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Disposition of Dietary Dieldrin in the Little Brown Bat and Correlation of Skin Levels with Body Burden

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Gray bats (*Myotis grisescens*), an endangered species, died of poisoning by dieldrin at two localities in Missouri in 1976-78 (Clark et al. 1978, 1980, in press) and in 1980-81 (Clark et al. 1983). Until banned in 1974, the parent compound, aldrin, was applied to corn to control cutworms (larvae of several moth species, family Noctuidae). Because dieldrin residues may persist in soil for years (Korschgen 1971), we assume the earlier use of aldrin is responsible for the bat mortality since the ban.

The present laboratory study was undertaken to understand better the mortality that was observed in Missouri. We wanted to define experimentally the range of lethal brain levels for dieldrin in both adult and juvenile little brown bats (*M. lucifugus*) and compare those levels with those found in dead gray bats in the field. This experiment and our interpretations of the data are based on the assumption that these two congeneric species respond similarly to dieldrin. The data from the experimental feeding allowed us to look at the rates of accumulation and loss and to compare estimates of body burden derived from analyses of small skin samples with results from whole carcass analyses, a potential technique for evaluating residue levels at death in bats stored in museum collections.

MATERIALS AND METHODS

On 16 July 1979, 56 female little brown bats were collected from a church attic in North East, Cecil County, Maryland, and brought to the Patuxent Wildlife Research Center, Laurel, Maryland. All bats were weighed and then caged individually in stainless steel wire mesh cages (18 X 22 X 37 cm) with excrement catch pans and were fed untreated mealworms (larvae of the beetle *Tenebrio molitor*). Subdued skylight entered the laboratory through two draped windows. Daily minimum and maximum temperatures averaged 23.3 and 31.7°C. Water was provided from rodent watering bottles.

During the first week of captivity and before dosing was begun, six bats died and three others were sacrificed because they were weak or did not eat well. On 23 July the remaining 47 bats (20 juveniles and 27 adults, age determined by epiphysis condition)

began receiving treated mealworms. The mealworms had been reared in wheat bran containing 1 ppm of dieldrin, and contained an average of 0.38 ppm (wet weight basis) dieldrin (Table 1).

Table 1. Dieldrin concentrations in samples of dosed mealworms and guano taken during the experiment.

Days after dosage began	Dosed mealworms		Guano ppm dry weight	
	ppm wet weight	ppm dry weight[a]	Adults	Juveniles
1	0.43	1.5		
16			0.08	0.11
19	0.40	1.4		
28			0.10	0.14
40	0.42	1.5		
53	0.27	0.95	0.09	0.17
Means	0.38	1.34	0.09[b]	0.14[b]

[a]Values obtained by converting ppm wet weight values through the experimental determination that mealworms are 71.8% water.
[b]Student's t for paired data = 3.273, not statistically significant, P = 0.08.

Mealworms reared in untreated bran contained no detectable dieldrin. Dosage lasted 52 days (final day, 13 September) after which the remaining bats were fed untreated mealworms. During dosage, 8 bats died and 15 were sacrificed. After dosage, 2 bats died and 22 were sacrificed. For sacrifice, bats were chosen randomly, usually one adult and one juvenile per week. The last remaining bats were sacrificed on 5 October, 74 days after dosage began. We hoped that differences in brain levels of dieldrin between sacrificed bats and bats that died would help define lethal levels. Daily mealworm rations were weighed and all bats fed the same weight of mealworms each day. However, all bats were weighed weekly and when their average weight changed, the daily mealworm ration was adjusted (it was either 0.9 or 1.0 g) to prevent any large change in average weight of the bats. Guano was collected for chemical analysis from the catch pans (dried urine was included) of all adult and juvenile bats on 8 and 20 August, and 14 September. Guano samples were desiccated with calcium carbonate at room temperature.

All samples were analyzed at the Patuxent Wildlife Research Center. Bats were dissected into brain and carcass components as described previously (Clark and Kroll 1977). Also, a 1 cm^2 sample of skin was removed from the ventral surface of the dried (10 months, ambient laboratory conditions) skin of each bat. All samples were ground with anhydrous sodium sulfate and extracted for 7 hrs with hexane in a paper extraction thimble on a Soxhlet

