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Mercury Contamination in Bats from the Central United States

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Abstract: Mercury (Hg) is a highly toxic metal that has detrimental effects on wildlife. We surveyed Hg concentrations in 10 species of bats collected at wind farms in the central United States and found contamination in all species. Mercury concentration in fur was highly variable both within and between species (range: 1.08–10.52 μg/g). Despite the distance between sites (up to 1200 km), only 2 of the 5 species sampled at multiple locations had fur Hg concentrations that differed between sites. Mercury concentrations observed in the present study all fell within the previously reported ranges for bats collected from the northeastern United States and Canada, although many of the bats we sampled had lower maximum Hg concentrations. Juvenile bats had lower concentrations of Hg in fur compared with adult bats, and we found no significant effect of sex on Hg concentrations in fur. For a subset of 2 species, we also measured Hg concentration in muscle tissue; concentrations were much higher in fur than in muscle, and Hg concentrations in the 2 tissue types were weakly correlated. Abundant wind farms and ongoing postconstruction fatality surveys offer an underutilized opportunity to obtain tissue samples that can be used to assess Hg contamination in bats. *Environ Toxicol Chem* 2018;37:160–165. © 2017 SETAC

Keywords: Bats; Mercury; Texas; Minnesota; Wind energy

INTRODUCTION

Mercury (Hg) is a highly toxic metal that has detrimental effects on wildlife [1–4]. Levels of Hg in the environment have increased as a result of anthropogenic emissions associated with coal burning, mining, and industrial activities [5]. Because of the long residence time of Hg in the atmosphere, deposition can occur both locally and at points far from emissions sites, which has resulted in a global contamination problem [5]. Inorganic forms of Hg deposited from the atmosphere are methylated by sulfate- and iron-reducing bacteria in aquatic ecosystems [5]. The methyl form of Hg biomagnifies and can reach high levels in aquatic consumers [6,7]. Aquatic insects that become contaminated with methylmercury (MeHg) as larvae have the potential to transfer MeHg to terrestrial predators when they emerge from aquatic ecosystems as adults [8,9].

Several recent studies have identified elevated concentrations of Hg in the tissues of insectivorous bats living near point sources of Hg discharge [1,4,10] and in bats far from known point sources of Hg pollution [10–14]. Prior studies of Hg contamination in bats from North America have focused on sites in the eastern United States [1,4,10] and southeastern Canada [12–14] while other regions of North America have not yet been assessed. Additional studies are needed to assess Hg concentrations in bats from other regions of North America. In the present study, we examined Hg contamination of bats collected in the central United States, a region that has high Hg deposition, large numbers of small ponds [15], and millions of foraging bats.

The objective of the present study was to assess Hg concentrations in 10 species of bats from the central United States and determine how fur and muscle Hg concentrations vary by species and with age and sex. Fur and muscle samples used in the present study were obtained from bat carcasses salvaged from utility-scale wind farms. Because bat fatalities occur in large numbers at wind energy facilities [16–18], many wind farms have fatality monitoring programs in place. Carcasses located during such monitoring provide an unprecedented opportunity to assess Hg concentrations in these terrestrial predators.

MATERIALS AND METHODS

Five hundred and seventy-six bat carcasses from 10 species were salvaged during postconstruction fatality surveys from

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2009 to 2014 at 2 wind farms in Texas (TX1 in north Texas and TX2 in south Texas) and 3 wind farms in southern Minnesota (MN1, MN2, and MN3; Figure 1 and Table 1). All bat species collected exhibit migratory behavior, and therefore it is not possible to determine their provenance. Bats collected at these sites are presumed to have been killed by the operation of the wind turbines. The time the carcasses were in the field prior to discovery is unknown, but because of frequent monitoring, it was less than 48 h in most cases. Carcasses were generally in good condition with no obvious signs of scavenging by vertebrate scavengers. Some carcasses were scavenged by ants before being collected but, ant scavenging was limited to the eyes and genitals. Carcasses were identified to species in the field [19] and frozen at –20 °C. We removed samples of fur from frozen carcasses for total Hg analysis. For carcasses collected at the north Texas (TX1) site, we also determined the age (juvenile or adult) [19] and sex [20] of all bats (Table 2). The method we used for aging bats (i.e., epiphyseal cartilage) can result in the misclassification of some juvenile bats as adults [19]. We collected breast muscle tissue, in addition to fur, for Hg analysis from a subset of adults from the most abundant species at the TX1 site (*Lasiurus borealis*, $n = 28$ and *Lasiurus cinereus*, $n = 26$). If bat muscle tissue exhibited evidence of scavenging by vertebrate or invertebrate scavengers, then these carcasses were not included in our analysis of muscle tissue.

