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Metal exposure and effects in voles and small birds near a mining haul road in Cape Krusenstern National Monument, Alaska

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Abstract Voles and small passerine birds were live-captured near the Delong Mountain Regional Transportation System (DMTS) haul road in Cape Krusenstern National Monument in northwest Alaska to assess metals exposure and sublethal biological effects. Similar numbers of animals were captured from a reference site in southern Cape Krusenstern National Monument for comparison. Histopathological examination of selected organs, and analysis of cadmium, lead, and zinc concentrations in liver and blood samples were performed. Voles and small birds captured from near the haul road had about 20 times greater blood and liver lead concentrations and about three times greater cadmium concentrations when compared to those from the reference site, but there were no differences in zinc tissue concentrations. One vole had moderate metastatic mineralization of kidney tissue, otherwise we observed no abnormalities in internal organs or DNA damage in the blood of any of the animals. The affected vole also had the greatest liver and blood Cd concentration, indicating that the lesion might have been caused by Cd exposure. Blood and liver lead concentrations in animals captured near the haul road were below concentrations that have been associated with adverse biological effects in other studies; however, subtle effects resulting from lead exposure, such as the suppression of the activity of certain enzymes, cannot be ruled out for some individual animals. Results from our 2006 reconnaissance-level study indicate that overall, voles and small birds obtained from near the DMTS road in Cape Krusenstern National Monument were not adversely affected by metals exposure; however, because of the small sample size and other uncertainties, continued monitoring of lead and cadmium in terrestrial habitats near the DMTS road is advised.

Keywords Metals · Birds · Mammals · Cape Krusenstern · Alaska · Environmental impacts

Introduction

Red Dog Mine, one of the world's largest producers of lead (Pb) and zinc (Zn) concentrates, is situated in northwest Alaska approximately

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50 km northeast of the boundary of the Cape Krusenstern National Monument (CAKR), one of several National Park Service (NPS) lands managed through the Western Arctic National Parklands (WEAR; Fig. 1). Despite the harsh climate of the Arctic region, the Red Dog Mine has produced Pb and Zn concentrates year-round since it began operation in 1989. After milling and flotation concentration at the mine, the powdered ore concentrates are transported 85 km by truck