apparatus. Hexane extracts of samples were cleaned by Florisil column chromatography, and the pesticides and PCB's (polychlorinated biphenyls) were separated on a SilicAR column (Kaiser et al. 1980). Residues were quantified with a gas-liquid chromatograph equipped with an electron-capture detector and a 1.5% OV-17/1.95% QF-1 column. Chemical identities in 4% of the samples were confirmed with a Finnigan 4000 gas-liquid chromatograph-mass spectrometer. Recovery of dieldrin from fortified tissue was 100%. Lipid levels were determined from weights of dried hexane extracts of carcasses. The lower limit of sensitivity was 0.1 ppm for dieldrin in carcasses, guano, mealworms, and skin, and 0.5 ppm for brains. Ppm values are on a wet (= fresh) weight basis, unless noted otherwise. Residues other than dieldrin measured in carcasses of dosed bats were DDE (100% of bats, maximum of 4.1 ppm), DDD (4%, 0.13 ppm), DDT (6%, 0.12 ppm), oxychlordane (53%, 0.61 ppm), *trans*-nonachlor (2%, 0.10 ppm), and PCB's, quantified as Aroclor 1260 (100%, 12 ppm). All of these concentrations are insignificant relative to known effect levels.

Regression lines were determined by the least squares method. Confidence bands of 95% were calculated according to Neter and Wasserman (1974).

RESULTS AND DISCUSSION

The results are presented in three ways (Fig. 1). Even though amounts of dieldrin in bats increased during dosage and decreased afterward, these changes did not appear as changes in average dieldrin concentration in the fat (ppm lipid weight) because the amount of fat was highly variable. Fat in the 47 carcasses averaged 9.43%, standard deviation 8.38%, coefficient of variation 88.8, and range 0.436-29.4%. However, when the data were restricted to bats with low (< 3%) fat, a significant increase could be seen (Fig. 1C). Uptake of dieldrin by juvenile bats was also significant ($r = 0.80$, $0.01 > P > 0.001$) but a covariance analysis showed no significant difference (slope $F = 0.063$, $P = 0.8$; elevation $F = 0.066$, $P = 0.8$) between regressions for juveniles and adults. The regression for loss of dieldrin by juveniles was not significant ($r = 0.35$, $P > 0.1$). Covariance analyses also showed no significant differences in uptake between bats that died and those that were sacrificed (slope $F = 1.015$, $P = 0.3$; elevation $F = 0.298$, $P = 0.6$); an analysis was not done for loss because only two bats died.

The data (Fig. 1) show that the buildup was continuing and equilibrium had not been reached when dosage was stopped. The average dieldrin concentration in the mealworm diet was 14.9 times the average concentration in guano from adult bats and 9.6 times that from juvenile bats (Table 1). The initial half-life for loss of dieldrin--the time required to reduce the average dieldrin concentration on day 53 by half according to the equation $Y = 4.40 - 0.04X$, Fig. 1B--is estimated at 24 days. This

