Heavy metal contamination in bats in Britain

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Abstract

Toxic metals are bioaccumulated by insectivorous mammals but few studies (none from Britain) have quantified residues in bats. We measured renal mercury (Hg), lead (Pb) and cadmium (Cd) concentrations in bats from south-west England to determine how they varied with species, sex, age, and over time, and if they were likely to cause adverse effects. Residues were generally highest in whiskered bats (*Myotis mystacinus*). Compared with other species, pipistrelle (*Pipistrellus spp)* and Natterer's bats (*Myotis nattereri*) had significantly lower kidney Hg and Pb concentrations respectively. Renal Hg increased over time in pipistrelles but the contributory sources are unknown. Kidney Pb did not decrease over time despite concurrent declines in atmospheric Pb. Overall, median renal metal concentrations were similar to those in bats from mainland Europe and 6 - >10 fold below those associated with clinical effect, although 5% of pipistrelles had kidney Pb residues diagnostic of acute lead poisoning.

Capsule: *Heavy metal contamination has been quantified in bats from Britain for the first time and indicates increased accumulation of Hg and no reduction in Pb*

Keywords: pipistrelle; brown long-eared bat; kidney; mercury; cadmium; lead; risk assessment

1. Introduction

The populations of many European bat species are thought to have declined and there has been considerable concern about the potential impact of contaminants on a range of species. This has mostly focussed on organochlorine insecticides, polychlorinated biphenyls and biocides used in remedial timber treatments (Clark and Shore, 2001). In comparison, few studies have considered the exposure and potential impacts on bats of toxic heavy metals, such as mercury (Hg), lead (Pb) and cadmium (Cd), even though these elements are readily transferred through insectivore foodchains (Ma and Talmage, 2001). Furthermore, many bat species are relatively long-lived (Arlettaz et al., 2002), and some metals, for example Cd, can be progressively accumulated with age (Walker et al., 2002). Thus, insectivorous bats may be at risk of accumulating high and potentially toxic tissue concentrations of some non-essential metals.

Several studies have reported Pb poisoning and potential Pb-mediated effects on reproduction in bats (Clark, 1979; Sutton and Wilson, 1983; Hariono et al., 1993). Sub-lethal concentrations of Cd, Pb, Hg, arsenic and essential trace elements in the body organs, fur and guano of various species have also been reported but most studies are from North America and Australia (Thies and Gregory, 1994; Méndez and Alvarez-Castañeda, 2000; Clark and Shore, 2001; Hickey et al., 2001; O'Shea et al., 2001). There have been very few reports of tissue metal concentration in bats from Europe, and, as far as we are aware, no published studies that report such data for bats from Britain. Most of the European studies have focussed on just one or two species or have analysed relatively small numbers of individuals within species (Gerell and Lundberg, 1993; Streit and Nagel, 1993a, b; Lüftl et al. 2003), thereby making it difficult to identify how toxic metal contamination varies within and between bat species.

In the current study, we examined heavy metal contamination in the kidneys from bat carcasses that had been collected opportunistically over approximately 15 years from the predominantly rural south-west (SW) region of Britain. Our aim was to use this large sample to: (i) determine how heavy metal concentrations in bats from Britain vary with species, sex, and age, and whether the levels of contamination are typical of those in bats from elsewhere; (ii) assess whether metal concentrations have changed over time; (iii) compare the measured tissue concentrations with critical residue levels associated with adverse effects in mammals.

2. Methods

2.1. Sample collection

A total of 272 bats, consisting of eight species, found dead or fatally injured between 1988 and 2003 were collected for analysis. All were from the counties of Cornwall and Devon in SW England. They were identified to species and sex, but were only separated by age class (adult/juvenile) from 2001 onwards. Juveniles were distinguished by the relative lack of ossification of their epiphyses. The bats were dissected, a post-mortem examination was carried out, and the body organs were excised for various studies. The kidneys were retained for quantification of metal contaminants and stored at -20ºC until analysed. Livers were retained for analysis of organochlorine insecticides and polychlorinated biphenyls, the data for which will be reported elsewhere.

2.2. Determination of Cd, Pb, and Hg in bat kidneys

The two kidneys in each bat were pooled so that the sample weight for analysis ranged between 0.008 g and 0.186 g wet weight (ww). Kidneys were dried to constant weight at 80ºC and solubilised in cold (Aristar grade) nitric acid (Merck Ltd, Poole, Dorset, UK) for at least 24 hours. Each acid digest was further solubilised by heating to 90 °C, adding 0.2 ml of hydrogen peroxide (to aid oxidation of lipids), and then heating to 120°C. Digestion proceeded at this temperature until digests had stopped fuming and were straw-coloured. Digests were then made up to fixed volume (typically 5 ml and always 20% acid).