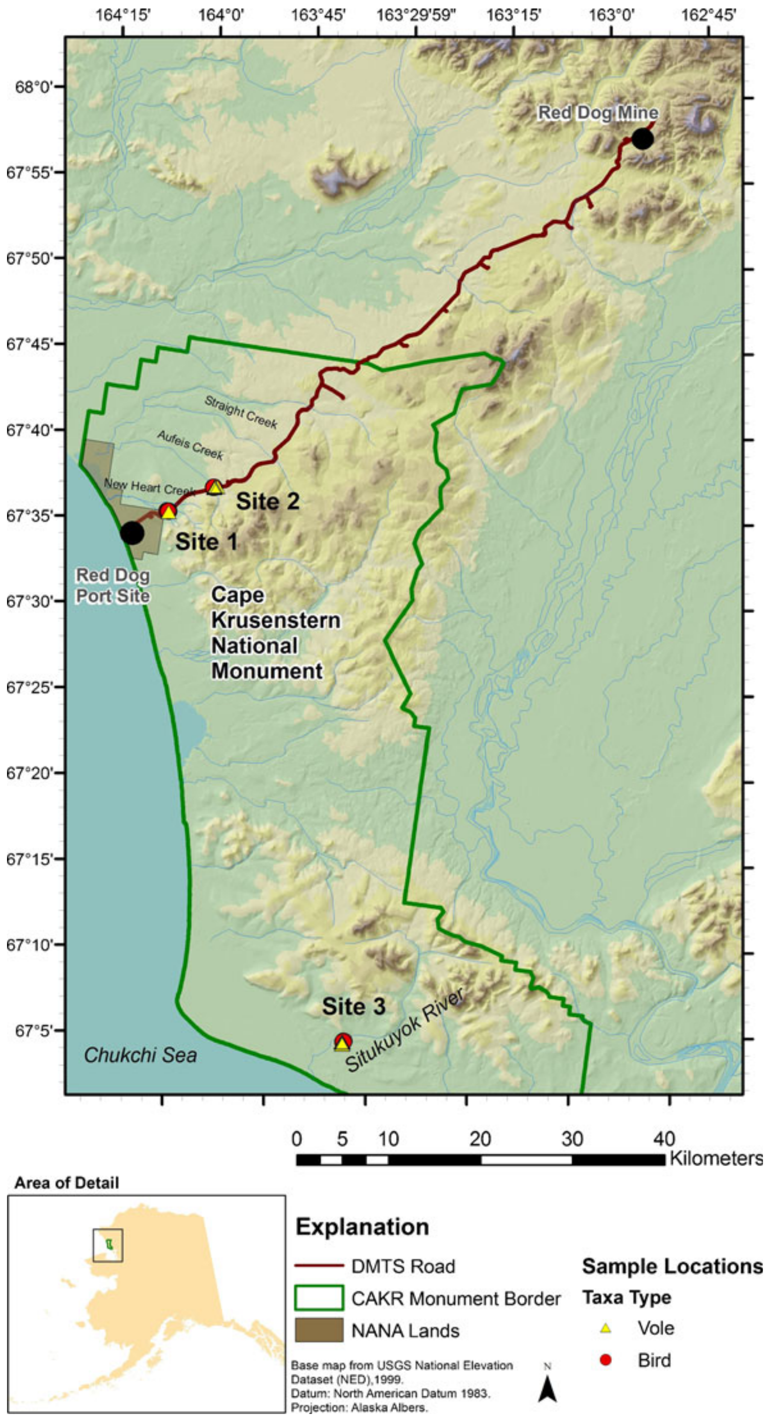

Fig. 1 Sampling locations for voles and small birds captured in Cape Krusenstern National Monument, Alaska, 2006

on a haul road to storage facilities adjacent to the Chukchi Sea. At the port site, the ore is stored until it can be distributed to various parts of the world during the brief ice-free shipping period from July to September. The haul road is the primary component of the Delong Mountain Regional Transportation System (DMTS) that also includes the mine and port facilities (Fig. 1); it traverses 32 km of CAKR land, for which a 100-year transportation easement was granted in 1985 by Congressional approval (Hasselbach et al. 2005).

Large trucks using the DMTS haul road to transport Pb and Zn concentrates from Red Dog Mine to the shipping facility at Red Dog Port are believed to have released quantities of finely powdered mine concentrates from their loads or exterior surfaces during transport (Alaska Department of Environmental Conservation 2007). Beginning in 1999, NPS researchers sampled moss (*Hylocomium splendens*) to document patterns of airborne heavy metal deposition on NPS lands from mining operations at Red Dog Mine. Moss sampled along the haul road corridor in 2000 contained elevated concentrations of cadmium (Cd), Pb, and Zn the source of which was attributed to escape of ore concentrate from trucks during transport (Ford and Hasselbach 2001). In a follow-up study, heavy metal concentrations in moss were measured throughout CAKR ($n = 226$) and geostatistical models were used to predict the extent and pattern of atmospheric deposition of Cd and Pb on NPS lands (Hasselbach et al. 2005). Spatial regression analyses indicated that heavy metal deposition decreased with distance from the haul road and the port site. Analysis of subsurface soil indicated that observed patterns of heavy metal deposition reflected in moss were not attributable to subsurface soils at the sample points. Furthermore, moss Pb concentrations throughout the northern part of the study area were greater than concentrations previously reported for other Arctic Alaska sites. Collectively, these findings indicated the presence of mine-related heavy metal deposition within the northern part of CAKR.

Based on the NPS moss studies, metal bioaccumulation resulting from mining activities could be a long-term issue of concern for managing the CAKR ecosystem. Between 2001 and 2004, an ecological risk assessment (ERA) on the effects of fugitive dusts associated with the DMTS was conducted (Alaska Department of Environmental Conservation 2007; Exponent 2007). In brief, the conclusions of that study were that ecological risks associated with transport of ore concentrates along the DMTS were low to aquatic organisms and to area wildlife populations (Exponent 2007).

In this study, additional data regarding natural resources within CAKR were collected to supplement work done in the ERA. Small animals that forage on terrestrial organisms or vegetation were targeted because they are likely to be at greatest risk from ingestion of fugitive dust either directly or indirectly after uptake by plants and insects. Risks associated with exposure by ingestion tend to be greater in smaller animals because weight-normalized food intake rates tend to increase with decreasing body weights (U.S. Environmental Protection Agency 1993). Areas along the haul road in the northwestern part of CAKR were targeted based on previous studies that indicated that metal deposition within CAKR was greatest along the road and nearest to the port site (Hasselbach et al. 2005). Areas north of the haul road are subject to greater contamination from fugitive dusts because of prevailing southerly winds. Fugitive dusts from transported mining concentrates were the most likely sources of Cd, Pb, and Zn.

Various metals, including Cd and Pb, are known to be clastogenic and strong correlations between DNA damage in animal tissues and exposure to these contaminants have been observed (Sharma and Taludker 1987; Hartwig 1994, 1995). Pancreatic lesions have been documented as a result of Zn poisoning in birds (Beyer et al. 2004); elevated concentrations of Cd could result in kidney damage in birds (Furness 1996); whereas a variety of gross internal lesions such as wasting of breast muscles, reduced visceral fat, impactions of esophagous, and proventriculus have been observed as a result of exposure to high concentrations of Pb in birds (Pain 1996). The objectives of this study were to assess biological effects of metals in voles and small passerine birds that could be associated with increased metals exposure near the DMTS haul road in CAKR, Alaska. We used flow cytometry to determine potential DNA damage associated with metal exposure, and examined for abnormalities in various tissues using histopathology.

