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Death in Bats from DDE, DDT or Dieldrin: Diagnosis via Residues in Carcass Fat

Donald R. Clark, Jr.

U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, MD 20811

Studies of birds and mammals have shown that accurate diagnoses of death from organochlorine pesticides can be based on analyses of brain tissue but not other tissues such as liver, carcass, plasma, kidney, or fat (DALE et al. 1963; STICKEL et al. 1966, 1969, 1970; STICKEL & STICKEL 1970). However, when residues exceed certain concentrations in the carcass lipids, they begin to appear in the brain, and from this point on brain and carcass lipid concentrations are significantly correlated. Such relationships have been demonstrated in bats (CLARK & PROUTY 1976, 1977; CLARK & KROLL 1977; CLARK et al. 1978a; CLARK et al. 1978b; CLARK & STAFFORD in press), in herons and egrets (OHLENDORF et al. 1979), and data discussed here show such relationships in two species of songbirds. Thus, it appears feasible to diagnose death caused by organochlorine pesticides by measurement of concentrations in carcass lipids if the minimum lethal brain concentration is known, if the brain-to-carcass lipid correlation has been measured accurately for the species involved, and if the correlation may be assumed applicable up to at least the species level.

This technique could be helpful, because certain circumstances preclude use of the brain itself. For example, when bats are found dead or dying, their brains are sometimes removed for rabies testing before thought is given to possible pesticide poisoning. Further, nearly all birds and mammals in museum collections are prepared as dried, cotton-filled skins from which both brain and body have been removed. Such skins contain carcass fat which other studies suggest would be abundant enough and representative enough of the total carcass fat to use for estimation of brain concentrations of organochlorine chemicals. Such analyses could be used to test bats or birds collected from die-offs that occurred even decades ago. Also, sometimes only dried fragments of bone, muscle, and skin are recovered from an individual of an endangered species (such as the California condor, *Gymnogyps californianus*), and authorities want to know whether pesticides were involved in the death. In such a situation, a parts per million (ppm) lipid value from the fragments could be compared with data derived from a related species (in this example perhaps the turkey vulture, *Cathartes aura*) and an evaluation made. Finally, there may be records (published or unpublished) of carcass lipid concentrations of pesticides for which it is now of interest to evaluate possible

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lethality. This could be done by measuring the brain-to-carcass lipid relationship for the species involved.

The objectives of the present paper are to present the brain-to-carcass lipid relationships for DDE, DDT, and dieldrin that are known for bats, to compare these relationships among bat species and to birds so that their breadth of taxonomic applicability can be evaluated, and to use them to estimate minimum concentrations of DDE, DDT, and dieldrin in carcass lipids that are diagnostic of death.

MATERIALS AND METHODS

Data for bats originated in three ways: DDE was fed to Mexican free-tailed bats, *Tadarida brasiliensis* (CLARK & KROLL 1977), big brown bats, *Eptesicus fuscus* (CLARK & PROUTY 1977), and little brown bats, *Myotis lucifugus* (CLARK & STAFFORD in press) in laboratory studies; DDT was sprayed on little brown bats in a New Hampshire roost (CLARK et al. 1978a); and gray bats, *Myotis grisescens*, in two Missouri colonies received high levels of dieldrin via the food chain (CLARK et al. 1978b; CLARK et al. 1980). Comparable data are available from a study in which free-tailed bats from the DDE-contaminated colony at Carlsbad Caverns, New Mexico, were removed to a laboratory, then exercised and starved to simulate the stress of migration (GELUSO, K. N. pers. comm.; GELUSO et al. 1976).

Comparable data from birds include dieldrin residues summarized for a composite sample of four species: great blue heron, *Ardea herodias*; black-crowned night heron, *Nycticorax nycticorax*; cattle egret, *Bubulcus ibis*; and great egret, *Casmerodius albus* (OHLENDORF, H. M. pers. comm.; OHLENDORF et al. 1979). Birds in this sample were all found dead or moribund. Other data for birds resulted from feeding of DDE to caged brown-headed cowbirds, *Molothrus ater* (STICKEL, W. H. pers. comm.; STICKEL et al. 1970) and common grackles, *Quiscalus quiscula* (STICKEL, W. H. pers. comm.).

