and feathers of Bald Eagles (*Haliaeetus leucocephalus*) banded as nestlings ($n = 17$), captured as free-flying ($n = 91$), or submitted for rehabilitation ($n = 29$) in southwestern Montana between December 2005 and April 2008 were sampled for mercury (Hg), selenium (Se), lead (Pb), seven other trace elements, and organochlorines. Hg concentrations in blood (hereafter “HgB”) did not differ between captured eagles and those submitted for rehabilitation, and HgB in both were higher than concentrations in nestlings ($P < 0.01$). Se concentrations in blood (“SeB”) were similar among groups. Pb concentrations in blood (“PbB”) were higher in captured eagles than in those submitted for rehabilitation ($P = 0.05$). No bird submitted for rehabilitation exhibited toxic levels of PbB, but 9% of captured eagles did. HgB and PbB in captured eagles declined as date of capture advanced from autumn to spring. Hg and Se concentrations in feathers (“HgF”; “SeF”) tended to increase with age-class. HgB and SeB, and HgB and HgF were correlated in nestlings and captured eagles ($P < 0.05$) but not in birds submitted for rehabilitation. Birds captured in autumn during this study had higher HgB ($P < 0.05$) than birds captured in autumn in the early 1990s, but SeB did not differ. HgB and SeB in birds captured in spring during this study were similar to those of birds captured in spring in the early 1990s, but PbB was lower. Five eagles were recaptured and resampled for contaminants up to 18 yr after initial banding and sampling but no time-trends were detected in contaminant concentrations due to small sample size. Other trace elements and organochlorines if detected in blood were at very low concentrations.

KEY WORDS: *Bald Eagle*, *Haliaeetus leucocephalus*; capture, DDE; lead; mercury; nestling; rehabilitation; selenium.

CONTAMINANTES AMBIENTALES EN TEJIDOS DE *HALIAEETUS LEUCOCEPHALUS* MUESTREADOS EN EL SUROESTE DE MONTANA, 2006–2008

RESUMEN.—Muestreamos sangre y plumas de águilas *Haliaeetus leucocephalus* anilladas como pichones ($n = 17$), capturadas en su ambiente ($n = 91$) o de individuos enviados para rehabilitación ($n = 29$) en el suroeste de Montana entre diciembre de 2005 y abril de 2008 para determinar las concentraciones de mercurio (Hg), selenio (Se), plomo (Pb), restos de otros siete elementos y compuestos organoclorados. Las concentraciones de Hg en la sangre (HgB) no difirieron entre las águilas capturadas y aquellas enviadas a rehabilitación, y la HgB en ambos grupos fue más alta que las concentraciones en los pichones ($P < 0.01$). Las concentraciones sanguíneas de Se (SeB) fueron similares entre los grupos. Las concentraciones sanguíneas de Pb (PbB) fueron más altas en las águilas capturadas que en aquellas enviadas a rehabilitación ($P = 0.05$). Ningún ave enviada a rehabilitación mostró niveles tóxicos de PbB, pero el 9% de las águilas capturadas sí mostró. HgB y PbB en las águilas capturadas disminuyeron a medida que progresó el día de captura desde el otoño hacia la primavera. Las concentraciones de Hg y Se en las plumas (HgF; SeF) tendieron a incrementar con las clases de edad. HgB y SeB, y HgB y HgF estuvieron correlacionadas en los pichones y en las águilas capturadas ($P < 0.05$), pero no en las aves enviadas a rehabilitación. Las aves capturadas en otoño durante este estudio tuvieron HgB más altas ($P < 0.05$) que las aves capturadas en otoño a principios de los 1990, pero SeB no difirió. En las aves capturadas durante este estudio en primavera, HgB y SeB fueron similares a las concentraciones de las aves capturadas en primavera a principios de los 1990, pero PbB fue más baja. Cinco águilas fueron recapturadas y muestreadas nuevamente en busca de contaminantes hasta 18 años después del anillado y muestreo inicial, pero no se detectaron tendencias temporales en las concentraciones de contaminantes debido a un tamaño de muestra pequeño. En aquellos casos en que se detectaron restos de otros elementos y de compuestos organoclorados en la sangre, éstos ocurrieron a concentraciones muy bajas.

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During winter 2005–06, debilitated Bald Eagles (*Haliaeetus leucocephalus*) submitted to Montana Raptor Conservation Center (MRCC), a raptor rehabilitation organization in Bozeman, Montana, were found to contain mercury (Hg) concentrations in blood considered above background levels for some piscivorous species (≥ 0.4 ppm wet weight; Burgess et al. 2005). Most were recovered within 30 km of the Upper Missouri River watershed in southwestern Montana. Staff at MRCC indicated that the number and morbidity of Bald Eagles submitted during this period was unusual and questioned whether there may be an emerging or chronic problem with Hg concentrations in the local environment. To address this concern, a pilot program was launched in May 2006 to monitor Hg concentrations in tissues of Bald Eagles submitted for rehabilitation (hereafter referred to as “rehab”) and wild nestling Bald Eagles in southwestern Montana. Scope of study expanded to include migrant and wintering eagles captured in southwestern Montana between autumn 2006 and spring 2008. Study methods permitted collection of tissues for analysis of other chemical elements and organochlorine compounds, in addition to Hg.

Study objectives were to determine the amount and extent of Hg, selenium (Se), lead (Pb), and organochlorines in tissues of nestling, rehab, migrant, and wintering Bald Eagles in southwestern Montana. Hg and Pb were emphasized because they are nonessential and toxic and have documented effects on avian health and reproduction (Eisler 1987, Boening 2000, Haruka et al. 2009). Hg is of primary concern in aquatic environments studied (e.g., Sorensen et al. 1990, Scheuhammer and Graham 1999) and Pb is of recent concern in upland habitats (Watson et al. 2009), both of which are frequented seasonally by Bald Eagles. Selenium was included because of its reported detoxification properties for Hg (Yoneda and Suzuki 1997, Odsjö et al. 2004, Yang et al. 2007, Berry and Ralston 2008). Methods also permitted screening for other potentially toxic chemical elements not typically reported (Burger and Gochfeld 2009).

STUDY AREA AND SAMPLE POPULATIONS

Nestling eagles were sampled along the Madison and Missouri rivers in southwestern Montana from the inlet to Ennis Lake near McAllister, Montana, to Holter Reservoir, northeast of Helena, Montana. Some nestlings from nests on the Gallatin and Jefferson rivers within 25 km of their confluence with

the Missouri River and the Yellowstone River near Big Timber, Montana, were also included. Free-flying eagles were captured between November and April 2006–07 and 2007–08 within the same geographical area where nestlings were sampled, but capture efforts were focused within 25 km of the headwaters of the Missouri River. Some eagles were captured in March near Ringling, Meagher County, Montana. Bald eagles classified in “rehab” group consisted of Bald Eagles submitted for rehabilitation to MRCC from throughout Montana, but most originated in the southwestern part of the state.