Methods

Sample collection

Animals were collected from two locations north of the haul road (within 200 m) along New Heart and Aufeis Creeks, and one reference location near the Situkuyok River in southern CAKR (Fig. 1). The reference area was chosen based on being a similar distance from the coast, having similar vegetative cover (willow shrub near waterways and mostly mixed-shrub tussock tundra elsewhere), and being far enough south of the DMTS road to effectively rule out the possibility that metal-enriched fugitive dusts might be reaching that area. Haul-road sites were sampled June 21–29, 2006 and the reference site was sampled July 2–5, 2006. The bird species collected included the common redpoll (*Carduelis flammea*, $n = 9$), savannah sparrow (*Passerculus sandwichensis*, $n = 6$), and American tree sparrow (*Spizella arborea*, $n = 5$). The small mammals collected included the northern red-backed vole (*Clethrionmys ritilus*, $n = 10$) and the tundra vole (*Microtus oeconomus*, $n = 2$). Birds collected from near New Heart Creek were dominated by savannah sparrow, whereas a mix of common redpoll and American tree sparrow were obtained from near Aufeis Creek and the Situkuyok River (Fig. 1). Three of the six voles from the reference site appeared to be juveniles; otherwise, all animals were adults.

Sherman traps baited with peanut butter and oats were used to capture voles, whereas mist nets were used for small birds. Traps were set with fresh bait in the evening and checked the following morning. Trap placement focused on mesic tussock/dwarf birch micro habitats where vole runs (trails) were evident. At the two DMTS road sites, approximately two-thirds of the traps were placed between 10 and 100 m from the road, and the remainder placed between 100 and 200 m away. Mist nets were set in the mornings and evenings to coincide with periods of greatest bird activity and were checked approximately every 10 min. Each captured bird was held in a nylon stocking for transport to a nearby tent for processing. Mist nets were set adjacent to the creeks by sites with taller willow and shrub vegetation, at nominal distances of 50, 100, 150, and 200 m from the DMTS road. All animals obtained from the two DMTS sites were captured between 12 and 200 m to the north of the haul road.

Voles and birds were processed inside a large tent set up about 150 m from the DMTS road and near the collection site in Situkuyok. Animals were weighed with a pesola scale and body mass and body condition were recorded. Between 0.5 and 1.0 mL of blood was collected from the jugular vein with a 1-mL tuberculin syringe and 25-gauge needle. In some instances a heart puncture (after euthanasia) using a 20-gauge needle was necessary to obtain blood. About five drops of whole blood were transferred to a cryogenic vial containing a commercially available sorter media and stored on dry ice for later determination of DNA damage by flow cytometry. Another five to six drops were dispensed into a pre-weighed, acid-cleaned, 10-mL borosilicate glass test tube fitted with a tetrafluoroethylene-lined polyethylene screw cap for metals determination (also placed on dry ice). Immediately after blood collection, birds were euthanized by cervical dislocation and mammals by thoracic compression. Each animal was then immediately opened for tissue collection following general procedures (Schmitt et al. 1999; MacDonald and Cook 2002). The animals were examined for evidence of gross lesions consistent with toxicity caused by high levels of heavy metal. These included the absence of body fat, muscle wasting, pallor, enlargement or shrinkage of the kidneys, enlargement or shrinkage of the liver, the absence of reproductive activity, and joint swelling (Munson 2007; Phalen 2003). Between samples, all instruments were wiped with a lint-free laboratory tissue and rinsed with isopropyl alcohol. All animal collection and handling procedures followed those described by the Ornithological Council (1999) or Gannon and Sikes (2007) and were approved by the US Geological Survey (USGS) Animal Use Committee.

A section of liver, kidney, skeletal muscle, ventriculus, femur or tibia, pancreas (three mammals), uterus (two mammals), stomach (mammals), and the whole spleen, ovary, or testes were transferred to vials containing 10% neutral buffered formalin at ambient temperature for histopathology analysis. Samples were thus preserved within 10 to 15 min of animal death. The remainder of each liver and kidney tissue, as well as a femur (voles) or tibia (birds) bone, were each transferred to a separate chemically cleaned cryogenic vial and stored on dry ice. The remaining carcass was placed in a heavy duty plastic bag, sealed with elimination of surrounding air, and stored on dry ice. All specimens except those in the liquid preservative were shipped with dry ice to the USGS laboratory in Columbia, Missouri, for archiving and analysis of metal contents. Accompanying each shipment of samples were completed USGS sample batch history and chain-of-custody forms.

Tissue histology

Histological observations were conducted on the liver, kidney, ovary or testes, spleen, skeletal muscle, and ventriculus from birds and mammals; and additionally in the pancreas of three and uterus of two mammals. Also, histological examination was done on stomachs from voles and evaluated in glandular and nonglandular regions, but histological preparations in these preliminary readings were limited to one region or the other in any given individual vole. The tissues were sectioned and prepared for examination for lesions or other abnormalities according to established procedures at the School of Veterinary Medicine, Texas A&M University (Saggese et al. 2007). Briefly, tissues were paraffin embedded, and sectioned at 4 μm. Deparaffinized tissues were then routinely stained with hematoxylin and eosin. All slides were read blind.