The accuracy with which diagnostic lethal brain concentrations of DDE, DDT, and dieldrin are known in bats (Table 1) probably varies according to the conditions under which the data were obtained. Lethal brain concentrations for DDE are probably the most accurate because they were obtained from laboratory feeding studies that allowed behavioral symptoms of poisoning to be observed and that minimized other mortality factors. Lethal brain concentrations for DDT were based on comparisons of brain levels in dead or dying bats versus apparently healthy bats. However, the sample of living bats probably included several that contained lethal concentrations and would have died within hours or days of capture. Also, other mortality factors were undoubtedly operating on this free-living population. In addition, breakdown products of another pesticide, chlordane, were present in these bats and there may have been

undetected additive or synergistic effects. Finally, lethal brain concentrations for dieldrin were based on published data for other species of mammals and birds. This was necessary because the animal involved was the endangered gray bat and collection of live individuals for comparison or for direct experimentation was not considered justified. These free-living gray bats were also exposed to other mortality factors and to residues of another pesticide, heptachlor, as well.

In the present study, all data for both bats and birds were limited either to adults or to juveniles except for herons and egrets (OHLENDORF et al. 1979) in which case the sample included both age groups. Samples for which a residue was measurable in the carcass but not in the brain were excluded because a wide range of carcass values may be associated with brain residue levels that are too low to measure for chemicals of low toxicity such as DDE (CLARK & PROUTY 1976).

With the exception of the free-tailed bats from Carlsbad (GELUSO et al. 1976), all chemical analyses mentioned in this report were performed at the Patuxent Wildlife Research Center or at Raltech Inc. of Madison, Wisconsin; the methodology was the same at both laboratories. The free-tailed bats from Carlsbad

TABLE 1

Estimated minimum lethal carcass lipid concentrations of DDE, DDT, and dieldrin in three bat species.

Species	Minimum Lethal Brain Concentration (ppm wet weight)	Estimated Minimum Lethal Concentration (with 95% confidence interval) in Carcass Lipids (ppm lipid weight)
	DDE	
Free-tailed Bat	460[1]	66,000 (55,000-80,000)
Little Brown Bat	540[2]	79,000 (65,000-97,000)
	DDT	
Little Brown Bat	12[3]	470 (360-660)
	Dieldrin	
Gray Bat	4.6[4]	390 (210-800)

[1] CLARK & KROLL (1977) [2] CLARK & STAFFORD in press [3] CLARK et al. (1978a) [4] CLARK et al. (1978b), CLARK et al. (1980)

were analyzed at the Denver Wildlife Research Center by a different methodology. Detailed chemical methodologies are presented in the papers from which the data were taken for use in the present paper.

Linear regressions were fitted by least squares methods, and pairs of regressions were compared by using covariance analysis (SNEDECOR & COCHRAN 1967). Confidence bands of 95% (Fig. 1) were calculated according to NETER & WASSERMAN (1974). Significance levels are: * = 0.05>P>0.01; ** = 0.01>P>0.001; *** = P< 0.001. NS designates a test result that was not significant.

RESULTS

Data from bats of three species that were fed DDE were plotted together and fitted with a single regression line (Fig. 1A) because results of covariance analyses (Table 2) showed that the individual relationships were not significantly

TABLE 2

Covariance comparisons among bat and bird species of the brain (ppm wet weight)-to-carcass (ppm lipid weight) relationship for residues of DDE and dieldrin.

