Arch. Environ. Contam. Toxicol. 49, 396–402 (2005)
DOI: 10.1007/s00244-005-7063-0

ARCHIVES OF
Environmental
Contamination
and Toxicology

Lead Contamination in American Woodcock (*Scolopax minor*) from Wisconsin

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Received: 25 February 2005 /Accepted: 8 May 2005

Abstract. An initial survey of lead levels in American woodcock (*Scolopax minor*) from Wisconsin was conducted in 1998 using wing bones from hunter-donated woodcock. The results of this initial survey indicated that young-of-year woodcock were accumulating extremely high levels of lead in their bones. Similar collections were made (using steel shot) between 1999 and 2001. The combined results of this collection indicated that 43.4% of young-of-year woodcock (range 1.5–220.0 μg/g dry wt) and 70% of woodcock chicks (range 9.6–93.0 μg/g dry wt) had bone lead levels in the elevated range (>20 μg/g dry wt). Blood samples were collected from chicks at a site considered elevated based on bone lead results (Mead Wildlife Area) and a site considered background (Navarino Wildlife Area). These samples were analyzed for lead concentration and aminolevulinic acid dehydratase activity. The mean blood lead concentrations of woodcock chicks from both sites did not reach levels that are considered elevated in waterfowl (>0.200 μg/ml). However, blood lead concentrations of chicks from the Mead Wildlife Area were significantly higher than lead levels in chicks from Navarino Wildlife Area ($p = 0.002$). Although the ultimate sources of lead exposure for Wisconsin woodcock currently remain unidentified, anthropogenic sources cannot be ruled out. Our results indicate that elevated lead exposure in Wisconsin woodcock is common and begins shortly after hatch.

Populations of American woodcock are declining across the species range, including Wisconsin. Declines are mainly attributed to degradation and loss of suitable breeding and wintering habitat (Kelley 2003); however, exposure to environmental contaminants, such as lead, may exacerbate these trends.

Research in Canada has indicated elevated bone lead concentrations in woodcock. (Scheuhammer *et al*. 1999). Higher bone lead concentrations occurred in birds from areas with acidic soil. Although the exact source of lead exposure was unclear, stable isotope analysis suggested that lead characteristics were not those of leaded gasoline, leaded agricultural pesticides, or from metal mining or smelting.

Lead has no biological function, and the adverse effects of lead exposure are well known. Lead adversely affects all body systems and inhibits enzymes required by all cells, such as aminolevulinic acid dehydratase (ALAD) (Dieter and Finley 1979). Lead modifies the function and structure of the kidney, bone, the central nervous system, and the hematopoietic system. It also produces adverse biochemical, histopathological, fetotoxic, teratogenic, and reproductive effects (Eisler 1988).

Although lethal and sublethal levels of lead in waterfowl are well established, it is unknown whether concentrations of lead observed in woodcock are sufficient to impact woodcock health. The objectives of this study were to determine the extent of lead contamination in young-of-year woodcock and woodcock chicks in Wisconsin, and to explore the potential impacts of sublethal lead exposure on woodcock. Focusing on young of year (<1 year) and woodcock chicks (prefledge) provides the best indication of the situation in Wisconsin.

Materials and Methods

Woodcock Sample Collection

An initial survey of lead concentration in the wing bones of Wisconsin woodcock was conducted in 1998 using woodcock wings donated from hunters throughout the state. A total of 84 individuals (young of year) were collected using lead shot. These young-of-year woodcock were grouped by sex and location (county) and analyzed as 13 separate pools (Figure 1). After reviewing results from this initial collection, woodcock were collected between 1999 and 2001 at specific locations from hunters using steel shot and analyzed individually (Figure 1). Birds were collected a minimum of 2 weeks prior to the regular hunting season (late September through early November) to increase the probability that only locally exposed birds (young of year) would be collected. Woodcock age and sex were determined via plumage characteristics as described by Sepik (1994).

At the time of dissection, the humerus and radius/ulna were excised and excess tissue and cartilage were removed. This process was followed for all woodcock collected, regardless of age or year collected. Liver tissue was collected for analysis and the GI tract was removed.