Most captured eagles were likely migrants originating in the boreal forests of western Canada because (1) many juvenile and other nonadult age classes of Bald Eagles produced in southwestern Montana leave in autumn to winter in coastal western states (Harmata et al. 1999) and (2) large numbers of migrant Bald Eagles move through Montana seasonally (Nijssen et al. 1985, McClelland et al. 1994, Miller et al. 1998, Harmata 2002). Further, over 300 Bald Eagles have been observed along the Madison–Missouri River system in southwestern Montana (Restani et al. 2000) in late autumn and winter and >200 in one 2-km² pasture within the study area in January 2008 (A. Harmata unpubl. data) and (3) only approximately 20 pairs of breeding Bald Eagles occur within the study area (Montana Fish, Wildlife, and Parks unpubl. data) with an estimated resident autumn/winter population of 40–50. Accordingly, the probability that a significant proportion of the captured eagles were produced or breeding in southwestern Montana was low.

METHODS

Montana Bald Eagle Working Group members and APEX Environmental, LLC, surveyed nesting activity of Bald Eagle breeding pairs in April and May. Nestling eagles were sampled when >6 wk of age. Sex was not assigned to nestling eagles because of uncertainty of hatching date and thus, stage in morphological development. Nestlings were considered a plumage class and a seasonal group for some comparative analyses.

Free-flying eagles were captured with a command-detonated, Coda net launcher (Coda Enterprises, Mesa, Arizona, U.S.A.). Road-killed ungulate carcasses, mostly white-tailed deer (*Odocoileus virginianus*), were used as bait, as were wild lagomorph and domestic bovine carcasses when available. The net launcher was usually not detonated when fewer than three eagles were within net range.

Date of capture was the sole criteria for classifying seasonal groups of captured eagles. Although a few captured eagles may have been associated with local nest sites, identification of local breeders was not possible. Eagles captured between 1 and 22 December were classified as autumn migrants; those captured between 23 December and 29 February classified as wintering; and those captured between 1 March and 15 April classified as vernal migrants. Both migrant classes and wintering eagles are referred to collectively as "captured" to distinguish them from nestlings and rehabs. Sex was assigned to captured and rehab eagles using the methods described by Bortolotti (1984) and Garcelon et al. (1985). Captured and rehab eagles were categorized in juvenile, immature, subadult, and adult plumage classes, which were related to advancing age (McCullough 1989). Nestling and captured eagles are also referred to collectively as wild.

Contaminant samples were collected only from eagles submitted live to MRCC for rehabilitation. Samples were collected at time of submission before any other palliative or rehabilitative care was administered.

One to 3 cc of whole blood and 250–500 mg of lower breast and/or abdominal feathers were collected from most eagles. MRCC staff collected, recorded, and shipped blood samples from rehab eagles. Blood is an appropriate medium for evaluating metal levels in birds (Kahle and Becker 1999) and half-life of Hg in avian blood is 1–3 mo (Stickel et al. 1977, Evers et al. 2005), permitting evaluation of seasonal differences in contamination (Tsao et al. 2009). Harvesting feathers is a noninvasive method for assessing metal contamination in birds (Burger and Gochfeld 2009) and breast feathers are the best indicator of whole-body burdens (Furness et al. 1986, Burger 1993). Metals ingested in food or water are excreted into feathers during a 2-wk to 1-mo period of development and are a profile of exposure during that time (Burger 1993, Burger and Gochfeld 2009). Heaviest molt in Montana Bald Eagles occurs during late summer (A. Harmata unpubl. data), thus breast feathers are an indicator of contaminant exposure in summer/nesting areas. Feathers were clipped near the skin surface with surgical scissors and deposited in plastic sandwich bags. All feathers were fully developed when harvested. Whole blood samples were divided on site; $\frac{2}{3}$ for organochlorine analysis and $\frac{1}{3}$ for analysis of Hg, Pb, Se and other toxic elements. Blood for organochlorine analysis was cooled for 24 hr, centri-

fuged, and sera withdrawn. Blood and serum samples were refrigerated and shipped with feather samples at season end.

Blood and feather samples were shipped for analysis to Michigan State University, College of Veterinary Medicine, Diagnostic Center for Population and Animal Health, Toxicology Section, Lansing, Michigan, U.S.A. (DCPAH). Whole blood was analyzed for the following chlorinated hydrocarbons with a detection limit of 1.0 part per billion (ppb): aldrin, alpha-benzene hexachloride (BHC), beta-BHC, delta-BHC, gamma-BHC, alpha-chlordane, gamma-chlordane, dichlorodiphenyldichloroethane (DDD), dichlorodiphenyldichloroethylene (DDE), dichlorodiphenyltrichloroethane (DDT), dieldrin, endosulfan I, endosulfan sulfate, endrin, heptachlor, heptachlor epoxide, trans-nonachlor, and oxychlordane. Reference standard chlorinated pesticides came as 2000 µg each in toluene/hexane (50:50) from Supelco, Inc., Bellefonte, Pennsylvania, U.S.A. Chlorinated pesticide analysis followed Price et al. (1986). Concentrations were reported in ppm (µg/ml). Two ml of whole blood were extracted (three times) with 6 ml hexane/acetone (9:1) by vortexing for 30 sec, centrifuging at $1430 \times g$ for 10 min, and transferring the hexane layer. Combined hexane fractions were evaporated under nitrogen and redissolved in 1 ml hexane for silica gel clean-up. Silica gel clean-up was accomplished using 9 mm chromaflex columns fitted with silanized glass wool that was prepared by pouring through 20 ml of hexane containing 5 g silica gel 60 followed by 2.54 cm of anhydrous sodium sulfate. The prepared column was layered with sample, including 1-ml hexane rinses as needed. Three fractions were collected: FI, from 10-ml hexane elution, FII from next 25-ml hexane elution, and FIII from the next 20 ml of benzene elution. Fractions FII and FIII were evaporated under nitrogen and resuspended in 2 ml ethanol/iso-octane, 20:80; aldrin elute in FII, whereas DDE and heptachlor elute in both FII and FIII, and the remaining chlorinated pesticides elute in FIII. One µl each of fractions FII and FIII were run on a Varian (Varian, Inc.) Gas Chromatograph (GC)—Electron Capture Detector (ECD; Varian Inc., Palo Alto, California, U.S.A.) on an initial screening set-up followed by a confirmatory set-up. Initial run utilized a DB-1701 15 m \times 0.30 mm \times 0.25 µ film thickness column with the injector temperature at 250°C and the detector at 300°C. The program was initially 150°C for 0.5 min, followed by a linear increase at 5°C/min to 280°C, then held at 280°C for 15 min. Confirmatory run used a DB-608

30 m × 0.32 mm × 0.5 μ film thickness column, with the injector temp at 250°C and the detector at 300°C. The program was initially held at 150°C, then increased linearly at 12°C/min to 280°C, then held at 280°C for 20 min. Blank solvent injections were run through the analyzer between samples. Sample extraction efficiency was judged on determination of recovery of compound standards from spiked blanks to verify that all recoveries exceeded a minimum of 60%. Blanks, spikes, standards, and specimens in singlicate were run together in the same sequence and GC peak identities were considered verified if peak retention times varied by no more than 0.1 min from those of standards.