Sub-samples of the digest were diluted with de-ionised water so that the analyte was within the calibration range and the final acid content was 10%. Hg was determined by cold vapour atomic absorption spectrophotometry (Solaar 969, ThermoUnicam, Cambridge, UK) using 10% tin chloride in 20% sulphuric acid as a reducing agent, nitrogen as a carrier gas, and an absorbance wavelength of 253.7 nm, as described by López Alonso et al. (2003). Lead and cadmium concentrations were determined by graphite furnace atomic absorption spectrophotometery using a Varian

SpectrAA 300 spectrophotometer and GTA-96 graphite furnace. A matrix modifier consisting of palladium, ammonium dihydrogenphosphate, magnesium nitrate and citric acid in a 1% HCl solution (Milton et al., 1998) was added to all standards and samples to allow the atomisation of the analyte at high temperature and to remove background interferences. The relative proportions of the different constituents in the modifier and the thermal programme used for the furnace were optimised separately for each metal. Certified reference materials of an appropriate matrix type and that had detectable Cd, Hg and Pb concentrations in sample masses equivalent to that of bat kidneys were not available. Analytical recoveries were therefore determined from spiked kidney samples and the mean (±SE) recovery was 101 ± 3.12 % (n=23) for Hg, 112 ± 5.22% (n=23) for Pb and 96.2 ± 4.01% (n=22) for Cd.

For all metals, the limit of detection (LoD) for the total amount of metal in the digest was calculated as three times the standard deviation of the mean blank value. There was minor variation in LoD between analytical batches and so the maximum batch LoD recorded was applied to all the samples. The LoD for the concentration in the kidney was derived by dividing the digest LoD by the mean kidney weight. Because kidney mass varies with body size and there were large size differences among the bat species analysed, the LoD for the kidney concentration varied some three- to four-fold among species. To eliminate biases in the statistical analyses, the LoD used for the smallest species was applied to all other species, which was 1.86 μg/g dry weight (dw) for Hg, 1.24 μg/g dw for Pb, and 0.162 μg/g dw for Cd.

2.3. Data analysis

Renal metal concentrations are presented on a dry weight basis. For statistical purposes, non-detected concentrations were assigned a value of half the limit of detection. Kidney metal concentrations were not usually normally distributed or variances among groups were not always homogenous. Differences among bat species were therefore determined by non-parametric analysis of variance (Kruskall-Wallis tests) and Dunn's *post-hoc* multiple comparison test; $P<0.05$ was taken as statistically significant in *post-hoc* tests. Because non-parametric approaches were used, summary statistics are given as medians and inter-quartile ranges.

Analysis of the variation in kidney metal concentrations with age and sex were usually conducted by analysis of variance using a general linear model (GLM: Minitab version 14.1, Minitab Inc., USA). In some instances, it was necessary to transform the data to ranks prior to analysis to ensure that the underlying assumptions of the model were met. Sex and age were usually entered as factors in the model and year of collection as a covariate. Associations between different renal metal concentrations and between kidney residues and body weight were determined using Spearman rank correlation coefficients. The analyses were restricted to bats with detectable residues only, as inclusion of animals with non-detected values can result in spurious correlations. Because of the relatively high proportion of non-detected renal metal concentrations in some species, temporal trends in contamination were determined in two ways. First, changes over time in the proportion of animals with detectable residues were analysed by binary logistic regression. Second, changes in the annual median renal concentrations of bats with detectable residues were assessed using backwards stepwise linear regression in which data from each year were weighted by the sample size for that year; year and sex were both used as terms in the model.

3. Results

3.1 Kidney Hg, Cd and Pb concentrations in different species

Four species comprised 96% of the bats analysed. Most (63%) were pipistrelles (*Pipistrellus* spp*)*, which were not separated into the recently recognised separate cryptic species of *P. pipistrellus* or *P. pygmaeus* (International Commission on Zoological Nomenclature, 2003). Brown long-eared (*Plecotus auritus*), whiskered (*Myotis mystacinus*) and Natterer's bats (*Myotis nattereri*) made up an additional 22%, 6% and 5% of the sample, respectively.

There were differences among species in their kidney metal concentrations (Table 1). Whiskered and Natterer's bats had the highest median renal Hg concentrations, and pipistrelles had significantly lower concentrations than any of the other three species. In contrast, kidney Pb concentrations did not vary significantly among pipistrelles, brown-long eared or whiskered bats but were significantly higher in these species than in Natterer's bats. Kidney Cd concentrations also varied significantly among species. Natterer's bats had the highest average renal concentration and the median concentration for brown long-eared bats was 1.5-7 fold lower than for the other species (Table 1). However, differences in kidney Cd between individual species were not statistically significant in *post-hoc* tests.

The kidneys from four barbastelle (*Barbastella barbastellus*), four lesser horseshoe (*Rhinolophus hipposideros*), three noctule (*Nyctalus noctula*) and one serotine (*Eptesicus serotinus*) bat were also analysed but, because of the limited sample sizes, the residue data were not included in any statistical tests. Median concentrations above the LoD were only detected for Hg in lesser horseshoes (1.62 μg/g dw) and for Pb in barbastelle bats (2.17 μg/g dw). Median kidney Cd concentrations exceeded the LoD in all four species and ranged between 0.28 and 2.13 μg/g dw.