The tissues were examined microscopically for evidence of acute heavy metal poisoning. Both lead and zinc poisoning would be expected to cause necrosis of the proximal tubules and a hemoglobin nephrosis. Hemosiderosis of the liver and spleen would also be expected (Hooser 2007; Garland 2007; Thompson 2007; Schmidt et al. 2003). Degranulation of pancreatic acinar cells would also be expected with zinc intoxication (Schmidt et al. 2003). Cadmium intoxication would cause metastatic mineralization of the kidneys and both hepatic and testicular apoptosis and necrosis. Chronic exposure to cadmium has been linked to an increased incidence of cancer (Hooser 2007).

Flow cytometry

The DNA of bird blood and mammal femur samples was analyzed by flow cytometry to detect possible DNA damage (Bickham 1990; Custer et al. 1994). Analyses were conducted at the Center for the Environment, Purdue University, Lafayette, Indiana. Briefly, thawed blood samples (50 μl) contained in the freezing media were added to a trypsin/citrate buffer solution and gently homogenized with a Teflon dounce. The reaction was terminated by the addition of trypsin inhibitor, then the sample solution was filtered through 30-μm nylon mesh and stained with propidium iodide. Stained nuclei were analyzed using a Beckman Coulter Epics Elite flow cytometer (Beckman Coulter, Fullerton, California) that measures the fluorescence of stained nuclei reflecting relative DNA content. The DNA content of 10,000 nuclei from the G1, or resting phase of the cell cycle, was measured and presented as a normally distributed histogram with a corresponding coefficient of variation [$CV = 100 \times$ standard deviation/mean of the histogram peak]. Half-peak coefficients of variation (HPCV) values were used for statistical analysis. A significantly higher HPCV value between samples from the haul road and those from the reference site would be indicative of potential DNA damage resulting from exposure to clastogenic chemicals, including the metals of concern.

Chemical analysis

Cadmium, Pb, and Zn were targeted for this study. The chemical procedures are described in detail in Brumbaugh et al. (2008). Briefly, liver and blood samples were lyophilized and then were pulverized with an acid-cleaned glass rod to a powder-like consistency. The dry samples were digested with nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). Analysis of the sample digestates for trace elements was conducted by inductively coupled plasma mass spectrometry for Cd, Pb, and Zn according to instrument manufacturer recommendations and United States Environmental Protection Agency (US EPA) quality assurance guidelines (U.S. Environmental Protection Agency 1996). Solutions of scandium, rhodium, and bismuth were used as internal standards. Quality-control measures included three to five method blanks (three with blood sample digestions; five with liver sample digestions), certified reference materials (freeze-dried liver tissue or blood), replicate samples, and fortified samples (pre-digestion spikes). Atomic masses monitored included ^{65}Zn, ^{68}Zn, ^{111}Cd, ^{114}Cd, and $^{206+207+208}$Pb. All Pb isotopes were summed and individual masses for Cd and Zn were reported. Either mass for Cd and Zn could have been used for accurate quantitation of these two elements. All liver and whole blood elemental concentrations are reported on a dry weight basis.

Mean recoveries of cadmium, lead, and zinc ranged from 92% to 112% in four certified reference tissues that included one each of liver, oyster, and whole fish tissue, and one synthetic gelatin matrix (National Institute of Standards and Technology SRM 1577: Bovine Liver, Gaithersburg, MD, USA; National Institute of Standards and Technology SRM 1566b: Oyster Tissue, Gaithersburg, MD, USA; International Atomic Energy Agency RM 407: Trace Elements and Methylmercury in Fish Tissue, Vienna, Austria; Eastman Kodak Gelatin Multi-component Trace Element Reference Material TEG-50-B, New York, USA). Recoveries of cadmium, lead, and zinc in three blood reference samples ranged from 89% to 109% (International Atomic Energy Agency A-13: Freeze-dried Animal Blood, Vienna, Austria; National Institute of Standards and Technology SRM 966: Toxic Metals in Bovine Blood, Gaithersburg, MD, USA; and ClinChek Whole Blood Control, Level II; Munich, Germany). The range of individual pre-digestion spike recoveries of Cd, Pb, and Zn added to a reference liver or blood sample was 91% to 105%. The method detection limits in micrograms per gram dry weight equivalents for blood and liver, respectively, were: Zn—1.0, 0.7; Cd—0.0008, 0.009; and Pb—0.003, 0.01.

Statistical analysis

There were 32 liver samples (20 for birds and 12 for mammals) and 22 blood samples (11 for birds and 11 for mammals) collected. To determine potential metal differences between the two sites sampled at the DMTS road, we compared concentration of liver Zn, Cd, and Pb in birds from Aufeis Creek and New Heart Creek with a two-sample *t* test. The data were normally distributed. Because of the limited sample size, no comparisons between these two sites were possible for bird blood or for liver and blood in mammals. Thus, liver and blood samples from each taxonomic class from both sites within the DMTS road were combined for comparisons with the reference site. We compared concentrations of Cd, Zn, and Pb between the DMTS road and the reference site with a two-sample *t* test. For the flow cytometry analysis, there were only 12 bird samples (blood) and ten mammal samples (femurs), and were compared similarly with a two-sample *t* test. All the statistical analyses were conducted with the use of SAS version 9.1 (SAS 2004). The P value was set at <0.05.