Metals in blood and feathers were analyzed by inductively coupled plasma mass spectrometry (ICPMS; Agilent 7500ce ICP-MS, Santa Clara, California, U.S.A.) also at the DCPAH (Goulette et al. 2005, Wahlen et al. 2005). Reportable quantization limits for metals were as follows: Pb, 1 ppb; Hg, 5 ppb; Se, 1 ppb; antimony (Sb), 1 pp; arsenic (As), 1 ppb; beryllium (Be), 5 ppb; cadmium (Cd), 5 ppb; chromium (Cr), 5 ppb; nickel (Ni), 1 ppb; thallium (Tl), 1 ppb; and vanadium (V), 1 ppb. Samples (0.2 ml each) were diluted with 5 ml of 0.05% EDTA, 1% NH₄OH, 0.05% Triton-X 100 and 2% n-butanol. Internal standards included 10–15 ppb scandium, germanium, rhodium and indium, each of which was associated with its nearest analyte by atomic weight. ICPMS was calibrated with 0, 1, 10, and 100 ppb standards, and sample concentrations were measured against standard reference solutions arranged in a linear relationship. Diluent blanks and Quality control (QC) materials were run with each sample sequence. QC was maintained by monitoring results obtained with Bio-Rad (Hercules, California, U.S.A.) Lypocheck Whole Blood Metals Controls Levels 1 and 2. Calibrations were also cross-checked against nitric acid-digested Standard Reference Materials obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, Maryland, U.S.A.), for example NIST Bovine Liver for various elements and NIST SRM 2976 mussel for mercury. Feathers were analyzed by ICPMS only for Hg and Se.

Organochlorine concentrations were not analyzed in eagles submitted for rehabilitation. Nestling eagles were included for organochlorine analysis because no historical data were available for southwestern Montana. Because origins of most captured eagles were presumed to be in Canada, and Henny et al. (1979) found low organochlorine concentra-

tions in migrant eagles as early as 4 yr after use of DDT was banned, only a few wintering/migrant Bald Eagles were tested for organochlorines. Organochlorines were measured only in adult females.

Contaminant concentrations were reported as parts per million wet weight (ppm) from DCPAH and units are retained here. Terms concentration(s), load(s), and level(s) are used interchangeably throughout. Geometric means are presented to promote comparison with other contaminant studies but geometric mean was not calculated if <50% of samples had concentrations below detection limit. When ≥50% of samples contained detectable levels of a contaminant, those with no detectable levels were assigned a concentration of half the detection limit of respective analytes for calculation of geometric mean. Arithmetic means are presented and compared for groups that had <50% of samples with no detectable levels and are so noted.

Contaminant data were transformed to common logarithms for parametric statistical tests. Pearson Product-Moment tests were used to test correlations (*r* values) between categories. Tukey's honestly significant difference tests for unequal *n* were used to detect differences among groups if ANOVA/MANOVA tests indicated differences. Multiple comparisons (Student's *t*-tests, ANOVA) with Bonferroni adjustments (i.e., 0.05/*n* tests) were conducted if all cells for MANOVA tests could not be filled. If log-transformations were inappropriate and raw data plots revealed curvilinear relationships or outliers, or Kolmogorov-Smirnov tests indicated nonnormal distribution, nonparametric tests were employed and Bonferroni adjusted for appropriate *P* value. *P* values ≤0.05 were considered significant. Season was excluded from any analyses involving feather contaminants because all eagles most likely developed their feathers in the summer. All statistical tests were performed and graphics produced in various modules of STATISTICA ver. 6.0 (StatSoft 2003).

Based on data presented by Kramer and Redig (1997) and Neumann (2009), Pb concentrations in blood of <0.2 ppm were considered background, 0.2 to 0.6 ppm were considered elevated ("sub-clinical" in Kramer and Redig 1997); >0.6 to 1.0 ppm as acute ("clinical" in Kramer and Redig 1997) and >1.0 ppm considered toxic ("fatal" in Kramer and Redig 1997). Similar exposure levels of Hg and Se in blood that potentially may affect health and reproduction in Bald Eagles have not been established (Burger and Gochfeld 1997, Spallholz and Hoffman 2002, Scheuhammer et al. 2008).

Table 1. Geometric mean concentrations (ppm wet wt.) of mercury (Hg), selenium (Se), and lead (Pb) in whole blood and feathers of Bald Eagles sampled in southwestern Montana, May 2006–April 2008.

| GROUP | Hg (<i>n</i>) | | Se (<i>n</i>) | | Pb (<i>n</i>) |
|------------|--------------------|---------------------|--------------------|-------------------------|--------------------|
| | BLOOD | FEATHERS | BLOOD | FEATHERS | BLOOD |
| Nestlings | 0.100 (17) | 3.04 (16) | 0.795 (17) | 1.927 (16) ^a | 0.037 (17) |
| Captured | 0.709 (88) | 13.038 (91) | 0.736 (88) | 1.538 (91) | 0.272 (88) |
| Autumn | 0.877 (23) | 13.769 (25) | 0.706 (23) | 1.524 (25) | 0.414 (23) |
| Winter | 0.728 (46) | 11.074 (47) | 0.823 (46) | 1.461 (47) | 0.264 (46) |
| Vernal | 0.514 (19) | 18.176 (19) | 0.592 (19) | 1.767 (19) | 0.177 (19) |
| Rehab | 0.670 (26) | 9.72 (11) | 0.835 (19) | 1.435 (11) | 0.132 (23) |
| All | 0.544 (131) | 10.415 (118) | 0.759 (124) | 1.427 (118) | 0.183 (128) |

^a Se concentrations in 2006 (geometric mean = 1.97 ppm), 2007 (geometric mean = 0.26 ppm).

RESULTS

Blood and feather samples were obtained from 17 nestling Bald Eagles in southwestern Montana, 12 in 2006 and 5 in 2007. Most samples (78%) were obtained from nests associated with free-flowing rivers (Gallatin, Jefferson, Madison, Yellowstone, Missouri). Four nestlings in nests associated with two reservoirs of the Missouri River (Canyon Ferry and Holter reservoirs) were also sampled.

Ninety-one Bald Eagles were captured, 30 in 2006–07 and 61 in 2007–08. Blood samples were obtained from 88 eagles; samples were not collected from three birds because ambient air temperature was $< -23^{\circ}\text{C}$. Feather samples were collected from 90 captured eagles and one juvenile eagle found recently (< 2 d) expired under a power line. More males (51) than females (40) were captured but sex distribution did not differ ($\chi^2 = 1.35$, $P > 0.24$). The most numerous plumage class represented among captured eagles was adult at 42%, followed by 32% juveniles, 20% immature, and 7% subadults.

Blood and feather samples were collected from 29 rehab eagles submitted to MRCC for rehabilitation between 10 December 2005 and 15 April 2008. Hg, Se, and Pb were analyzed in blood of most rehab eagles (early submissions) but a few also were tested for additional elements plus Hg and Se in feathers. Rehabs included one subadult male eagle captured and sampled during this study, and recaptured alive 30 d later due to electrocution. Most rehabs (72%) were found in southwest Montana, including the Upper Missouri River watershed, and most (62%) rehabs were males. The most numerous plumage class represented was subadult (52%); 17% were adults, 17% immatures, and 14% juveniles. Most (75%) died or were euthanized. Two eagles banded

as nestlings in Montana were among the rehab sample. No others were known to have bred or hatched in Montana.

