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The Uptake and Effects of Lead in Small Mammals and Frogs at a Trap and Skeet Range

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Abstract. This study was performed to evaluate the bioavailability and effects of lead in wildlife at a trap and skeet range. The total lead concentration in a composite soil sample (pellets removed) was 75,000 μg/g dry weight. Elevated tissue lead concentrations and depressed ALAD activities in small mammals and frogs indicate that some of the lead deposited at the site is bioavailable. Mean tissue lead concentrations (μg/g dry wt.) in white-footed mice (*Peromyscus leucopus*) at the range liver = 4.98, kidney = 34.9, femur = 245) were elevated ($P < 0.01$) 5- to 64-fold relative to concentrations in mice from a control area. Tissue lead concentrations in the only shorttail shrew (*Blarina brevicauda*) captured at the range (liver = 34.1, kidney = 1506, femur = 437) were elevated 35- to 1038-fold. Femur lead concentrations in green frogs (*Rana clamitans*) at the range (1,728 μg/g) were elevated nearly 1000-fold, and the lead concentration in a pooled kidney sample (96.2 μg/g) was elevated 67-fold. There was significant depression of blood ALAD activity in mice ($P = 0.0384$) and depression of blood and liver ALAD activity in frogs ($P < 0.001$). Hematological and histopathological lesions associated with lead toxicosis were observed in some animals. Hemoglobin concentrations were reduced 6.7% in mice ($P = 0.0249$), but hematocrit was not significantly affected in mice or frogs. Intranuclear inclusions were present in the renal proximal tubular epithelium of two of the mice and the shrew that were captured at the range, and necrosis of the tubular epithelium was also evident in one of the mice. Kidney:body weight ratios were similar in range and control mice. Soil ingestion may be a significant route of lead uptake in small mammals at the range. However, the tendency of lead to concentrate in the bones rather than in more digestible soft tissues may minimize food chain uptake of lead by predators, especially raptors that regurgitate undigestible material.

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Soils and sediments at trap and skeet ranges contain large quantities of spent lead shot (Jørgensen and Willems 1987; Stansley *et al.* 1992). Once shot is deposited on soil, the pellets are slowly oxidized, resulting in the release of lead compounds into the soil (Jørgensen and Willems 1987). Manninen and Tanskanen (1993) reported little uptake of lead by plant foliage at a shooting range with soil pH of 5.6–5.9. However, Ma (1989) reported strongly elevated tissue lead concentrations in both herbivorous and carnivorous small mammals at an abandoned shooting range with acid soils (pH 3.9). Shrews (*Sorex araneus*) and bank voles (*Clethrionomys glareolus*) from the range also had increased kidney:body weight ratios, a possible indication of lead poisoning (Goyer *et al.* 1970).

At some ranges, shot may be deposited into surface waters or wetlands. The hazard to feeding waterfowl posed by spent lead shot has long been recognized (Bellrose 1959), and lead poisoning has been reported in northern pintail ducks (*Anas acuta*) and Canada geese (*Branta canadensis*) feeding at trap and skeet ranges (Roscoe *et al.* 1989, 1990). Elevated lead concentrations have also been reported in surface waters at trap and skeet ranges (Stansley *et al.* 1992), although the potential hazards to aquatic biota in water contaminated with lead shot have not been studied.

The current paper describes a study of small mammals and frogs at a trap and skeet range. Tissue lead concentrations, kidney:body weight ratios, and hematological and histopathological findings are presented. The potential for lead uptake via soil ingestion is also discussed.

