Organochlorine Residues in Females and Nursing Young of the Big Brown Bat (*Eptesicus Fuscus*)

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A previous study described placental transfer of PCB (Aroclor® 1260) and DDE residues in big brown bats (*Eptesicus fuscus fuscus*) and implicated PCB as the cause of 5 of 51 bats being born dead (CLARK and LAMONT in manuscript). Present data, taken from a second population, allow examination of the subsequent transfer of residues that occurs through milk during lactation and nursing.

MATERIALS AND METHODS

On 20 June 1973, 18 big brown bats were captured in the attic of a house at 14921 Dufief Mill Road in Gaithersburg, Montgomery County, Maryland. Six females had seven young associated with them and the remaining five females were recently parturient without young. All 18 were frozen on the day of collection.

Bats were thawed and weighed before dissection. In dissection, wings, feet, and skin were removed first. The head was severed at the base of the skull, and the gastrointestinal tract was removed. The brain was removed after clipping away the top of the cranium with iris scissors. The major masses of head musculature were added to the remaining portion of the body, which was analyzed as "carcass." Stomachs of four young contained milk, which was pooled for chemical analysis. Similarly, ground insects from stomachs of three adults were pooled for analysis as a single sample. Carcasses and brains were placed individually into preweighed clean glass jars, weighed, and refrozen until just before being ground.

The occlusal tip width of the upper left canine of adult bats was measured using a 30X dissecting microscope and ocular micrometer. This measurement served as an indicator of age (CHRISTIAN 1956).

All samples were analyzed at the Patuxent Wildlife Research Center, Laurel, Md. Personnel immunized against rabies performed the initial preparation of the tissue under a bacteriological hood

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in an isolated area of the laboratory. After extraction, no special precautions were required. The material was thawed, then ground with anhydrous sodium sulfate to remove moisture. The resultant mixture was transferred to a paper extraction thimble and extracted with hexane on a Soxhlet apparatus for approximately 7 hours. The extract was cleaned up on a Florisil column. Pesticides and PCB's were separated into three fractions on a Silicar column and analyzed by electron capture chromatography on a 4% SE-30/6% F-1 column. The lower limit of sensitivity was 0.10 parts per million (ppm). Samples were analyzed for p,p'-DDE, p,p'-DDD, p,p'-DDT, dieldrin, heptachlor epoxide, mirex, oxychlordane, *cis*-chlordane and/or *trans*-nonachlor, *cis*-nonachlor, hexachlorobenzene (HCB), toxaphene, and PCB. PCB's were quantified using the standard whose peak pattern most closely resembled the pattern in the analytical sample. Standards included Aroclor 1248, 1254, and 1260. Average percentage recoveries from spiked mallard duck tissues ranged from 96 to 110, except for HCB which was 69%. Residue data were not adjusted on the basis of these recoveries. Residues in 12% of the samples were unequivocally confirmed on a combined gas chromatograph-mass spectrometer. A more detailed description of the analytical procedure is presented by CROMARTIE et al. (in press). The letters "ppm" indicate parts per million by weight of fresh (or "wet") sample.

Because the residue data were positively skewed, they were log transformed prior to all statistical testing. Also, geometric means are given for residue data rather than arithmetic means. Arithmetic means (where used) are accompanied by their standard errors. Significance levels: * = $0.05>P>0.01$; ** = $0.01>P>0.001$; *** = $P<0.001$. Regression lines were fitted by the least squares method.

RESULTS

Summary of residues. Levels of the principal organochlorines, PCB (Aroclor 1260) and DDE, found in the 18 bat carcasses are summarized in Table 1. The young bats contained total amounts (micrograms) of chemical that did not differ significantly from amounts in their parents. (Tests were made for the five females with single young, using a t-test for paired data). However, young bats weighed much less than their parents, and the concentrations of chemicals, expressed as ppm, were significantly higher in young. (DDE, $t = 5.100$**; and possibly PCB, $t = 2.404$, $0.1>P>0.05$).

The micrograms of PCB and DDE in the six females with young did not differ significantly from the amounts in the five without young.

Four other chemicals were found in some of the adult bats, in amounts less than 0.25 ppm. These were: DDT in four bats; DDD in

TABLE 1

PCB and DDE in carcasses of 18 big brown bats collected in Gaithersburg, Maryland. Both chemicals were found in all 18 bats.