3.2. Variation in kidney residues in pipistrelle and brown long-eared bats

The effects of age, sex and time on renal metal concentrations were determined only for pipistrelle and long-eared bats as they were the only species for which sample sizes were sufficiently robust. Potential age and sex related effects were examined first using the subset of data (bats collected between 2001 and 2003) for which age data were available; age class and sex were factors in the GLM and year of collection was a covariate. In brown long-eared bats, there was no significant effect of age, sex or year on renal concentrations of any of the three metals; however, the number of bats analysed was relatively small. In pipistrelle bats, adults had significantly higher kidney Cd concentrations than juveniles ($F_{1,39} = 9.51$, $P<0.005$) but kidney Cd did not vary with sex or year (Figure 1). Renal Pb concentrations did not vary significantly with age, sex or year. Age likewise did not significantly affect renal Hg concentrations in pipistrelles. Mercury was not detected in any adult or juvenile females and, among males, there was also a high proportion (69%) of non-detected concentrations and no significant effect of age.

When changes in renal metal concentrations over time were examined, there were no significant progressive changes in the proportion of brown long-eared bats with detectable Cd, Pb, or kidney Hg residues (Figure 2). Median kidney Hg, Cd and Pb concentrations in individuals with detectable residues likewise did not vary significantly over time (Figure 2), nor did they vary significantly between males and females. In pipistrelles (Figure 3), median renal Hg concentrations in bats with detectable concentrations did increase significantly over time ($F_{1,10} = 6.05$, $P=0.034$); there was no significant difference between males and females, nor any change in the proportion of bats with detectable Hg residues (Figure 3). Although the proportion of pipistrelle bats with detectable Pb concentrations appeared to decline over time (Figure 3), this was not statistically significant ($G_{(1)} = 0.613$, $P = 0.434$). Similarly, there was no significant change over time in the proportion of bats with detectable kidney Cd residues or in annual median renal concentrations of Pb or Cd in bats with detected residues. Detectable kidney Pb concentrations did not vary with sex either, but median renal Cd concentrations were slightly, but significantly, higher in females than males (2.10 vs 1.72 µg/g dw, $F_{1,18} = 5.80$, $P = 0.027$). This was unexpected as there was no sex effect on kidney Cd in the pipistrelles of known age class collected between 2001 and 2003. This apparent sex difference in pipistrelles may have been an artefact that could have arisen if there was a difference in the age structure of the males and females in the sample.

3.3 Associations among kidney metal concentrations and body weight in pipistrelle and brown long-eared bats

Relationships between kidney concentrations of different metals and between renal metal concentrations and body weight were examined to determine if there was evidence of co-exposure and of potential adverse effects, respectively. There was a significant positive relationship between kidney Cd and Pb residues in pipistrelle bats (Table 2, Figure 4) and a similar relationship in brown-long-eared bats, although the sample size was smaller and the trend was not statistically significant (Table 2). There was no significant relationship between renal Hg and Pb or between Hg and Cd in either bat species (Table 2). When body weight was related to kidney metal concentrations, (Table 3), there was a significant negative relationship for Hg in pipistrelles (Table 3, Figure 5). However, a similar relationship was not detected in brown long-eared bats (Table 3, Figure 5). There were no other significant relationships between kidney metal concentrations and body weight (Table 3).

4. Discussion

Both average and maximum kidney Hg, Cd and Pb concentrations in the bats from SW England were similar to those in the same and other species collected over the same time period in Austria (Lüftl et al., 2003) and Germany (Streit and Nagel, 1993a, b), and were approximately two-three times higher than reported in the scant data available for kidney concentrations of metals in other bat species from outside Europe (Miura et al., 1978; Clark et al., 1986). Thus, metal contamination in bats from this rural region of Britain appears to be similar to that found in bats elsewhere in Europe. However, pipistrelle bats from close to an industrial area in Sweden had higher renal metal concentrations than individuals from a remote area (Gerell and Lundberg, 1993). Thus, it is possible that contamination in bats in SW England may be lower than in individuals from more industrialised areas of Britain.

Our results demonstrated that there was significant variation in kidney metal concentrations among pipistrelle, brown long-eared, whiskered and Natterer's bats collected from the same region. Average renal metal concentrations were generally highest in whiskered bats. Lüftl et al. (2003) also observed that whiskered bats in Austria tended to accumulate higher renal toxic metal concentrations than other species, and suggested this was because they are relatively long lived. However, renal Hg and Pb concentrations have not been found to increase with age in various other terrestrial mammals (Massie et al., 1993; Shore and Rattner, 2001; López Alonso et al., 2003) and we found no evidence of renal accumulation of Pb and Hg with age in bats from SW England. High accumulation of toxic metals by whiskered bats compared with other species is more likely to reflect differences in dietary exposure, although it may also result from inter-species variation in assimilation. The low accumulation of mercury by pipistrelles from Devon and Cornwall (Table 1) most probably reflected low levels of exposure, as pipistrelles do not seem to be intrinsically poor accumulators of Hg; renal Hg concentrations in pipistrelles from Austria were similar to those in other bat species (Lüftl et al., 2003). It seems likely that transfer rates (and possibly pathways) differ not only between bat species but also between toxicants, otherwise pipistrelles in the current study would have been expected to accumulate low kidney residues of Pb and Cd as well as Hg, but this was not the case.