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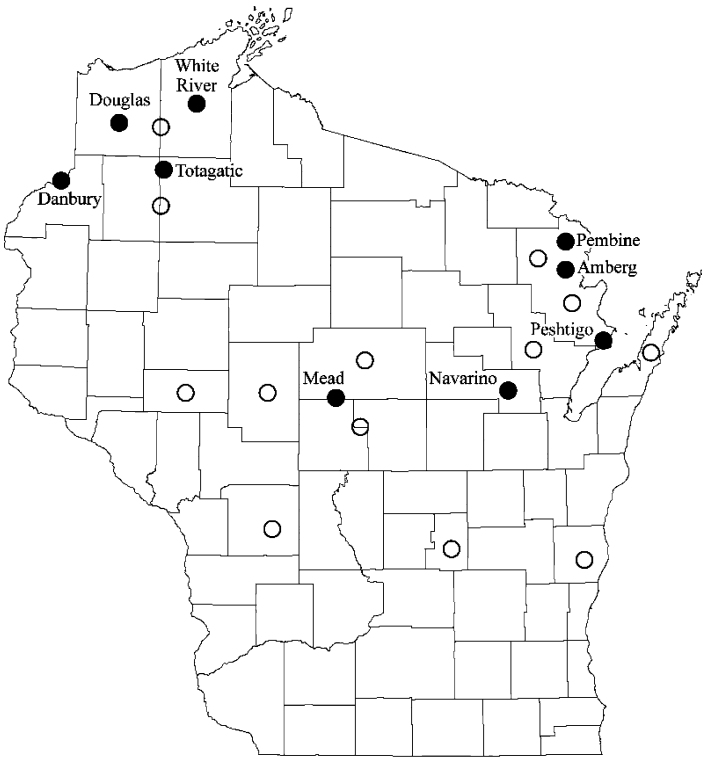

Fig. 1. Woodcock collection sites 1998–2001

The GI tracts of all woodcock, regardless of age, were radiographed at a local veterinary clinic. The radiographs were reviewed for any radiodense objects. The GI tracts were also dissected and visually inspected for lead pellets or metallic particles. Sex of the animal was also confirmed at the time of dissection.

Blood Sample Collection

In 2001, sample collection was restricted to Mead Wildlife Area and Navarino Wildlife Area (Figure 1). These areas were selected based on habitat type, accessibility, concentration of birds, and previous data on bone lead concentrations. Based on previous analyses (Strom *et al.* unpublished data), birds from Mead Wildlife Area were considered to have elevated levels of lead, whereas birds from Navarino Wildlife Area were within background ranges. The brood/chick collections took place between April and May 2001. All federal and state permits were obtained prior to any field collection.

Pointing dogs were used to assist in finding woodcock nests and broods. When a brood was found, one chick was randomly selected and collected by hand for a blood sample, which was obtained via jugular venipuncture with a heparinized syringe. Blood was immediately transferred to a heparinized tube and inverted. Three to four drops of blood were placed in a clean microfuge tube for ALAD analysis and immediately placed on dry ice until transport to a –70°C freezer. Remaining blood was kept cool until transport to the lab for residue analysis. The chick was then euthanized via cervical dislocation for tissue analysis as described above.

Upon return to the lab, capillary tubes were filled approximately half-full with blood for hematocrit measurement. Samples were analyzed in duplicate on a hematocrit centrifuge and recorded as the ratio of red blood cell volume/total volume. ALAD analysis was performed by the Canadian Wildlife Service using the activity ratio method described in Scheuhammer (1987). The ALAD activity ratio is the ratio between activated and nonactivated enzyme activity. The enzyme is reactivated by the addition of factors that remove the effects of any potential inhibitors. A ratio below 1.3 is considered ''background,'' whereas a ratio above 1.3 is considered indicative of lead exposure. A ratio of 2 indicates that activity is only 50% of maximal. A level in this range is indicative of elevated lead exposure and may possibly be associated with other signs of lead poisoning.