Mercury, Selenium, and Lead in Tissues. Hg, Se, and Pb were detected in the blood of all Bald Eagles sampled (Table 1). Concentrations of Hg, Se, and Pb in blood (hereafter HgB, SeB, and PbB, respectively) of nestlings did not differ between 2006 and 2007 ($P > 0.37$) nor between 2006–07 and 2007–08 ($P > 0.22$) in captured eagles and no trends were detected in HgB, SeB, or PbB relative to date of admittance for rehab eagles. Thus, years were pooled for further analysis. HgB was lowest in nestling eagles ($P < 0.01$) but did not differ between captured and rehab eagles ($P = 0.79$). SeB did not differ among all groups ($P > 0.78$). PbB was lower in nestling eagles than in captured and rehab eagles ($P < 0.01$) and PbB of captured eagles was higher than that of rehab eagles ($P = 0.05$; Fig. 1). HgB and PbB were lower in nestlings than all other plumage classes of wild eagles ($P < 0.01$), but no differences in HgB, SeB, or PbB associated with plumage class were detected in captured eagles ($P = 0.71$; Fig. 2, top). In rehab eagles, PbB of immatures was lower than that of juveniles ($P = 0.04$) but not subadults or adults and no differences in HgB or SeB among rehab plumage classes were detected ($P > 0.21$; Fig. 2, bottom). Both HgB and PbB declined as capture date progressed (Fig. 3), but SeB remained stable across all seasons. PbB of captured eagles was higher in males (geometric mean = 0.34 ppm) than in females (geometric mean = 0.21 ppm; $P = < 0.007$) but no difference between sexes was detected for HgB, SeB, or PbB in rehab eagles ($P = 0.411$).

Proportion of eagles with PbB > 0.2 ppm (above background; Fig. 4) differed among groups ($\chi^2 =$

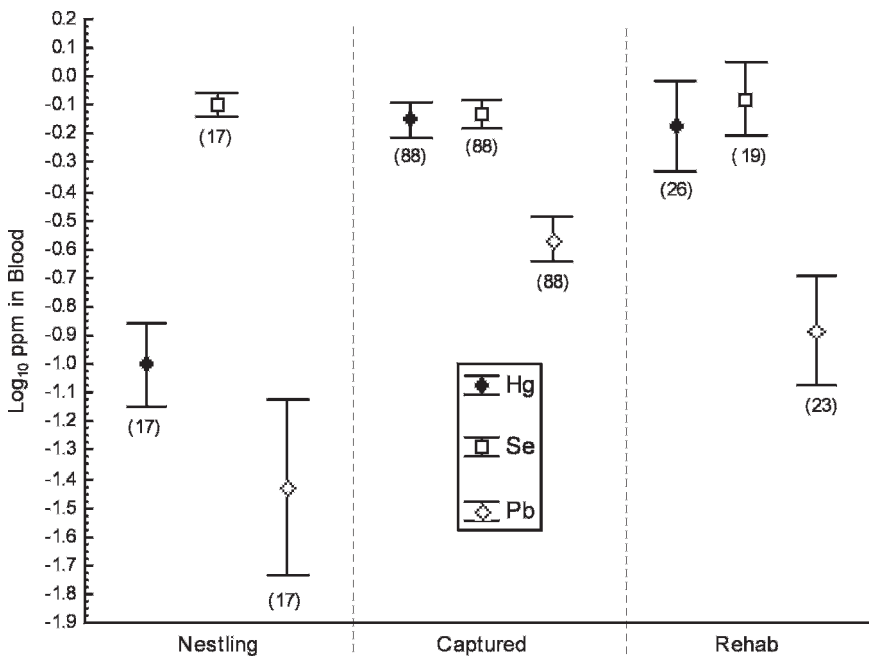


Figure 1. Mean (point) and 95% confidence interval (whisker) of mercury (Hg), selenium (Se), and lead (Pb) concentrations (wet wt.) in blood of nestling, captured, and rehabilitated (rehab) Bald Eagles in southwestern Montana, 2006–08.

26.5, $P < 0.001$). No nestling or rehab eagle, but 9% of captured eagles had PbB above toxic threshold, although none appeared sick, debilitated, or injured. Mild-to-severe symptoms that might have been attributable to heavy metal poisoning (Gilsleider and Oehme 1982) especially from Pb (Locke and Thomas 1996), were recorded for 60% of rehabs, but the severity of symptoms was not correlated to HgB, SeB, or PbB (R. Key pers. comm.). Symptoms included most or some of a suite of symptoms including drooping wings and head, inability to stand, clenched toes, tremors, discolored (green) excreta, unresponsiveness, half-closed eyelids, depression, foul-smelling breath, nonregenerative anemia, vomiting, diarrhea, ataxia, blindness, and epileptiform seizures (Kramer and Redig 1997). HgB, SeB, or PbB of rehab eagles that died or were euthanized ($n = 17$) did not differ ($P > 0.132$) from those of birds that were eventually released ($n = 6$).

HgB and SeB were positively correlated in nestlings and captured eagles (Fig. 5), but not in rehabs ($n = 19$, $r = 0.34$, $P = 0.157$). SeB was negatively correlated with PbB in captured eagles ($n = 88$, $r = -0.26$, $P = 0.016$) but not in nestlings ($n = 17$, $r = -0.05$, $P = 0.851$) or rehab eagles ($n = 17$, $r = 0.178$, $P = 0.494$).

Hg concentrations in feathers (“HgF”) and Se concentrations in feathers (“SeF”) were detectable in all eagles tested (Table 1). HgF and SeF in nestlings were both higher in 2006 than in 2007 ($P < 0.03$). Eagles captured in 2006–07 may have had lower ($P = 0.054$) HgF (geometric mean = 10.0 ppm) than eagles captured in 2007–08 (geometric mean = 14.9 ppm) but SeF did not differ between years (geometric means = 1.44, 1.58 ppm, $P = 0.06$). HgF and SeF in younger plumage classes of wild eagles were different than older plumage classes ($P < 0.01$) and concentrations tended to increase with age (Fig. 6). No difference in HgF and SeF among plumage classes of rehab eagles were evident, nor were there differences in HgF and SeF between sexes for captured eagles ($P > 0.150$) or rehab eagles ($P > 0.541$).

HgB was positively correlated with HgF in nestlings and captured eagles ($r > 0.45$, $P < 0.001$) but not in rehabs ($r = 0.29$, $P = 0.454$). SeB and SeF were correlated for captured eagles ($r = 0.25$, $P = 0.02$) but not for nestlings or rehabs ($r = 0.23$, $P = 0.55$).

Historical Comparisons. HgB and PbB of eagles captured in autumn of 2006 and 2007 in southwestern Montana (Table 1) were higher ($P \leq 0.05$) than