Accumulation of renal metal residues has been found to vary with age and sex in bats, but not consistently across species (Gerell and Lundberg, 1993; Lüftl et al., 2003; this study), other than kidney Cd is accumulated with age as also reported in other mammals (Shore and Rattner, 2001). We observed that kidney Cd was accumulated with age in pipistrelles in our study, but renal Pb and Hg concentrations did not vary with age class, and kidney Pb, Hg and Cd residues did not vary with sex.

There was also no evidence that Pb or Cd residues varied significantly over time. The lack of any statistically significant detectable decline in kidney Pb in pipistrelle or brown long-eared bats contrasts with the marked decline over the same time period in Pb petrol emissions and atmospheric Pb (Environment Agency, 2006). Our results suggest that reductions in petrol Pb emissions have not significantly affected the bioavailability of Pb to bats in this region of Britain. However, it may be expected that declines in Pb contamination in terrestrial and aquatic food chains would lag behind declines in atmospheric Pb levels.

The positive relationship between kidney Pb and Cd residues in bats in the present study suggests individuals exposed to higher levels of Pb are likewise exposed to relatively high amounts of Cd. In contrast, the lack of a significant relationship between renal Hg concentrations and either kidney Pb or Cd suggests that habitat contamination and/or transfer pathways for Hg differ from those for Cd and Pb. Furthermore, unlike Cd and Pb, kidney Hg concentrations increased significantly between 1988 and 2003 in pipistrelles. This suggests that exposure to bioavailable Hg increased during this period and may reflect increased diffuse atmospheric inputs of mercury and/or increased exposure to point sources. No significant time trend for Hg was observed in brown long-eared bats but annual and total sample sizes were smaller. As far as we are aware, there are no other time-trend data for Hg in bats in Britain or elsewhere, nor for insectivorous mammals or birds in Britain, and so it is impossible to assess whether the trend in pipistrelles is typical for other bat species or other insectivores in Britain or elsewhere.

Heavy metal renal concentrations in the bats from SW England were generally lower than those associated with adverse effects in mammals. The maximum kidney residues measured in this study did not exceed the critical concentrations of 20-30 μg/g ww (approximately 70-100 μg/g dw) for Hg (Thompson, 1996) and 105 μg/g dw for Cd (Chmielnicka et al., 1989) that are associated with adverse effects, while median concentrations were more than an order of magnitude lower. Mercury and Cd-mediated adverse clinical effects in bats in SW England would not therefore be expected. We found a negative relationship between renal Hg concentration and body weight in pipistrelles but this is not necessarily evidence of a direct adverse effect. For example, such a relationship could arise simply from differences in the prey (and its associated level of contamination) taken by individuals of different size.

However, further investigation of the health status of contaminated pipistrelles is warranted, especially given that accumulation of mercury by pipistrelles in SW England appears to be rising. As with Hg and Cd, median renal Pb concentrations were 6-20-fold lower than the 25 μg/g dw suggested as diagnostic of acute lead poisoning in mammalian wildlife (Ma, 1996). However, approximately 5% of pipistrelles had renal Pb residues that exceeded this concentration. Some of these individuals may have suffered lead-induced effects. Ideally, we would have supported our chemical analysis with histolopathological examinations of the kidneys to determine whether there were lesions consistent with or suggestive of Pb poisoning in the bats with high Pb residues. However, the kidneys were too small for both histological and chemical contaminant analysis and, in many cases, the bats were too autolysed for histopathological examination.

5. Conclusion

This study is the first evaluation of toxic heavy metal concentrations in bats in Britain. Our results suggest that accumulation of Pb, Cd and Hg in individuals from the south-west is similar to that measured in bats from elsewhere in Europe and is mostly well below that associated with adverse clinical effects in other orders of wild mammals. However, little is known about the interactions between metal contamination in bats and other stressors such as disease. Furthermore, there is no evidence that accumulation of toxic metals by bats has declined over the last 15-20 years and, to the contrary, accumulation of Hg by pipistrelles appears to be increasing. Continued monitoring of the long-term trends in metal accumulation and associated health status in bats, together with exploration of the extent to which contamination may be greater in individuals from more industrialised regions, is merited.

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Table 1
Median, inter-quartile range (IQR), and maximum dry weight renal concentrations of mercury, lead, and cadmium (μg/g dw), and number of samples below limit of detection (ND)

		Species[a]				
		brown long-eared	Natterer's bat	pipistrelle	whiskered bat	Kruskal-Wallis statistic[d]
	N[b]	59	13	172	17	
Hg	Median	1.52^{B}	2.65^{AB}	0.93^{C}	3.00^{A}	63.5***
	IQR	0.93 - 2.22	0.93 - 4.74	0.93 - 0.93	2.16 - 5.50	
	Max	8.14	7.87	5.08	8.01	
	ND[c]	24	4	133	2	
Pb	Median	3.38^{A}	1.16^{B}	2.45^{A}	4.05^{A}	17.4**
	IQR	1.83 - 5.08	0.62 - 1.98	1.26 - 4.85	2.43 - 9.61	
	Max	25.0	5.68	69.7	24.3	
	ND	0	4	37	1	
Cd	Median	0.830^{A}	6.27^{A}	1.42^{A}	1.61^{A}	7.96*
	IQR	0.24 - 2.75	0.29 - 13.92	0.34 - 3.38	0.63 - 4.31	
	Max	12.5	18.5	29.1	16.0	
	ND	7	0	29	0	

