

# Assessment of methylmercury availability to bats on the North Fork of the Holston River, Virginia - 2005

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## Abstract

Based on findings from the pilot assessment for bats in 2005, there is sufficient information to conclude that bats from near-downstream areas of the NFHR have potentially harmful body burdens of Hg. When comparing bat Hg levels between the reference and near-downstream site, blood and fur Hg levels are significantly higher in the near-downstream area. None of the bats in the reference area exceeded 20 ug/g, fw. Of the 15 individual bats sampled in the near-downstream area, 14 were considered to contain fur Hg burdens that exceeded levels of concern (i.e. 20 ug/g, fw). Ten of the 15 individuals exceeded the 47 ug/g, fw mortality threshold documented in mink. Bat fur Hg levels in the near-downstream area regularly exceeded those from studies in Arkansas, Japan, and Ontario where researchers considered their bat populations at risk. Downstream areas of the NFHR are particularly important for determining injury in three species of federally endangered species – the Townsend's big-eared bat, gray bat, and Indiana bat. An individual of the Indiana bat was sampled and showed a fur Hg level of 58.7 ug/g, fw. Although the effects of methylmercury on bats is unknown, Hg effect levels from other mammalian species indicate that Indiana bats and other associated species are likely at risk.



Northern Long-eared Bat



Mist nets on the North Fork of the Holston River, VA

## Introduction

Mercury and other aquatic-based persistent bioaccumulative contaminants are prevalent in freshwater environments. Mercury availability to fish and wildlife varies inter-regionally because its availability is strongly influenced by biogeochemistry and hydrology. The North Fork Holston River (NFHR), Virginia is a waterbody that was highly contaminated by a waterborne point source of mercury through contamination by a chlor-alkali plant in Saltville, Virginia. Although operations at the chlor-alkali plant ended in the late 1970s, contamination of the river by existing stockpiles of mercury may continue. Similar investigations by BRI of other riverine areas with historical waterborne point sources (e.g., Nyanza Superfund Site on the Sudbury River, Massachusetts) indicate continued contamination of aquatic-based biota can occur in river habitats for many years and for many miles downstream of the point source. To interpret environmental exposure in wildlife established benchmarks are needed. Standardized sampling of high-risk biosentinel provides a method for making informed comparisons and definitive interpretations, thereby helping assess risks to wildlife and allow landscape-level extrapolations of the hazards.

More than half of the species of bats in the U.S. can be characterized as at least occasionally foraging over water and on emergent insects. There have been very few exposure investigations of exposures of bat to metals. Examination of guano in studies has shown trace amounts of metals but this requires finding roost sites usually using telemetry. Researchers have shown that aquatic nymphs of flying insects from a Virginia river polluted by a point source had elevated mercury. Insectivorous Eastern Pipistrelles (*Pipistrellus subflavus*) showed elevated mercury levels in livers and muscle tissue, but reference areas were not sampled. Other research has shown that, in comparison with reference areas, mercury was elevated in muscle, kidney, liver, brain, and fur of bats collected over or near streams in areas of Arkansas with fish consumption warnings.

Our objective was to develop a mercury exposure profile for the North Fork of the Holston River bat populations based on fur and blood samples obtained from mist netting. This profile serves as the basis for the small mammal component of the North Fork of the Holston River project in which we would examine whether the various bat populations are at risk.

## Methods and Study Area

### Study Site

The study sites are located along the North Fork Holston River (NFHR), which flows through Smyth and Washington Counties, in southwest Virginia. The NFHR joins the South Fork Holston River to form the main stem Holston River near Virginia's southern border with Tennessee. In 2005, two study sites were selected for this pilot assessment. One site, at mile 80, was immediately downstream of Saltville, where Saltville NPL was in operation. A second site served as a reference condition and was located on an upstream stretch from Saltville at mile 92 (Figure below-map).

### Capture and Sample Collection

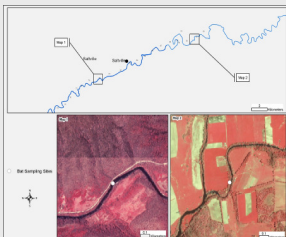
Bat capture and sampling occurred at two different sites in the middle of July 2005. Six to ten mist nets at each site were deployed. Two triple high mist nets and a couple of single high mists were used at both sites.