Materials and Methods

Study Site and Sample Collection

The study site, a trap and skeet range that has been in continuous operation for at least 30 years, consists of a mixture of wooded upland and freshwater wetland habitats. The shot density in the upper 7.5 cm of soils/sediments in water-covered areas at the range (3.7×10^9 pellets/ha) was the highest of eight ranges sampled in a previous survey (Stansley *et al.* 1992). White-footed mice (*Peromyscus leucopus*) and

shorttail shrews (*Blarina brevicauda*) were live-trapped from the shot fall zone and a nearby control area. Green frogs (*Rana clamitans*) were collected by hand. Collection of blood samples was attempted from those animals that were alive upon return to the laboratory. Small mammals were weighed to the nearest 0.1 g and blood was collected in heparinized tubes following decapitation. An aliquot of blood was removed for hematocrit and hemoglobin determination and the remaining blood was frozen in sealed microhematocrit tubes at −40°C for determination of delta-aminolevulinic acid dehydratase (ALAD) activity. The right kidney was removed and placed in 10% buffered neutral formalin and the liver and left kidney were weighed to the nearest 0.1 mg and frozen. The kidney:body weight ratio was calculated as (kidney weight/body weight) ×100. One femur was also removed and frozen for lead analysis, and the animal's sex was recorded.

The head-body length of the frogs was determined and blood was collected in heparinized microhematocrit tubes by cardiac incision following brain pithing. The livers were removed and frozen at −40°C for determination of ALAD activity. One kidney was removed from each of four frogs from the shot fall zone and five frogs from the control site. The kidneys were bisected, one half preserved in formalin and the other in 3% glutaraldehyde. The remaining kidney from each frog was frozen for lead analysis. The sex and length of each animal was recorded, the carcass was frozen, and a femur was dissected later for lead determination.

Soil samples were collected along the trapline transects in the shot fall zone and the control area. Thirty soil samples were taken at each site to a depth of 7.5 cm (excluding the litter layer) and composited. The composite samples were screened through a 1.0 mm plastic sieve prior to analysis to remove lead shot.

Tissue Lead and Soil Chemistry Analyses

Small mammal kidneys and portions of liver weighing 0.15 g (wet wt.) were analyzed individually. Frog kidneys were pooled. Tissue samples were dried overnight at 103°C, digested in nitric acid, and analyzed by flameless atomic absorption spectrophotometry. All lead concentrations are expressed on a dry weight basis. Digestion blanks and samples of oyster tissue standard reference material (National Institute of Standards and Technology, Bethesda, MD) were included in each batch of samples. Significant lead contamination was detected in two blanks. The results for samples associated with these blanks (4 liver samples and 3 kidney samples) are not reported. The mean lead concentration determined in 14 samples of the standard reference material was 0.44 μg/g (SD = 0.08). The certified concentration is 0.371 ± 0.014 μg/g. The mean percent recovery for 11 spiked digests was 111%.

Femurs were dried overnight at 103°C in glass culture tubes, digested in nitric acid, diluted with distilled water, and analyzed by flameless atomic absorption spectrophotometry. Digestion blanks and samples of bone meal standard reference material were also analyzed. Lead was below the detection limit in all digestion blanks. The mean lead concentration determined in 8 samples of the standard reference material was 1.24 μg/g (SD = 0.12). The certified concentration is 1.335 ± 0.014 μg/g. The mean percent recovery for 7 spiked digests was 102%.

Total lead, pH, and total organic carbon in soil samples were determined by standard EPA methods (*USEPA* 1990).

Body Burden Estimation and Soil Ingestion Model

The body burden of lead in adult white-footed mice was estimated by summing the lead burdens in bone, kidney, and liver, the primary tissues for lead accumulation (Talmage and Walton 1991). Contributions from other tissues were assumed to be negligible. Lead concentration in the femur was assumed to be similar to concentrations in other bones. The proportional weight of the skeleton of the house mouse (*Mus musculus*; 8.4% of body weight) reported by Welcker and Brandt (1903) was used to estimate total bone mass.

