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Toxicity of Lead-Contaminated Sediment to Mute Swans

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Abstract. Most ecotoxicological risk assessments of wildlife emphasize contaminant exposure through ingestion of food and water. However, the role of incidental ingestion of sediment-bound contaminants has not been adequately appreciated in these assessments. This study evaluates the toxicological consequences of contamination of sediments with metals from hard-rock mining and smelting activities. Lead-contaminated sediments collected from the Coeur d'Alene River Basin in Idaho were combined with either a commercial avian maintenance diet or ground rice and fed to captive mute swans (*Cygnus olor*) for 6 weeks. Experimental treatments consisted of maintenance or rice diets containing 0, 12 (no rice group), or 24% highly contaminated (3,950 μg/g lead) sediment or 24% reference (9.7 μg/g lead) sediment. Although none of the swans died, the group fed a rice diet containing 24% lead-contaminated sediment were the most severely affected, experiencing a 24% decrease in mean body weight, including three birds that became emaciated. All birds in this treatment group had nephrosis; abnormally dark, viscous bile; and significant ($p \leq 0.05$) reductions in hematocrit and hemoglobin concentrations compared to their pretreatment levels. This group also had the greatest mean concentrations of lead in blood (3.2 μg/g), brain (2.2 μg/g), and liver (8.5 μg/g). These birds had significant ($\alpha = 0.05$) increases in mean plasma alanine aminotransferase activity, cholesterol, and uric acid concentrations and decreased plasma triglyceride concentrations compared to all other treatment groups. After 14 days of exposure, mean protoporphyrin concentrations increased substantially, and mean δ-aminolevulinic acid dehydratase activity decreased by more than 95% in all groups fed diets containing highly contaminated sediments. All swans fed diets that contained 24% lead-contaminated sediment had renal acid-fast intranuclear inclusion bodies, which are diagnostic of lead poisoning in waterfowl. Body weight and hematocrit and hemoglobin concentrations in swans on control (no sediment) and reference (uncontaminated) sediment diets remained unchanged. These data provide evidence that mute swans consuming environmentally relevant concentrations of Coeur d'Alene River Basin sediment developed severe sublethal lead poisoning. Furthermore, toxic effects were more pronounced when the birds were fed lead-contaminated sediment combined with rice, which closely resembles the diet of swans in the wild.

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Sediments in the Coeur d'Alene River Basin are greatly contaminated with lead and other metals from decades of mining and smelting activity in the South Fork Coeur d'Alene River drainage (Neufeld 1987; Hornig *et al.* 1988). Mining wastes deposited in over 4,000 ha of wetlands in lateral lakes along the lower Coeur d'Alene River have increased sediment lead concentrations to the thousands of μg/g (Kreiger 1990; Campbell *et al.* 1999), as far as 50 km downstream from the Bunker Hill Superfund site, near Kellogg, ID. These mine wastes also contain high concentrations of zinc, cadmium, arsenic, copper, mercury, and iron. Various wildlife, such as osprey (*Pandion haliaetus*) (Henny *et al.* 1991), other raptors (Henny *et al.* 1994), and songbirds (Johnson *et al.* 1999) have been affected by the lead, but effects have been especially severe on tundra swans (*Cygnus columbianus*) and other waterfowl (Kreiger 1990; Blus *et al.* 1991, 1999; Audet *et al.* 2000). Reports of repeated waterfowl die-offs within this area were first reported in 1924 (Chupp and Dalke 1964) and continue to the present (Audet *et al.* 2000).

In general, lead poisoning of waterfowl has been associated with ingestion of lead shot or sinkers (Bellrose 1959; Pain 1996), but in the Coeur d'Alene River Basin ingestion of contaminated sediment and plant material was suggested to be responsible for most of the lead-poisoning deaths (Chupp and Dalke 1964; Benson *et al.* 1976; Blus *et al.* 1991). Aquatic plants favored by waterfowl accumulate some lead but in general are unlikely to accumulate sufficient quantities of lead to poison waterfowl (Behan *et al.* 1979; Beyer *et al.* 1997). Field studies have shown that sediment ingestion seems to be the principal route of lead exposure for waterfowl in the Coeur d'Alene River Basin (Blus *et al.* 1991; Beyer *et al.* 1998; Audet *et al.* 2000). An examination of digesta from wood ducks (*Aix sponsa*) in the Coeur d'Alene River Basin demonstrated that the amount of lead ingested was proportional to the amount of sediment ingested, and that ducks that

ingested little sediment ingested little lead (Beyer *et al.* 1997). A comprehensive survey and analysis of about 650 waterfowl feces has reinforced the relationship between sediment ingestion and lead exposure in other waterfowl in the Coeur d'Alene River Basin (Beyer *et al.* 1998).

Because previous laboratory studies on lead poisoning of waterfowl were based on ingestion of either a solid lead artifact (*i.e.*, shotshell pellet or fishing sinker) or a compound of lead added to a diet without sediment, we lacked the means to accurately evaluate the toxicity of the Coeur d'Alene River Basin sediments to waterfowl. The chemical form of the lead and the presence of sediment presumably affects its bioavailability to waterfowl (Kreiger 1990). Lead in soil or sediment is less bioavailable than more soluble forms of lead, such as lead acetate, that are commonly fed in laboratory studies. For example, the bioavailability of soilborne lead relative to lead acetate, when fed to rats and measured in blood, was reported as 18% (Freeman *et al.* 1994), 20% (Freeman *et al.* 1992), and 40% (Schoof *et al.* 1995). The lead in the mine tailings is bound primarily as sulfides and is only sparingly available, but the lead that moves downriver is more available, primarily in secondary minerals, such as iron-manganese-lead oxides, which adhere to detritus (Rantala *et al.* 1996). The other contaminants in the sediment and the sediment itself undoubtedly influenced lead's toxicity. Consequently, we concluded that the sediments in question should be fed to waterfowl rather than to try to predict toxic effects from laboratory studies based on other forms of lead.

Captive mute swans (*Cygnus olor*), a congeneric surrogate for tundra swans, were selected for this study based on similarities in diet and foraging behavior and because of the availability of eggs. Mute swan eggs were collected from relatively uncontaminated areas of the Chesapeake Bay, and cygnets were raised under laboratory conditions to ensure that test subjects had no significant prior exposure to environmental contaminants. Related laboratory studies investigated the toxicity of similar sediments to mallards, *Anas platyrhynchos* (Heinz *et al.* 1999; Hoffman *et al.* 2000a), Canada geese, *Branta canadensis* (Hoffman *et al.* 2000b), and northern bobwhite quail, *Colinus virginianus* (Connor *et al.* 1994).

Harrison Slough, located near the entry of the Coeur d'Alene River into Lake Coeur d'Alene, was selected as the source of contaminated sediments for this study. The sediments from this slough are appropriate for detailed study because the slough attracts large numbers of waterfowl for feeding, because waterfowl mortality has been observed there for numerous years (Neufeld 1987; Audet *et al.* 2000), and because estimates of the sediment and lead concentrations in swan feces from this site were available (Beyer *et al.* 1998). However, sediment lead concentrations in some upriver wetlands and lakes are substantially greater than those from Harrison Slough (Neufeld 1987; Campbell *et al.* 1999), potentially posing a much greater hazard to wild waterfowl. Reference sediment was collected from Round Lake, in the nearby St. Joe River, which has similar areas of waterfowl feeding habitat but is hydrologically distinct from the Coeur d'Alene River (Audet *et al.* 2000). It was possible that drying the sediments before incorporating them into the pelletized diets might affect the availability of lead. However, a complementary study (Day unpublished data) demonstrated that the drying of the sediments in preparing the diets had no effect on the bioavailability of the lead in the sediment.