Nets were strung directly in front of ledge outcroppings or between trees along small access roads in the middle of the river in such a way that funneled the bats into the nets. Roads were chosen that led towards water (with the assumption that bats would fly towards the water to drink and feed after leaving their daytime roosts), and which, were surrounded by mature trees or ledges that would provide good roosting habitat. Nets were set at dusk and monitored until at least 2330 h and if bats were being captured, nets were left up until 0100 h. All bats captured were identified to species, checked for reproductive status, sexed, and aged. Small blood samples were collected by puncturing a vein in the wing with a 26 gauge needle. Fur samples were also collected. All bats were released unharmed at the site.

### Sample Analysis

Thirty fur and 30 blood samples were analyzed for total mercury using Cold Vapor Atomic Absorption (CVAA) methods. Laboratory analysis was conducted by Texas A&M Trace Element Research Lab (TERL), College Station, Texas. TERL has conducted BRI's mercury analysis for bird tissues (blood, feathers, and eggs), fish, and crayfish for the past ten years. Mercury level results are given as fresh weight (fw) for fur and wet weight (ww) for blood. Instead of analyzing methylmercury (MeHg) levels we focused on total Hg because it is (1) less costly, (2) generally correlated with MeHg in most tissues, and (3) reflective of fur Hg levels because >95% of the Hg is methyl.

### Bat sampling locations on North Fork of the Holston River, Virginia, 2005.



### Big Brown Bat sampled on NFHR in Virginia, 2005.



**Acknowledgements:** This effort involved many researchers from Virginia Department of Game and Inland Fisheries, U.S. Fish and Wildlife Service, Olin Corporation and MACTEC and was funded by the U.S. Fish and Wildlife Service and Olin Corporation.

## Results

### Sampling effort

We captured 30 bats in 2005 on the North Fork of the Holston River. Sixteen of the 30 bats were caught below the contaminated site at approximately mile 80. Two species, Northern Long-eared bat (*Myotis septentrionalis*, n=15) and Indiana bat (*Myotis sodalis*, n=1) were caught at the site during three nights of trapping.

Fourteen of the 30 bats were caught above the contaminated site at approximately mile 92. Four species; Northern Long-eared bat (*Myotis septentrionalis*, n=4), Big Brown bat (*Eptesicus fuscus*, n=2), Eastern Pipistrelle (*Pipistrellus subflavus*, n=5), and Eastern Red bat (*Lasiurus borealis*, n=3), were caught at the site during two nights of trapping.

### Mercury exposure in bats

Mean fur Hg concentrations from river mile 80 and 92, were 49.9 ppm (+/- 4.54) and 5.02 ppm (+/- 5.24), respectively (Table 1). Fur Hg concentrations from river mile 80 and 92 ranged between 1.6 ppm and 89 ppm and between 1.08 ppm and 9.43 ppm, respectively (Table 1). We used a t test to examine difference in mean fur Hg concentrations from each river mile. Bat fur from mile 80 had significantly higher Hg concentrations than fur from mile 92 (t=-6.48, p<0.0001, df=26, Figure 1).

Mean blood Hg concentrations from river mile 80 and 92, were 0.272 ppm (+/- 0.025) and 0.041 ppm (+/- 0.029), respectively (Table 2). Blood Hg concentrations from river mile 80 and 92 ranged from 0.125 ppm to 0.464 ppm and from 0.013 ppm to 0.119 ppm, respectively (Table 2). We used a t test to examine difference in mean blood Hg concentrations from each river mile. Bat blood from mile 80 had significantly higher Hg concentrations than blood from mile 92 (t=6.12, p<0.0001, df=26, Figure 2). Bats from river mile 80 exhibited 6 times higher blood Hg concentrations than river mile 92. Also river mile 80 exhibited 10 times higher fur Hg concentrations than from river mile 92.

