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Trace Elements in the Fur of Bats (Chiroptera: Vespertilionidae) from Ontario and Quebec, Canada

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A large literature reporting the concentrations and effects of anthropogenic contaminants on wildlife has developed over the past several decades (Hughes et al. 1997). These studies have documented geographic (Bishop et al. 1995) and temporal (Frank et al.1983) variation in the concentrations of contaminants, as well as their associated health effects (Bishop et al. 1998) in a wide range of species. Species at the tops of food chains, especially birds of prey, have received a great deal of attention because they accumulate high concentrations of mercury and fat-soluble contaminants like polychlorinated biphenyls (PCBs) and other organic compounds (Hughes et al. 1997).

Bats are among the most common vertebrates in many urban and agricultural settings (Brigham and Fenton 1986) and they accumulate contaminants (Clark 1981). However, relatively little attention has been paid to the concentrations of anthropogenic contaminants in bats and other insectivores compared to other taxa. Most of the existing data on contaminant concenrations in bats have focused on organic compounds (Thies et al. 1996) and to our knowledge, there are no published data on the concentrations of metals in Canadian bats.

Five features of their life histories and biology make bats obvious species of interest with respect to contaminants. First, bats are long-lived, small and mobile. Second, bats typically consume between 40% and 100 % of their body mass in prey each night (Hickey and Fenton 1996). Third, some species feed heavily on emerging insects such as Trichoptera that spend their larval stages in sediments (Brigham and Fenton 1991) where contaminants from past industrial activities may have accumulated. Fourth, bats often coexist with humans in urban and agricultural settings (Brigham and Fenton 1986), potentially exposing them to contaminants (Clark 1981). Fifth, aquatic insects and bats in urban environments may provide a pathway for the movement of contaminants from aquatic sediments to terrestrial ecosystems (Currie et al. 1997).

The purpose of this study was to assess variation in the concentrations 24 elements including mercury and several other metal contaminants in the fur of bats captured at sites in eastern Ontario and adjacent Quebec, Canada. In addition to documenting patterns of variation, we also tested two hypotheses:

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1) concentrations of contaminants in the fur of bats would be highly variable (within and among species), perhaps reflecting varying quality of their foraging habitats, and 2), concentrations of mercury in the fur of bats would be higher than expected based on their position in the food web, reflecting the propensity of bats to live in urban environments and consume large amounts of food relative to their body mass. Mammals with insectivorous or omnivorous diets and humans eating consuming uncontaminated food have mercury concentrations in hair or fur that are usually below 1 mg/kg (Burger et al. 1994, Saeki et al. 1996). Carnivores and humans eating large amounts of mercury-contaminated fish typically have mercury concentrations in hair or fur exceeding 10 mg/kg (Halbrook et al. 1994, Kehrig et al. 1997).

For most of the other trace elements there is insufficient information to make specific predictions about the concentration we expected to find in bat fur.

MATERIALS AND METHODS

In August 1997 and 1998, we collected fur from bats at sites in eastern Ontario and adjacent regions of Quebec, Canada (Table 1). Bats were trapped while swarming at hibernation sites, at summer maternity roosts, or along flyways leading to foraging areas. We captured bats with mist-nets, harp traps, or hand nets, depending on the situation. The fur was clipped from the mid-dorsal region of each bat, directly into pre-cleaned centrifuge tubes which were labeled with a unique identification number. Samples were stored at room temperature until they were shipped to the laboratory for analysis.

In 1997, we conducted preliminary sampling by collecting individual or pooled samples from two species at four locations (Table 1). Pooled samples were composed of fur from 2 to 38 bats. In 1998 we collected individual fur samples from 49 bats (*Myotis lucifugus*, *M. leibii*, *M. septentrionalis* and *Eptesicus fuscus*) at 4 locations (Table 1).

Fur samples were shipped to a private certified and accredited laboratory (Philip Analytical Services, Halifax, Canada) for analysis. The laboratory conducting the analysis was provided with the sample identification numbers, species, and date of capture, but not the rest of the information we recorded, nor were they aware of the purpose of the study or of the hypotheses we were testing.