Results

Birds

Concentration of liver Zn, Cd, and Pb were not significantly different between birds from the two locations along the DMTS road (Aufeis Creek and New Heart Creek). Similarly, concentrations of Zn in liver of birds from the DMTS sites were not significantly different from those at the reference site; however, concentrations of Cd ($t = 4.3$, $P = 0.0014$) and Pb ($t = 9.04$, $P = 0.0001$) were significantly higher in liver of birds from the DMTS road sites than in those from the reference site (Table 1). In blood, there were no significant differences in concentrations of Zn in birds collected along the DMTS road or those from the reference site. However, Cd and Pb were significantly higher in samples from the DMTS road than in those from the reference site ($t = 3.0$, $P = 0.05$ and $t = 5.5$, $P = 0.015$ for Cd and Pb, respectively).

Table 1 Concentrations (mean ± SD, μg/g dw) of selected metals in blood and liver of birds and mammals from Cape Krusenstern National Monument, Alaska (different letters between DMTS and Situkuyok values indicate significant differences)

	Location	Species	*n*	Liver			*n*	Blood			*n*	HPCV DNA
				Zn	Cd	Pb		Zn	Cd	Pb		
Birds	Aufeis (site 2)	American tree sparrow	1/1	72.2	1.13	2.18	1/1	26.4	0.140	0.52	0/1	–
		Common redpoll	3/3	90.2 ± 13.3	0.98 ± 0.24	0.53 ± 0.04	1/3	21.6	0.004	0.06	1/3	3.36
		All	4	85.7 ± 22	1.0 ± 0.5	0.94 ± 0.83	2/4	24 ± 3.4	0.009 ± 0.006	0.29 ± 0.32	1/4	3.36
	New Heart (site 1)	American tree sparrow	2/2	97.7 ± 6.1	1.42 ± 0.8	3.38 ± 4.1	1/2	30.8	0.014	0.49	2/2	5.83 ± 3.05
		Savannah sparrow	6/6	82.3 ± 6.5	1.7 ± 0.5	2.4 ± 1.2	5/6	27.7 ± 10.8	0.02 ± 0.02	0.38 ± 0.14	3/6	6.84 ± 3.45
		All	8	88.4 ± 8.3	1.6 ± 0.5	2.64 ± 1.9	6/8	23.2 ± 7.3	0.018 ± 0.005	0.45 ± 0.27	5/8	6.44 ± 2.93
	DMTS	All	12	87.5 ± 13.3 A	1.4 ± 0.55 A	2.08 ± 1.8 A	8/12	23.4 ± 6.2 A	0.02 ± 0.01 A	0.41 ± 0.27 A	6/12	5.9 ± 2.9 A
	Situkuyok (site 3)	American tree sparrow	2/2	81.8 ± 10.7	0.78 ± 0.15	0.04 ± 0.0	1/2	3.8	0.004	0.003	2/2	7.44 ± 5.05
		Common redpoll	6/6	93.1 ± 17.2	0.65 ± 0.13	0.12 ± 0.04	2/6	19.1	.007	0.03	4/6	7.04 ± 2.74
		All	8	90.3 ± 11.5 A	0.7 ± 0.14 B	0.10 ± 0.05 B	3/8	23 ± 6.9 A	0.006 ± 0.002 B	0.02 ± 0.02 B	6/6	7.2 ± 3.1 A
Mammals	Aufeis (site 2)	Tundra vole	2/2	93.8 ± 8.8	0.14 ± 0.05	0.58 ± 0.08	1/2	21.5	0.002	0.5	2/2	1.69 ± 1.28
		Red-backed vole	3	77.1 ± 7.7	2.5 ± 1.6	1.64 ± 0.65	3	24.8 ± 6.9	0.02 ± 0.01	0.60 ± 0.17	3/3	3.19 ± 0.21
	New Heart (site 1)	Red-backed vole	1	120.0	0.56	0.94	1	22.0	0.002	0.88	1/1	2.67
	DMTS	All	6	89.8 ± 18.0 A	1.39 ± 1.59 A	1.17 ± 0.67 A	5	23.6 ± 5.2 A	0.01 ± 0.01 A	0.64 ± 0.19 A	6/6	2.6 ± 0.94 A
	Situkuyok (site 3)	Red-backed vole	6	82.0 ± 13.7 A	0.15 ± 0.15 B	0.07 ± 0.02 B	6	25.2 ± 5.2 A	0.003 ± 0.002 A	0.04 ± 0.01 B	4/6	2.7 ± 0.89 A

n ratios indicate number of samples with measurable amount of sample/total number of samples

Mammals

There were no significant differences in liver Zn concentrations between the DMTS road and the reference site; however, Cd and Pb concentrations were significantly different between both locations ($t = 2.7$, $P = 0.04$ and $t = 9.8$, $P < 0.0001$, respectively) and were higher at the DMTS road than at Situkuyok. In blood of mammals, concentrations of Zn and Cd were not significantly different between the DMTS road and the reference site; however, Pb was significantly higher in blood of mammals from the DMTS road sites than from Situkuyok ($t = 7.9$, $P = 0.001$).