The potential importance of lead uptake via the ingestion of soil at the range was examined using a simple uptake model that does not include lead uptake from food or water or lead uptake prior to reaching adult size. The animals were assumed to feed at the site 100% at the time. The rate of soil ingestion (expressed as a percentage of the total diet) necessary to account for the mean body burden of lead in adult mice was calculated as follows:

$$SI = [BB/(FI \times Pb_S \times A_{Pb} \times T)] \times 100$$

where: SI = soil ingestion (% of diet), BB = lead body burden (μg), FI = food intake (g dry wt./day), Pb_s = soil lead concentration (μg/g dry wt.), A_{Pb} = fraction of ingested lead absorbed, and T = exposure time (days).

Food intake (4.6 g/day) is the mean value calculated from data presented by Sealander (1952). The fraction of ingested lead that is absorbed (0.0065) is based on data for Wistar rats that received large doses of lead in food (Barltrop and Khoo 1975). Since complete turnover probably occurs annually in white-footed mouse populations (Lackey *et al.* 1985), a maximum life span of one year was assumed. The exposure period (300 days) for adult mice was calculated as the life span minus the time required to attain adult size (~40–70 days, Lackey 1978).

Kidney Histopathology

Formalin-preserved kidney tissues were processed, embedded in paraffin, sectioned at 6μm, and mounted on glass slides. Two tissue slides were prepared for each sample. Tissue sections on one set of slides were stained with hematoxylin and eosin (H&E), and the other set was stained with the Ziehl Neelsen acid fast procedure at the Northeastern Research Center for Wildlife Diseases (NERCWD), University of Connecticut, Storrs, CT. The slides were examined under light microscopy.

Glutaraldehyde-preserved frog kidney tissues were submitted to the NERCWD for electron microscopic examination. The magnification of electron micrographs ranged from 7,000 to 28,750 ×.

Hematology and Liver ALAD Assays

Dried blood smears from mice and frogs were fixed and stained using the LeukoStat Stain Kit (Fisher Scientific, Orangeburg, NY) and examined by light microscopy. At least two thousand red blood cells were examined under oil immersion (1000 ×) for each animal. Suitable blood smears were not obtained from shrews.

Hematocrit was measured on a microhematocrit capillary tube reader following 5 min of centrifugation on a clinical centrifuge. Because only a small amount of blood was available from some small frogs, hematocrit and ALAD activity were determined from the same sample. Immediately after determining the hematocrit, the open end of the capillary tube was sealed and the blood was frozen at −40°C. Hemoglobin concentrations were measured to the nearest 0.5 mg/dl on a hemoglobinometer.

Blood ALAD activity in mice was measured before and after reactivating with dithiothreitol (DTT) by the method of Granick *et al.* (1973). Freeze/thawing was used to hemolyze blood samples because of analytical interference caused by Triton X-100. The ALAD activity in frozen blood samples was stable for at least four weeks. ALAD activity in frog blood was determined by modifications to the method of Hodson (1976). Unless otherwise noted, all reagents were prepared as described for the original method. Microhematocrit tubes containing the previously centrifuged blood samples were thawed and the blood volume determined by calculation from the length of the liquid column and

the inside diameter of the tube. The accuracy of the volume calculation method was verified by comparison with gravimetric determinations using water-filled tubes. Blood volumes used for the assays ranged from 44 to 58 µl. Each blood sample was dispensed into a microcentrifuge tube containing sufficient substrate solution (containing 0.5% Triton X-100) to achieve a total volume of 360 µl. The tube was thoroughly mixed and a 120 µl aliquot was transferred to a blank tube containing 300 µl of TCA solution. After incubating for 2 h at 25°C, the reaction was stopped by adding 600 µl of TCA solution to the sample tube, and the tubes were centrifuged for 5 min at 2500 × g. The amount of porphobilinogen produced in the reaction was determined as outlined in the original method using one 350 µl aliquot of blank supernatant and two 350 µl aliquots of sample supernatant.