Hair samples were cleaned three times in acetone and then washed with Triton X® detergent (1:400 detergent/reagent grade water). Hair samples were then rinsed three times in reagent grade water and dried at 60 °C. Hair samples were analyzed following standard analytical methods for mercury and trace elements.

For trace element analysis, the hair samples were cleaned, dried and then weighed into pre-cleaned 50 ml polypropylene centrifuge tubes. Approximately 2 ml of high purity HNO_3 was added and the sample was allowed to stand over night. The

Table 1. Sampling locations and species from which samples were obtained in 1997 and 1998.

1997 Pooled Samples			
Site Name	Location	Species	N
Lafleche Cave	Val-des-monts, Quebec 45° 39' N, 75° 39' W	*M. lucifugus*	2 (10,10)
Tyendinaga Cave	Tyendinaga, Ontario 44° 16' N, 77° 15' W	*M. lucifugus*	1 (38)
Fly Creek	Cornwall, Ontario 45° 02' N ,74° 43' W	*M. lucifugus*	1 (2)
Cornwall	Cornwall, Ontario 45° 01' N, 74° 43' W	*E. fuscus*	1 individual

1998 Samples from Individual Bats			
Site Name	Location	Species	M/F
Lafleche Cave	Val-des-monts, Quebec	*M. lucifugus*	3/2
		M. septentrionalis	4/1
		E. fuscus	0/1
Westport	Westport, Ontario	*M. lucifugus*	0/13
Hunt Mine	Dacre, Ontario	*M. lucifugus*	6/15
		M. leibii	0/2
		E. fuscus	1/0
Trinity Church	Cornwall, Ontario	*E. fuscus*	0/1

N = the number of pooled or individual samples from each site (1997). The number of bats contributing to the pooled samples are in brackets. For 1998 samples F = number of females and M = number of males.

samples were warmed until the digestion reaction subsided and subsequently heated in a boiling water bath until the acid volume was reduced to less than 1 ml. Another 2 ml of HNO_3 was added and the samples were further evaporated until the volume was less than 1 ml. Approximately 1 ml of additional HNO_3 and 10 ml of distilled, de-ionized water were added to the samples which were gently heated for 20 minutes. After the samples had cooled, they were diluted to a final volume of 25 ml. The samples were finally diluted 10-fold and analyzed directly using a Sciex/Perkin-Elmer Elan 5000 ICP-MS.

For mercury analysis, cleaned and dried hair samples were weighed into pre-cleaned 35 ml centrifuge tubes. Sulfuric acid was added to the samples which were allowed to stand overnight. The samples were then digested for 2 hours at 60°C in a convection oven. After cooling, 2 ml of nitric acid was added to each sample and the samples were digested for another 2 hours at 60°C.

After cooling, an excess of potassium permanganate was added to ensure that all samples remained in an oxidized state. Each sample was then transferred to a sparging vessel and the excess potassium permanganate was destroyed by the addition of hydroxylamine hydrochloride. Mercury in the samples was reduced to its atomic state by the addition of stannous chloride. Samples were analyzed for mercury using an LCD Analytical UV Detector (Fixed Wave Mercury Monitor).

We also included control tubes that contained no fur samples but were left uncapped in the field for the approximate time that it took us to collect a fur sample. Standard quality assurance and quality control procedures (including duplicate samples, spikes and blanks) were employed by the laboratory conducting the analyses. Detection limits varied because of small quantities of sample material available for analysis. The ranges of the detection limits for each element were: Hg, 0.01-0.12; Al, 52-1300; Sb, 10.0-260; As, 10.0-260; Ba, 32.0-770; Be, 32.0-770; B, 32.0-770; Cd, 1.7-41.0; Cr, 10.0- 260; Co, 4.2-100; Cu, 10.0- 260; Fe, 100.0-2600; Pb, 4.4-63.0; Mn, 10.0-260; Mo, 10.0-260; Ni, 10.0- 260; Se, 10.0- 260; Ag, 2.5-62.0; Sr, 32.0- 770; Tl, 0.4-10.0; Sn, 10.0-260; U, 0.4-10.0; V, 10.0-260; Zn, 26-2600.