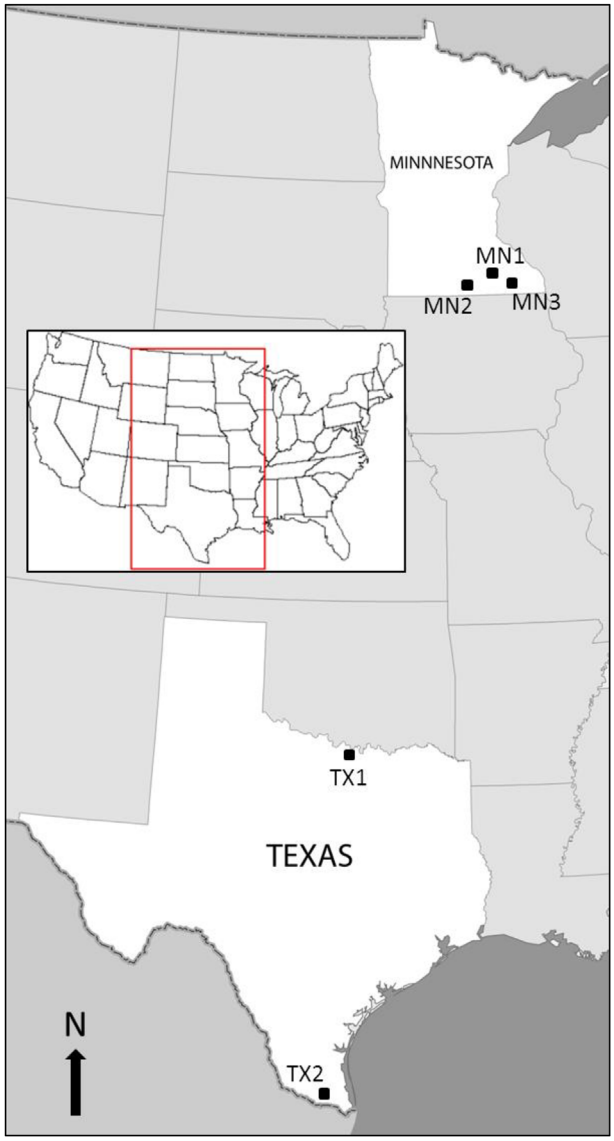

FIGURE 1: Map indicating the location of 5 wind facilities from which bat fur and tissue samples were collected during postconstruction fatality surveys.

TABLE 1: Summary of fur samples in the present study

Species	Site			Total
	MN	TX1	TX2	
Big brown bat *(Eptesicus fuscus)*	7	–	–	7
Eastern red bat *(Lasiurus borealis)*	64	152	–	216
Hoary bat *(Lasiurus cinereus)*	50	110	–	160
Southern yellow bat *(Lasiurus ega)*	–	–	5	5
Northern yellow bat *(Lasiurus intermedius)*	–	–	12	12
Silver-haired bat *(Lasionycteris noctivagans)*	14	5	–	19
Little brown bat (*Myotis lucifugus)*	35	–	–	35
Evening bat (*Nycticeius humeralis)*	–	51	5	56
Tri-colored bat (*Perimyotis subflavus)*	–	21	–	21
Mexican free-tailed bat *(Tadarida brasiliensis)*	–	19	26	45

MN = combination of 3 wind farms in Minnesota; TX1 = wind farm in north Texas; TX2 = wind farm in south Texas.

Prior to Hg analysis, fur samples were cleaned using a procedure developed to remove external contamination from feathers [21]. Specifically, fur was washed with a 30:1 detergent solution, rinsed 3 to 6 times with deionized water and dried overnight, washed again with 2:1 chloroform:methanol, and then dried overnight. Breast muscle was dried in a 60 °C oven for at least 48 h prior to total Hg analysis.