The presumed main route of exposure of waterfowl to lead in the Coeur d'Alene River Basin is through sediment ingestion. Lead shot, a potential alternative source of lead, was ruled out as the main source of exposure because historically few of the lead-poisoned waterfowl collected in the Coeur d'Alene River Basin contained shot (Chupp and Dalke 1964; Benson *et al.* 1976; Blus *et al.* 1991). A recent investigation of waterfowl die-offs in the Coeur d'Alene River Basin reported that nonartifactual lead poisoning (no lead pellets or sinkers present in the digestive system) was the cause of death for 77% of the 285 waterfowl carcasses found sick or dead between 1992 and 1997. Of the 239 lead-poisoned waterfowl collected during this period, only 8% were found to have ingested lead shot (Sileo *et al.* 2001). Chemical analyses had shown that the lead concentrations in plant samples collected from the Coeur d'Alene River Basin were very low (Beyer *et al.* 1997) compared to those in the surrounding sediments (Kreiger 1990). Much of the lead measured in waterfowl food items was associated with soil particles adhering to the surface of the plant material rather than lead incorporated into the plant tissues (Beyer *et al.* 1997; Audet *et al.* 2000). Lead poisoning resulting directly from sediment ingestion was further demonstrated by the examination of tundra swan feces from Harrison Slough, which showed the close relation between dietary lead exposure and the rate of sediment ingestion (Beyer *et al.* 1998). The estimated mean sediment ingestion rate of tundra swans (n = 93) from the Coeur d'Alene River Basin and a reference area was 9% and the 90th percentile was 22% (Beyer *et al.* 1998). Consequently, treatment levels of 12% and 24% sediment were selected.

The effects of diet quality on the bioavailability of sediment-bound lead were examined by combining the two types of sediment with either a highly nutritious commercial waterfowl maintenance diet or a ground rice diet. Rice (*Oryza sativa*) is more typical of the diet of wild waterfowl in the Coeur d'Alene River Basin and is considered nutritious (Martin *et al.* 1951) and adequate for waterfowl to maintain their weight (Loesch and Kaminski 1989). However, rice is less nutritious than the fortified commercial maintenance diet because of its low protein content and its lower, unbalanced mineral content. The selection of a rice diet is biologically relevant because sediment ingestion by tundra swans may be greatest when they are feeding on wild rice, *Zizania aquatica* (Beyer *et al.* 1998).

Materials and Methods

Diet Preparation

Experimental treatments consisted of a commercial waterfowl maintenance diet (Zeigler Bros, Gardners, PA) or a ground whole-kernel rice diet (Rollison Seed, Dewitt, AR) combined with either lead-contaminated or uncontaminated (reference) sediment in the following proportions:

Maintenance diets

- I—control 0% sediment
- II—24% reference sediment
- III—12% Pb sediment
- IV—24% Pb sediment

Rice diets

- V—control 0% sediment
- VI—24% reference sediment
- VII—24% Pb sediment

Bulk collections of the two sediment types were processed and stored separately to avoid cross-contamination. To facilitate incorporation of the water-saturated sediments into grain-based diets, the wet sediments were thoroughly air-dried and homogenized on an outdoor concrete slab prior to diet formulation. The dried sediments were screened through a 1-mm sieve to remove coarse debris and any lead artifacts that might have been present. No shotgun pellets or fishing sinkers were found. Three composite samples of each sediment were saved for chemical analysis. The experimental diets were formulated by adding the appropriate amounts of sieved sediments to the basal maintenance or rice diets and rehomogenized with a large Hobart mixer. Previous feeding trials using dried sediment incorporated into granular diets had documented preferential ingestion of the grain portion of unconsolidated diets by mallards (Heinz personal communication). To avoid any uncertainty in exposure rates in this study, all diets were pelletized by a commercial feed house (Zeigler Bros.) to ensure that the swans could not sort the basal diet from the sediment. Total feed consumption over the course of the experiment was monitored for each treatment group by recording the total amount of feed that was provided to the birds corrected by the amount that was periodically removed from the feeders by investigators to ensure the availability of fresh food. A daily consumption rate was estimated by averaging the total feed provided per treatment/number of birds per treatment/number of days. Three composite samples, representing a subsample of each bag of feed used, were collected and stored frozen for residue analysis.

Composite samples of the laboratory basal diets and several natural waterfowl food items (Horsetail rush, *Equisetum fluviatile*; pondweed, *Potamogeton spp*.; water potato tubers, *Sagittaria latifolia*; and wild rice, *Zizania aquatica*) from the Coeur d'Alene River Basin were collected for nutritional analysis. A sample of whole-kernel corn, a common waterfowl food, obtained from Zeigler Bros., was included for comparison.

Birds and Treatments

Mute swan eggs were collected from nests of wild swans along the Chesapeake Bay in spring 1995, under the provisions of an appropriate state collection permit. The eggs were maintained at 37.5°C (dry bulb) and ≥ 32.2°C (wet bulb) in Petersime incubators and hatched between May 1–May 21. Hatchlings were uniquely identified using aluminum web tags (National Band and Tag Co.) and placed in semi-enclosed 1.5 × 3.0 m outdoor pens with supplemental heat and continuous-flow water troughs for drinking and loafing. A commercial waterfowl starter mash (Zeigler Bros. I) high in crude protein (30%) was provided *ad libitum.* The diet was supplemented with live mealworms (*Tenebrio molitor*) for several days to encourage eating. Cygnet weights were recorded daily, and individuals that were slow to gain weight were fed by hand.

At 10 days of age, the cygnets were thermocompetent and rapidly gaining weight. The cygnets were evaluated for any obvious physical deformities and fecal samples were screened for the presence of endoparasites. Suitable candidates were banded with flexible, plastic leg bands (National Band and Tag Co., Newport, KY) and relocated to six outdoor pens containing constructed wetlands with grassy upland borders. Cygnets were captured, examined, and weighed weekly. The swans were fed a waterfowl grower diet containing 24% crude protein while approximately 30–60 days old, and an adult maintenance diet (16% protein) thereafter. When approximately 120 days old, the swans were examined and 3 ml of blood was collected for a preexposure health screening. Any birds with abnormal hematocrit, hemoglobin, or protoporphyrin concentrations were eliminated from the experimental candidate pool. A second fecal sample was screened for the presence of endoparasites; no prophylactic treatment was required.

Swans were randomly assigned to treatment groups according to a randomized block design resulting in a maximum of two individuals originating from the same clutch per treatment group. Eight swans were assigned to each group with the exception of group V, which due to a shortage of pen space, had six swans. Group V was included solely to determine if swans could maintain their weight and health on a diet of 100% rice; consequently, residue analyses were not performed on this group at the conclusion of the feeding trial. Three weeks prior to initiation of the study, the swans were placed one per pen, except group V, in the same 1.5 × 3 m outdoor pens used previously to house the newly hatched cygnets. Each pen contained a 1 m^2 plastic flow-through water trough suitable for bathing and loafing, but no supplemental heat. Group V birds were housed three per pen in similar 2.6 × 6.6 m pens with continuous-flow water troughs and feed pans large enough to accommodate multiple birds. During this acclimation period, the birds were fed a pelletized diet of either 100% maintenance or ground rice, according to treatment assignment. Behavior and weight change were monitored frequently and the individual swans that did not adapt well to the experimental conditions were replaced.

The initiation of exposure to experimental diets was staggered over 3 days to facilitate collection and processing of blood samples. The swans were checked daily for signs of illness and were weighed at weekly intervals. Three-milliliter blood samples were collected by jugular venipuncture biweekly. A pooled fecal sample for each treatment group was collected after 3 weeks of exposure. After 6 weeks, the birds were weighed, bled, euthanized by CO_2, then thoroughly examined at necropsy. Samples of blood, liver, and brain were saved for residue analysis, and an aliquot of blood was retained for biochemical evaluation. Segments of liver, kidney, heart, bone marrow, sciatic nerve, proventriculus, uropygial gland, and pancreas were fixed in 10% neutral buffered formalin and processed for histopathological examination. Necropsy and histopathological examinations were performed without prior knowledge of treatment assignment.