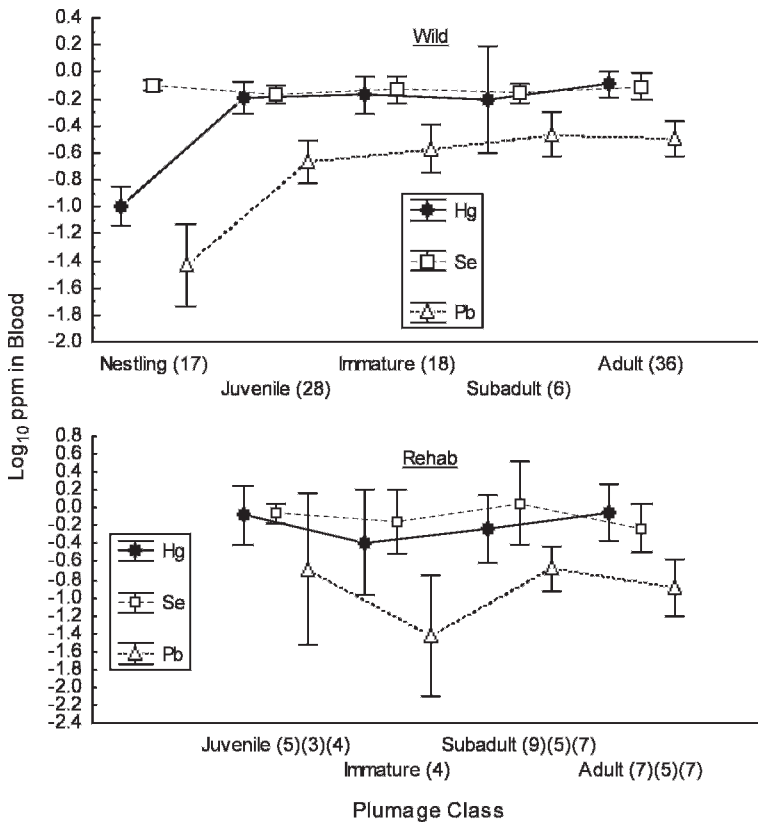


Figure 2. Mean (point) and 95% confidence interval (whisker) of mercury (Hg), selenium (Se), and lead (Pb) concentrations (wet wt.) in blood of wild (top) and rehabilitated (bottom) Bald Eagle plumage classes sampled in southwestern Montana, 2006–08. Unequal sample sizes for respective Hg, Se, and Pb in rehabs follow plumage class labels.

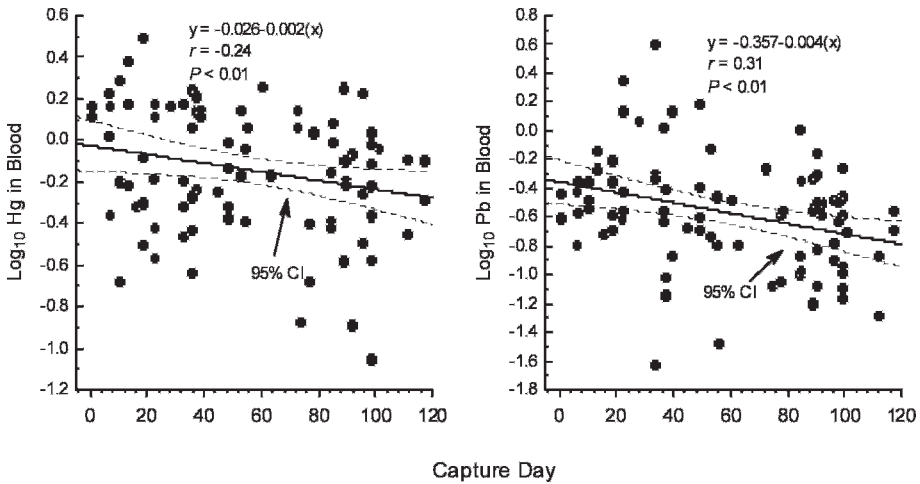


Figure 3. Concentrations (ppm wet wt.) of mercury (Hg) and lead (Pb) in blood of Bald Eagles captured in southwestern Montana by advancing date of capture. Data were pooled for both December–April 2006–07 and 2007–08. Capture Day 1 was December 1 for both years.

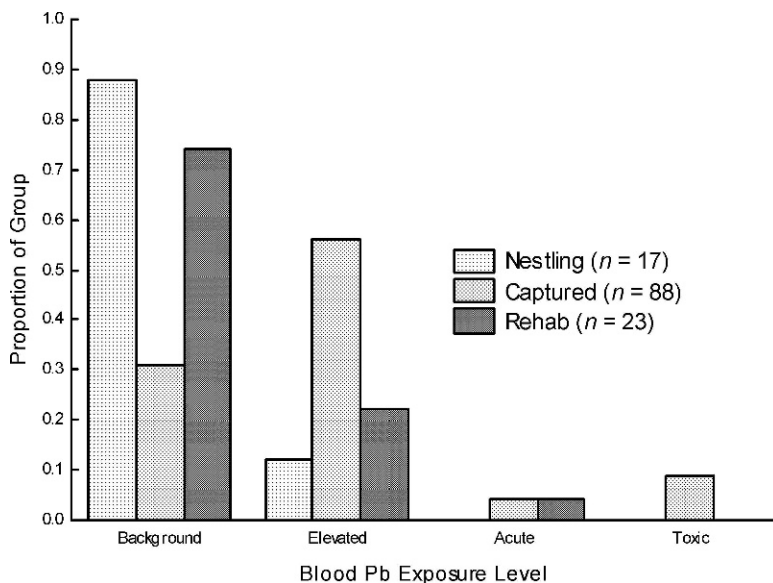


Figure 4. Exposure levels of lead (Pb) in blood of Bald Eagle sample groups in southwestern Montana, 2005–08. Background = <math><0.2</math> ppm wet wt., elevated = $>0.2 \le 0.6$ ppm, acute = $>0.6 \le 1.0$ ppm, toxic = >1.0 ppm.

those of autumn migrants captured at Hauser Lake in Montana in the early 1990s (Hauser Lake HgB geometric mean = 0.50 ppm; PbB geometric mean = 0.256; M. Restani and A. Harmata unpubl. data; Fig. 7). SeB did not differ (Hauser Lake: geometric mean = 0.609 ppm). Geometric mean and maximum concentration of HgB in vernal migrants captured in southwestern Montana from 2006–08 (Table 1) were virtually identical to those of vernal migrants captured in central Montana between 1987 and 1995 (0.54, 1.7 ppm, respectively; Harmata and Restani 1995) and detection rates were not different (100% vs. 94%; $P = 0.52$). Vernal migrants captured in southwestern Montana between 2006–08 had similar geometric mean SeB (0.59 ppm), detection rate (100%), and maximum detected concentration (3.17 ppm) as vernal migrants captured by Harmata and Restani (1995) between 1987 and 1995 (0.55 ppm, 94%, 2.8 ppm, respectively). Geometric mean PbB of vernal migrants captured in southwestern Montana in the late 2000s (Table 1) was lower than that reported for vernal migrants captured in central Montana between 1987 and 1995 (0.32 ppm; Harmata and Restani 1995), but detection rate was similar (97%).

HgB and SeB of five Bald Eagles were measured both at the time of the original banding and at a subsequent encounter (Table 2). Time between samplings ranged from 5 mo to 18 yr and all en-

counters occurred within 57 km of original capture sites. No trends in contamination were evident among eagles that were encountered after banding.

Other Blood Contaminants. Other chemical elements if detected in blood of Bald Eagles were at low concentrations (Table 3). No captured eagle exhibited signs of toxicity or teratogenic or mutagenic effects. Only one nestling exhibited signs of morbidity, but no blood was drawn from this bird due to its debilitated condition. Symptoms of toxicity manifest in rehabs were attributed to heavy metals and were likely not influenced by other elements detected. No differences in blood concentrations of other chemical elements (Table 3) among groups were detected ($P > 0.05$).

DDE residues in blood were detected in 36% of nestlings and nearly 60% of captured eagles tested (Table 3). No differences were found in DDE concentrations or detection rates among seasonal groups of captured birds.

DISCUSSION

Mercury. The primary impetus for initiation of this study was concern that Bald Eagles in southwestern Montana may have been exposed to Hg at levels that would affect survival and reproduction of the local population. Reproduction is the most sensitive endpoint of Hg toxicity in birds (e.g., Toschik et al.