Samples	F values	
	Slope	Elevation
Bats Dosed with DDE		
Free-tailed Bats vs. Big Brown Bats	0.04NS	0.05NS
Free-tailed Bats vs. Little Brown Bats	0.25NS	0.04NS
Big Brown Bats vs. Little Brown Bats	0.06NS	0.61NS
DDE-dosed Bats vs. other Bats and vs. DDE-dosed Birds		
Carlsbad Free-tailed Bats	0.16NS	55.55***
Brown-headed Cowbirds	9.56**	9.62**
Common Grackles	5.62*	12.34***
Birds Dosed with DDE		
Brown-headed Cowbirds vs. Common Grackles	24.64***	0.01NS
Dieldrin in Field-collected Bats and Birds		
Gray Bats vs. Herons and Egrets	12.66**	0.61NS

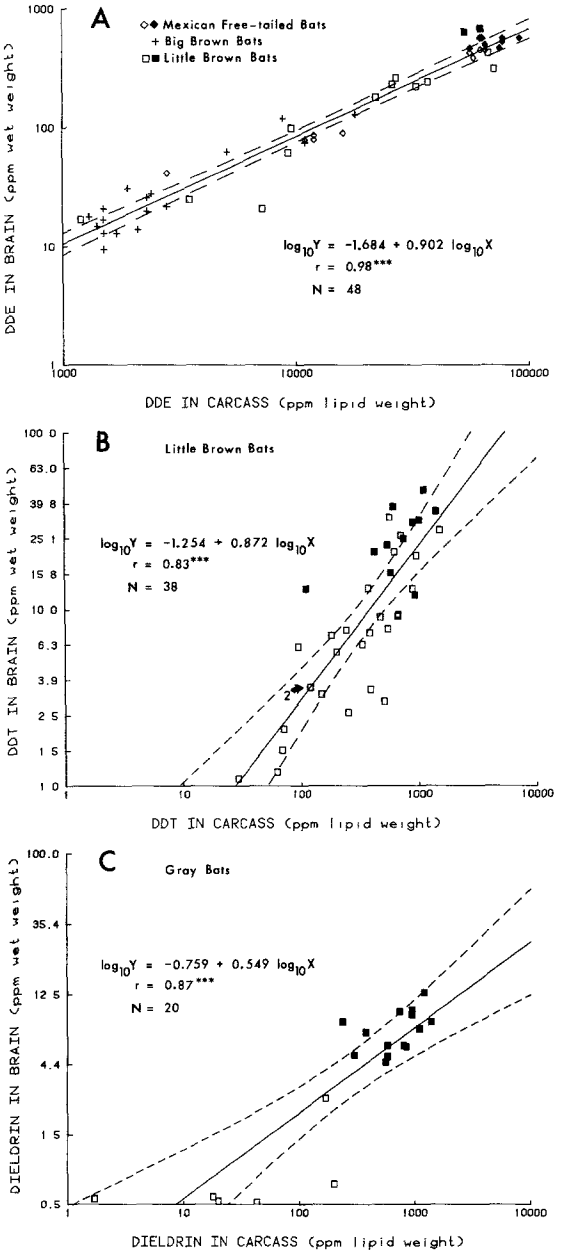

FIGURE 1. Brain (ppm wet weight)-to-carcass (ppm lipid weight) relationships in bats for residues of DDE, DDT, and dieldrin. Open symbols represent bats known or believed to have survived the toxicant; solid symbols represent bats known or believed to have died of the toxicant. There were no deaths among big brown bats. Dashed lines indicate 95% confidence bands.

different. Plots for DDT (Fig. 1B) and dieldrin (Fig. 1C) were based on single species. The equations for these regressions were used to estimate concentrations in carcass lipids that correspond to the minimum lethal levels in the brain (Table 1). Among the little brown bats exposed to DDT (Fig. 1B), nearly half of those with at least 400 ppm in carcass lipids (10 of 21, 47.6%) were dead and two-thirds of those with at least 700 ppm in carcass lipids (6 of 9, 66.7%) were dead.

Even though DDE data from three bat species were not different, similar data from young-of-the-year free-tailed bats that were collected at Carlsbad Caverns then starved and exercised (GELUSO, K. N. pers. comm.; GELUSO et al. 1976) produced a regression ($\log_{10}Y = -1.566 + 0.959 \log_{10}X$, $r = 0.93^{***}$, $N = 10$) that was significantly different in elevation (Table 2).