Residue Analysis

All lead analyses were conducted at the Wisconsin Veterinary Diagnostic Laboratory, Madison, Wisconsin. Bone samples were forced-air dried overnight at 100°C to remove water, finely crushed in a mortar, and extracted with diethyl ether to remove fat prior to weighing for lead analysis. All sample tissues (bone, liver, and blood) were digested in the same manner. Briefly, samples were weighed into Teflon microwave vessels and digested under pressure with nitric acid in a microwave. After the samples cooled to room temperature, the contents were transferred to a volumetric flask and brought to volume with purified water. Samples were analyzed on a Perkin-Elmer 5100ZL graphite furnace atomic absorption spectrometer. A tissue and/or blood control sample was tested with each analytical run to ensure accuracy. Bone lead concentrations >20 μg/g dry weight were considered to be elevated (Pain 1996). Likewise, liver lead concentrations >1.0 μg/g wet weight and blood lead concentrations >0.200 μg/ml were considered elevated (Pain 1996).

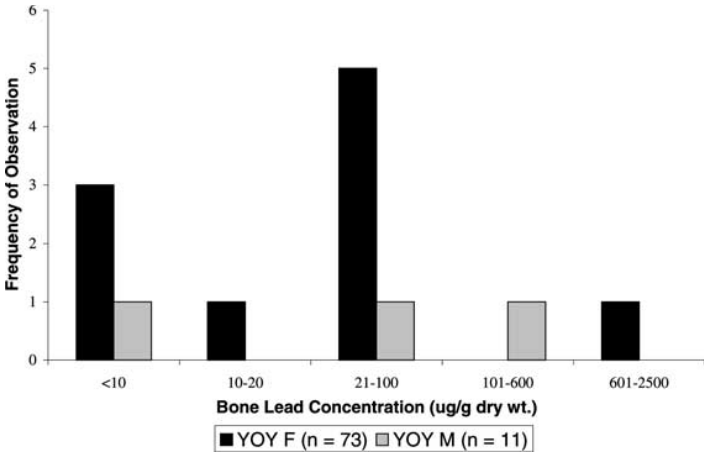

Fig. 2. Frequency of occurrence of lead in wing bone samples, pooled by age, sex and location, in 1998

Table 1. Bone lead concentrations (μg/g dry wt) in Wisconsin woodcock (1999–2001)

Collection site	n	Mean	Median[a]	Standard deviation	Range
Navarino (2000–2001)					
Young-of-year	12	18.4	11.3A	18.0	<3.0–48.0
Chick	7	30.1	27.9A	22.5	9.6–72.0
Mead (2000–2001)					
Young-of-year	13	39.2	29.1A	50.0	4.5–198.0
Chick	3	76.0	87.0B	24.4	48.0–93.0
Peshtigo (1999–2000)					
Young-of-year	6	67.5	40.5A	80.7	6.9–222.0
Pembine(1999–2000)					
Young-of-year	6	18.5	19.7A	11.3	<3.0–33.0
Danbury(1999–2000)					
Young-of-year	5	12.5	5.7A	14.8	4.8–39.0
Douglas (1999–2000)					
Young-of-year	4	10.5	7.4A	8.9	3.9–23.4
White River (1999)					
Young-of-year	2	9.7	9.8A	1.5	8.7–10.8
Amberg (1999)					
Young-of-year	3	38.1	11.7A	50.2	6.6–96.0
Totagatic (1999)					
Young-of-year	2	84.0	84.0A	72.1	33.0–135.0

[a] For each age class, medians sharing the same letter are not significantly different between locations.

Data Analysis

For statistical analyses, samples with metal concentrations below the detection limit (0.02 μg/g in bone, 0.01 μg/ml in blood, and 0.01 μg/g in liver) were assigned a value equal to one-half the detection limit. All statistical analyses were carried out using Excel and SYSTAT software packages. Nonparametric methods (Kruskal-Wallis tests) were utilized to determine differences between tissue lead concentrations from woodcock collected at various sites across the state. The level of statistical significance was set at $\alpha = 0.05$.

Results

Bone

The initial wing collection from 1998 produced a total of 84 samples from young-of-year woodcock, which were then pooled into 13 groups (based on age, sex and location collected). Results of this initial survey (Figure 2) revealed that young-of-year woodcock from areas throughout the state were accumulating significant levels of lead. The results also indicated that many young-of-year woodcock have bone lead concentrations in a range considered elevated in waterfowl (>20 μg/g dry wt). There was one pooled sample that was extraordinarily high (>600 μg/g). It is possible that this sample was contaminated with metallic lead particles as a result of using lead shot for collection.