We performed all statistical analyses using Systat 7.0 for Windows. Since data were not normally distributed, they were log transformed before statistical analysis or non-parametric tests were used. Data are reported as geometric means and 95% confidence intervals. All tests were two-tailed and the rejection level was set at $\propto = 0.05$.

RESULTS AND DISCUSSION

Some of the fur samples from the four species of bats we captured (*M. lucifugus, M. leibii, M. septentrionalis* and *E. fuscus*) had detectable concentrations of mercury, zinc, selenium, lead, aluminum, and iron. The other elements were not detected.

For the 1998 data, we calculated geometric means and performed statistical tests using half the detection limit for Hg and Zn when these elements were below the detection threshold. Since the other elements (Se and Pb) were detected infrequently we report only ranges.

We detected mercury, zinc, selenium, lead, aluminum, and iron in all of the pooled samples obtained in 1997 (Table 2.). In 1998 we detected mercury, zinc, selenium and lead in fur samples from individual bats. Mercury and zinc were detected most frequently (Hg: 48 of 49 samples; Zn: 47 of 49 samples), whereas selenium and lead were detected in 6 and 4 of the 49 samples, respectively (Table 2.).

In 1998, the concentrations of mercury and zinc varied significantly among species (ANOVA; Hg: $F = 2.81$, d.f.= 3, $P=0.05$; Zn: $F = 9.28$, d.f.= 3, $P<0.001$)

Table 2. Concentrations of metals in the fur of bats from eastern Ontario and adjacent Quebec, Canada.

1997 Location	Metal Concentration (mg/kg)					
	Hg	Zn	Se	Pb	Al	Fe
Lafleche-1 (10 *M. lucifugus*)	2.0	140	17	2.5	67	220
Lafleche-2 (10 *M. lucifugus*)	2.8	200	13	3	70	220
Tyendinaga (38 *M. lucifugus*)	7.6	130	22	1.6	41	79
Fly Creek (2 *M. lucifugus*)	3.9	130	69	6.2	27	72
Cornwall (1 *E. fuscus*)	4.6	160	9.5	8.8	4.8	100
Control	0.01	0.5	0.5	0.18	2.5	0.5

1998 Species	Metal Concentration (mg/kg)			
	Hg	Zn	Se	Pb
M. lucifugus	1.5	101.4	ND-26.9	ND-11.3
(n=39)	(1.3-2.5)	(94.9-110.1)	-	-
M. septentrionalis	4.4	107.6	-	-
(n=5)	(-1.2-10.2)	(94.4-121.6)	-	-
Eptesicus fuscus	1.5	101.3	-	ND-6.1
(n=3)	(-5.0-15.4)	(105.1-154.8)	-	-
Myotis leibii	5.3	314.6	-	-
(n=2)	(-61.0-76.2)	(-4513-5523)	-	-

Some of the samples from 1997 are pooled. The species and number of individuals comprising the samples are given in brackets. Data from 1998 are geometric means (95% confidence intervals are given in brackets). For 1998 data, one half the detection limit was used to compute geometric means and confidence intervals for Hg and Zn. Since Se and Pb were detected in only a few samples we report only ranges.