Residue Analysis

Lead and other elements in sediments, tissues, feces, and diets were analyzed at Research Triangle Institute (Research Triangle Park, NC), in accordance with quality control and quality assurance provisions established by the Patuxent Analytical Control Facility within the U.S. Fish and Wildlife Service (USFWS). Metals data were validated using quality control criteria specified in a project specific quality assurance plan (USFWS and EcoChem 1995). Blood samples were digested in concentrated nitric acid followed by microwave concentration of the digestates before spectroscopic quantification of acid soluble metals. All other samples were freeze-dried and pulverized prior to undergoing nitric acid/microwave digestion. Mercury was quantified by cold-vapor atomic absorption, arsenic and selenium by graphite furnace atomic absorption, potassium by flame atomic absorption, and the other elements by inductively coupled plasma emission spectroscopy. Brain tissues were analyzed for lead and zinc by graphite furnace atomic absorption to achieve lower detection limits. Organochlorine pesticide concentrations in sediments were analyzed at Mississippi State Chemical Laboratory (Mississippi State, MS), under the quality control and quality assurance procedures of the Patuxent Analytical Control Facility. Sediments were extracted with acetone, followed by petroleum ether. Extracts were purified on florisil and silicic acid columns, and quantified by electron capture gas chro-

Table 1. Plasma chemistries measured in mute swans

Parameter	Method	Source (Application No.)
Albumin	Doumas's bromocresol green method	Trace[a] (PI360040.01)
Alkaline phosphatase	IFCC method using 4-nitrophenylphosphate	Trace (PI111040.03)
ALT	Henry and Bergmeyer's modification of Wroblewski and LaDue	Trace (PI185040.03)
AST	Henry's modification of Karmen's procedure	Trace (PI175040.02)
Calcium	Arsenazo III method	Trace (PI292040.03)
Cholesterol	Roeschlau's modification of Allain's method	Trace (PI132040.04)
CK	Rosalki and Szasz's modification of Oliver's procedure	Trace (40140)
Creatinine	Jaffe's picric acid method	Trace (40350)
γ-GT	Method of Persijn and van der Slik	Trace (PI191040.03)
Glucose	Hexokinase method	Trace (PI150040.02)
Inorganic phosphorus	Wang's modification of Daly and Ertingshausen	Trace (40300)
LDH-L	Method of Gay, McComb, and Bowers	Trace (PI200040.02)
Total protein	Biuret method	Trace (P1340040.02)
Triglycerides	GPO-Trinder method	Trace (222040.02)
Uric acid	Trinder method	Trace (40241)

[a] Trace America, 7260 NW 58th Street, Miami, FL 33166, USA.

matography. Detection limits were 0.05 μg/g (dry weight) for toxaphene and total PCBs and 0.01 μg/g for other compounds. Nutritional analysis of experimental diets and dietary items collected from wetlands throughout the Coeur d'Alene River Basin were performed at Mississippi State Chemical Laboratory using AOAC (1995) methods.

Blood and Plasma Chemistries

Hematocrit was estimated from a microhematocrit capillary tube reader, following centrifugation of heparinized capillary tubes on a clinical hematocrit centrifuge at 7,500 rpm for 5 min. Hemoglobin concentrations were determined by the cyanmethemoglobin method. Red blood cell δ-aminolevulinic acid dehydratase (ALAD) activity was determined using 0.1-ml aliquots of whole blood, in duplicate, according to Pain (1987), but with unit activity expressed as an increase in absorbance of 0.100 at 555 nm with a 1.0-cm light path/ml erythrocytes/h at 38°C, as in Burch and Siegel (1971). Approximately 0.5 ml of each blood sample was retained in the syringe and refrigerated at approximately 4°C for 48 h before free erythrocyte protoporphyrin concentration was measured (Franson *et al.* 1986). Each 20-μl sample of blood was thoroughly mixed for 1 min on a cover slip and inserted into an AVIV hematofluorometer modified for determining free protoporphyrin in samples of avian blood (Roscoe *et al.* 1979). The blood was stirred slowly between measurements and the fluorescence measured at three 30-s intervals and then at 2.5 min; the average of the highest protoporphyrin value in each of two aliquots was calculated. The hematofluorometer was checked against standards each time samples were run. The final readings were adjusted after the fact by a correction curve developed by Western Ecosystems Technology (John Kern personal communication) to standardize values from different studies based on control mallard blood.

Plasma chemistry measurements were selected that have been indicative of general developmental toxicity in previous studies with birds (Hoffman *et al.* 1982, 1985, 1987, 2000a, 2000b; Rattner *et al.* 1987). The following plasma enzyme activities were measured on a centrifugal analyzer (Centrifichem 500; Baker Instrument, Allentown, PA): alkaline phosphatase (EC 3.1.3.1), alanine aminotransferase (ALT; EC 2.6.1.2), aspartate aminotransferase (AST; EC 2.6.1.1), creatinine phosphokinase (CK; EC 2.7.3.2), γ glutamyl transferase (γ-GT; EC 2.3.2.2), and lactate dehydrogenase-L (LDH-L; EC 1.1.1.27) as previously described in Hoffman *et al.* (1985, 1987). Other plasma constituents measured included were albumin, calcium, cholesterol, creatinine, glucose, inorganic phosphorus, total protein, triglycerides, and uric acid. Assays and corresponding methods are summarized in Table 1.

Statistical Analysis

Differences in metals concentrations between the two sediment types were measured by two-tailed *t*-tests ($\alpha = 0.05$); elements where one of three measurements was below the detection limit ($n = 3$) were assigned a value of one-half the detection limit; statistical comparisons between the sediments for elements with $\geq$ 2 values below the detection limit were not run. Statistical comparisons among treatment groups were determined *a priori*. Initially, the effects of adding relatively uncontaminated sediment (24% reference sediment) to the basal diets were evaluated to determine the role of sediment ingestion independent of the presence of lead. Differences in whole blood and plasma chemistry, lead and zinc concentrations in tissues (maintenance only), and weight changes were compared between treatment groups that consumed a control diet (100% maintenance or rice) versus the group fed the same basal diet mixed with 24% reference sediment. This comparison was made by way of a two-tailed *t*-test with a level of significance placed at $\alpha = 0.05$. Subsequent evaluations of dietary effects were made by comparisons between treatment groups receiving various concentrations of lead-contaminated diets and their matched reference group (basal diet with 24% reference sediment). The three groups fed maintenance diets containing sediment were compared with an ANOVA and Dunnett's multiple comparison test with $p \leq 0.05$ considered significant. The two treatment groups fed rice diets containing sediment were compared to each other using two-tailed *t*-tests ($\alpha = 0.05$). Finally, to determine the effect of the basal diet on lead and zinc availability, a comparison of the two groups fed 24% lead-contaminated sediment (rice and maintenance) was made employing a two-tailed *t*-test ($\alpha = 0.05$). Data were log transformed when necessary to meet the assumptions of homogeneous variances.

Results

Soil and Feed Residues

No organochlorine pesticides or polychlorinated biphenyls (PCBs) were detected in the sediments from either site. The mean lead concentration in Harrison Slough sediment was 3,950 μg/g dry weight, compared to 9.7 μg/g in Round Lake sediment (Table 2). Concentrations of 14 of the 23 metals quantified differed statistically between the two sites. Metal concentrations of primary concern, specifically lead and zinc, were 408 and 46 times as high in Harrison Slough sediment as in Round Lake sediment. Concentrations of arsenic, cadmium, copper, and manganese were also greatly elevated in the Harrison Slough sediment.

Based on the lead concentration (3,950 μg/g) in the Harrison Slough sediment, the lead concentration in the formulated diet containing 12% sediment (III) was expected to be about 470 μg/g and about 950 μg/g in the two 24% lead-sediment diets (IV and VII). The actual lead concentrations measured in the experimental diets were 455 μg/g in III, 850 μg/g in IV, and 702 μg/g in VII. Lead levels were below the detection limit of 2.0 μg/g in the two control diets and were 5.8 μg/g (II) and 4.4 μg/g (VI) in the diets containing reference sediment (Table 3).