[a] Data for different sexes and ages within species were pooled; [b]N = Number of samples analysed ; [c]Values given to samples below the LoD were 0.929, 0.622 and 0.081 μg/g dw for Hg, Pb and Cd, respectively; [d]Values with different superscripts in the same row are significantly different:* = P<0.05, ** = P<0.001, *** = P<0.0001

Table 2
Spearman rank correlation coefficients (r_s) between renal concentrations of different metals in pipistrelle and brown long-eared bats

Species	Parameter	Comparison		
		Hg vs Pb	Hg vs Cd	Pb vs Cd
pipistrelle bat	r_s	-0.067	0.026	0.308
	N[a]	37	37	120
	P-value	0.694	0.877	<0.001
brown long-eared bat	r_s	0.162	0.105	0.218
	N	35	52	36
	P-value	0.353	0.458	0.202

[a] N = Number of pairs included in analysis. Only pairs in which both metal concentrations were above the limit of detection were included in the analysis

Table 3

Spearman rank correlation coefficients (r_s) between body weight and renal concentrations of Hg, Pb and Cd in pipistrelle and brown long-eared bats

Species	Parameter	Hg	Pb	Cd
pipistrelle bat	r_s	-0.376	-0.120	0.082
	N[a]	37	133	142
	P-value	0.022	0.169	0.333
brown long-eared bat	r_s	0.040	-0.118	0.173
	N	34	57	50
	P-value	0.821	0.382	0.230

[a] N = Number of pairs included in analysis. Only pairs in which metal concentrations were above the limit of detection were included in the analysis.

Figure 1. Median and inter-quartile range (IQR) concentrations of Hg, Pb and Cd in the kidneys of 9 juvenile (□) and 35 adult (■) pipistrelle bats. ** indicates a significant difference (P<0.01) between adult and juvenile bats.

Figure 2. Scatterplots of changes over time in: the proportion of brown long-eared bats with detectable renal metal residues (left hand graphs); the annual median renal metal concentration in males (●) and females (o) that had detectable residues (right hand graphs).

Figure 3. Scatterplots of changes over time in: the proportion of pipistrelle bats with detectable renal metal residues (left hand graphs); the change in annual the annual median renal metal concentration in males (●) and females (o) that had detectable residues (right hand graphs). The line showing the change in annual median renal Hg concentration over time is the weighted linear regression line for males and females pooled (regression equation: renal Hg = 0.060year - 118).

Figure 4. Scatterplots of relationship between kidney Cd and Pb concentrations in brown-long-eared and pipistrelle bats. Only bats with detectable kidney residues of both metals were included in the analysis.

Figure 5. Scatterplots of relationship between kidney Hg concentrations and body weight in brown-long-eared and pipistrelle bats. Data for all bats (left hand graphs) and only bats with detectable kidney Hg residues (right hand graph) are shown.