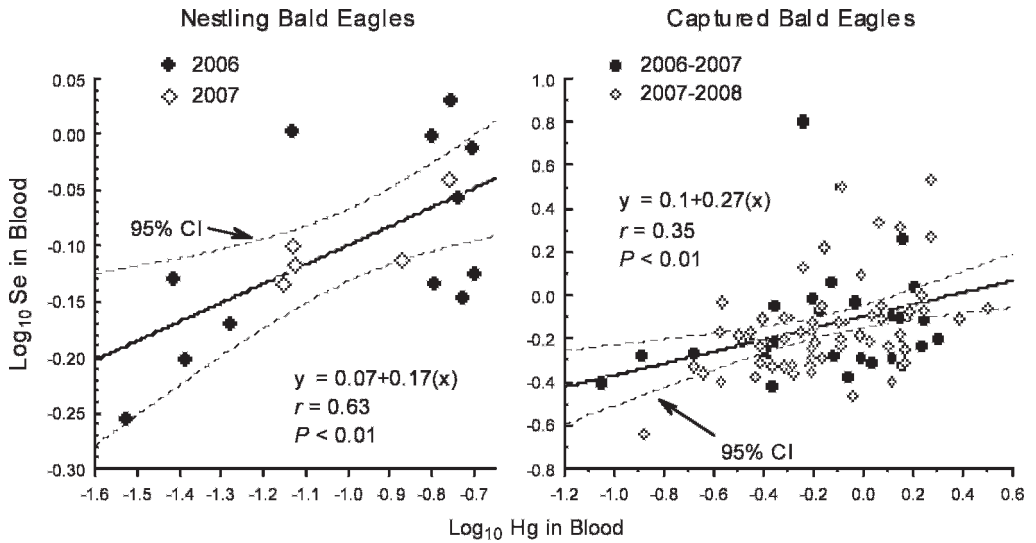


Figure 5. Relationship of selenium (Se ppm wet wt.) and mercury (Hg ppm wet wt.) in blood of nestling (left) and captured (right) Bald Eagles in southwestern Montana, 2006–08.

2005, Sheuhammer et al. 2007, Burgess and Meyer 2008). Concentrations of Hg in blood of nestlings in Montana were similar to those in nestlings in Florida (geometric mean = 0.13, $n = 48$) that Wood et al. (1996) considered to be “baseline” and lower than those in Maine (0.53), where DeSorbo and Evers (2005) felt Hg did not have a major impact on reproduction.

Deposition of Hg in developing feathers is important in excretion of total body burdens (Furness et al. 1986, Braune and Gaskin 1987, Wolfe et al. 1998). Feather concentrations may be more representative of Hg in the local environment in summer, where all feather development occurred in a localized area (i.e., nest site) over an extended period. Bowerman et al. (1994) considered HgF in Bald Eagles of the Great Lakes (geometric mean = 21.1 ppm, range: 3.6–48) as merely “elevated.” Odsjö et al. (2004) found geometric mean concentration of HgF in juvenile Osprey (*Pandion haliaetus*) in Sweden was 5.25 ppm dry weight, which they considered low. Wood et al. (1996) considered geometric mean HgF of 3.23 (ppm wet wt; $n = 61$) in Florida Bald Eagle nestlings as “background.” HgF in Montana nestlings were well below concentrations found in these studies and although nestlings displayed 30-fold more HgF than HgB, this difference was intermediate among several studies in the continental U.S. where Bald Eagle production has not been affected (Weech et al. 2006). Hg is clearly

not limiting reproduction of Bald Eagles in Montana. The Bald Eagle nesting population in Montana has grown from 19 nesting pairs in 1980 (Flath et al. 1991) to nearly 500 pairs in 2009 and continues to grow at about 10% annually (Hammond 2010).

Montana nestlings and captured juveniles were essentially the same plumage/year class but HgB of nestlings were much lower than those of juveniles. However, HgF of nestlings and juveniles were not different (Fig. 7), suggesting similar contamination profiles. Differences in contaminant concentrations in blood between these age classes may be more a reflection of time-trend in bioaccumulation rather than geographical origin, as juveniles were sampled 5 to 6 mo later in life than nestlings. Breast feathers of most eagles were probably developed in summer/nesting grounds. Higher HgB in juveniles than nestlings may be indicative of Hg accumulated in juvenile natal areas during summer and sequestered in other tissues (liver, kidney), then mobilized in migrant eagles under food or physiological stress as a result of rigors of migration. Declining HgB from autumn to spring in captured eagles (Fig. 4) suggested that food items were more contaminated with Hg farther north, or at least earlier in late summer/early fall than in spring.

Atmospheric aerosols now are considered to be the primary mechanism by which Hg contaminates aquatic environments on which Bald Eagles depend

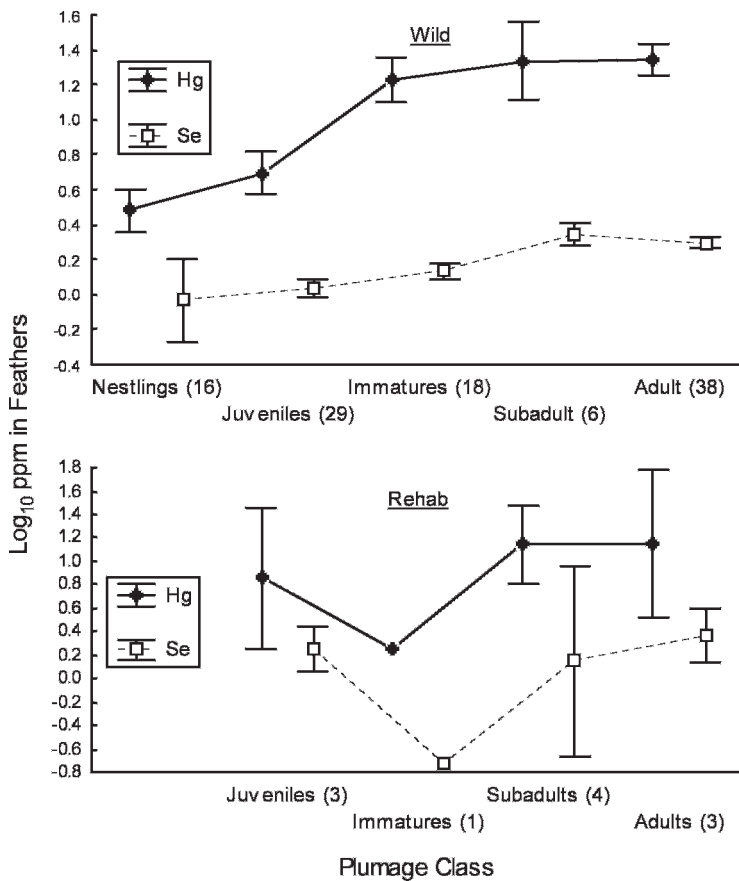


Figure 6. Mean (point) and 95% confidence interval (whisker) of mercury (Hg) and selenium (Se) concentrations in feathers of plumage classes of wild (top) and rehabilitated (bottom) Bald Eagles sampled in southwestern Montana, 2006–08.