Table 2. Concentrations of elements (μg/g, dry weight; mean ± SE; n = 3[a]) in sediment from Round Lake on the St. Joe River and Harrison Slough on the Coeur d'Alene River

Element	Round Lake	Harrison Slough
Aluminum	12,600 ± 120	4,900 ± 50*
Arsenic	2.3 ± 0.13	310 ± 6.9*
Barium	90 ± 0.3	170 ± 6.5*
Beryllium	0.58 ± 0.01	0.34 ± 0.09
Boron	7.2 ± 0.15	32 ± 0.3*
Cadmium	ND[b]	35 ± 1.7[#]
Calcium	2,600	2,000
Chromium	13 ± 0.3	6.1 ± 0.24*
Copper	18 ± 0.2	110 ± 0.7*
Iron	14,400 ± 170	68,300 ± 710*
Lead	9.7 ± 1.1	4,000 ± 170*
Magnesium	4,700 ± 40	3,200 ± 40*
Manganese	140 ± 1.4	6,700 ± 70*
Mercury	ND	2.2 ± 0.03[#]
Molybdenum	ND	ND
Nickel	10 ± 0.1	13 ± 0.8*
Phosphorus	510	400
Potassium	1,200	400
Selenium	ND	ND
Sodium	300	ND
Strontium	9.4 ± 0.05	6.6 ± 0.25*
Vanadium	21 ± 0.2	7.6 ± 0.15*
Zinc	75 ± 9.9	3,500 ± 70*

* Harrison Slough sediment was significantly different from Round Lake sediment by a two-tailed *t*-test ($\alpha = 0.05$).

[#] A *t*-test was not run because two or more values within one group were below the detection limit.

[a] n = 1 for Ca, K, Na, and P; no statistical comparison was run.

[b] ND = two or more of the three values were below the detection limit: 0.2 μg/g of Cd; 0.1 μg/g of Hg; 5 μg/g of Mo; 30 μg/g of Na; and 0.5 μg/g of Se. When only one value was below the detection limit, a value of one-half the detection limit was assigned for statistical purposes.

Nutritional Analysis

Proximate and mineral content analyses were performed on both experimental basal diets and several natural waterfowl feed items collected from the Coeur d'Alene River Basin. Although wild waterfowl rarely forage exclusively on one food item for extended periods of time, individual food items were compared separately to enable a better understanding of the impacts of lead exposure in conjunction with a particular food type. Three nutritional comparisons were of particular interest to this study: (1) cultivated rice versus commercial diet; (2) cultivated rice versus wild rice; and (3) wild rice versus other natural forage items. The nutritionally fortified commercial diet was a better source of protein, calcium, and phosphorus than the other diets (Table 4). In addition to having a high caloric content, the commercial diet had a 2:1 ratio of dietary calcium (20,600 μg/g) to phosphorus (10,000 μg/g) considered optimal for proper metabolic activity (Robbins 1983). Cultivated rice and wild rice were nutritionally very similar. Both forms of rice grains were relatively high in crude protein and caloric content but contained little calcium (260 μg/g and 160 μg/g, respectively) and had unbalanced calcium to phosphorus ratios of 1:12 and 1:19, respectively. Water potato tubers, a preferred food item of tundra swans in the Coeur d'Alene Basin, contained a similarly low calcium concentration (380 μg/g), unbalanced calcium to phosphorus ratio (1:16), and had low nutritional value. Horsetail rush and pondweed samples had much higher concentrations of calcium, resulting in more balanced calcium to phosphorus ratios (1.2:1 and 3.6:1) but were poor sources of protein and, due to relatively low caloric content, were considered nutritionally deficient. Corn contained the lowest calcium concentration and lowest calcium to phosphorus ratio (1:72), but highest caloric content of all food items measured (Table 4).

Survival and Weight Changes

All swans survived the 6-week study period. Three birds in the group fed 24% lead-contaminated sediment in rice (VII) were observed to be lethargic and ataxic after 3 weeks. These three swans became emaciated, and by the time of necropsy had lost an average of 28% of their body weight. Although all treatment groups had similar mean body weight at the initiation of the feeding trial, the mean weight loss in group VII was 24% after 6 weeks. This contrasted significantly from all the other groups which maintained similar mean body weights throughout the study (Figure 1). The mean liver to body weight ratio was increased 34% in the group fed 24% lead-sediment with maintenance (IV) compared to the group fed 24% reference sediment with maintenance (II) and increased 36% between similar pairings within the rice basal diet (VII versus VI). Testis size was not significantly different among treatments.

Table 3. Concentrations of metals (μg/g, dry weight) in experimental diets and feces of mute swans

	Maintenance Diet								Rice Diet					
	I Control		II 24% Ref. Sed		III 12% Pb Sed		IV 24% Pb Sed		V Control		VI 24% Ref. Sed		VII 24% Pb Sed	
Element	Feed[a]	Feces[b]	Feed	Feces	Feed	Feces	Feed	Feces	Feed	Feces	Feed	Feces	Feed	Feces
Aluminum	33	120	3,300	7,800	770	1,900	1,300	3,100	26		2,900	4,900	980	2,300
Barium	4.8	15	23	56	23	61	41	100	1.4		19	36	30	74
Beryllium	ND[c]	ND	ND	0.34	ND	ND	ND	ND	ND		ND	0.28	ND	ND
Boron	6.8	15	7.0	12	10	19	12	24	ND		ND	6.8	8	21
Cadmium	ND	ND	ND	ND	4.2	12	7.2	19	ND		ND	0.30	6.6	15
Chromium	1.1	1.4	14	19	8.2	9.5	9.5	14	1.4		14	29	8.5	21
Copper	11	31	8.7	21	16	40	27	65	3.4		6.0	12	20	77
Iron	200	740	3,400	8,400	7,500	17,700	13,800	36,300	86		3,200	5,700	10,900	38,500
Lead	ND	ND	5.8	6.8	460	1,200	850	2,000	ND		4.4	4.3	700	1,500
Magnesium	1,400	3,400	2,100	4,400	1,600	3,400	1,700	3,200	890		1,700	2,800	1,200	2,300
Manganese	62	180	76	180	810	2,200	1,400	3,800	88		100	170	1,200	3,000
Molybdenum	1.3	2.4	ND	1.5	1.0	1.9	ND	2.0	ND		ND	1.8	0.9	1.8
Nickel	1.5	2.9	4.0	7.8	3.1	5.6	4.2	8.8	ND		3.8	7.1	3.8	12
Strontium	9.5	31	7.6	20	8.2	25	7.2	20	0.72		2.1	5.2	1.5	4.0
Vanadium	ND	ND	5.2	13	1.0	3.2	1.9	4.6	ND		5.6	9.2	1.4	3.8
Zinc	59	190	49	130	470	1,300	780	2,200	20		26	49	630	1,500

[a] n = 3 for all feed values.
[b] n = 1 for all fecal values. Feces collected after 3 weeks.
[c] ND = two or more of the three values below detection limits; 4.0 μg/g of B, 0.2 μg/g of Be and Cd, 0.99 μg/g of Mo, 1.0 μg/g of Ni, 2.0 μg/g of Pb, 0.96 μg/g of V.