Chemical	Residue Level Parts per Million	Residue Level Total Micrograms
	Adult Females without Young (N = 5)	
PCB (Aroclor 1260)		
Geometric mean	0.71	6.66
95% conf. int.	0.39-1.29	3.97-11.20
Range	0.5-1.2	4.7-11
DDE		
Geometric mean	0.67	6.30
95% conf. int.	0.26-1.74	2.52-15.82
Range	0.25-1.5	2.4-12.8
	Adult Females with Young (N = 6)	
PCB (Aroclor 1260)		
Geometric mean	0.68	6.31
95% conf. int.	0.28-1.64	2.73-14.59
Range	0.24-2.5	2.2-22
DDE		
Geometric mean	0.45	4.13
95% conf. int.	0.20-1.01	1.95-8.73
Range	0.19-1.1	1.9-9.4
	All Adult Females (N = 11)	
PCB (Aroclor 1260)		
Geometric mean	0.70	6.47
95% conf. int.	0.44-1.08	4.26-9.84
Range	0.24-2.5	2.2-22
DDE		
Geometric mean	0.54	5.01
95% conf. int.	0.32-0.90	3.08-8.14
Range	0.19-1.5	1.9-12.8
	Young (N = 7)	
PCB (Aroclor 1260)		
Geometric mean	1.43	3.57
95% conf. int.	0.87-2.35	2.22-5.74
Range	0.5-2.4	1.4-7.4
DDE		
Geometric mean	1.85	4.55
95% conf. int	1.44-2.36	3.68-5.62
Range	1.2-2.5	3.6-6.3

three; oxychlordane in three; and dieldrin in two. Four other chemicals also occurred in some of the young bats, in amounts less than 0.40 ppm. These were: DDT in six bats; oxychlordane in six; dieldrin in six; and heptachlor epoxide in one.

The pooled milk sample contained 2.3 ppm of DDE, 0.58 ppm of DDT, and 8.8 ppm of PCB (Aroclor 1254). The pooled sample of ground insects contained 13 ppm of PCB (Aroclor 1248).

Of the 18 brains, only those of three young contained detectable residues. One had 8.2 ppm of DDE, another 4.8 ppm of PCB (Aroclor 1260), and the third 11 ppm of PCB (Aroclor 1248).

Body condition and residues in brains. In free-tailed bats (*Tadarida brasiliensis*) at Bracken Cave in Texas, residues of DDE in brains increased as body condition (expressed as an index calculated as weight in g/forearm length in mm X 100) declined (CLARK et al. 1975). A similar relationship is suggested in the present sample where body condition indexes of the three young with detectable levels of residues in the brain were 19.4, 20.0, and 24.3; these were the lowest, next to lowest, and fifth from lowest condition indexes among all 18 bats. The significance of these levels of chemicals in the brain cannot be evaluated until lethal brain levels have been determined experimentally.

Residues compared with age of female. Younger females contained higher levels of PCB and DDE than did older ones. However, amounts appeared to begin rising again with age, as shown by increasing residues in females with greater tip widths. Increases appeared to begin at tip width near 0.6 mm (Fig. 1).

Figure 1 illustrates the similarity in residue levels between females with young and those without. Greater residues would have been expected among females without young if they were reproductively more advanced and had weaned their young. Examination suggested that this was not true, however. One female that lacked young had a greatly enlarged uterus (each horn 10 mm across) indicating recent parturition. Also, her nipples were furred and did not appear to have been nursed. Perhaps her young had been born dead. Two other females without young showed enlarged uteri (similar to those of some females with young) and could have still been nursing young at the time of capture.

Residues and growth of young. Forearms of the seven nursing young ranged from 17.6 to 31.1 mm (mean = 25.5±1.9) suggesting ages of perhaps 1 to 13 days (KUNZ 1974). The transfer of residues from parent to young during this interval is shown in Figure 2. (The regressions, r = 0.953* for PCB and 0.940* for DDE, were computed using only the five single young because they were strictly comparable. However, inclusion of the other two young produced r values with the same significance level.) The figure suggests that DDE was transferred more readily than PCB during lactation, and that more total residue was transferred by