The results of the initial survey, along with other habitat criteria, were used to select sites for further sample collection. Locations were compared regardless of the year collected. Young of year data revealed no significant differences in bone lead concentrations between any of the areas sampled (Table 1, $p = 0.12$). However, the combined data from all the hunts indicated that 23 of 53 (43%) young-of-year woodcock had bone lead levels in the elevated range.

Table 2. Liver lead concentrations (μg/g wet wt) of Wisconsin woodcock in 2001

Collection site	n	Mean	Median[a]	Standard deviation	Range
Navarino					
Young-of-year	9	0.13	0.11A	0.17	<0.01–0.54
Chick	7	0.16	0.03A	0.21	<0.01–0.47
Mead					
Young-of-year	13	0.28	0.22A	0.31	<0.01–1.05
Chick	3	0.06	0.03A	0.08	<0.01–0.15

[a] For each age class, medians sharing the same letter are not significantly different at $\alpha = 0.05$ between locations.

Table 3. Blood lead concentrations (μg/ml) of Wisconsin woodcock in 2001

Collection site	n	Mean	Median[a]	Standard deviation	Range
Navarino					
Young-of-year	7	0.20	0.1A	0.24	0.054–0.72
Chick	9	0.042	0.034A	0.044	<0.01–0.13
Mead					
Young-of-year	3	0.084	0.69A	0.031	0.064–0.12
Chick	5	0.11	0.096B	0.037	0.072–0.15

[a] For each age class, medians sharing the same letter are not significantly different at $\alpha = 0.05$ between locations.

In 2001, only Mead Wildlife Area and Navarino Wildlife Area were sampled. The decision to focus on these two locations was based on the previous (2000) data in addition to the quality of habitat, known populations of woodcock, and knowledge of the area. During this period, concentrated efforts were made to find woodcock chicks (prefledge). The premise behind this decision was that sampling woodcock chicks would give the best indication of local lead levels. The concentration of lead in the wing bones from Mead chicks was significantly different than those from Navarino (Table 1, $p = 0.03$).

Radiographs of the GI tracts of the woodcock collected did not reveal any lead pellets or metallic particles. Furthermore, no lead pellets or metallic objects were observed during the visual inspection of the GI tracts.

Liver

For young-of-year woodcock, no significant differences were observed between the two different sites (Table 2, $p = 0.21$). Similarly, no significant differences were observed in liver lead concentrations of woodcock chicks between the two study areas ($p = 0.81$). Of all woodcock sampled, only one (a young-of-year from Mead Wildlife Area) had a liver lead concentration (1.05 μg/g wet wt) that would be considered elevated (>1.0 μg/g wet wt).

Blood

There was no significant difference between blood lead concentrations of young-of-year woodcock from Mead and young-of-year woodcock from Navarino (Table 3, $p = 0.42$). Conversely, the median blood lead concentration of woodcock chicks from Mead was significantly different from blood lead concentrations of chicks from Navarino ($p = 0.03$). However, none of the observed concentrations reached a level considered to be elevated (>0.200 μg/ml) in waterfowl.

Hematocrit (Packed Cell Volume)

The median hematocrit of woodcock chicks from the Mead Wildlife Area and Navarino Wildlife Area were not significantly different ($p = 0.62$) (Table 4). The percent change in mean hematocrit between Navarino and Mead woodcock chicks was 7.1%, with no chicks from Mead having significantly reduced hematocrit (had hematocrits below 2 standard deviations of reference mean).

Aminolevulinic Acid Dehydratase (ALAD)

Activity ratios of ALAD were compared to determine whether enzyme activity of blood samples from Mead woodcock was significantly different from Navarino woodcock (Table 4). A significant difference was not found to exist between the ALAD activity ratios of young-of-year woodcock from Navarino and young-of-year woodcock from Mead ($p = 0.54$). Similarly, significant differences were not observed in the ALAD activity ratios of woodcock chicks ($p = 0.12$). None of the woodcock, in either age class, had an activity ratio >1.62.