Liver ALAD activity in frogs was determined by modifications of the method of Hodson (1976). Frog livers were homogenized in 0.1 M, pH 6.2 phosphate buffer at a rate of one mL of buffer per 100 mg of tissue. One hundred µL aliquots of homogenate were added to three tubes containing 100 µL of substrate solution (without Triton X-100). A 300 µL aliquot of TCA solution was added to one tube for use as a blank, and the sample and blank tubes were incubated at 25°C for two h. The amount of porphobilinogen produced was determined as outlined in the original method. Protein concentration in the liver homogenates was measured using a commercially available kit (total protein micro Lowry method, Sigma Chemical Co., St. Louis, MO).

Statistical Analyses

All statistical analyses were performed using Statistical Analysis Software for Personal Computers (*SAS* 1987). White-footed mice were roughly divided into adult and non-adult (sub-adult and juvenile) age groups based on body weight. Animals ≥16 g were classified as adults, based on site-specific body mass criteria used to define adults in *Peromyscus* populations in four locations in the eastern and central United States (15–17 g, Krohne *et al.* 1988). Tissue lead concentrations and blood parameters in white-footed mice from the range and control site were analyzed by three-factor analysis of variance (PROC GLM; factors = site, sex, and age group) in order to account for the possible effects of sex- and age-related differences on between-site comparisons.

The relationships between tissue lead concentrations and blood parameters in white-footed mice were examined by linear regression (PROC REG). The kidney:body weight ratios in male mice from the range and control site were compared by two-factor analysis of variance (factors = site and age group), and the relationship between the kidney:body weight ratio and kidney lead concentration was examined using Spearman's rank correlation (PROC CORR). Femur lead concentrations and ALAD activities in green frogs were compared by analysis of covariance (PROC GLM) using length as the covariable and site and sex as the class variables.

The distributions of the residuals from the GLM analyses were examined by means of the Shapiro-Wilk statistic (PROC UNIVARIATE) to determine if data transformations were necessary. Log transformations were applied to white-footed mouse tissue lead data and blood ALAD ratio data, and a reciprocal transformation was applied to frog femur lead data. Mean values presented in tables and figures are arithmetic means computed from raw data. Interactions among the main effects in the model were tested, when possible, and analyses were repeated excluding those interactions that were not significant. Sample sizes for hematological parameters in white-footed mice were not sufficient to test for three-way interactions, and these interactions were assumed to be non-significant. All probabilities reported are those associated with the Type III sum of squares. Unless otherwise stated, main effects and interactions were not significant.

Table 1. Soil measurements

Parameter	Control site	Range
pH	4.9	6.3
Total organic carbon (µg/g)	2,200	2,000
Total lead (µg/g)	74	75,000

Table 2. Tissue lead concentrations in white-footed mice, shorttail shrews, and green frogs

	Lead concentration (µg/g dry weight)		
	Mean (n) (range)		
Site	Liver	Kidney	Femur
White-footed mice			
Trap range	4.98[a] (22) (0.31–38.6)	34.9[a] (22) (1.37–275)	245[a] (20) (3.55–1656)
Control site	0.98 (16) (0.17–3.93)	2.42 (14) (0.90–4.28)	3.80 (17) (0.48–12.5)
Shorttail shrews			
Trap range	34.1 (1)	1506 (1)	437 (1)
Control site	0.46 (2) (0.31–0.62)	1.45 (4) (0.81–1.99)	12.3 (4) (6.27–16.4)
Green frogs			
Trap range	Not analyzed	96.2[b]	1728[a] (11) (18.9–3643)
Control site	Not analyzed	1.44[c]	1.79 (12) (0.84–2.73)

[a] Significantly different from control site (P = 0.0001). Statistical comparisons were not performed on tissue lead data from shorttail shrews or kidney lead data from green frogs
[b] Pooled sample from four individuals
[c] Pooled sample from five individuals

Results

Soil Chemistry and Tissue Lead Concentrations

Soils: The total lead concentration in soil at the range was 1000-fold higher than the concentration at the control site (Table 1). Soil pH was also higher at the range, and total organic carbon concentrations were similar at both sites.