but not among locations (ANOVA; $F<0.6$, d.f.= 4, $P>0.6$ for both Hg and Zn). The conclusions were identical wether we performed ANOVAs on log transformed data or non-parametric (Kruskal-Wallis) equivalents on the actual values. For mercury, *M. lucifugus* differed significantly from *E. fuscus* (Tukey's test $F=4.647$, $P=0.02$) whereas all other paired comparisons were not significant ($P>0.22$ in all cases). For zinc, *M leibii* differed from all other speices (Tukey's test; $F=10.6$, $P<0.01$ in all cases) whereas the other three species (*E. fuscus, M. septentrionalis* and *M. lucifugus*) did not differ from one another ($P>0.46$ in all cases). There were no significant differences in the concentrations of mercury or

zinc among locations, whether we used the whole data set or just the data from *M. lucifugus* (ANOVA Hg: $F = 1.866$, $P = 0.17$, $n = 39$; Zn: $F = 1.141$, $P = 0.33$, $n = 39$). We did not have a sufficient sample size to make comparisons among locations for the other three species but we include data from these limited samples because of the scarcity of contaminant data from these species (i.e., *M. leibii* and *M. septentrionalis*).

The concentrations of contaminants did not differ significantly between male and female *M. lucifugus* (ANOVA Hg: $F = 0.11$, $P = 0.917$, $n = 39$; Zn: $F = 1.014$, $P = 0.31$, $n = 39$). For the other species (*E. fuscus, M. leibii* and *M. septentrionalis*) our sample sizes precluded comparison of metal concentrations between genders.

QA/QC: Spiked mercury samples (n=2) yielded recovery rates of 97 and 99% respectively. Method blanks (empty tubes subjected to the same treatment as the samples) yielded non-detectable values (<0.01mg/kg). Duplicate samples (n=2 bats) yielded values results that differed by <0.05 mg/kg.

Mercury in the hair of bats we captured ranged in concentration from ND - 13 $mg.kg^{-1}$, values similar to those in the fur of bats from Japan (Miura et al. 1978). The concentrations of mercury we measured in bats are within the range of those reported from the hair of people consuming mercury-contaminated fish (3.48 ∓ 3.01, Flemming et al. 1995; 0.9-3.0 $mg.kg^{-1}$, Saeki et al.1996; 1.0-59.4 $mg.kg^{-1}$, Kehrig et al. 1997; 0.8-94 $mg.kg^{-1}$, Barbosa 1998) and carnivorous mammals (e.g., 0.49-54.37 for *Lutra canadensis*, Halbrook et al. 1994). The values we report for bats are an order of magnitude higher than those reported for the fur of Opossums, an omnivore that includes insects in its diet (0.228 $mg.kg^{-1}$, Burger et al. 1994). Some of the individual bats (of all four species) we measured had hair mercury concentrations that approached or exceeded 10 mg/kg, the threshold at which detrimental effects have been detected in humans (Murata et al. 1999) and rodents (Burton et al.1977).

Zinc concentrations in the fur of the bats we sampled varied from ND - 900 mg/kg, within the range reported by Streit and Nagel (1993) from the fur of European bats. The other metals were detected in too few samples to make appropriate comparisons.

Although we detected significant inter-specific differences in the concentrations of metals in the fur of bats, our data are characterized by large standard deviations and our sample sizes for some species and locations were small. Variation among species could reflect foraging behaviour and patterns of habitat use or physiological differences among species. We did not find differences in mercury or zinc concentrations among bats from different sites. This is not surprising because some of our sampling locations were hibernation sites where bats from a wide area congregate to spend the winter. Data collected from bats at summer feeding sites are needed to address the question of geographic variation in more detail.

The concentrations of mercury we report are among the highest concentrations reported for bats, and are within the range typical for top predators consuming mercury-contaminated prey. Our results suggest that bats consuming large quantities of prey that spend their larval stages in sediments may be exposed to levels of mercury sufficient to cause sub-lethal biological effects.

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REFERENCES

Barbosa AC, Silva SRL, Dórea JG (1998) Concentration of mercury in hair of indigenous mothers and infants from the Amazon basin. Arch Environ Contam Toxicol 34:100-105.

Bishop CA, Koster MD, Chek AA, Hussell DJT, Jock K (1995) Chlorinated hydrocarbons and mercury in sediments, red-winged blackbirds (*Agelaius phoeniceus*) and tree swallows (*Tachycineta bicolor*) from wetlands in the Great Lakes-St. Lawrence River basin. Environ Toxicol and Chem 14:491-501.