Table 4. Concentrations of minerals (μg/g, dry weight) and other nutritional components (%) in experimental basal diets and natural waterfowl food items

	Commercial Diet	Cultivated Grains		Natural Forage			
	Maintenance (I) (n = 1)	Rice (V) (n = 2)	Corn (n = 1)	Horsetail (n = 14)	Pondweed (n = 12)	Water Potato (n = 12)	Wild Rice (n = 12)
Minerals							
Calcium	20,600	260	76	7,300	8,700	380	160
Copper	8.5	3.9	2.3	16	4.2	6.3	5.3
Iron	230	84	37	470	1,400	3,000	48
Magnesium	1,800	1,100	1,900	3,900	5,900	1,300	930
Manganese	53	130	11	1,300	2,100	71	38
Phosphorus	10,000	3,000	5,500	6,100	2,400	6,100	3,100
Potassium	6,600	2,900	5,100	24,700	14,200	28,100	3,300
Sodium	1,400	25	ND[a]	820	6,300	210	26
Zinc	89	26	33	250	130	77	45
Other variables							
Crude protein	16	7.1	7.8	3.0	1.9	3.2	7.5
Crude fat	3.1	1.6	4.6	0.2	0.3	0.2	0.6
Crude fiber	2.6	11	3.5	2.3	2.7	0.7	5.4
Ash	5.1	5.7	1.5	1.5	1.0	1.5	2.6
Calories[b]	340	290	350	36	34	77	200

[a] ND = below detection limit of 15 μg/g.
[b] cal/100 g.

Swans in the two control groups (I and V), consumed feed at similar rates, 387 and 363 g/bird/day, while maintaining or slightly increasing their weight (Figure 1). The swans in the group fed a maintenance diet containing 24% reference sediment (II) consumed 36% less feed (minus the sediment) than the control group but were able to maintain their weight. In contrast, the swans fed a diet containing 24% lead-contaminated sediment with rice (VII) consumed feed at a similar rate (203 g/bird/day) to group II (249 g/bird/day) but experienced severe weight loss.

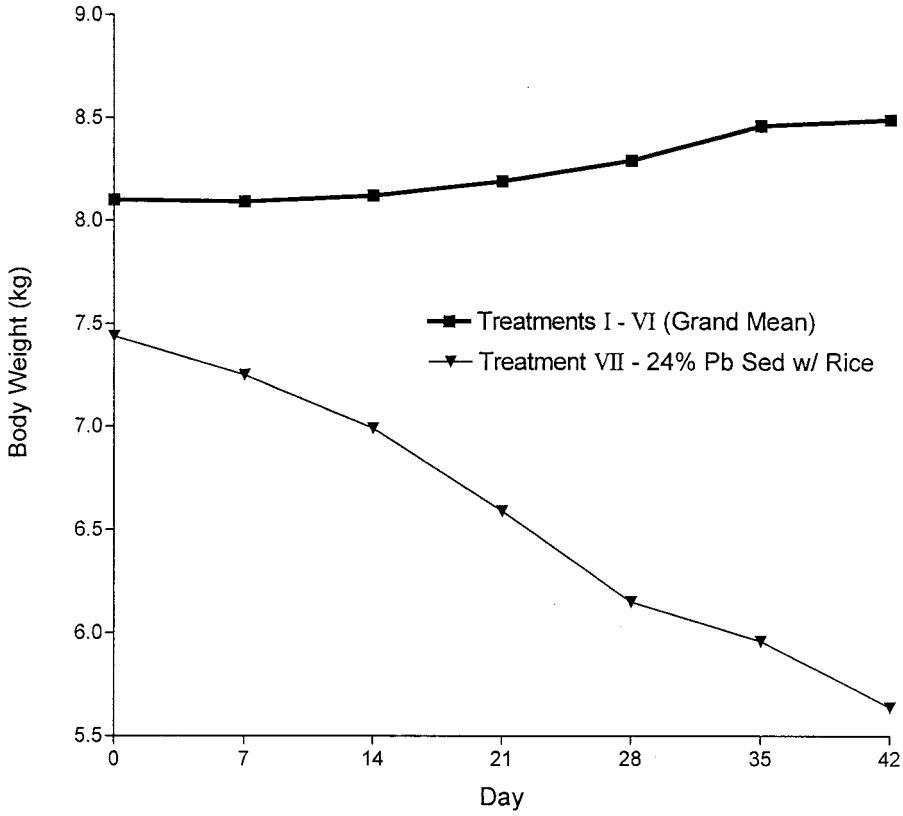

Fig. 1. Body weights of all mute swans in treatment groups I–VI (grand mean) compared to group VII which was fed a ground rice diet containing 24% lead-contaminated sediment. Treatment means did not differ significantly ($p \leq 0.05$) at day 0. Sample size for all groups was 8, except the rice control group, where $n = 6$

Blood Chemistries and Blood Residues

Ingestion of lead-contaminated diets significantly affected blood chemistry. Whereas the mean hematocrits of swans fed diets containing 24% reference sediment were not significantly different from dose groups fed matched control diets without sediment (II versus I, VI versus V), the mean hematocrit was reduced 11% in swans (IV) fed the maintenance diet containing 24% lead-contaminated sediment (compared to II) and 16% in swans (VII) fed the diet of ground rice containing 24% lead-contaminated sediment (compared to VI) after 6 weeks of exposure. Effects on hemoglobin were similar but more pronounced. The mean hemoglobin concentration after 6 weeks of exposure was reduced 21% in IV compared to II and 26% in VII compared to VI (Table 5). All three lead-contaminated sediment groups had severely depressed ($> 95\%$) red blood cell ALAD activity relative to controls after 2 weeks of exposure (Figure 2). ALAD activity was also significantly reduced in swans fed diets containing 24% reference sediment (II and VI), confirming the sensitivity of this bioindicator to exposure of low (< 6 μg/g) concentrations of lead in the reference diets (Table 5). All three lead-contaminated sediment groups had significantly elevated free protoporphyrin concentrations compared to their respective control groups after 2 weeks of exposure (Figure 3). Protoporphyrin concentrations increased by 3.3-fold in the 12% lead-contaminated sediment group (III) and 5.8-fold in the group fed the commercial diet with 24% lead-contaminated sediment (IV) compared to II, and 4.8-fold in the group fed the rice diet with 24% lead-contaminated sediment (VII) compared to VI (Table 5).

For most of the biochemical responses measured, the addition of sediment to the basal diets had little effect on plasma chemistry (Table 6). However, alkaline phosphatase activity was significantly lower in the group fed 24% lead-contaminated sediment in rice (VII) than in all other groups. Alanine aminotransferase activity was significantly reduced in group II compared to group I, but greatly increased (83%) in group VII compared to group VI. Plasma cholesterol was significantly increased only in group VII. Triglyceride levels increased somewhat in the 12% Pb sediment group (III) but were reduced 34% in the group fed 24% lead-contaminated sediment with maintenance (IV) compared to group II and reduced 54% in the matched rice diet (VII) compared to its reference group (VI). Uric acid was significantly increased in group VII verses group VI.

Blood lead was significantly elevated in all three groups fed lead-contaminated diets relative to the matched reference sediment groups. The mean blood lead concentration was nearly twice as high in the group fed 24% lead-contaminated sediment (IV) as in the group fed 12% lead-contaminated sediment (III). The mean blood lead concentration in the group fed 24% lead-contaminated sediment with rice (VII) was 39% higher than the concentration in the group fed the same proportion of lead-contaminated sediment with maintenance (IV) (Table 5). Blood zinc, in contrast, varied little among treatment groups. The highest mean blood zinc occurred in the group fed the rice diet containing 24% lead-contaminated sediment (VII) (Table 5).