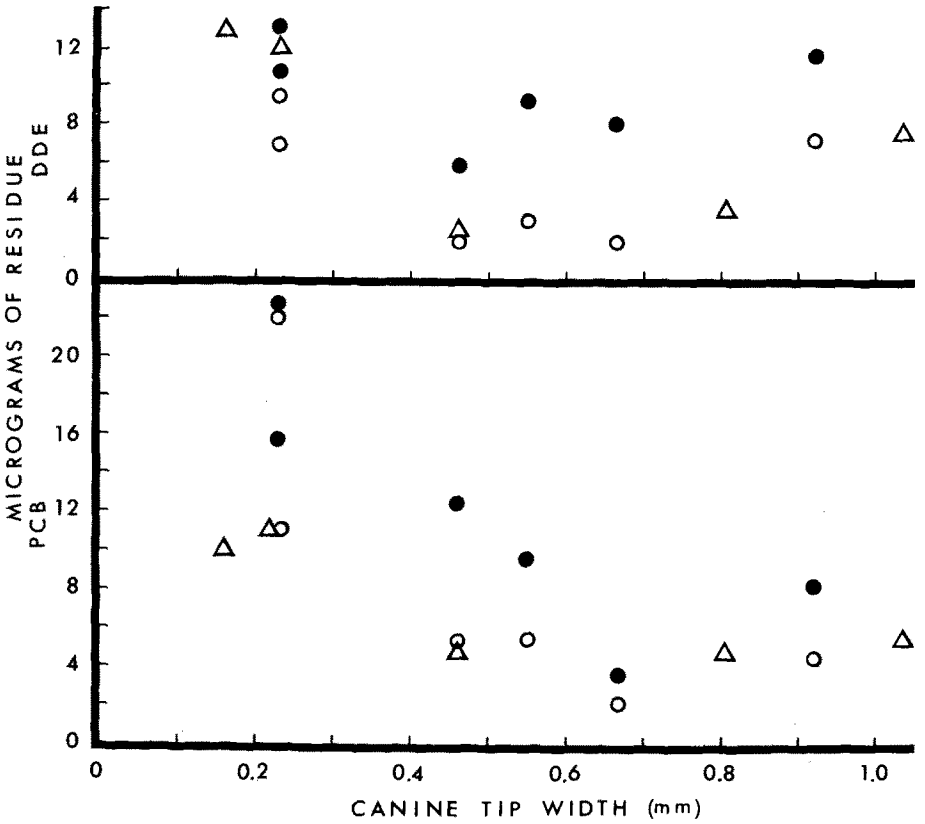

Figure 1. Residues of PCB and DDE versus canine tip width for 11 adult female big brown bats. Solid circles indicate total residues (female plus young) for six females with young (average residues in young were used for the single female with two young); open circles indicate residues for the same six females alone; and triangles represent five females that lacked young.

lactation than through the placenta. This extends the results of our earlier study of *E. fuscus*, which showed the transfer of chemicals from parent to young through the placenta and excluded transfer by nursing. In that study, two groups of neonates (which were analyzed whole rather than as carcasses) contained 16.8 and 31.8% as much PCB as their parents but only about 9% as much DDE.

DISCUSSION

Five of 51 neonate *E. fuscus* from a Laurel, Maryland, population are thought to have been born dead because of residues of PCB that were transferred across the placenta (CLARK and LAMONT