DNA HPCV and histological analysis of avian and mammal tissue

For birds and mammals, there were no significant differences in the DNA HPCV values between the DMTS road and the reference site. With one exception, histological analysis revealed no abnormalities in either the bird or mammal tissues that could be related with metal exposure. The lone abnormality was moderate multifocal mineralization with moderate diffuse degeneration of proximal tubules in the kidney of one red-backed vole from Aufeis Creek.

Regarding the reproductive status of the animals collected, all the male birds were producing sperm and there was evidence of folliculogenesis in the ovaries of all female birds. In contrast, spermatogenesis was not occurring in three of five male mammals, but two of those which were from the reference site might have been juveniles All female mammals had developing follicles or one or more corpus lutea suggesting that they were reproductively active at the time they were collected.

Discussion

Animals captured from near the road were assumed to be exposed to elevated concentrations of metals including cadmium, lead, and zinc. Previous measures of these three metals in mosses collected at distances of 3, 50, 100, 250, and 1,000 m from the road indicated mean concentrations of lead as 430, 299, 159, 71, and 33 mg/kg dw; cadmium as 12, 7.2, 4.1, 1.8, and 0.8 mg/kg dw; and zinc as 1960, 1250, 763, 370, and 187 mg/kg dw, respectively (Ford and Hasselbach 2001). Soils of this arctic region lie underneath a thick mat of peat and living moss, and most of the soil horizon is in a state of permafrost; consequently, in most areas metals are not elevated appreciably in the soil itself (Hasselbach et al. 2005). Thus, the animals in this area presumably are exposed mostly by metals that reside in the peat, moss, and other vegetation, as opposed to the underlying soil.

Lead was the most elevated metal measured in animals captured near the DMTS road. Both voles and small birds captured from near the DMTS road had blood and liver Pb concentrations that were greater on average by factors of about 16 to 20 as compared with the reference location. Cadmium was approximately three times greater in tissues of birds and voles captured near the DMTS road than at the reference site. Liver cadmium concentrations in individual voles obtained from near the DMTS road varied substantially (range of 0.1 to 4.0 μg/g dw) and red-backed voles had much greater Cd concentrations than tundra voles (Table 1). The one vole with the kidney lesion also had the greatest blood and liver Cd concentrations (0.036 and 4.0 μg/g dw, respectively) and the liver concentration was more than 25 times greater than the mean in voles from the reference site (0.15, Table 1). Therefore, Cd exposure, which is known to cause this type of kidney lesion (Scheuhammer 1987), might have been at least partly responsible.

Mean blood Pb concentrations of animals obtained near the road were about 30% greater in voles than in small birds, but the opposite trend was observed for liver Pb concentrations. Lead concentrations in livers were much more variable among individual birds than in voles, perhaps because not all of the birds arrived to the breeding area at the same time or because of potential differences in diet or foraging locations among the three species collected. Most migratory birds typically arrive in this region sometime in mid-May (Anderson 1994); therefore, the birds probably only had inhabited the area for about 6 weeks before their capture in late June. The finding of elevated liver Pb concentrations in small birds that presumably had been present in the region for only about 6 weeks suggests rapid assimilation of Pb by birds near the DMTS road. It is surprising that birds had greater liver Pb levels than voles since voles are resident and do not hibernate; thus, they should have foraged periodically throughout the winter (Osborne 1994). Dietary preferences and greater food consumption rates might partly explain the apparent greater assimilation of Pb in the small birds as compared to the voles, and why tundra voles had much lower Cd concentrations than red-backed voles. Ma et al. (1991) observed that common shrews (*Sorex araneus*), which had consumed mostly earthworms, spiders, and beetles, had accumulated much greater body burdens of Cd and Pb from metal-contaminated soils as compared to field voles (*Microtus agrestis*), which had consumed mostly grasses and mosses. During the breeding season, a large fraction of the diet of sparrows typically consists of insects (Anderson 1994; Knox and Lowther 2008; Naugler 1993; Wheelwright and Rising 1993) whereas voles, particularly the *Microtus* genus, typically consume mostly vegetation (Osborne 1994). Other factors that may help explain differences between birds and mammals include differences in absorption rates of metals due to different pH of the stomach and preference for grit and soil by some bird species (Beyer et al. 1994).

Mean liver Pb concentrations measured in voles and small birds collected from the CAKR reference site (0.073 and 0.097 μg/g dw, respectively) were considerably less than concentrations previously reported at several reference sites in other regions. For example, voles from several reference locations in the USA, Canada, and Europe had liver Pb concentrations that ranged from 2.8 to 12 μg/g dw (*Clethrionomys glareolus*), and 1 to 6 μg/g dw (*Microtus* spp; Talmage and Walton 1991). Getz and others (1977) reported mean liver Pb concentrations (0.6 μg/g dw) in rural house sparrows (*Passer domesticus*), about six times greater than in sparrows obtained from the reference location in CAKR. Liver Pb concentrations in voles and sparrows at reference locations for these pre-1990 studies might have been affected by residual atmospheric Pb originating from historic uses as a gasoline additive through the 1970s, whereas the remoteness of the reference location in southern CAKR might have been less affected by any anthropogenic sources of Pb. In addition, recent improvements in ultra-trace preparation procedures and instrumentation have led to increased accuracy for lower concentrations and smaller tissue samples. For example, in a recent study with deer mice (*Peromyscus maniculatus*) Pb liver concentrations of laboratory-raised control animals were reported as only about 0.05 μg/g dw (McBride 2007).