ALAD activity ratios were plotted against blood lead concentrations for each age class of woodcock in order to determine the accuracy and reliability of the ALAD assay and to determine how well blood lead concentrations predict ALAD values in woodcock. When ALAD activity ratios were plotted against blood lead concentration for woodcock chicks (Figure 3a), a strong, positive correlation was observed ($p = 0.01$, $r = 0.69$). Similarly, for young-of-year woodcock (Figure 3b), a strong, statistically significant relationship was observed between ALAD activity ratios and blood lead concentration ($p = 0.03$, $r = 0.66$). However, two data points influence the relationship. When these outliers are removed from analysis, the relationship was not statistically significant ($p = 0.12$, $r = 0.59$). These data indicate a strong relationship between

Table 4. Blood characteristics of Wisconsin woodcock from the Mead and Navarino Wildlife areas in 2001

	ALAD activity ratio					Hematocrit				
	n	Mean	Median[a]	Standard deviation	Range	n	Mean	Median[a]	Standard deviation	Range
Navarino										
Young-of-year	8	1.26	1.24A	0.19	0.97–1.58	NA	NA	NA	NA	NA
Chick	8	1.21	1.17A	0.18	1.03–1.53	5	36.8	38.0A	7.2	27.0–44.0
Mead										
Young-of-year	3	1.35	1.39A	0.30	1.03–1.62	NA	NA	NA	NA	NA
Chick	5	1.36	1.27A	0.17	1.20–1.54	4	34.2	34.5A	0.96	33.0–35.0

[a] For each age class, medians sharing the same letter are not statistically significant at $\alpha = 0.05$ between locations.
NA = not analyzed.

ALAD activity ratio and blood lead concentration in woodcock chicks. However, this relationship is somewhat inconsistent with young-of-year woodcock. Nonetheless, the general trend suggests that an increase in blood lead concentration is reflected by an increased ALAD activity ratio.

Discussion

Bone Lead

Our study strongly indicates that young of year and woodcock chicks are experiencing significant lead exposure at various locations throughout the state of Wisconsin. Scheuhammer *et al*. (1999) documented a high incidence of elevated lead levels (>20 μg/g dry wt) in wing bones from adult (52%) and young-of-year woodcock (29%) from eastern Canada. Results of the current study are even more intriguing because we have documented elevated lead levels in the wing bones of 43% of young-of-year woodcock and 64% of woodcock chicks sampled. Furthermore, the geometric mean concentration of bone lead in young-of-year woodcock from Wisconsin (17.5 μg/g) was greater than that reported in the Canadian study (11.9 μg/g). The sample size of the current study was much smaller than that of the Canadian study and provides limited statistical power; however, this does not negate the significance of the observed results. Of particular concern was the high mean concentration observed in woodcock chicks (30.1 μg/g). Although our sample size was small ($n = 10$), mean concentrations this high have not been observed elsewhere, and are a cause for concern.

Bone lead concentrations observed in Wisconsin woodcock are comparable to bone lead concentrations found in mourning doves from game management areas (Kendall and Scanlon 1979), and mottled ducks collected during the lead shot era of waterfowl hunting (Merchant *et al*. 1991). However, there are no known studies that have investigated bone lead concentrations in chicks or nestling birds. Lead concentrations in bone tissue of woodcock chicks observed in this study are similar to those observed in waterfowl during the period when lead shot was allowed. Results of the current study suggest that woodcock chicks from Wisconsin have the ability to quickly accumulate high concentrations of lead.

Bone lead concentration is a good indicator of the relative degree of lifetime lead exposure, because lead has a high affinity for mineralized tissue and readily accumulates in bone