We used total Hg as a proxy for MeHg because most of the Hg in fur is MeHg [10]. We examined total Hg in all samples (~0.004 g fur, ~0.02 g muscle) with a direct Hg analyzer (DMA-80) that uses thermal decomposition, gold amalgamation, and atomic absorption spectrometry [22]. Quality assurance included reference and duplicate samples. Specifically, we analyzed samples of National Research Council Canada reference materials approximately every 10 samples, and the mean percentage recovery was 102% ($n = 79$). We analyzed duplicate samples approximately every 20 samples, and the mean relative percentage difference was 14.8% ($n = 39$). All

TABLE 2: Detailed summary of fur samples from the Texas 1 (TX1) site

Species	Age			Total
	Juvenile	Adult	Unknown	
Eastern red bat *(Lasiurus borealis)*	52	98	2	152
Hoary bat *(Lasiurus cinereus)*	12	87	11	110
Silver-haired bat *(Lasionycteris noctivagans)*	0	3	2	5
Evening bat (*Nycticeius humeralis)*	7	38	6	51
Tri-colored bat (*Perimyotis subflavus)*	2	13	6	21
Mexican free-tailed bat *(Tadarida brasiliensis)*	3	14	2	19

total Hg values are reported in μg/g dry weight unless otherwise noted.

A preliminary analysis revealed that fur Hg concentrations of bats from the 3 Minnesota sites (each site <150 km from the others) were not significantly different from one another (data not presented), so we treated them as a single pooled site for all analyses. We constructed 2 general linear models (GLMs) in Minitab Ver 17 to determine which factors explained variation in mean concentrations of Hg in fur. Model 1 utilized data from all sites and included site and species as fixed factors and year as a random factor. Model 2 was limited to samples from the north Texas site (TX1), the only site for which age and sex data were available, and included species, sex, and age as fixed factors. Where necessary, GLMs were followed by simultaneous post hoc Tukey–Kramer tests with a family $\alpha = 0.05$ or 2-sample *t* tests with Bonferroni corrections for multiple comparisons. To investigate the relationship between fur and muscle concentrations for individuals from which we sampled both tissue types, we conducted Pearson's correlation analysis and a paired *t* test ($\alpha = 0.05$). Mean Hg concentration was log transformed prior to all analysis to meet the normality requirements of the tests. To aid in interpretation of results, untransformed Hg concentrations are presented in the tables and figures.

RESULTS

Average Hg concentrations in fur ranged from 1.08 to 10.52 μg/g in the 10 species examined and were highly variable both within and between species (Figure 2). For bats collected from all sites, we examined the effect of species, site, and year of collection on Hg concentration in fur (model 1). Model 1 indicated that species and collection site had an effect on Hg concentrations of fur (GLM, species: $F_{9,560} = 16.41$, $p < 0.001$; GLM, site: $F_{2,560} = 12.70$, $p < 0.001$), but not year of collection (GLM, year: $F_{5,560} = 0.64$, $p = 0.672$; Figure 2). Post hoc tests revealed that *Lasiurus ega*, *Tadarida brasilensis*, *L. cinerus*, and *L. borealis* had significantly lower concentrations of Hg in fur than *Nycticeius humeralis*, *L. noctivagans*, *Perimyotis subflavus*, *Myotis lucifugus*, and *Eptesicus fuscus* (Tukey–Kramer, $p < 0.05$). Post hoc tests revealed that of the 5 species found at more than one site, only 2 (*L. borealis* and *T. brasiliensis*) had fur Hg concentrations that were significantly different between sites (*L. borealis*, $t = 5.53$, $df = 138$, $p < 0.001$; *T. brasiliensis*, $t = -3.02$, $df = 41$, $p = 0.004$; for all other species $p > 0.07$; Figure 3).

At the north Texas site (TX1) we examined the effect of species, year of collection, sex, and age on the concentration of Hg in fur (model 2). Model 2 indicated that species and age had a significant effect of Hg concentrations in fur (GLM, species: $F_{5,513} = 13.08$, $p < 0.001$, GLM, age: $F_{1,313} = 70.77$, $p < 0.001$). Sex and collection year did not have a significant effect on Hg concentrations in fur (GLM, sex: $F_{2,313} = 0.40$, $p = 0.528$; GLM, year: $F_{5,313} = 0.27$, $p = 0.93$). Post hoc tests revealed that *T. brasilensis*, *L. cinerus*, and *L. borealis* had significantly lower fur Hg concentrations than *N. humeralis* and *P. subflavus* (Tukey–Kramer, $p < 0.05$). Mean fur Hg concentration in adults was higher than in juveniles in all species, although after Bonferroni correction the difference was only significant for *L. borealis* and *L. cinereus*, most likely because of the limited number of juvenile samples for the remaining species (*L. borealis*, $t = 8.42$, $df = 135$,