Small Mammals: Liver, kidney, and femur lead concentrations in white-footed mice from the trap range were elevated 5- to 64-fold ($P < 0.01$) relative to mice from the control site (Table 2). Females had significantly higher liver lead concentrations than males ($P = 0.0034$), and adults had significantly higher femur lead concentrations than non-adults ($P = 0.0171$). Only one shorttail shrew was captured at the trap range, and tissue lead concentrations were 35- to 1038-fold higher than the corresponding mean concentrations in control animals.

The estimated mean body burden of lead in adult white-footed mice from the range was 374 µg (range: 3.67 − 2054, n = 15), and bone accounted for 98% of the total amount. Based on the uptake model, the mean total body burden could be accounted for by soil ingestion alone if 0.06% of the diet consisted of soil.

Table 3. Hematocrit (Hct), hemoglobin concentration (Hb), blood ALAD activity, the ratio of activated:unactivated ALAD activity, and kidney:body weight ratios (males only) in white-footed mice. ALAD activity is expressed as nmoles PBG/mL RBCs/h

Parameter	Trap range	Control site	P value
Hct (%)			
mean (n)	47 (11)	49 (12)	0.1083[a]
(range)	(37–55)	(42–56)	
Hb (mg/dL)			
mean (n)	14.0 (11)	15.0 (12)	0.0249[a]
(range)	(11.5–16.0)	(12.5–17.0)	
ALAD activity			
mean (n)	169 (9)	323 (11)	0.0384[a]
(range)	(78–247)	(164–592)	
ALAD ratio			
mean (n)	3.0 (9)	1.2 (11)	0.0035[a]
(range)	(1.3–6.9)	(1.1–1.3)	
Kidney:body weight ratio			
mean (n)	0.64 (14)	0.59 (7)	0.2538[b]
(range)	(0.52–0.76)	(0.49–0.72)	

[a]Row means compared by three-factor ANOVA (factors = site, sex, and age group)
[b]Row means compared by two-factor ANOVA (factors = site and age group)

Frogs: Femur lead concentrations in green frogs from the trap range were nearly 1000-fold higher (P = 0.0001) than concentrations in control frogs (Table 2), and concentrations were higher in males than in females (P = 0.0426). The lead concentration in a pooled kidney sample from the range frogs was 67-fold higher than the corresponding concentration in control frogs.

Kidney Histopathology and Kidney:Body Weight Ratios

Mice: Two mice (kidney Pb = 50.8 and 237 μg/g) out of 23 examined from the range had acidophillic (H&E) and acid-fast (Ziehl-Neelsen) intranuclear inclusion bodies and marginated chromatin in the renal proximal convoluted tubular epithelium. One of these mice (kidney Pb = 50.8 μg/g) had focal accumulations of brown-black pigment surrounding inclusions which were free in the lumen of renal collecting tubules at the interface of the cortex and medulla. In this mouse, karyomegaly of epithelial cells from foci of tubular necrosis was associated with or in the absence of intranuclear inclusions. Renal edema was evident in these foci of tubular necrosis. A few focal accumulations of brown-black pigment in interstitial macrophages and free in the tubular lumen were present in a total of four range mice. Of these four, two had intranuclear inclusions (described above) and two did not (kidney Pb = 16.8 and 275 μg/g). No intranuclear inclusions, focal tubular necrosis, or pigment-cellular debris accumulations were present in the twelve control mice. One range mouse with none of the previously described pathology had *Cryptosporidia* sp in the proximal and distal convoluted renal tubular epithelium.

There was no significant difference (P = 0.2538) in kidney:body weight ratios between males from the range and control site (Table 3). Kidney:body weight ratios tended to increase with increasing kidney lead concentration, but the correlation was not statistically significant (P = 0.0867). Kidney:body weight ratios were not tested for females because of the possible confounding effects of pregnancy-related increases in body weight.