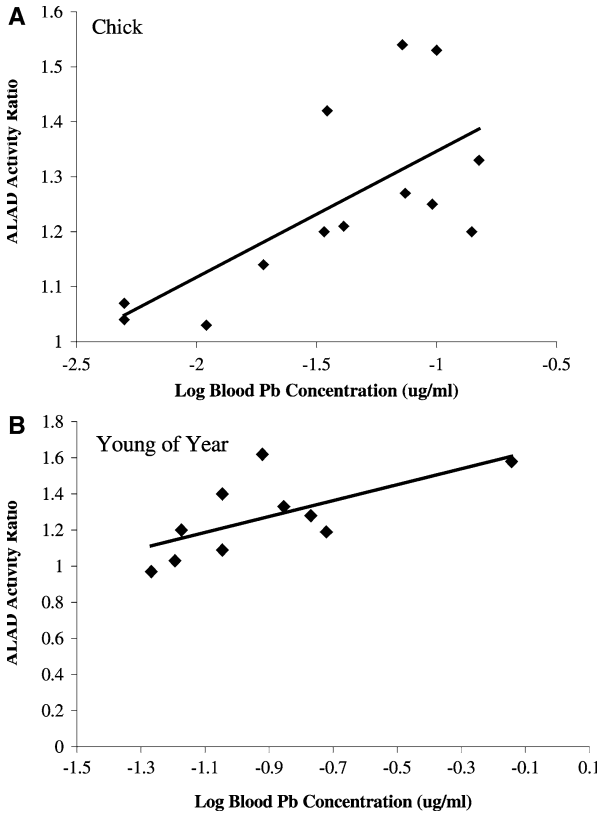

Fig. 3. Relationship between ALAD activity ratio and blood lead concentration (μg/ml) in woodcock chicks (A) and young-of-year woodcock (B)

(Scheuhammer and Dickson 1996). Lead has an extremely long half-life in bone tissue and after exposure, a bird can exhibit an elevated bone lead level for the rest of its life. Theoretically, juvenile birds, unlike adult birds, should have uniformly low bone lead concentrations (<2.0 μg/g) unless recently exposed to lead (Scheuhammer and Dickson 1996). This would suggest that woodcock (especially young of year and chicks) in Wisconsin are being exposed to high levels of lead and/or quickly accumulating lead in their bones.

The actual exposure pathway for Wisconsin woodcock is not clear. Scheuhammer *et al*. (1999) suggest that lead exposure in Canadian woodcock is through the ingestion of lead

shot pellets. Although there are no known documented cases of shot ingestion by woodcock, their feeding behavior and probing habits could make them susceptible to occasional shot ingestion. Ingestion of lead shot has been observed in other species related to woodcock such as godwits, dowitchers, and snipe (Hall and Fisher 1985; Pain 1990). However, all woodcock collected for the current study were radiographed in order to identify any lead shot present in their GI tracts. No lead pellets were identified in any of the woodcock radiographed and sampled. The absence of lead pellets from the GI tracts does not necessarily eliminate lead shot as a potential source, because ingested pellets may be rapidly excreted in birds that do not possess a muscular gizzard.

Liver and Blood Lead

The results of the present study revealed that elevated levels of lead in the wing bones of chicks and young-of-year woodcock were not reflected by similarly elevated levels of lead in either liver tissue or blood. A significant difference was observed between blood lead levels in chicks from Navarino versus Mead. However, the observed blood lead concentrations in these chicks were not at a level considered elevated in waterfowl. The explanation for the elevated levels of lead in woodcock bone combined with low levels of lead in liver and blood is unclear, especially considering the age of the woodcock chicks. It appears that woodcock chicks may have the ability to transfer lead from liver and blood to bone tissue very quickly. More research involving the physiology of woodcock in relation to metal exposure and accumulation is needed to answer such questions.

ALAD

Enzyme activity of ALAD is extremely sensitive to inhibition by lead and may result in a reduction of porphyrin synthesis not only for hemoglobin production, but also for production of respiratory heme-containing enzymes (Allen 1971; Brace and Altland 1956). Furthermore, lead toxicity and inhibition of ALAD in the liver and brain of birds and mammals may affect critical processes such as synthesis of protoporphyrins that support detoxification in the liver (Hoffman *et al*. 1981; Dieter and Finley 1979; Buchet *et al*. 1976). Unfortunately, the biological significance of reduced ALAD activity over a long period of time is not understood.

The value of measuring ALAD activity in avian blood as an indicator of environmental lead exposure has been well established (Strom *et al*. 2002; Blus *et al*. 1995; Grue *et al*. 1984; Kendall and Scanlon 1982). The correlation between blood lead and blood ALAD enzyme activity has been demonstrated for other species (Strom *et al*. 2002; Blus *et al*. 1995; Murase *et al*. 1993; Dieter and Finley 1979; Finley and Dieter 1976). The present study suggests that woodcock ALAD activity ratio may be an accurate and sensitive indicator of environmental lead exposure.