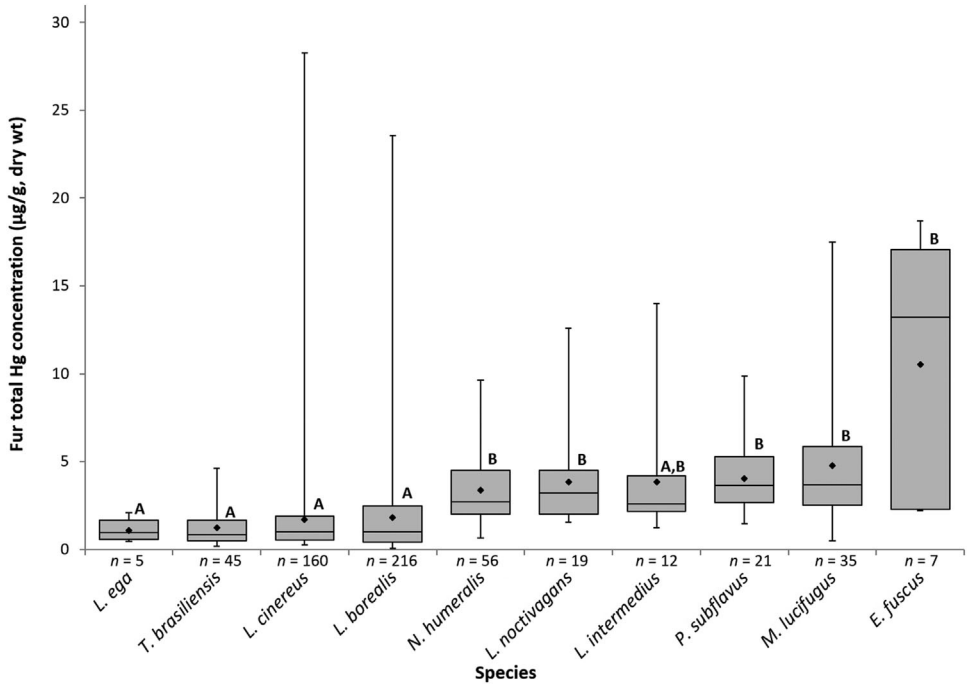

FIGURE 2: Fur mercury (Hg) concentrations for all species studied. Boxes represent first quartile, median, and third quartile, and whiskers represent the range. Diamonds reflect mean fur Hg concentrations, and numbers below bars indicate sample sizes. Letters above bars represent the results of the simultaneous post hoc Tukey–Kramer test with a family $\alpha = 0.05$: bars that share common letters do not differ significantly.

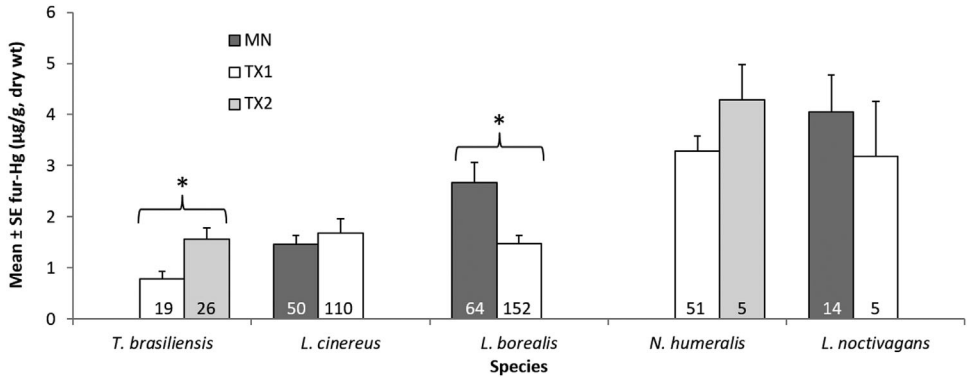

FIGURE 3: Mean (± standard error [SE]) fur mercury (Hg) concentrations for species collected at >1 site. Asterisks indicate significant differences between sites (2-sample *t* tests with Bonferroni correction, $\alpha = 0.01$, $p \leq 0.004$). MN = combination of 3 wind farms in Minnesota; TX1 = wind farm in north Texas; TX2 = wind farm in south Texas.