Concentrations of Zn in livers and blood of voles or birds were not significantly different between sites. Previously, it was reported that Zn concentrations were greatly elevated in mosses near the DMTS road (Ford and Hasselbach 2001), thus, we anticipated that Zn concentrations might be elevated in animals collected near the DMTS road. However, Zn tissue concentrations are regulated internally by most vertebrates unless the Zn dose is extraordinary (Talmage and Walton 1991); furthermore, acute Zn poisoning in wildlife seldom has been documented. Waterfowl suffering from pancreatitis had liver concentrations of at least 280 μg Zn/g dw, presumed to be caused by consumption of mine wastewater containing elevated concentrations of Zn (Sileo et al. 2004). Liver Zn concentrations in these waterfowl were about three times greater than liver Zn concentrations of passerine birds from CAKR.

Critical tissue concentrations for sub-clinical effects (lesions, for example) in small mammals have been suggested to be about 20 ug/g dw for Pb in liver and about 120 ug/g dw for Cd in kidney (Ma et al. 1991; Cook and Johnson 1996). The maximum liver Pb concentration in voles from our study (2.2 ug/g dw) was well below the sub-clinical benchmark for Pb. The kidney is the "target" organ for Cd and is where effects would be expected to be first observed (Cook and Johnson 1996); though Scheuhammer (1987) suggested that the liver is preferred for biomonitoring because kidney Cd concentrations may begin to decrease in animals exposed at high doses. We did not measure kidney metal concentrations, but the ratio of Cd in kidney/liver in voles and mice reportedly ranges from about 2 to 8 (Cook and Johnson 1996). Therefore, assuming a conservative ratio of 8, the maximum corresponding kidney Cd concentration among voles from our study would be about 32 ug/g dw, also well below the sub-clinical benchmark.

Analysis for DNA damage in blood is another approach for assessing sub-clinical effects of pollutants (Bickham 1990), particularly clastogenic contaminants such as cadmium. We did not find any DNA damage in tissues of animals collected along the haul road as determined by the lack of differences in DNA HPCV values between species collected along the DMTS road and those from the reference site. Both Cd and Pb are known to produce DNA damage although generally at concentrations greater than those observed in our study (Hartwig 1994). However, in at least one study, DNA damage observed in black kite nestlings (*Milvus migrans*) captured from near a mining accident in Spain was positively correlated with maximum blood Cd concentrations of only about 0.1 μg/g dw (Baos et al. 2006). The authors of that study emphasized that their analysis indicated that other contaminant stressors likely contributed to the observed DNA damage; therefore, blood Cd concentrations of those birds cannot be assumed to represent thresholds for DNA damage. Furthermore, Cd residues in healthy bird populations can vary by several orders of magnitude among different species (Furness 1996). Nevertheless, given the high toxicity and carcinogenicity of Cd, it is noteworthy that some birds in our study had elevated blood Cd concentrations and that one vole had a kidney lesion associated with elevated Cd concentrations.

Comparison of Pb and Cd concentrations to other studies

Although Cd and Pb were elevated in animals captured near the DMTS road, vole and small bird liver and blood concentrations were less than concentrations at which sub-clinical effects were evident in other studies. For example, rodents, including mice and gophers, captured near metal smelters that displayed histopathological effects from metal toxicosis had mean Pb concentrations of between 5 and 15 μg/g dw in the liver and >30 ug/g dw in the kidney (Stansley and Roscoe 1996; Damek-Poprawa and Sawicka-Kapusta 2003; Reynolds et al. 2006). Mean liver Cd concentrations in affected rodents of those same studies ranged from about 4.0 to 9.0 μg/g dw. Effects in the livers of those animals included decreased glycogen content, interstitial fibrosis, increased number of pyknotic nuclei, and necrosis; whereas effects in kidneys included the presence of intranuclear inclusions, hyperplasia of tubules, atrophy of glomeruli, interstitial fibrosis, and necrosis. In our study, only one abnormality (mineralization in kidney tissue) was found, but the Cd liver concentration (4.0 μg/g dw) of that animal was consistent with the lower range of liver Cd concentrations associated with the onset of kidney lesions and other effects in deer mice (Damek-Poprawa and Sawicka-Kapusta 2003). Cook and Johnson (1996) suggested that one of the major difficulties in understanding the effects of Cd in wildlife is whether measurable kidney damage is related to ecological fitness. This is because the kidney has spare function and considerable regenerative capacity; furthermore, some effects apparently can be tolerated through the life of an organism and do not necessarily lead to eventual renal failure. Thus, a kidney lesion may not necessarily cause direct health effects; however, individuals affected in this manner may be more likely to contract other diseases (Cook and Johnson 1996).