Shrews: Homogeneously staining (H&E), acidophillic, spherical to oval shaped intranuclear inclusion bodies with a brush-like perimeter were present in 81 of 100 proximal convoluted renal tubular epithelial cells from the shrew taken at the range (kidney Pb = 1506 μg/g). Yellow refractile pigment granules were present in the cytoplasm of some cells with intranuclear inclusions. Chromatin was marginated in all nuclei containing inclusions. These inclusions were acid fast with the Ziehl-Neelsen method. No renal lesions were observed in the four control site shrews.

Frogs: No intranuclear inclusion bodies typical of those described for lead poisoned rats (Goyer *et al.* 1970) and mallards (Locke *et al.* 1966) were observed in renal tubular epithelium of five control site frogs and four range frogs either by light microscopy or by electron microscopy. One range frog and one control frog had coccidia (*Isospora* sp.) in the renal tubular epithelium and tubular lumens. A mild cellular inflammatory response was characterized by eosinophil infiltration of the tubular epithelium.

Hematology

Mice: No lesions of the erythrocytes suggestive of lead toxicosis were observed in peripheral blood smears from the 11 mice collected on the range and 12 mice from the control site.

Blood ALAD activity in mice from the trap range was depressed 48% relative to activity in controls, and the mean ALAD ratio was elevated 2.5-fold (Table 3). Hemoglobin and hematocrit data from one control animal were excluded from statistical analysis because the measured values (9.0 mg/dL and 27%, respectively) were approximately five standard deviations below the mean value for all other control animals. These differences were assumed to be the result of a disease condition unrelated to lead, since tissue lead concentrations were not elevated. Hemoglobin concentrations in mice from the trap range were depressed 6.7% (P = 0.0249) relative to concentrations in controls (Table 3). Hematocrit was similar in mice from the range and control site. Hematocrit and hemoglobin were inversely related to femur lead concentration in males ($r^2 = 0.75$, P = 0.0052, n = 9 and $r^2 = 0.65$, P = 0.0015, n = 9, respectively), but not in females (P > 0.2, n = 11).

Frogs: Erythrocytes from two of the four range frogs were parasitized by malaria (*Plasmodium* sp.). No erythrocytic lesions were observed in the blood smears from five frogs at the control site.

Blood ALAD activities in male and female frogs were compared separately because of a significant interaction between sex and body length. Blood ALAD activity was depressed 73% in females (P = 0.0004) and 86% in males (P = 0.0004) relative to activities in controls. Liver ALAD activity was depressed 64% (P = 0.0001). The difference in hematocrit was not statisti-

Table 4. Hematocrit (Hct), blood ALAD activity (nmoles PBG/mL RBCs/h), and liver ALAD activity (nmoles PBG/g protein/h) in green frogs

Parameter	Trap range	Control site	P value
Hct (%)			
mean (n)	19 (11)	23 (10)	0.1295[a]
(range)	(12–26)	(15–31)	
Blood ALAD activity: females			
mean (n)	290 (7)	952 (6)	0.0004[b]
(range)	(88–870)	(615–1265)	
Blood ALAD activity: males			
mean (n)	160 (4)	1112 (3)	0.0004[b]
(range)	(99–288)	(854–1202)	
Liver ALAD activity:			
mean (n)	1062 (11)	2974 (12)	0.0001[a]
range	(395–2470)	(868–5072)	

[a]Row means compared by analysis of covariance (factors = site and sex, covariable = length)
[b]Row means compared by analysis of covariance (covariable = length)

cally significant (P = 0.1295; Table 4). Hemoglobin concentrations were usually below the range of the hemoglobinometer and were not determined.