Two studies in which DDE was fed to birds (STICKEL, W. H. pers. comm.; STICKEL et al. 1970) resulted in regressions (cowbirds, $\log_{10}Y = -0.597 + 0.684 \log_{10}X$, $r = 0.92^{***}$, $N = 27$; grackles, $\log_{10}Y = -2.106 + 1.024 \log_{10}X$, $r = 0.97^{***}$, $N = 46$) that were significantly different in both slope and elevation from that for bats (Table 2). These two regressions for birds also differed significantly from each other in slope (Table 2). The regression for dieldrin in gray bats (Fig. 1C) was found to be significantly different in slope (Table 2) from that ($\log_{10}Y = -1.876 + 0.985 \log_{10}X$, $r = 0.91^{***}$, $N = 21$) for herons and egrets (OHLENDORF, H. M. pers. comm.; OHLENDORF et al. 1979; Table 2).

DISCUSSION AND CONCLUSIONS

Because the regression for DDE was the same for three bat species belonging to two families and because one of the species was the free-tailed bat, it was surprising that data for free-tailed bats from Carlsbad Caverns (GELUSO, K. N. pers. comm.; GELUSO et al. 1976) did not fit this regression. Two explanations seem possible. First, all of Geluso's bats were young-of-the-year, whereas all other bats were adults. To determine whether such an age difference could account for different regressions, I compared the regression for DDT in adult little brown bats (Fig. 1B) with data from eight young-of-the-year little brown bats that were part of the same study. Unfortunately, the regression for these young bats was not statistically significant so a valid test was not possible. Similarly, data for dieldrin in four adult gray bats were compared with data for the juveniles (Fig. 1C), but again the adult data did not show a significant regression. The second possible explanation is that the difference was a result of different chemical methodologies.

Because neither of these possibilities can be ruled out at this time, it would be prudent to use these relationships (Fig. 1) for diagnosis only if bats were of the same age category and only if analyses were performed by the same methodology. The data do suggest, however, that when these conditions are met, the

relationships may apply to several species. Thus, conditions for use of carcass lipid concentrations for diagnosis of organochlorine poisoning seem to be no more restrictive than those for use of brain concentrations. Therefore, the estimated minimum lethal concentrations in carcass lipids (Table 1) could be used to evaluate the likelihood that DDE, DDT, or dieldrin caused mortality when chemical analyses of these species are presented as ppm in carcass lipids.

The fact that the DDE relationships for cowbirds and grackles differed from that for bats and from each other (Table 2), even though these two bird species belong to the same family (Icteridae), indicates that it is best to assume that each relationship is species specific unless wider applicability is demonstrated.

It appears theoretically possible to evaluate whether birds and mammals prepared as dried, cotton-filled skins in museum collections died of organochlorine poisoning. However, it has yet to be shown that sufficient fat could be obtained from such specimens without undo damage or that residues from such fat would be representative of the carcass fat as a whole. On the first point, PEAKALL (1974) extracted enough fat from the dried membranes and inside surfaces of peregrine falcon eggs, *Falco peregrinus*, collected in 1948 to quantify DDE, and it seems likely that small pieces (1 cm^2) of skin from museum specimens of birds or mammals would contain at least as much fat. On the second point, samples of renal, omental, subcutaneous, and scrotal fat from steers were not significantly different in the amounts of DDT that they contained (BLUNT & SAUNDERS 1978). A definitive answer will require direct experimentation, but the evidence presented here suggests a valid basis for such testing.

The described technique broadens our capabilities for evaluating the role of organochlorine pesticides in deaths of bats. Because one species of endangered bat, the gray bat, and another agriculturally important bat, the Mexican free-tailed bat, have both been shown to have serious organochlorine pesticide contamination problems at certain localities (CLARK et al. 1978b; CLARK et al. 1980; GELUSO et al. 1976), the ability to evaluate the impact of these chemicals is important.

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