In gophers, a blood Pb concentration greater than about 1.0 μg/g dw was associated with suppressed ALAD enzyme activity (Reynolds et al. 2006). A similar threshold for effects on ALAD activity in relation to blood Pb concentration was reported for deer mice (McBride 2007). Suppression of ALAD activity is perhaps the most sensitive biochemical indicator of Pb exposure, but mild suppression usually is not associated with adverse biological effects (Dieter 1979). Liver Pb concentrations of voles near the DMTS road in CAKR were about three to five times less than liver concentrations associated with histopathological effects in field studies of mice or gophers, but their blood Pb concentrations were only slightly less than the concentration reported to be associated with an onset of suppressed ALAD enzyme activity in gophers or deer mice.

According to Pain (1996), Franson (1996), and Friend and Franson (1999), biological functions can be impaired and external signs of poisoning may be observed in birds when blood Pb concentrations are between 0.5 to 1.0 μg/mL (about 2.6 to 5.2 μg/g dw), or when liver Pb concentrations are between 6–15 μg/g wet weight (about 20–50 μg/g dw). The US Fish and Wildlife considers adult waterfowl with blood Pb concentrations greater than 0.2 μg/mL (about 1.3 μg/g dw) to be above "background" (U.S. Fish and Wildlife Service 1986). In comparison, the mean Pb concentration for all 12 birds from the DMTS road sites in CAKR was 0.41 μg/g dw in blood and 2.1 μg/g dw in liver. The greatest Pb concentration measured among these birds was in a savannah sparrow captured about 50 m from the DMTS road at New Heart Creek (0.93 μg/g dw in blood; 6.2 μg/g dw in liver)—concentrations that were about three times less than the reported thresholds for effects.

Beyer et al. (2004) investigated concentrations of metals in several species of birds from the Tri-state lead–zinc mining district in Oklahoma, Kansas, and Missouri, including mourning doves (*Zenaida macroura*), bank swallows (*Riparia riparia*), northern cardinals (*Cardinalis cardinalis*), and American robins (*Turdus migratorius*). Except for the doves, mean liver concentrations in the other passerine birds reported by Beyer were 1.6 to 3.5 times greater in Pb and 2.0 to 4.2 times greater in Cd than the concentrations measured for sparrow species captured near the DMTS road. Notably, Beyer et al. (2004) reported greater than 50% inhibition of blood ALAD associated with increased Pb concentrations in the songbirds from that study, but found no effects that could be attributed to Cd exposure. Song sparrows (*Melospiza melodia*) from the Coeur D'Alene River Basin, a region contaminated with Pb and Zn, had a mean liver Pb concentration of about 6.7 μg/g dw (Johnson et al. 1999) or about 2.5 times greater than that of sparrows near the DMTS road. Custer and others (2002) reported 50% or greater reduction in ALAD activity in 25% of tree swallows nesting at a location where stream sediments contained increased concentrations of Pb. The geometric mean Pb liver concentration of the tree swallows was 0.54 μg/g dw, which was less than the geometric mean of 1.47 μg/g dw for all small birds captured from near the DMTS road. Thus, unless sparrows are more tolerant of Pb than swallows, those data indicate that subtle biochemical effects such as inhibition of ALAD enzyme activity might be possible for some of the birds nesting nearest the DMTS road.

Although we found no toxicological effects, our study shows that small passerine birds nesting near the DMTS road accumulated Pb, and to a lesser extent, Cd, during the breeding season. It is of some concern that tissue concentrations of these metals could increase throughout the summer resulting in potentially greater toxic effects on the young and adults at the time when the birds are preparing to migrate south for the winter. Some studies indicate that animals in the field may be more sensitive to metal exposure than predicted from laboratory tests (Shore and Rattner 2001). Thus, potentially greater tissue Pb and Cd concentrations during bird migration might have a greater impact on birds than what we observed at the beginning of the nesting season.

Conclusions

In this investigation we demonstrated that concentrations of Pb and Cd were elevated in small birds and mammals of the DMTS relative to the reference site. Blood and liver metal concentrations of animals captured near the DMTS road were about 20 times greater for Pb and about three times greater for Cd, but there were no differences for Zn. Blood and liver Pb concentrations of voles and small birds inhabiting areas near the DMTS road were below critical tissue levels associated with sub-clinical effects (Ma 1996; Pain 1996). Accordingly, Pb concentrations in these animals were below concentrations associated with the onset of adverse biological effects in other studies; however, subtle effects on blood chemistry resulting from Pb exposure, such as the suppression of the activity of certain enzymes, cannot be ruled out for some individuals. Liver Cd concentrations of voles and birds from near the DMTS road also were below critical levels reported to be associated with sub-clinical effects (Cook and Johnson 1996; Furness 1996). One vole exhibited moderate metastatic mineralization of kidney tissue, otherwise, we observed no abnormalities within internal organs or DNA damage in blood in any of the animals. The affected vole also had the greatest liver and blood Cd concentrations, indicating that the lesion might have been caused by Cd exposure. Results from our 2006 reconnaissance-level study indicate that overall, voles and small birds captured from near the DMTS road in Cape Krusenstern National Monument were not adversely affected by metals exposure; however, because of the small sample size and other uncertainties, continued monitoring of lead and cadmium in terrestrial habitats near the DMTS road is advised.

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