Table 5. Effects of sediment ingestion on blood chemistry and on Pb and Zn tissue concentrations (mean ± SD and range) in mute swans after 6 weeks (n = 8 except group V, where n = 6)

	Maintenance Diet				Rice Diet		
Group treatment	I Control	II 24% Ref. Sed	III 12% Pb Sed	IV 24% Pb Sed	V Control	VI 24% Ref. Sed	VII 24% Pb Sed
Whole blood							
Hematocrit (%)	47.9 ± 4.0 [38.5–51.0]	46.2 ± 1.8 [42.5–48.0]	46.2 ± 1.2 [44.0–47.5]	40.9 ± 5.2* [30.0–49.0]	51.8 ± 1.8 [49.0–54.5]	52.6 ± 2.4 [50.0–57.0]	43.6 ± 3.4* [39.0–48.0]
Hemoglobin (g/dl)	17.0 ± 2.1 [11.9–18.4]	17.0 ± 0.77 [15.6–18.0]	16.0 ± 0.87 [14.8–17.0]	13.4 ± 2.8* [7.1–16.4]	17.8 ± 0.93 [16.5–18.9]	18.3 ± 0.80 [17.4–19.6]	13.6 ± 1.5* [11.3–15.5]
ALAD (units)	174 ± 24 [125–200]	139 ± 24[#] [114–183]	3.9 ± 1.6* [1.9–6.4]	8.8 ± 3.7* [2.7–15]	228 ± 49 [177–318]	125 ± 39[#] [93–215]	4.4 ± 3.2* [1.2–10]
Protoporphyrin (μg/dl)	85 ± 18 [65–124]	92 ± 18 [63–114]	306 ± 83* [194–402]	530 ± 100* [402–671]	87 ± 19 [65–117]	96 ± 16 [75–123]	465 ± 90* [369–633]
Tissue residue							
Blood lead (μg/g wet weight)	0.19 ± 0.008 [0.17–0.19]	0.20 ± 0.015[#] [0.19–0.24]	1.28 ± 0.22* [0.98–1.53]	2.30 ± 0.68* [1.37–3.53]		0.20 ± 0.008 [0.18–0.21]	3.20 ± 0.76* [2.36–4.30]
Blood zinc (μg/g wet weight)	3.93 ± 0.78 [2.42–5.23]	4.54 ± 0.84 [3.23–5.42]	4.14 ± 0.27 [3.54–4.38]	4.74 ± 0.80 [3.57–5.57]		5.10 ± 1.14 [3.57–6.27]	5.32 ± 1.11 [3.71–7.49]
Brain lead (μg/g wet weight)	0.027 ± 0.03 [0.005–0.09]	0.038 ± 0.014 [0.02–0.05]	1.13 ± 0.32* [0.73–1.72]	1.76 ± 0.37* [1.36–2.33]		0.032 ± 0.023 [0.01–0.08]	2.21 ± 0.46* [1.60–2.97]
Brain zinc (μg/g wet weight)	7.44 ± 0.53 [6.50–8.05]	7.60 ± 0.75 [6.69–8.61]	7.38 ± 0.52 [6.27–7.96]	7.66 ± 1.00 [6.17–9.04]		7.16 ± 0.94 [5.84–8.27]	8.21 ± 0.19* [7.92–8.42]
Liver lead (μg/g wet weight)	0.15 ± 0.008 [0.14–0.17]	0.16 ± 0.004 [0.15–0.16]	1.5 ± 0.37* [0.95–2.0]	3.8 ± 1.0* [1.9–5.2]		0.16 ± 0.005 [0.15–0.16]	8.5 ± 2.9* [5.0–13]
Liver zinc (μg/g wet weight)	29 ± 5.2 [23–35]	30 ± 3.2 [26–35]	25 ± 3.6 [21–33]	46 ± 14* [33–76]		32 ± 3.3 [28–39]	150 ± 44* [90–204]

[#] Treatment groups fed reference sediment diets were significantly different from their respective control groups (II versus I or VI versus V) by a two tailed *t*-test ($\alpha = 0.05$).

* Treatment groups fed lead-contaminated diets were significantly different from their respective reference sediment groups (III or IV versus II) by an ANOVA and Dunnett's multiple comparison test ($p \leq 0.05$); or VII versus VI by a two tailed t-test ($\alpha = 0.05$).

Liver and Brain Residues

Mean liver lead concentrations were significantly elevated in all three groups fed diets containing lead-contaminated sediment relative to matched reference sediment groups (Table 5). A significant 2.5-fold increase in liver lead concentrations was measured between the group fed 12% lead-contaminated sediment (III) and group fed the maintenance diet with 24% lead-contaminated sediment (IV). The group fed rice containing 24% lead-contaminated sediment (VII) had a mean liver lead concentration that was more than double that of the group fed maintenance diet containing 24% lead-contaminated sediment (IV). Liver zinc concentrations were slightly elevated (×1.5) in group IV but greatly elevated (×4.7) in group VII relative to matched reference sediment groups (Table 5). Similar to lead concentrations, zinc concentrations were significantly greater in the group fed 24% contaminated sediment in rice than in the group fed the same proportion of contaminated sediment combined with the maintenance diet (Table 5).

Brain lead concentrations were significantly elevated in all three groups fed lead-contaminated diets relative to matched reference sediment groups and significantly higher in the group fed rice with 24% lead-contaminated sediment (IV) compared to the group fed a similar proportion of Pb sediment with maintenance (Table 5). Brain zinc concentrations were significantly higher in the group fed 24% lead-contaminated sediment with rice (VII) than in group VI. Other brain zinc concentrations did not differ statistically among groups (Table 5).

Pathology

Body conditions of all swans ranged from fair to excellent when examined at necropsy, except for four swans from group VII (24% Pb sediment with rice), which were in poor or emaciated body condition. One swan in group III (12% Pb sediment with maintenance) and five swans in group VII had abnormally viscous bile compared to the normal watery bile found in the rest of the swans. Acid fast renal intranuclear inclusion bodies were detected in kidneys of all swans in group IV (24% lead sediment with maintenance) and group VII, but in no other groups. All eight of the swans in group VII, six of the swans in group IV, and two of the swans in group I had hepatic hemosiderosis. Four of the swans in group VII, six of the swans in group IV, and two of the swans in group I had nephrosis.

Discussion

The main route of exposure of waterfowl to lead in the Coeur d'Alene River Basin is through sediment ingestion (Beyer *et al.* 1998). Lead artifacts, a potential alternative

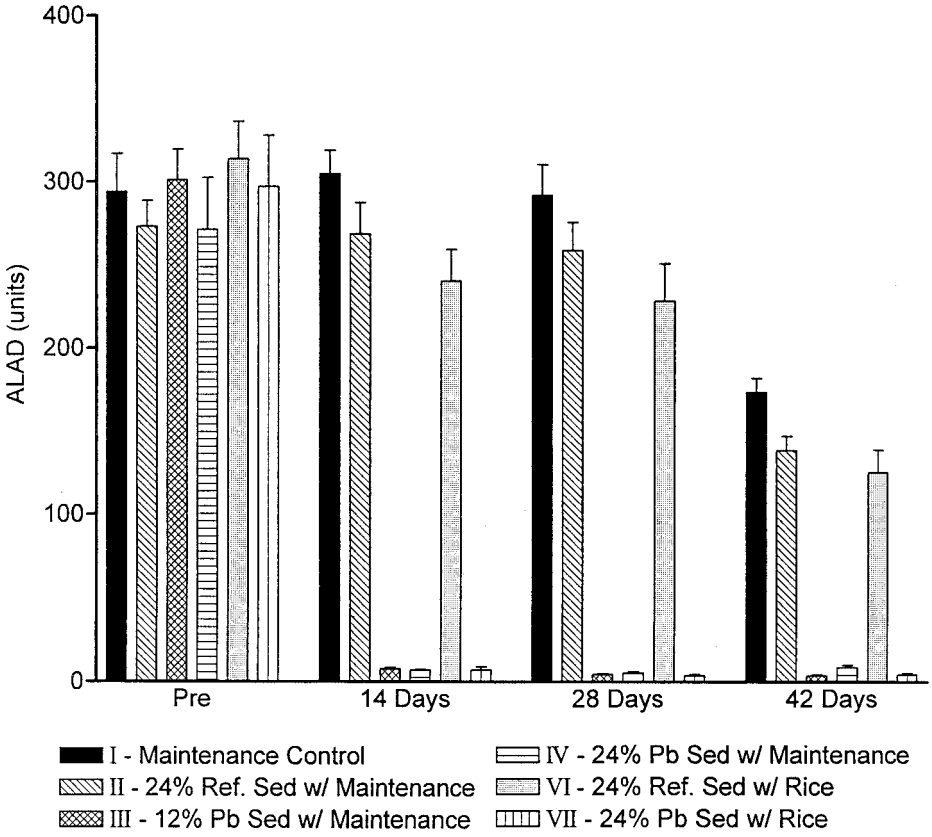

Fig. 2. δ-Aminolevulinic acid dehydratase (ALAD) activity levels in blood of mute swans fed commercial avian maintenance diets containing 0% (group I), 12% (III), or 24% lead-contaminated sediment (IV), or a ground rice diet containing 24% lead-contaminated sediment (VII). The height of each bar represents the arithmetic mean, with one standard error shown above. Sample size for all groups was 8