Figure 1

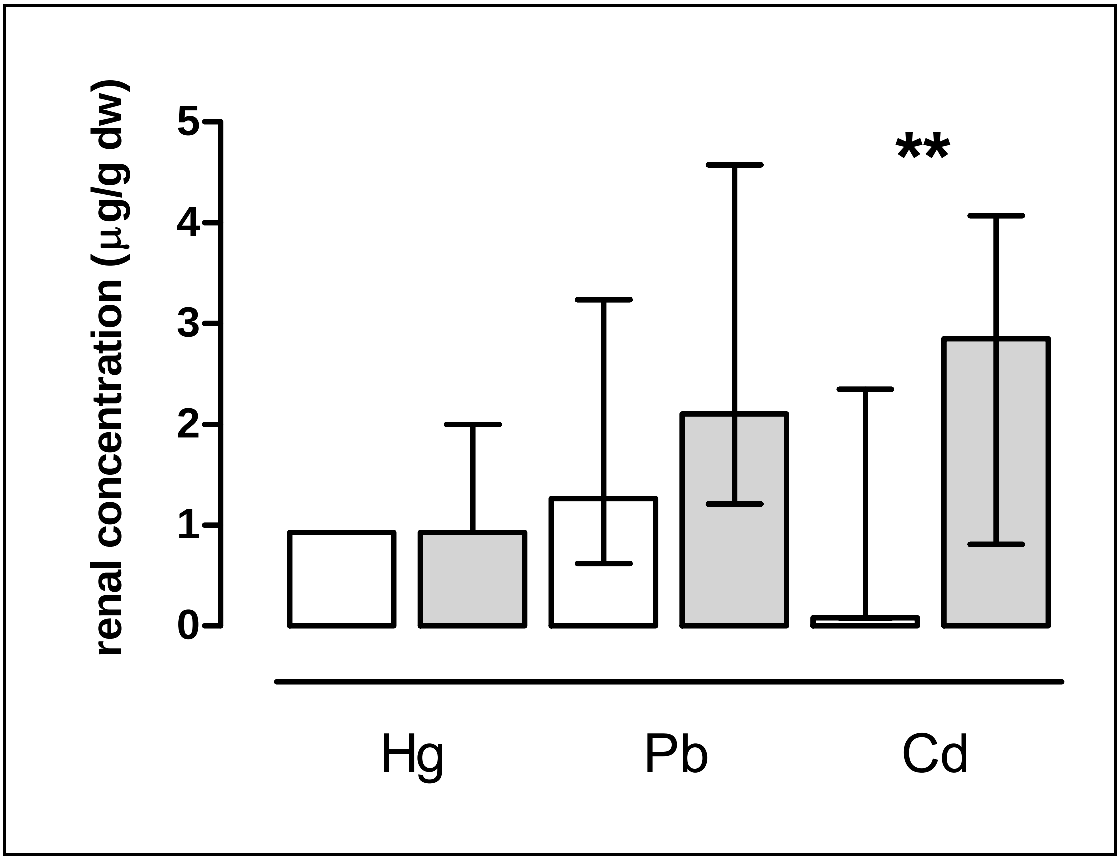


Figure 2

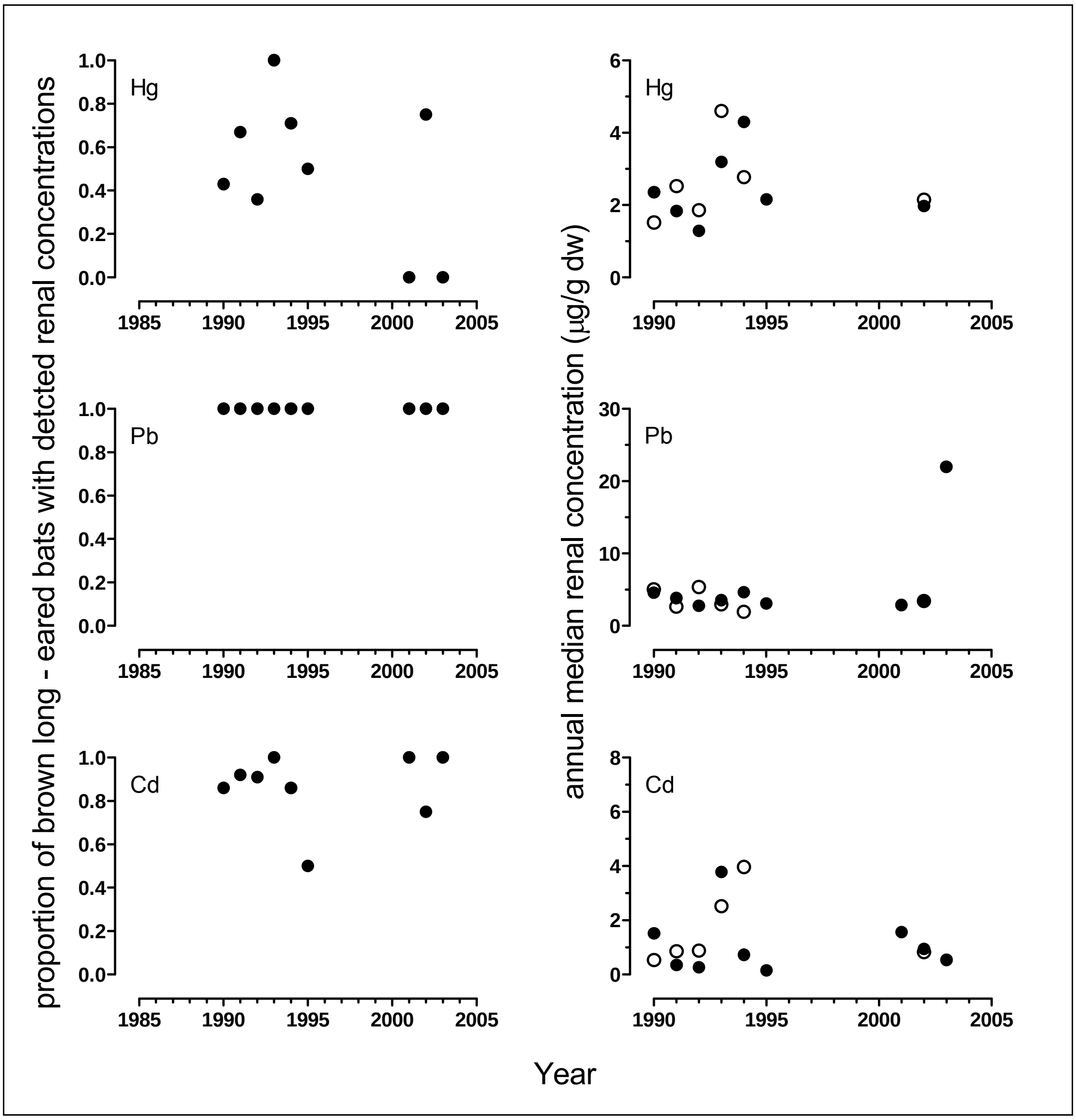

proportion of brown long - eared bats with detcted renal concentrations
annual median renal concentration (µg/g dw)
Hg
Pb
Cd
1.0
0.8
0.6
0.4
0.2
0.0
6
4
2
0
30
20
10
8
1985
1990
1995
2000
2005
Year