(Engstrom et al. 1994, Fitzgerald et al. 1998, 2005, Hammerschmidt and Fitzgerald 2006, Lamborg et al. 2002, Wiener et al. 2006). Although anthropogenic sources such as mine effluents, burning of fossil fuels, and cement production contribute to atmospheric loads (Pacyna et al. 2010), recent focus has been on the role of natural and human-caused wildfires in deposition of Hg in aquatic systems (Friedli et al. 2001, 2003, Turetsky et al. 2006). Large, intense forest fires in the continental U.S. (e.g., Yellowstone fires of 1988) and Canadian boreal forest (Witt et al. 2009) release Hg into the atmosphere not only from trees consumed, but especially peat that absorbed disproportionate amounts of atmospheric Hg emitted during the industrial age (Grigal 2003, Friedli et al. 2003, Turetsky et al. 2006). Higher HgB of eagles captured in southwestern Montana early in the 21st century

as compared to those captured late in the 20th century (Fig. 7) suggest environmental Hg may be increasing. Advancing global climate change and associated desiccation and ignition of temperate and boreal forests (Sigler et al. 2003), exacerbated by extensive clear-cutting (Garcia and Carignan 1999, 2000) and projected increases of industrial effluents (Pacyna et al. 2010), may intensify poisoning of aquatic ecosystems with Hg, hence the need for continued, periodic monitoring.

Selenium. Se-induced mortality has been documented in waterfowl, but effects are primarily teratogenic or manifested in reduced natality or productivity (Eisler 1985, Ohlendorf et al. 1986, Spallholz and Hoffman 2002). No nestling, captured, or rehab Bald Eagle exhibited any physical symptom of Se poisoning. Se concentrations in tissues were similar for all groups of eagles (Fig. 1, 2,

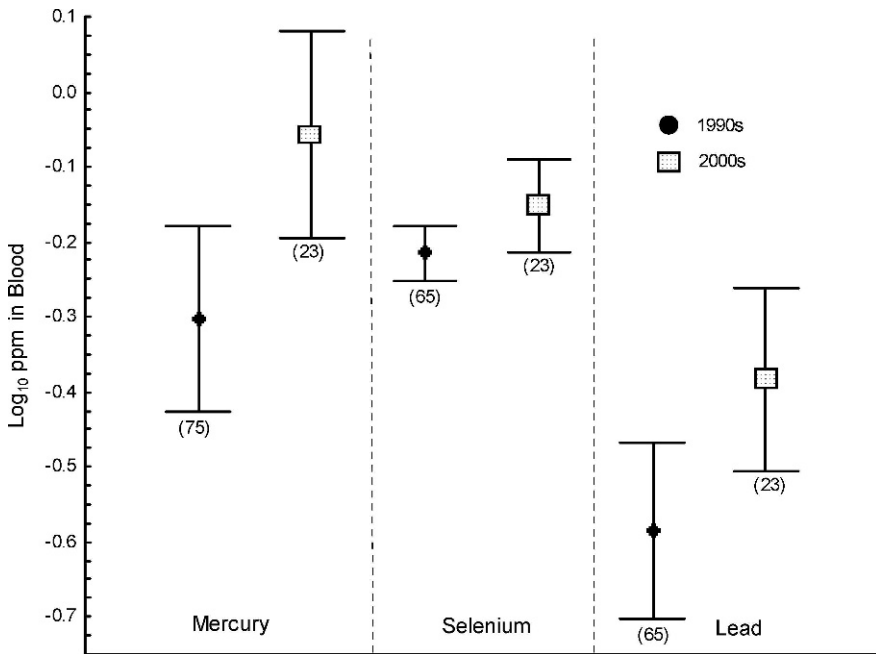


Figure 7. Mean and 95% confidence interval (whiskers) of mercury, selenium, and lead in blood of Bald Eagles captured in autumn at Hauser Lake, Montana, in the early 1990s (dot; M. Restani and A. Harmata unpubl. data) and in autumn in southwestern Montana in the late 2000s (box).

5, 6) and it was unlikely that these concentrations represent anything more than benign background or even beneficial levels. Se is considered efficacious for detoxifying Hg (Yoneda and Suzuki 1997, Odsjö et al. 2004) in organisms and Hg toxicity in birds may be highly dependent upon the availability of dietary Se (Weech et al. 2003). Strong correlations of HgB and SeB of nestling and captured eagles (Fig. 5) may indicate an active, antagonistic metabolic process with Hg, as additional Se is absorbed

to mitigate the effect of Hg (Rudd et al. 1980, Eisler 1985, Chen et al. 2006). Lack of correlation of HgB and SeB in rehabs and dissimilar trends in HgF with ostensibly healthy eagles (nestlings and captured) may indicate a malfunction of the detoxification mechanism or lack of Se in food or water available to rehabs.

Se is present at high concentrations in some arid areas of the western U.S. (Crampton and Harris 1969). Dissolution of Se from soils and accumula-

Table 2. Mercury (Hg), selenium (Se), and lead (Pb) concentrations (ppm wet wt.) in blood (B) and feathers (F) of Bald Eagles at initial banding and subsequent encounter in southwestern Montana.

| BAND | AGE ¹ | DATE | BANDING | | | | | ENCOUNTER | | | | | | | |
|-------|------------------|-------|---------|------|------|------|------|-----------|-------------------|---------|------|------|------|------|------|
| | | | Hg | | Se | | Pb | DATE | TIME ² | WHY | Hg | | Se | | Pb |
| | | | B | F | B | F | B | | | | B | F | B | F | B |
| 23653 | Nest | 6/07 | 0.07 | 1.40 | 0.74 | 0.21 | 0.12 | 6/08 | 1.2 yr | Impact | 0.49 | 13.7 | 0.75 | 1.70 | 0.30 |
| 23655 | Nest | 6/07 | 0.17 | 5.69 | 0.91 | 0.29 | 0.02 | 10/07 | 5 mo | Impact | 1.32 | 4.75 | 0.88 | 1.53 | 0.01 |
| 32087 | Juv | 3/91 | 0.80 | | ND | | 0.20 | 2/08 | 18 yr | Capture | 0.62 | 41.7 | 0.52 | 1.82 | 0.70 |
| 37359 | Juv | 11/92 | 0.57 | | 0.32 | | 0.12 | 3/07 | 16 yr | Capture | 0.86 | 32.8 | 0.42 | 1.74 | 0.31 |
| 00030 | Ad | 1/08 | 1.40 | 32.2 | 0.66 | 2.2 | 0.75 | 3/08 | 2 mo | Electro | 0.92 | | 0.35 | | 0.20 |

¹ By plumage.

² Time from banding to encounter.

