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Estimating Pesticide Burdens of Bats from Guano Analyses

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Bat guano was first collected and analyzed for environmental pollutants in the early 1970's (REIDINGER 1972:53-64; PETIT & ALTENBACH 1973). Later analyses of both guano and bats from two roosts indicated a correspondence between levels of organochlorine residues in guano and in bat carcasses from the same colony (CLARK & PROUTY 1976). Gray myotis (*Myotis grisescens*) were found dead with lethal brain levels of dieldrin in two colonies where dieldrin levels in guano were high relative to other colonies (CLARK et al. 1978, 1980). We collected and analyzed both guano samples and bats found dead or killed accidentally during handling from numerous roosts of several bat species. The objective of this study was to determine whether a correlation existed between organochlorine residues in guano and in bat carcasses.

MATERIALS AND METHODS

Guano samples were collected from 48 roosts of gray myotis in Missouri, Tennessee, and Alabama, from 6 roosts of Indiana myotis (*Myotis sodalis*) in Missouri, Kentucky, and Indiana, from 2 roosts of little brown myotis (*Myotis lucifugus*) in Maryland, from 1 roost of big brown bats (*Eptesicus fuscus*) in Maryland, and from 1 roost of Brazilian free-tailed bats (*Tadarida brasiliensis*) in Texas. Data for both guano and carcasses were obtained from 19 aggregations of bats at 18 of these collection sites (Table 1).

Two colonies of gray myotis were sampled twice in different years (samples A and B, C and D); one of these colonies changed roost sites (samples A and B). Samples of fresh, surface guano and bats were collected in the same year for most sites (Table 1). We selected dieldrin, heptachlor epoxide, and DDE for study because they were the most commonly recovered residues.

Chemical analyses were done at the Patuxent Wildlife Research Center by methods described elsewhere (CLARK et al. 1975; CLARK & PROUTY 1976; CLARK et al. 1978, 1980). The term "carcass" refers to that part of the body remaining after the wings, feet, skin, head, and gastrointestinal tract are removed. All guano samples collected in 1975 through 1978 were desiccated at room temperature for about 30 days with calcium sulfate. Because most of these samples contained 50-80% water, drying was necessary for accurate parts per million (ppm) comparisons. The three guano samples

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TABLE 1

Nineteen samples of bat guano and carcasses analyzed for organochlorine chemical residues.

Location	Colony Type[a]	No. of Bats[b]	Date Collected (month/year) Bats	Guano
Gray Myotis				
Bat C.[c] No.2, Franklin Co., Mo.(A)[d]	M	12(0)	7/76	3,7/76[e]
Bat C. No.3, Franklin Co., Mo.(B)	M	13(5)	7/78	7/78[f]
Roaring Springs C., Franklin Co., Mo.(C)	M	6(1)	6,7/76	6/76[e]
Roaring Springs C., Franklin Co., Mo.(D)	M	3(0)	7/78	7/78[f]
Beck C., Hickory Co., Mo.(E)	M	6(2)	7/76	9/78[f]
Bat C., Dent Co., Mo.(F)	M	12(0)	6/76	9/76[g]
Mary Lawson C., Laclede Co., Mo.(G)	M	2(2)	7/78	5/78[h]
Mauss C., Camden Co., Mo.(H)	M	1(0)	8/78	5/78[f]
Cave Springs C., Morgan Co., Ala.(I)	M	4(0)	7/78	8/76[f]
Onyx C., Crawford Co., Mo.	T	2(2)	10/75	4/76[f]
Moles C., Camden Co., Mo.	T	5(0)	8/78	6/78[f]
Saloon C., Crawford Co., Mo.	T	14(8)	6-8/76	7,9/76[h]
Coffin C., Laclede Co., Mo.	H	2(2)	12/78	12/78[f]
Chimney C., Shannon Co., Mo.	H	1(1)	3/76	10/75[i]
Indiana Myotis				
Great Scott C., Washington Co., Mo.	B	3(3)	4,9/76	4/76[f]
Little Brown Myotis				
Montpelier Barn, Prince Georges Co., Md.	M	6(5)	4,7/73	11/73[g]
North East Church, Cecil Co., Md.	M	17(17)	5,10/73	5/73[g]
Big Brown Bat				
Montpelier Barn, Prince Georges Co., Md.	M	16(15)	4-10/73	1/76[g]
Brazilian Free-tailed Bat				
Bracken C., Comal Co., Tex.	M	20(20)	6/73	6/73[f]

[a]M = maternity, T = transient, H = hibernation, B = bachelor. [b]Number in parentheses is number of adult bats in sample, all others were juveniles. [c]C = cave. [d]Letters identify samples as plotted in Fig. 1. [e]Three analyses were performed, 2 of 1 sample and 1 of another. [f]Two analyses of 1 sample were performed. [g]One analysis of 1 sample was performed. [h]Two analyses were performed, 1 each of 2 samples. [i]Four analyses were performed, 2 each of 2 samples.

collected in 1973 were already as dry as if they had been put in the desiccator when collected.