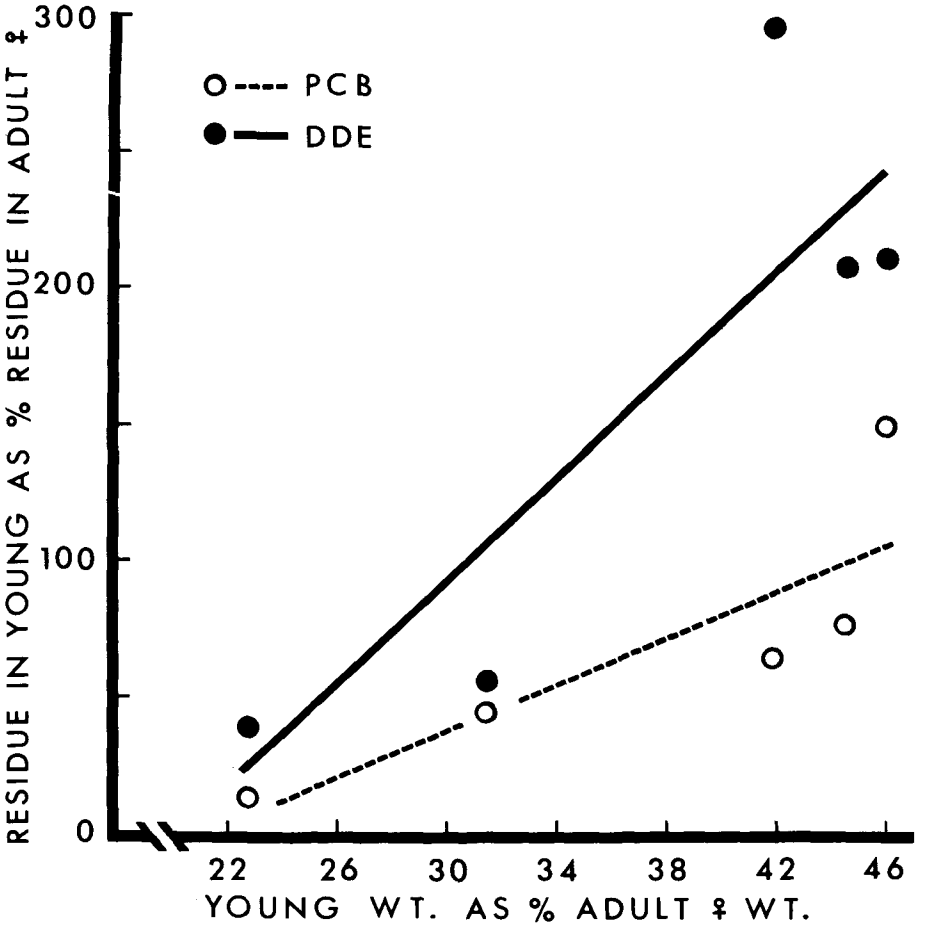

Figure 2. Comparison of residues in female parent and young in relation to size of young. Total residues (micrograms) in young are expressed as a percentage of the total residues in the parent and plotted against the weight of the young expressed as a percentage of the weight of the parent.

in manuscript). Data from the present study show that even greater amounts of PCB may be transferred to young by lactation and nursing. Thus the potential exists for additional deaths of young due to PCB in milk.

In our earlier study, residues of PCB in 27 adult females (plus litters) declined linearly with increasing age, from 28.3 micrograms for the youngest bats to 8.5 micrograms for the oldest (CLARK and LAMONT in manuscript). In the present series (Fig. 1), PCB residues declined until approximately 4 micrograms remained,

and appeared to begin increasing thereafter. It may be that the residues in bats in the earlier study declined continuously because initial amounts were higher and chemical uptake did not compensate for loss within the age span sampled. Free-tailed bats sampled from Bracken Cave, Texas, clearly showed the pattern of residue loss and gain that is suggested by the big brown bats of the present study (CLARK et al. 1975). The data for free-tailed bats were numerous and the pattern was clear. Not clear are the kinetics of residue uptake, storage, and loss that result in this pattern. In any event, there do not appear to be large increases in residues associated with increasing age of the female bats so far sampled. Males remain to be studied.

Residues in 11 milk samples from stomachs of young free-tailed bats averaged 2.7 ppm of DDE but had no detectable amount of PCB (CLARK et al. 1975). The single pooled sample of milk from the present study contained 2.3 ppm of DDE but also 8.8 ppm of PCB. Ground insects from the stomach of an adult free-tailed bat contained only 0.3 ppm of DDE, whereas the pooled sample of insects from three big brown bats contained 13 ppm of PCB and no detectable DDE. It is evident that PCB's are abundant pollutants in local big brown bats but are of no apparent consequence to the population of free-tailed bats of Bracken Cave, Texas.

SUMMARY

Carcasses and brains of 18 big brown bats from Gaithersburg, Maryland, were analyzed for residues of organochlorine insecticides and PCB's. Eleven bats were adult females, and six of these had seven nursing young associated with them.

Young bats resembled their parents in microgram amounts of PCB and DDE present in carcasses. However, concentrations of chemicals (expressed as ppm) were significantly higher in young. Brains of three young contained detectable residues of PCB and DDE.

Younger adult females contained higher levels of PCB and DDE than did older ones. However, among the oldest females, amounts appeared to begin rising again. This pattern resembles that in free-tailed bats from Bracken Cave, Texas, but differs from the continuous linear decline seen in a Laurel, Maryland population of big brown bats, in which initial levels among younger females were higher than those in the Gaithersburg population.

DDE was transferred from female to young more readily than was PCB by nursing. Five of 51 neonate big brown bats from the Laurel population were thought to have been born dead because of residues of PCB that were transferred across the placenta. Present data show that even greater amounts of PCB may be transferred to young by lactation and nursing.

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