source of lead, were previously ruled out as a significant source of exposure because an examination of feces of tundra swans from Harrison Slough showed the close relation between lead and sediment ingestion (Beyer *et al.* 1998) and because few of the lead-poisoned waterfowl collected in the Coeur d'Alene River Basin contained artifacts (Audet *et al.* 2000). Plants from the site had lead concentrations that were low compared to those in the sediment (Beyer *et al.* 1998). Consequently, the incorporation of Coeur d'Alene sediments into the experimental diets of captive mute swans approximated the lead exposure of wild waterfowl at that site. Tundra swan feces from Harrison Slough had an average of 1,300 μg/g of lead, and swan feces collected throughout the lower Coeur d'Alene River Basin, from Killarney Lake to Harrison Slough, had an average of 880 μg/g and a 90th percentile of 2,700 μg/g of lead (Beyer *et al.* 1998). The fecal lead concentrations (2,000, 1,500 μg/g) in groups IV and VII, respectively (Table 3) were below the 90th percentiles for the basin. This means that many tundra swans feeding in the Coeur d'Alene River Basin are exposed to higher concentrations of dietary lead than those used in this study, depending on the particular wetlands within the Coeur d'Alene River Basin where feeding occurs and on the season (Beyer *et al.* 1998).

When exposed to Harrison Slough sediment at realistic dietary concentrations, mute swans became sick and developed signs of lead poisoning. The severity of the injury depended on the amount of sediment ingested and the quality of the diet. Ingestion of a similar amount of uncontaminated sediment in both maintenance (II) and rice diets (VI) failed to induce any significant ill effects. When examined histopathologically, all of the swans in groups IV and VII were diagnosed as lead poisoned. Although some of the signs recorded at necropsy are only indicative of lead poisoning (rather than specific for it), the renal tubular acid fast intranuclear inclusion bodies observed in all swans fed diets containing 24% lead-contaminated sediment are considered diagnostic of lead poisoning (Sileo *et al.* 2001).

The severity of the lead poisoning may be evaluated by several variables that were included in this study. After 6 weeks of exposure, the swans in group VII (24% Pb sediment with rice) were severely ill. The mean weight loss of 24% for the group, the finding of emaciation in three birds at necropsy, and the finding of low mean triglycerides (Table 6) suggests that several of the swans were close to death. Plasma triglycerides are known to decrease in nutritionally stressed waterfowl (Rattner *et al.* 1987). The elevated plasma cholesterol in group VII (Table 6) might also be caused by emaciation (Ritchie *et al.* 1994) or by the low protein content of the diet (Mori and George 1977). The elevated tissue lead concentrations provide similar evidence of severe poisoning. In evaluating lead poisoning in mute swans in Sweden, Frank and Borg (1979) considered hepatic concentrations above 5 μg/g "highly elevated indicating lead poisoning." Hepatic lead concentration in all individuals in group VII were measured at or above this suggested criterion. The mean hepatic lead concentration (8.5 μg/g) also falls within Pain's (1996) range of 6–15 μg/g, which is associated with clinical poisoning (external signs of

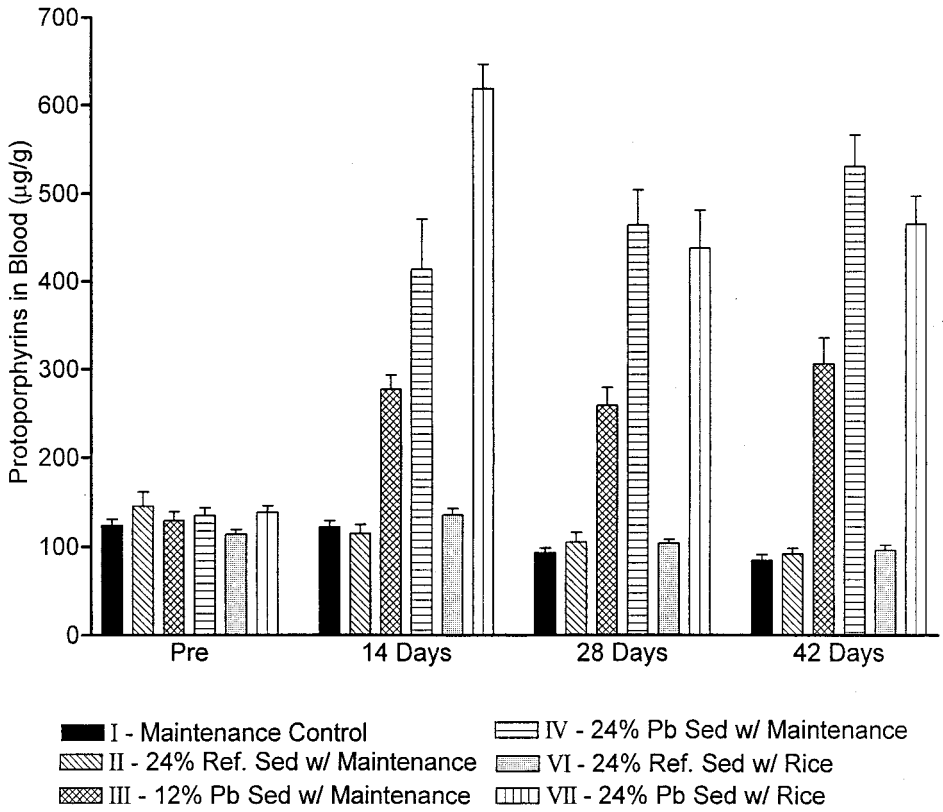

Fig. 3. Protoporphyrin concentrations in blood of mute swans fed commercial avian maintenance diets containing 0% (group I), 12% (III), or 24% lead-contaminated sediment (IV) or a ground rice diet containing 24% lead-contaminated sediment (VII). The height of each bar represents the arithmetic mean, with one standard error shown above. Sample size for all groups was 8

poisoning and impaired biological functioning) in waterfowl. The swan blood contained a mean of 3.2 μg/g of lead, more than the 1 μg/dl of lead that Pain (1996) concluded was the lower bound for severe clinical poisoning in waterfowl. The mean protoporphyrin concentration (465 μg/dl) was also high; when mallards were fed a chronic dose of lead sufficient to kill half of the birds in a treatment group, the blood of the survivors had a median protoporphyrin concentration of 943 μg/dl (Beyer *et al.* 1988). The swans were slightly anemic, as demonstrated by reduced hematocrits and hemoglobin concentrations (Table 5). Lead is known to cause hemolytic anemia (Cotran *et al.* 1989), which increases production of intensely stained bile that distends the gallbladder (Jubb *et al.* 1986). These lesions were noted at necropsy in several of the swans in group VII. Hepatic hemosiderosis was observed in eight of the swans in group VII and six of the swans in treatment group IV. This results from excess iron from catabolized hemoglobin stored as hemosiderin (Schalm *et al.* 1975). An increase in LDH-L is considered a nonspecific result of tissue damage (Woerpel and Rosskopf 1984), and the observed increase (Table 6) in this study may be another result of hemolysis. The increased plasma uric acid concentrations could be a sign of renal disease (Woerpel and Rosskopf 1984); several of the swans developed nephrosis. Alternatively, the increased concentrations might be a consequence of protein catabolism (Mori and George 1977; Rattner *et al.* 1987) related to the emaciation.