$p < 0.001$; *L. cinereus*, $t = 3.62$, $df = 19$, $p = 0.002$; for all other species $p \geq 0.025$, Figure 4).

We measured Hg concentration in muscle tissue from 64 bats belonging to 2 species collected from TX1. We found significantly lower concentrations of total Hg in muscle than in fur (paired *t* test: $t = 20.14$, $df = 55$, $p < 0.001$; *L. borealis*, mean ± standard error: 0.11 ± 0.01 µg/g, range: 0.05–0.27 µg/g; *L. cinereus*, mean = 0.20 ± 0.04 µg/g, range: 0.04–0.95 µg/g). The concentration of Hg in muscle was not correlated with the concentration of Hg in fur for *L. borealis* ($r = 0.18$, $p = 0.362$; Figure 5), but was correlated for *L. cinereus* ($r = 0.53$, $p = 0.004$; Figure 5). In *L. cinereus*, a single bat with an unusually high fur Hg concentration strongly influenced this relationship, and removal of this individual weakened the correlation ($r = 0.44$, $p = 0.025$; Figure 5).

DISCUSSION

The concentrations of Hg in fur observed in the present study all fell within the previously reported ranges for their respective species, although the bats collected in the present study often had lower maximum values than have been reported previously [10,12–14]. For a given species, we may have observed lower maximum values in the present study because none of our study sites are located near point sources, as was the case in previous studies [10]. All of our sites are located in areas where atmospheric deposition is presumed to be the primary source of Hg [23].

The Hg concentrations observed in most of the bats examined in the present study were well below levels known to cause lethal effects in mammals [3]; however, the nonlethal effects of lower concentrations of Hg in mammals have not been well studied [3,24]. There are few studies investigating the relationship between Hg exposure and adverse effects in wild bats (but see Becker et al. [25]) and no published studies to date that investigate the impacts of Hg exposure on bats in a laboratory setting. Mercury impacts the endocrine, neurological, immune, and reproductive systems, resulting in altered behavior, reduced productivity, and increased infections in wild mammals [3,24]. For example, Hg levels as low as 7.8 µg/g in

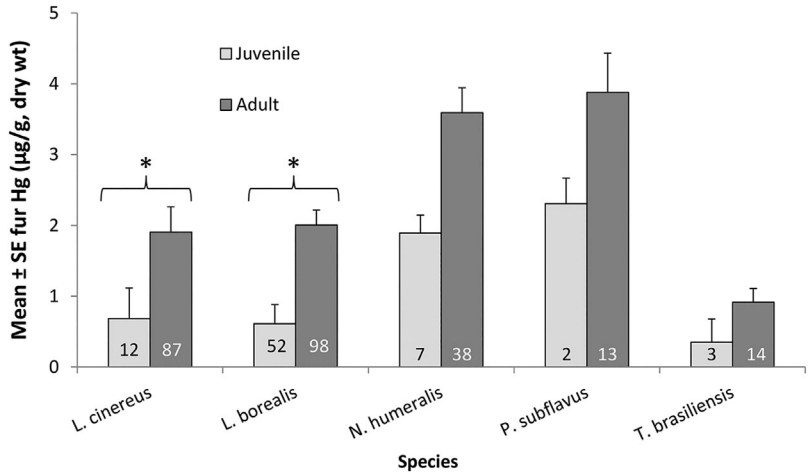

FIGURE 4: Comparison of mean (± standard error [SE]) fur mercury (Hg) in adults and juveniles collected at the north Texas site (TX1). Numbers in bars indicate sample size. Asterisks indicate statistically significant differences between adults and juveniles (2-sample *t* tests with Bonferroni correction, $\alpha = 0.01$, $p \leq 0.002$).

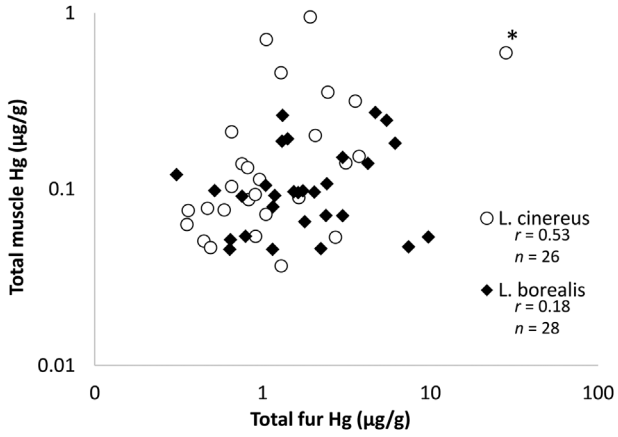

FIGURE 5: The relationship between mercury (Hg) concentration in fur and muscle from *Lasiurus borealis* and *Lasiurus cinereus* collected at the north Texas site (TX1). One *Lasiurus cinereus* with high fur Hg concentration is indicated by the asterisk.