Geometric means were used for residues in carcasses because the data were positively skewed. Residue levels reported as "not detected" were considered zero; means of data series that included zeros were calculated on data transformed as $\log_{10}(x + 0.01)$; 0.01 was subtracted from the retransformed means. The carcass means were normally distributed and yielded higher r values without transformation. The calculations (Table 2, Fig. 1) used geometric means without transformations. Residue concentrations in guano were most commonly determined by duplicate analyses of halves of single guano samples (Table 1). Results were based on one to four analyses when duplicate analyses were run for two guano samples (Table 1). An arithmetic average was calculated for duplicate analyses; this average was then averaged with results from a second sample if available.

Samples showed variation typical of field measurements of organochlorine residues. For example, coefficients of variation for dieldrin in the seven samples of bat carcasses with N > 10 (Table 1) were 28, 28, 80, 310, 580, 90, and 57. For guano, variation inherent in the chemistry procedures was measured by the percentage difference between results for the 14 samples done in duplicate. For dieldrin these differences ranged from zero to 29%

TABLE 2

Concentrations of organochlorine chemicals in bat guano versus bat carcasses expressed as correlation coefficients (r) and regression equations.

Chemical	Gray Myotis: Maternity Roosts (N = 9)	Gray Myotis: All Roosts (N = 14[a])	All Bat Roosts (N = 19[a])
Dieldrin			
r	0.91	0.87	0.78
Equation	Y=0.133+0.034X	Y=0.156+0.033X	Y=0.157+0.033X
Heptachlor epoxide			
r	0.84[b]	0.85	0.82
Equation	Y=0.034+0.036X	Y=0.018+0.038X	Y=0.008+0.038X
DDE			
r	0.99	0.99	0.99
Equation	Y=-0.060+0.146X	Y=-0.059+0.146X	Y=-0.068+0.146X

[a]Sample sizes are N - 1 for heptachlor epoxide. [b]Significant at 0.01 > P > 0.001; all other r's are significant at P < 0.001.

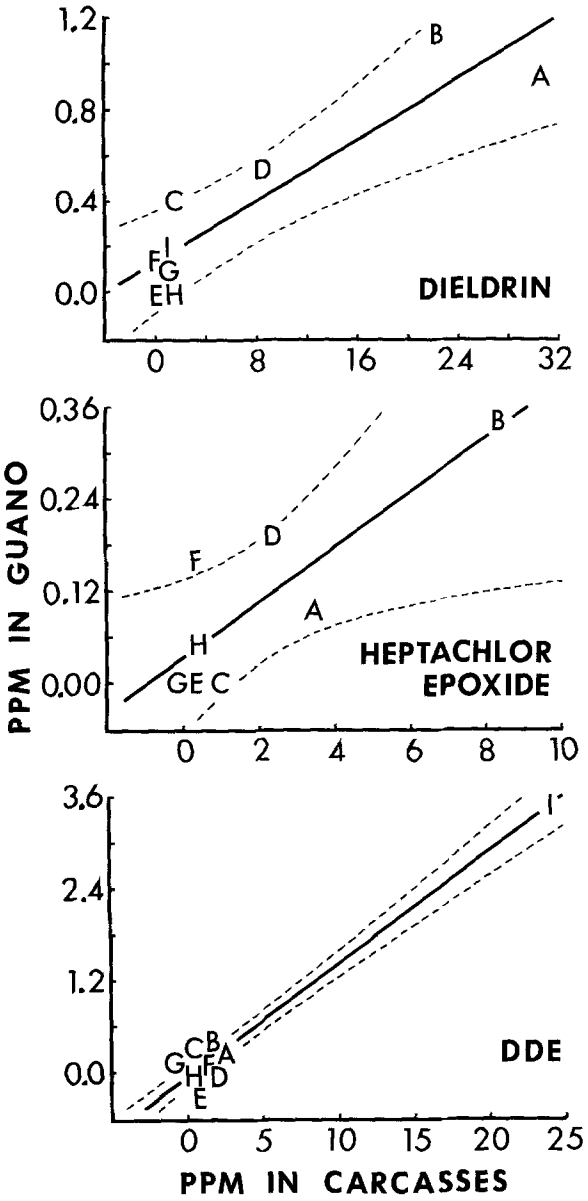

FIGURE 1. Concentrations of organochlorine pesticide residues in guano (ppm dry weight) versus bat carcasses (ppm wet weight) from maternity colonies of gray myotis. Letters refer to samples described in Table 1. Correlation coefficients and regression equations are given in Table 2. Dashed lines indicate 95% confidence intervals.

and averaged 9.2%. Two different guano samples were collected at eight of the sites. Percentage differences in dieldrin for these ranged from zero to 57% and averaged 25%. If about 9% of this is attributable to variation in the chemistry, the remaining 16% can be attributed to real differences within the roost.