Several studies have utilized the ALAD assay to investigate potential cases of environmental lead toxicity in birds (Custer *et al*. 2003; Strom *et al*. 2002; Grue *et al*. 1984, 1986; Kendall and Scanlon 1982). In a study monitoring sublethal effects in mallard ducks fed low, chronic levels of lead, Finley *et al*. (1976) found that ALAD activity was significantly reduced in ducks when blood lead concentrations approach 100 ng/ml. In another study, wild canvasback ducks exhibited a 75% decrease in ALAD activity when blood lead concentrations exceeded 200 ng/ml (Dieter *et al*. 1976). Similar results occurred in an experimental study where mallards were given sublethal doses of lead shot (Finley *et al*. 1976; Dieter and Finley 1975). Although only a few woodcock had blood lead levels more than 200 ng/ml, numerous woodcock had blood lead levels more than the 100 ng/ml level.

Results indicated that significant differences in ALAD activity ratios did not exist between Mead and Navarino woodcock. There was no significant difference for both age classes of woodcock. Even though significant differences in ALAD activity ratios were not observed, the ratios of several individual woodcock suggest elevated exposure to lead.

The relationship between ALAD activity ratio and blood lead concentration in woodcock is similar to that in other studies. Strom *et al*. (2002) observed highly significant relationships between ALAD activity and blood lead concentration in both adult and nestling American dippers (*Cinclus mexicanus*). The reasons for the inconsistent relationship in young-of-year woodcock are unclear, but the small sample size may have played a role. The data suggest that ALAD activity ratio is a strong, sensitive indicator of lead exposure in woodcock and could be a valuable biomarker to assess the impacts of lead exposure in woodcock.

Source of Lead Contamination

Stable lead isotopes have been used to discriminate between potential sources of lead exposure in several wildlife species. Scheuhammer *et al*. (2003) observed that lead isotope ratios in wing bones of woodcock with elevated lead exposure is consistent with, though not proof of, lead shot ingestion because the range of ratios for wing bones overlapped with ratios of lead shot pellets. The lead isotopic data for Canadian woodcock were not consistent with ratios for either past gasoline combustion or Precambrian mining or smelting, suggesting that these are not the sources. Ingestion of lead shot used for upland game bird hunting cannot be ruled out as the primary source of high bone lead accumulation in woodcock from eastern Canada. Preliminary results of stable lead isotope analysis on a small number of bone samples from Wisconsin young-of-year woodcock produced inconclusive results. The observed ratios in Wisconsin woodcock overlapped with lead isotope ratios of both lead shotgun pellets and Precambrian lead (Strom *et al*. unpublished data). More research is needed to further elucidate the source of elevated lead in Wisconsin woodcock.

Conclusion

The results of the present study carry significant implications. It is clear that young woodcock in Wisconsin are being exposed to an unidentified source of lead and are accumulating lead in bone tissue at levels that may have toxic consequences

for the animals. Although the exact source of lead exposure has not been identified, the data suggest that exposure was occurring locally, and was beginning very early in life. Additional stable isotope or lead speciation analyses may further elucidate the exact source of lead exposure. More detailed research is also needed to better understand the physiology of woodcock as it relates to lead exposure and more specifically, the absorption, distribution, and excretion of lead in woodcock. Likewise, further investigation is also needed to elucidate what impact (if any) elevated lead exposure, as indicated by elevated bone lead levels, has on woodcock.

Acknowledgments. Funding for this study was provided by the Webless Migratory Game Bird Research Program (U.S. Fish and Wildlife), the Wisconsin Department of Natural Resources Wildlife Health Program, and the Ruffed Grouse Society. Brian Beard, University of Wisconsin-Madison, Department of Geology and Geophysics, conducted stable isotope analysis. Thanks to Matt Verdon for his assistance with the manuscript graphics. Dave Zoromski, Wisconsin Veterinary Diagnostic Laboratory conducted all lead analyses. Special thanks go out to all the people who helped with this labor-intensive project and volunteered their (and their dogs') time: John Huff, Brian Dhuey, Rich Wissink, Tom Meier, Ken Blomberg, Missy Sparrow, and Larry Gregg.

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