Figure 3

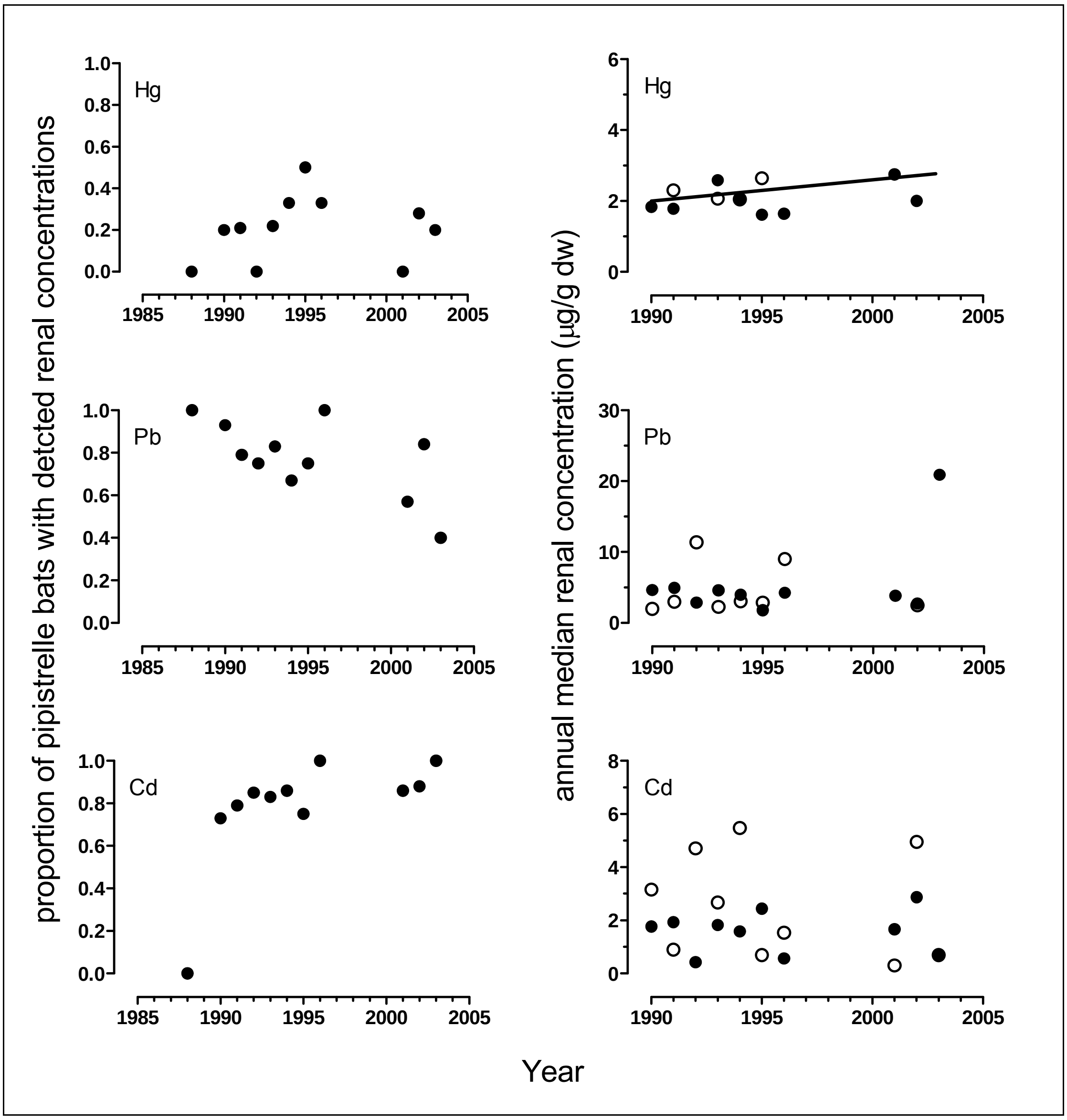
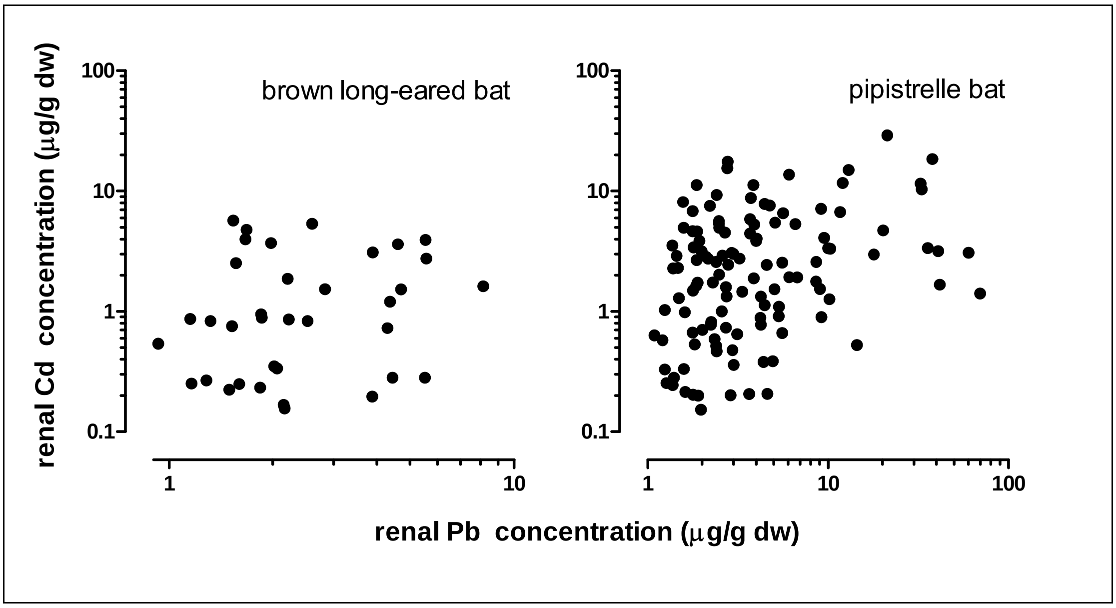
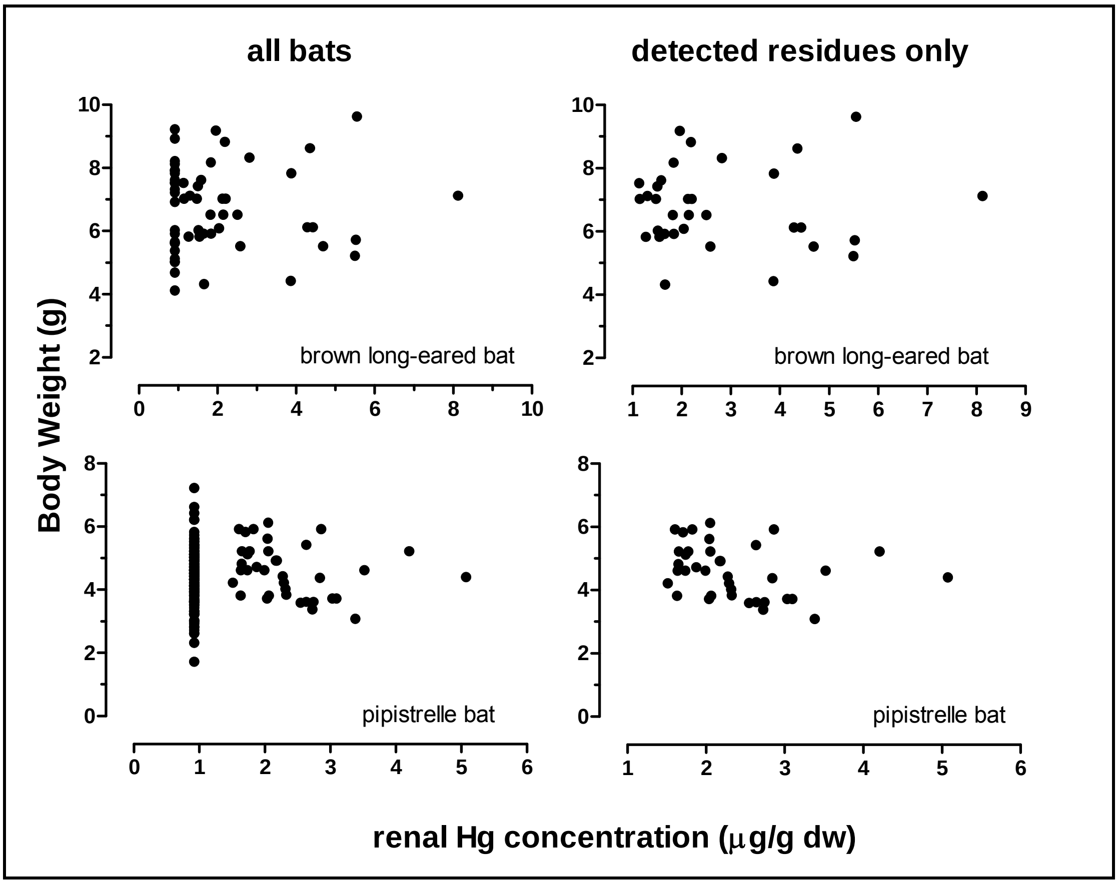

Hg
Pb
Cd
proportion of pipistrelle bats with detcted renal concentrations
annual median renal concentration (µg/g dw)
Year

Figure 4



brown long-eared bat
pipistrelle bat
renal Cd concentration (µg/g dw)
100
10
1
0.1
100
10
1
0.1
1
10
1
10
100
renal Pb concentration (µg/g dw)

Figure 5



all bats
detected residues only
Body Weight (g)
brown long-eared bat
brown long-eared bat
pipistrelle bat
pipistrelle bat
renal Hg concentration (μg/g dw)