Table 3. Concentrations (ppm wet wt.) of dichlorodiphenyldichloroethylene (DDE) in sera and trace elements (see Methods for anagram definition) in whole blood of Bald Eagles sampled in southwestern Montana, May 2006–April 2008. Bold values are geometric means, otherwise arithmetic means are presented. ND = None Detected. Beryllium and 17 other organochlorines (see Methods) were also tested but not detected. Number detected over number tested is shown parenthetically.

| GROUP (n) | DDE | Sb | As | Cd | Cr | Ni | Tl | V |
|---------------------|---------------------------|---------------------------|--------------|----------------------|---------------------------|---------------------------------|---------------------------------|---------------------------|
| Nestlings (17) | 0.006 (4/11) ¹ | ND (17) | 0.005 (3/17) | ND (17) | 0.009 (5/17) ² | 0.005 (5/5) ² | 0.004 (17/17) | ND (5/5) |
| Fall migrant (23) | 0.004 (2/6) ¹ | 0.006 (4/23) | 0.004 (8/23) | 0.004 (10/23) | 0.009 (8/23) ² | 0.016 (9/23) | 0.009 (8/23) ² | 0.004 (2/23) ² |
| Wintering (47) | 0.009 (10/14) | 0.009 (1/46) ² | 0.003 (7/46) | 0.003 (7/46) | 0.008 (10/46) | 0.02 (8/46) | 0.008 (9/46) | 0.01 (1/46) |
| Spring migrant (19) | 0.003 (14/14) | ND (17) | 0.002 (4/19) | 0.003 (10/17) | 0.005 (3/17) | 0.025 (9/9) ² | 0.007 (9/9) ² | ND (19) |
| Rehab (31) | | 0.002 (1/1) | 0.002 (1/1) | 0.006 (2/5) | 0.009 (1/1) | ND (0/1) | 0.003 (1/5) | ND (0/1) |

¹ All in 2006.

² All in 2007.

tion in ecosystems is accelerated by irrigation (Eisler 1985). Southwestern Montana is a mosaic of irrigated and dry cropland interspersed with native habitats. The region is most likely high in Se, as demonstrated by relatively common occurrence of milk vetch (*Astragalus* spp.), most species of which are indicators of Se-rich soils (Beath et al. 1939). Geographic regions with low soil Se have higher Hg-bioaccumulation, e.g., northern Canada (Ralston 2005), where at least some eagles in this study probably originated. Seasonal residence in southwestern Montana for Bald Eagles therefore may have therapeutic value because of high concentrations of environmental Se, which may help neutralize effects of Hg acquired in other areas of the continental U.S. or Canada.

Lead. Lead concentrations in Montana eagles were low, surprisingly even for those submitted for rehabilitation (Table 2). Low PbB in nestlings probably reflects the seasonal diet of local Bald Eagles, which mainly feed on fish during the nesting season in southwestern Montana (Swenson et al. 1986). Fish are unlikely to contain sinkers or shot, and less likely than ungulates, waterfowl, or squirrels to contain Pb fragments from rifle or shotgun pellets. Autumn migrants and wintering eagles had geometric mean PbB above background concentrations (Table 1). Autumn concentrations most likely reflect seasonal contamination or residency in areas farther north (see Miller et al. 1998). Eagles captured in autumn likely have more recently arrived from northern latitudes than eagles captured in winter or spring. Lower winter levels suggest elimination or at least less ingestion of Pb in southwestern Montana. Declining detection rates and blood concentrations of toxic elements with season of capture suggest that residency in southwestern Montana may provide food and water less contaminated with Hg and Pb than areas where captured eagles originated. Concentrations of Pb considered toxic according to Kramer and Redig (1997) were found in 9% of captured eagles. Theoretically, they should have been dead, or at least moribund (P. Redig pers. comm., Kramer and Redig 1997), but all appeared healthy and unaffected and plumage was in good condition. However, these apparently healthy eagles may become sick later in the year and simply not be found. No rehab birds exhibited toxic concentrations of Pb (Fig. 4). These metals may be purged from the system prior to recovery, whereas indirect effects or symptoms may linger. Perhaps other preemptive causes of morbidity such as for-

aging ineptness, disease, or injury predispose eagles to suffer toxic effects of metals, or metal presence may be merely an identifiable proximate rather than the ultimate cause of debilitation in rehabs.

Historically, etiology of Pb poisoning in eagles focused on effects of residual Pb shotgun pellets in waterfowl (Pattee and Hennes 1983). As a result, the U.S. and Canada imposed bans on Pb shot for waterfowl hunting by the 1990s in response to large-scale poisonings of eagles. However, concentrations of Pb in blood of Bald Eagles in Montana apparently did not decline. Detection rate (100%) and PbB of autumn migrants (Table 1) captured in southwestern Montana during this study were higher than those of autumn migrants tested in northern Montana before the Pb ban (1980, 1981; <50% and 0.072 ppm; Wiemeyer et al. 1989) and seemingly higher than those of Bald Eagles captured at Hauser Lake in Montana in the early 1990s (Fig. 7). Like HgB, PbB declined in samples from autumn through spring (Fig. 3), indicating eagles were feeding on a less-contaminated food base as seasons progressed.

Recent attention on the etiology of Pb poisoning in eagles has been focused on effects of residual fragments from Pb-core center-fire rifle bullets in big game carcasses (Hunt et al. 2006, Watson et al. 2009). Higher PbB in captured juveniles may reflect this phenomenon. Increased scavenging by inexperienced young birds might expose them to more Pb in carcasses than that available to older eagles more adept at catching live, uncontaminated prey (waterfowl, fish). Perhaps the source of much Pb in Montana eagles always has been residual fragments from rifle bullets (including small caliber rim fire ammunition; Harmata and Restani 1995) in terrestrial nongame such as lagomorphs, ground squirrels (*Spermophilus* spp.), prairie dogs (*Cynomys* spp.), and big game carcasses rather than from shotgun pellets in waterfowl or upland game birds. Continued monitoring of both Pb in eagles and degree of hunter compliance with voluntary Pb-free ammunition campaigns may confirm relationships of Pb contamination and use of Pb-core projectiles.

Organochlorines. DDE (a metabolite of DDT) was the primary contaminant reducing reproductive success of Bald Eagles in North America, with the majority of exposure apparently derived from the avian portion of the diet (Wiemeyer 1991). Low concentrations found in this study reflect the 1972 ban on DDT and subsequent decline in use. Despite low levels of contamination, detection in nestlings, wintering birds, and vernal migrants reflects long-term persis-

tence of the compound. Lowest concentration was detected in a nestling sampled farthest upstream in the Upper Missouri River watershed, and the highest concentration was found in a nestling sampled farthest downstream. A continual decrease in DDE residues can be expected barring unforeseen changes in legalization and use.

Other Trace Elements. Low concentrations and detection rates of trace elements other than Hg, Se, and Pb (Table 3) in eagles during this study indicated that these elements are currently of little concern in population management of Bald Eagles in western North America. However, expense and effort to continue monitoring is minimal, especially when samples for other blood-borne contaminants are obtained. Likewise, once birds are in hand, the collection of feather samples from eagles is minimally invasive, as well as informative, and if the feathers are not immediately analyzed, they can be easily archived for future inspection.

Detection rates of contaminants suggested that Bald Eagles residing in southwestern Montana, regardless of origin, were exposed to a variety of potentially toxic substances. However, low contaminant concentrations in nestling blood and feathers indicated that Bald Eagles in southwestern Montana lived and reproduced in a relatively clean environment. Seasonally resident eagles in Montana have been shown to originate in Canada, the greater Yellowstone ecosystem, Arizona (Hunt et al. 2009), and southern California (Harmata 1992). Analysis of blood and feathers of captured eagles outside the nesting season suggested that nonresident eagles arrived in southwestern Montana more contaminated than resident breeders. Further, declining detection rates and contaminant concentrations in eagles captured as autumn progressed through spring suggested that local environments in southwestern Montana provided clean foods that assisted in reducing overall body burdens of deleterious chemicals and compounds. Although contaminants currently may not be of major concern in the health of local Montana Bald Eagle populations, numbers and mortality rates of rehabs may indicate that other anthropogenic mortality or morbidity pressures, exacerbated by contaminants, may be.

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