Bishop CA, Ng P, Pettit KE, Kennedy SW, Stegeman JJ, Norstrom RJ, Brooks RJ (1998) Environmental contamination and developmental abnormalities in eggs and hatchlings of the common snapping turtle (*Chelydra serpentina serpentina*) from the Great Lakes-St Lawrence River basin (1989-91). Environ Pollut 101:143-156.

Brigham RM, Fenton MB (1986) The influence of roost closure on the roosting and foraging behavior of *Eptesicus fuscus* (Chiroptera: Vespertilionidae). Canadian J Zool 64:1128-1133.

Brigham RM, Fenton MB (1991) Convergence in foraging strategies by two morphologically and phylogenetically distinct nocturnal aerial insectivores. J Zool (London) 223:475-489.

Burger J, Marquez M, Gochfeld M (1994) Heavy metals in the hair of Opossum from Palo Verde, Costa Rica. Arch Environ Contam Toxicol 27: 472-476.

Burton GV, Alley RJ, Rasmussen L, Orton, P, Cox V, Jones P, Graff D (1977). Mercury and behavior in wild mouse populations. Environ Res 14:30-34.

Clark DR Jr. (1981) Bats and environmental contaminants: a review. U.S. Department of the Interior. Special Scientific Report–Wildlife No. 235. 27 pp.

Currie RS, Fairchild WL, Muir DCG (1997) Remobilization and export of cadmium from lake sediments by emerging insects. Environ Toxicol Chem 16:2333-2338.

Fleming LE, Watkins S, Kaderman R, Levin B, Ayyar DR, Bizzio M, Stephens D,

Bean JA (1995) Mercury exposure in humans through food consumption from the Everglades of Florida. Water Air Soil Pollut 80:41-48.

Frank R, Lumsden H, Barr JF, Braun HE (1983) Residues of organochlorine insecticides, industrial chemicals, and mercury in eggs and in tissues taken from healthy and emaciated common loons, Ontario, Canada, 1968-1980. Arch Environ Contam Toxicol 12:641-654.

Halbrook RS, Jenkins JH, Bush PB, Seabolt ND (1994) Sublethal concentrations of mercury in river otters: monitoring environmental contamination. Arch Environ Contam Toxicol 27:306-310.

Hickey, MBC, Fenton MB (1996) Behavioural and thermoregulatory responses of female hoary bats, *Lasiurus cinereus* (Chiroptera: vespertilionidae), to variations in prey availability. Ecoscience 3:414-422.

Hughes KD, Ewins PJ, Clark KE (1997) A comparison of mercury levels in feathers and eggs of osprey (*Pandion haliaetus*) in the North American Great Lakes. Arch Environ Contam Toxicol 33:441-452.

Kehrig HA, Malm O, Akagi H (1997) Methylmercury in hair samples from different riverine groups, Amazon, Brazil. Water Air Soil Pollut 97: 17-29.

Miura T, Koyama T, Nakamura T. (1978) Mercury content in museum and recent specimens of Chiroptera in Japan. Bull Environ Contam Toxicol 20:696-701.

Murata K, Weihe P, Araki S, Budtz-Jorgensen E, Grandjean P (1999b). Evoked potentials in Faroese children prenatally exposed to methylmercury. Neurotoxicol Teratol 21:471-472.

Saeki K, Fujimoto, M, Kolinjim, D, Tatsukawa, R. (1996) Mercury concentrations in hair from populations in Wau-Bulolo area, Papua New Guinea. Arch Environ Contam Toxicol 30:412-417.

Streit B, Nagel A. 1993. Element assessment in tissue samples from European bats (Microchirpotera). Fresenius Environ Bull 2:162-167.

Thies ML, Thies K, McBee, K. (1996) Organochlorine pesticide accumulation and genotoxicity in Mexican free-tailed bats from Oklahoma and New Mexico. Arch Environ Contam Toxicol 30:178-187.