Data analyses for heptachlor epoxide (Fig. 1, Table 2) excluded the Cave Springs Cave, Alabama, locality because the concentration in guano exceeded that in the carcass sample. We suspect a decline in environmental levels of heptachlor epoxide occurred between 1976 when the guano was collected and 1978 when the bats were collected.

Regression lines (Fig. 1) were fitted by the least squares method. Confidence bands of 95% (Fig. 1) were calculated according to NETER & WASSERMAN (1974:149-152).

RESULTS

There were highly significant correlations between guano and carcass concentrations of dieldrin, heptachlor epoxide, and DDE (Fig. 1, Table 2). The plotted data (Fig. 1) were limited to the nine maternity colonies of gray myotis because regression analyses (Table 2) showed that these data had the strongest relationships. The data available from other colonies (Table 1) did not extend the upper residue limits or enhance these regressions because levels of pollutants were zero or low in most cases.

Dieldrin levels (Fig. 1) included concentrations at which mortality occurred in the colonies. Some bats in each of the four samples with highest dieldrin levels had lethal brain concentrations (i.e., 10 of 12 in sample A, 12 of 13 in B, 2 of 4 in C, and 2 of 3 in D). When the sample was increased to include all gray myotis roosts (N = 14) and all bat roosts (N = 19), the correlation coefficient (r) decreased but the slope and y-intercept of the regression equation changed only slightly (Table 2).

The highest concentrations of heptachlor epoxide (Fig. 1, sample B) were near levels where mortality could be expected (CLARK et al. 1980). However, the lethal levels of dieldrin in the bats of this sample precluded any conclusion. The scatter of points for heptachlor epoxide was greater than for dieldrin. The y-intercept of the equation changed markedly as the sample was made more inclusive (Table 2).

The function for DDE (Fig. 1) is poor because the data were inadequately distributed. The highest concentration was associated with brain levels up to only 27 ppm, which is far below the several hundred ppm necessary to cause mortality in the two bat species for which lethal brain levels have been measured (Brazilian free-tailed bat, CLARK & KROLL 1977; little brown myotis, CLARK & STAFFORD 1981). The slope and y-intercept of the

DDE equation remained relatively constant (Table 2), probably because the function was controlled by a single elevated value.

DISCUSSION

The relationship for dieldrin between mean concentration in bat carcasses and concentration in guano (Fig. 1) contained substantial variation and should not be used to predict residue levels in bats. Nevertheless, the function can serve to alert investigators to colonies with potential dieldrin problems.

The dieldrin data (Table 2) indicate that restriction to one species and one type of colony produced a stronger correlation. The most restricted data (N = 9) still lacked uniformity in that they included adult as well as juvenile bats, males and females, bats collected alive or found dead, and guano and bat samples collected at different times. Also, sample sizes for carcass means were as small as one, and there was variation in different guano samples from the same cave. Despite these problems, the correlation was strong (Table 2). The equation changed only slightly as data for other types of colonies and other species were added; therefore it may be applicable to most bat colonies. The data for heptachlor epoxide and DDE are less useful and were included primarily to show that such relationships probably exist for all organochlorine pollutants.

As a survey technique, the collection and analysis of a single guano sample has three advantages over analyses of bats themselves. First, it probably provides a more accurate, longer-term measure of the contaminant condition of the colony tested because a single guano sample contains feces from many times more bats than could be reasonably analyzed separately, and the time period represented (assuming the guano was taken from the surface) is days or weeks rather than a single instant. Second, because only one analysis (or, as a precaution against analytical error, two analyses of subsamples) is required, the expense is minimized. Third, it is not necessary to collect bats.

When a roost is found where organochlorine residues in guano seem high, the investigator can return later to look for mortality. Mortality may be most likely when young bats begin to fly and metabolize fat stored during nursing (CLARK et al. 1978, 1980). Analyses of brains and carcasses of bats found dead will confirm whether the chemical in question caused the mortality.

Commercial analytical laboratories routinely perform organochlorine analyses. However, when bat tissues or guano are to be analyzed, the possibility of human exposure to rabies virus must be controlled. The most practical solution is for the investigator (previously immunized) to grind, extract, and dry the samples before shipment to the analytical laboratory. The extraction process kills rabies virus. Procedures for preparation and shipment of samples can be obtained from the Patuxent Center.

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