Discussion

Based on the shot density previously measured at the trap and skeet range (Stansley *et al.* 1992) and assuming, for the sake of simplicity, that all of the spent pellets are No. 8 shot with a mean weight of 72 mg (Buerger *et al.* 1986), approximately 266,000 kg lead shot/ha is present in the upper 7.5 cm of soils and sediments in the shot-fall zone. The high lead concentration in the soil (pellets excluded) observed in this study is similar to that reported by Manninen and Tanskanen (1993), who measured total lead concentrations as high as 54,000 mg/kg in humus samples from a shooting range after the spent shot was removed. These findings are consistent with those of Jørgensen and Willems (1987), who reported that oxidized lead compounds form on the surface of spent shot and are subsequently released into the soil. The high tissue lead concentrations in small mammals and frogs at the site indicate that some portion of this lead is bioavailable.

Food-chain uptake through contaminated plants is a potential source of lead in white-footed mice, since they are omnivorous (Lackey *et al.* 1985). At a shooting range with acidic soils (pH 3.9), Ma (1989) reported strongly elevated lead concentrations in both herbivorous and carnivorous small mammals. He concluded that lead was mobilized in the acid soil environment and entered the food chain through plants as well as soil fauna. However, Manninen and Tanskanen (1993) reported little uptake of lead by plant foliage in shooting range soils with a pH of 5.6–5.9. This suggests that the higher soil pH (6.3) observed in the present study may limit food chain uptake of lead through plants.

Wildlife ingest soil intentionally or inadvertently when feeding, and soil ingestion may therefore be a significant route of exposure for some environmental contaminants (Beyer *et al.* 1994). Soil ingestion is undoubtedly an important source of lead in shortfall shrews since they feed on soil invertebrates (Talmage and Walton 1991) including earthworms, which typically contain 20–30% soil (Beyer *et al.* 1994). White-footed mice, however, ingest relatively little soil. Beyer *et al.* (1994) estimated that soil comprises <2% of the total diet of this species. Nonetheless, the soil uptake model predicts that ingestion of <0.1% soil could account for the total estimated body burden. There are considerable uncertainties associated with such model estimates, particularly with regard to assumptions about the fraction of ingested lead that is absorbed (Pascoe *et al.* 1994). However, the absorption factor used in the model (0.65% of the ingested dose) is much more conservative than the value of 10% that is typically used for dietary lead absorption in humans (Klassen and Rozman 1991). Even if the absorption factor was an order of magnitude lower (0.065%), ingestion of <1% soil could account for the total body burden. This suggests that incidental ingestion of soil in the shot-fall area might be a significant exposure route, even in animals that ingest relatively little soil. Some species ingest considerably more soil than do white-footed mice (Beyer *et al.* 1994), and could potentially accumulate substantial amounts of lead via this route, regardless of lead concentrations in plants or prey animals.

The chemical form of lead in shooting range soils may also contribute to lead uptake via soil ingestion. Jørgensen and Willems (1987) reported that hydrocerrusite, or basic lead carbonate ($Pb_3(CO_3)_2(OH)_2$), is one of the major transformation products of lead shot in soil. Barltrop and Meek (1975) found that the dietary uptake of basic lead carbonate by Wistar rats was greater than the uptake of seven other lead compounds that they tested, and was twelve-fold greater than the uptake of metallic lead.

Soil ingestion may also be important in frogs. Beyer *et al.* (1994) suggested that high lead concentrations in tadpoles inhabiting highway drainages (Birdsall *et al.* 1986) are probably associated with soil ingestion, since aquatic prey such as insect larvae may contain significant amounts of soil.

The very high femur lead concentrations measured in green frogs are consistent with the findings of Niethammer *et al.* (1985), who reported that bullfrogs (*Rana catesbeiana*) collected in a lead mining area had higher concentrations of lead, cadmium, and zinc than four other species of riparian vertebrates tested. These findings support the use of frogs as biomonitors for lead and other heavy metals.