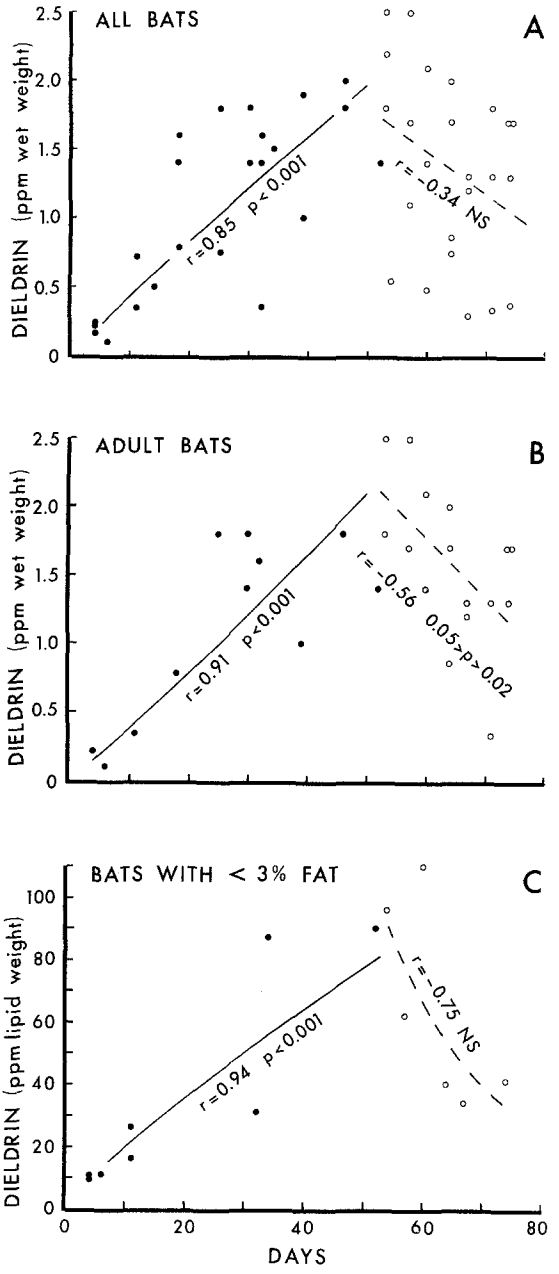

Figure 1. Dieldrin residues in carcasses of dosed little brown bats. Circles represent bats that died or were sacrificed while on dieldrin dosage (solid), or on clean food after dosage (open). Significant regressions ($P < 0.05$) for increase: (A) $\log_{10}Y = -1.32 + 0.95 \log_{10}X$, N = 23; (B) $\log_{10}Y = -1.51 + 1.08 \log_{10}X$, N = 11; (C) $\log_{10}Y = 0.44 + 0.85 \log_{10}X$, N = 8; for decrease (B) $Y = 4.40 - 0.04X$, N = 16.

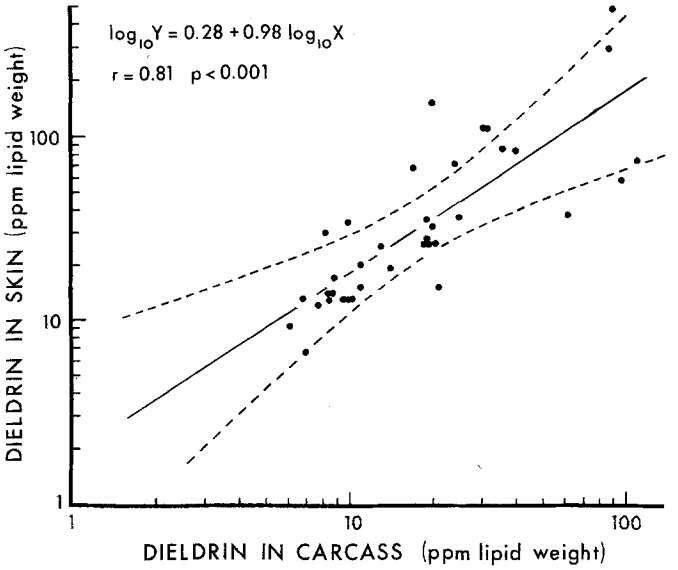

Figure 2. Correlation of dieldrin residues (ppm lipid weight) in skin with residues in carcass of dosed little brown bats. Dashed lines are 95% confidence bands.

half-life interval is generally longer than values reported for dieldrin in other mammals: 14 days in short-tailed shrews *Blarina brevicauda* of both sexes (Blus 1978); 10 days in male rats *Rattus norvegicus* (Robinson et al. 1969); 4.5 days overall but about 8 days for the initial half-life in male rats (Baron and Walton 1971); and 7 to 10 days in male rats and three times that long in female rats (Matthews et al. 1971).

Whereas the lowest carcass levels of dieldrin found in 36 gray bats with lethal brain concentrations were 4.3 ppm wet weight and 239 ppm lipid weight (Clark et al. 1978, 1980, 1983, in press), the highest carcass levels produced in the feeding study were 2.5 ppm wet weight and 110 ppm lipid weight. Whether lethal levels would have been reached at this dosage level with additional feeding is an open question.

We do not know the actual average dietary levels of dieldrin in Missouri gray bats, but samples of prey insects collected where bats from affected colonies were feeding contained as much as 3.1 ppm, or about 8 times the dietary level of our experiment. Also, lactation concentrates residues, and levels of dieldrin in milk from Missouri gray bats were as high as 89 ppm (Clark et al. in press); many of the dead gray bats were young still nursing.

Measurable dieldrin was found in brains of only 6 of 47 bats. The concentrations and days after start of dosage were 0.67, 32; 1.3, 34; 0.92, 52; 0.80, 54; 0.51, 57; and 0.61 ppm, 60 days. These concentrations are well below lethal levels, which begin at

about 5 ppm (Clark et al. 1978). They occurred in bats with low fat levels (2.7% or less) that died or were killed during the middle part of the experiment when body burdens were highest.

Concentrations of dieldrin, as ppm of lipid, were measured in both skin and carcass of 37 dosed bats. The two concentrations were highly correlated; on average, levels in skin exceeded those in carcass (Fig. 2). This correlation suggests that it is possible to test museum specimens and judge whether the bats carried high dieldrin residues at the time they died or were killed. For dieldrin in which the residue relationship between levels in carcasses and brains has been described (Clark 1981), it would be possible to speculate whether the levels had been lethal. There is much variation in the data and evaluations would be subjective, but if several specimens from a die-off contained high levels, the residue data would probably be compelling.

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