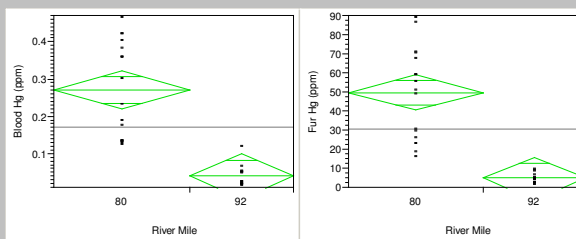


Figure 1. One-way ANOVA of bat blood Hg by river mile.

Figure 2. One-way ANOVA of bat fur Hg by river mile.

Table 1. Mean fur Hg concentrations and ranges by river mile.

River Mile	N	Mean Hg	SE +/-	Min.	Max.	Lower 95%	Upper 95%
80	16	49.88	4.54	15.8	89	-5.75	59.20
92	12	5.02	5.24	1.08	9.43	-5.75	15.78

Table 2. Mean bat blood Hg concentrations and ranges by river mile.

River Mile	N	Mean Hg	SE +/-	Min.	Max.	Lower 95%	Upper 95%
80	16	0.272	0.025	0.125	0.464	0.221	0.323
92	12	0.041	0.029	0.013	0.119	-0.017	0.099

Fur and blood Hg levels were significantly correlated (r<sup>2</sup>=0.82, p<0.001, n=28) (Figure 3). Blood and fur represent multiple species.

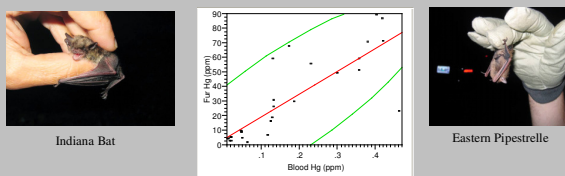


Figure 3. Bat blood and fur Hg levels.

## Discussion

There have been very few investigations on exposure of bats to metals.

\*Researchers in Japan examined various species of Chiroptera from areas sprayed with mercury fungicides. They measured total fur Hg in 1965 and 1966 and found 33.0 ppm (+/-6.3) and 33.7 ppm (+/-4.2), respectively. The fur Hg concentrations found in Chiroptera from the contaminated area of North Fork of the Holston (mean Hg 49.9 +/- 10.3ppm) exceeded these values from Japan.

\*In Arkansas, researchers examined various Chiroptera species from rivers in Arkansas that were under fish consumption advisories and found Hg concentrations ranging from 1 to 30 ppm in fur. They concluded that Hg accumulation had exceeded the hazard criteria set by USFWS and that Hg accumulation in the bats is a serious problem that warranted further investigation.

\*In eastern Ontario and Quebec, researchers in 1997 pooled samples from 5 sites and found Hg concentrations ranging from 2.0 to 7.6 ppm in fur. In 1998, they sampled the same sites and found fur Hg concentrations that approached or exceeded 10 ppm.

\*A risk assessment for aerial insectivorous wildlife on the Clinch River, TN (Oak Ridge Reservation) modeled dose levels for the NOAEL and LOAEL for little brown bats are 0.114 and 0.56 ppm, respectively. Bats experiencing exposure equal or greater than the LOAEL were found to display impaired growth, reproduction, and offspring viability.

Because there was a significant difference between the reference and contaminated site for Hg levels in blood and fur, bats appear to be appropriate indicators for Hg contamination. When we compare bat mean fur Hg concentrations to Maine's mink and otter mean fur Hg concentrations we find that mean fur Hg levels in bats from the contaminated site exceed mortality thresholds for mink and otter (Figure 4). Thus, if bats are as sensitive to Hg as mink and otter, Hg levels in *Myotis sp.* in the North Fork of the Holston River are a cause for concern.

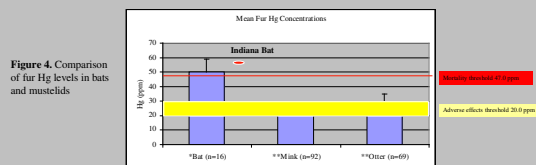


Figure 4. Comparison of fur Hg levels in bats and mustelids

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