In white-footed mice, most of the lead burden was present in bone, in agreement with other studies of small mammals (Talmage and Walton 1991). The combined lead burden in the liver and kidneys amounted to only 2% of the estimated amount of skeletal lead. The same general pattern appears to hold true for frogs, since the lead concentration in the femur was 18-fold higher than the concentration in the kidney. As noted by others, lead deposited in bone is relatively unavailable to predators (Roberts and Johnson 1978), especially raptors that regurgitate bones and other undigestible material (Henny *et al.* 1994). Therefore, only a fraction of the lead present in green frogs and white-footed mice is likely to be transferred to higher trophic levels.

Increased kidney:body weight ratios due to renal edema have been reported in rats exposed to lead in the laboratory (Goyer *et al.* 1970; Bankowska and Hine 1985) and in small mammals at an abandoned trap and skeet range (Ma 1989). In the current study, kidney:body weight ratios in white-footed mice were not

significantly elevated relative to controls. However, the capture of some relatively uncontaminated animals in the shot-fall area may have influenced between-site comparisons. Five males captured at the range had kidney lead concentrations that were within the 95% CI for controls. The low lead concentrations in these individuals may be the result of marginal overlap between the animal's home range and the shot-fall area or recent recruitment from uncontaminated areas. When data from these animals were excluded from analysis, the between-site difference in kidney:body weight ratios approached statistical significance ($P = 0.0561$).

The presence of renal inclusions is considered the most sensitive indicator of lead-induced renal changes (Talmage and Walton 1991). Our findings regarding the presence of intranuclear inclusions are in general agreement with those of Goyer *et al.* (1970), who reported that the lowest kidney Pb concentration that resulted in inclusions in laboratory rats was 8.1 μg/g wet wt. (≈30 μg/g dry wt). In the present study, three of the four small mammals with kidney lead concentrations in excess of 30 μg/g had intranuclear inclusions. However, the white-footed mouse with the highest kidney lead concentration (275 μg/g) did not have intranuclear inclusions.

Depressed blood ALAD activity in both mice and frogs at the range is an indication of recent lead exposure but is not, by itself, an indication of toxic exposure, since ALAD is not the rate limiting enzyme in heme synthesis (Mayer *et al.* 1992). Liver ALAD activity was also found to be an indicator of lead exposure in frogs. It is especially useful for measurements in small frogs since it can be difficult to collect an adequate blood sample from such animals.

The lower hemoglobin concentrations in mice from the range are an indication that heme synthesis was affected. The reduction of hemoglobin concentrations (6.7%) in mice with normal hematocrits was apparently not severe enough to produce discernible hypochromic erythrocytes in peripheral blood smears. The finding that hematocrit and hemoglobin concentration are related to the degree of lead contamination (as indicated by femur lead concentration) in males but not in females indicates that the sex of the animal should be considered when interpreting such data. The different results could be due to depressed hematocrit and hemoglobin concentrations related to pregnancy or lactation, which have been reported in small mammals from widely separated, apparently uncontaminated habitats (Sealander 1964). Alternatively, or in addition, lead may be mobilized from bone during pregnancy and lactation (Thompson *et al.* 1985; Manton 1985), which could differentially affect hematopoiesis, hematocrit, and hemoglobin concentrations. Mobilization of bone lead might also be a contributing factor in the significantly higher liver lead concentrations observed in female white-footed mice.

Conclusions

A large amount of lead remained in the soil after the spent shot was removed. Depressed ALAD activity and elevated tissue lead concentrations in small mammals and frogs indicate that some portion of the lead deposited at the trap and skeet range is bioavailable. Based on a simple uptake model, it appears that incidental ingestion of even relatively small amounts of soil may contribute significantly to the lead body burden of small mammals at the site. However, the potential for food chain uptake by predators, especially raptors, appears to be low due to the tendency for lead to concentrate in bone rather than in more digestible soft tissues of prey animals. In small mammals, lead concentrations were high enough in some individuals to produce histopathological indications of lead toxicosis, and hemoglobin was depressed in white-footed mice.

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