The bioaccumulation of lead and the resultant effects on blood chemistry exhibited by mute swans under controlled conditions were consistent with the findings of several investigations of lead poisoning in wild tundra swans within the Coeur d'Alene River drainage. For instance, the accumulation of lead in the blood, brain, and liver tissues of mute swans was similar but not quite as severe as the lead poisoning of wild tundra swans collected dead or moribund from the Coeur d'Alene River Basin. Whereas swans in group VII had from 5 to 13 μg/g of hepatic lead, lead-poisoned wild tundra swans found dead had from 6.4 to 40 μg/g of hepatic lead (Blus *et al.* 1991). Blood lead (3.3 μg/g) and ALAD activity (9.9 units) of wild swans found moribund were similar to those in group VII. The mean hematocrit (36.3%) and mean hemoglobin (10.9 g/dl) were also reduced in the tundra swans, and their mean protoporphyrin was elevated (154 μg/dl). Lead concentrations in brain tissues (0.7–3.0 μg/g) were elevated in all groups fed a diet containing lead and were similar to those measured in wild tundra swans found dead in the Coeur d'Alene River Basin in 1974, which averaged 8.9 μg/g (Benson *et al.* 1976). The finding of the dark, viscous bile in the wild tundra swans was also similar to the findings in the experimentally poisoned swans. Swans maintained under favorable experimental conditions are presumably under less environmental stress than the wild tundra swans. Cold, nutrition, and disease may further influence toxicity (Hoff-

Table 6. Effects of sediment ingestion on plasma enzyme activity and other plasma constituents (mean ± SD) in mute swans after 6 weeks (n = 8 except group V, where n = 6)

	Maintenance Diet				Rice Diet		
Group Treatment	I Control	II 24% Ref. Sed	III 12% Pb Sed	IV 24% Pb Sed	V Control	VI 24% Ref. Sed	VII 24% Pb Sed
Plasma enzyme activities							
Alkaline Phosphatase (IU/l)	156 ± 43	138 ± 40	153 ± 45	150 ± 48	140 ± 22	165 ± 59	105 ± 41*
ALT (IU/l)	17 ± 7.9	10 ± 4.3#	11 ± 3.7	13 ± 4.3	14 ± 4.8	12 ± 4.3	22 ± 15*
AST (IU/l)	23 ± 6.4	18 ± 4.6	21 ± 6.6	16 ± 3.7	24 ± 9.7	27 ± 6.5	39 ± 16
CK (IU/l)	83 ± 19	73 ± 16	108 ± 107	66 ± 22	80 ± 14	95 ± 24	110 ± 22
Gamma-GT (IU/l)	22 ± 9.4	9.4 ± 3.8#	9.9 ± 5.6	7.0 ± 5.2	10 ± 7.2	11 ± 11	6.0 ± 5.2
LDH-L (IU/l)	109 ± 20	94 ± 18	101 ± 33	83 ± 19	96 ± 29	115 ± 31	151 ± 41
Other plasma constituents							
Albumin (g/dl)	2.2 ± 0.34	2.0 ± 0.11	2.1 ± 0.29	2.3 ± 0.37	2.1 ± 0.21	2.0 ± 0.12	2.1 ± 0.26
Calcium (mg/dl)	12.2 ± 1.6	12.3 ± 1.1	11.9 ± 0.9	12.3 ± 1.8	12.4 ± 1.1	12.2 ± 1.5	12.6 ± 2.2
Cholesterol (mg/dl)	241 ± 33	219 ± 38	255 ± 17	256 ± 70	228 ± 47	275 ± 51	325 ± 40*
Creatinine (mg/dl)	34 ± 17	39 ± 6.2	35 ± 8.6	41 ± 25	44 ± 12	26 ± 12#	37 ± 9.6
Glucose (mg/dl)	212 ± 29	205 ± 29	217 ± 38	230 ± 27	205 ± 21	219 ± 22	213 ± 40
Inorganic phosphorus (mg/dl)	3.5 ± 0.44	3.9 ± 0.64	3.5 ± 0.77	3.8 ± 0.71	3.8 ± 0.75	4.1 ± 0.83	3.4 ± 0.75
Total protein (g/dl)	5.3 ± 0.90	4.6 ± 0.74	5.2 ± 0.78	5.7 ± 1.1	4.8 ± 0.75	4.8 ± 0.71	4.8 ± 0.51
Triglycerides (mg/dl)	460 ± 79	449 ± 109	529 ± 92*	362 ± 116*	534 ± 160	515 ± 67	238 ± 101*
Uric acid (mg/dl)	7.0 ± 1.9	5.5 ± 0.88	6.8 ± 0.99	6.8 ± 2.7	9.6 ± 3.0	9.4 ± 1.9	13.6 ± 3.2*

Treatment groups fed reference sediment diets were significantly different from their respective control groups (II versus I or VI versus V) by a two tailed *t*-test ($\alpha = 0.05$).

* Treatment groups fed lead-contaminated diets were significantly different from their respective reference sediment groups (III or IV versus II) by an ANOVA and Dunnett's multiple comparison test ($p \leq 0.05$); or VII versus VI by a two tailed *t*-test ($\alpha = 0.05$).

man *et al*. 1990; Rattner and Heath 1995); based on observations of lead poisoned birds in the field, it is unlikely that the emaciated swans in group VII would have survived in the wild.

Swans in group IV (24% Pb-sed with maintenance) accumulated lower concentrations of tissue lead than swans in group VII, approximately 45% of the hepatic lead and 72% of the blood lead (Table 5), even though the swans in group IV consumed twice as much feed containing slightly higher concentrations of lead. This suggests that diet quality, particularly the amount of calcium and the ratio of calcium to phosphorus in the diet strongly influences the accumulation of dietary lead. Scheuhammer (1996) demonstrated a 400% increase in the accumulation of lead and increased inhibition of ALAD by zebra finches (*Poephila guttata*) when fed a diet low (0.3%) in calcium compared to a group fed a diet containing 3% calcium. Swans in group III (12% Pb-sed with maintenance) accumulated about half the hepatic and blood lead as did swans in group IV. Swans in both of these groups maintained their weights but were exposed to toxic concentrations of lead, as demonstrated by the decreased ALAD activity and the elevated protoporphyrin concentrations (Table 5).

Zinc from the Coeur d'Alene River Basin sediments may also be affecting waterfowl directly or through interactions with lead (Hoffman *et al.* 2000a, 2000b). Zinc concentrations in blood and tissue tend to be closely regulated in animals even when exposed to high concentrations (Beyer and Storm 1995). The hepatic zinc concentrations in swans from group VII were about five times those of the controls (Table 5), and we may conclude only that the exposure was above the range in which the swans can regulate zinc.

Summary

This study and three related studies (Heinz *et al.* 1999; Hoffman *et al.* 2000a, 2000b), are unique in wildlife toxicology because waterfowl were experimentally exposed to a contaminant through sediment ingestion. Soil ingestion has been widely studied and accepted as an important means of exposure to lead for humans and domestic animals (Chaney and Ryan 1994) but has not been adequately appreciated in wildlife toxicology. The results of this study clearly indicate that swans can accumulate sufficient concentrations of lead to cause severe health consequences when they ingest contaminated sediment as part of their diet. Incidental consumption of contaminated sediment, not biomagnification of contaminants up the food chain, may represent the most critical pathway of exposure to poorly absorbed contaminants, such as lead. In addition, the availability of the sediment-bound contaminant may be exacerbated by the low nutritional quality of the diet. Therefore, we suggest that to fully characterize the toxicological risks to waterfowl inhabiting a contaminated wetland, consideration must be given to all components of their diet.

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