fur can cause behavioral deviations and decreased ambulatory activity in wild mice [26]. Twenty-two bats (3.8%) in the present study exceeded this concentration. More recently, Eccles et al. [27] conducted a meta-analysis of 6000 mink and otter samples from 16 studies and 96 sampling sites and recommended a screening guideline of 15 µg/g in fur for sensitive piscivorous mammals [27]. Seven bats (1.2%) in the present study exceeded 15 µg/g in fur. Additional research is needed to understand the impact of the levels of Hg observed in the present study and how chronic exposure may interact with other threats to negatively influence bat survival. Concern is warranted given that many bat populations are in decline [28,29] because of numerous and often multifaceted factors [16,17,29].

The Hg concentrations varied between species in the present study. Between-species variation in Hg concentrations of bats is often because of differences in diet [11]. For example, insectivorous bats in Malaysia had higher Hg levels than frugivorous bats [11]. The species of bats examined in the present study feed exclusively on invertebrates in terrestrial habitats and were likely exposed to Hg via emergent insects that develop as larva in Hg-contaminated aquatic ecosystems and emerge as terrestrial adults [8,9,15]. Bats whose diets consist of a high proportion of emergent aquatic insects (midges, dragonflies) would be expected to have higher Hg concentrations than those that prefer terrestrial prey (moths). Differences in prey preference and foraging strategies could explain the species-specific differences observed in the present study. However, the diets of the species examined in the present study are not understood with enough resolution to test this hypothesis.

Differences between species concentrations of Hg might also be explained by variation in movement patterns of bats. Because the majority of the bats from the present study were killed during the peak of fall migration, our samples are composed of a mixture of resident bats and migrants [30]. The concentrations of Hg in fur reflect the Hg concentrations in bat diets at the time that the hair was grown, usually during late summer/early autumn before migration begins [4,10,30,31]. The concentration of Hg in fur can also be influenced by local point sources of contamination [10] and has even been shown to vary in resident bats at sites relatively close to one another (e.g., between sites in Nova Scotia [14]). In the present study, the concentration of Hg in the fur of migrant bats likely reflects the Hg contamination at sites across the central United States, and the presence of migrants from a few Hg hotspots could explain the wide range of Hg concentration values we observed within a single species in the present study.

Age was an important determinant of the concentration of Hg in fur; juvenile bats had lower concentrations of Hg in fur than adults in all species, which is consistent with time-related bioaccumulation and with age-based shifts in prey choice [4,9,10,32]. For example, Rolseth et al. [32] found that juvenile *L. cinereus* consumed more Chironomidae (which tend to have low Hg tissue concentrations) and fewer Odonata (which tend to have higher Hg tissue concentrations) than adults from the same area [9].

The present study afforded an opportunity to sample muscle Hg concentrations in bats, which have been relatively unstudied to date. Mercury concentrations in fur from *L. borealis* and *L. cinereus* were almost 17 and 8.5 times higher than concentrations of Hg in muscle, respectively. Previous studies have found strong correlations between blood Hg levels and fur Hg levels [10]. Our study found that the correlation between muscle Hg and fur Hg levels was much weaker than that observed with blood. At the time of fur growth, which occurs during late summer/fall when most of our samples were collected, Hg is shifted into the new fur from muscle and organs [10,31,33]. Variation in the depuration of Hg among individuals may be responsible for the weak correlation between Hg concentrations in muscle and fur that we observed.

In the present study, tissues were obtained from wind farms, which provided an unprecedented opportunity to assess concentrations of Hg in bats across the central United States. Abundant wind farms and ongoing mortality surveys offer an underutilized opportunity to obtain tissue samples to aid efforts to monitor Hg contamination in bat populations. Together with data collected using traditional sampling methods (e.g., mist netting), tissues collected during mortality surveys at windfarms could help scientists assess the extent of Hg contamination in bats.

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Data availability—Data, associated metadata, and calculation tools are available from the corresponding author (m.m.chumchal@tcu.edu).

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