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Uptake of Dietary PCB by Pregnant Big Brown Bats (*Eptesicus fuscus*) and Their Fetuses

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In a previous study (CLARK and LAMONT 1976), 26 pregnant big brown bats (*Eptesicus fuscus*) were captured in late May to early June and caged until their litters were born. All bats were fed clean mealworms (that is, larvae of the beetle *Tenebrio molitor* that were free of organochlorine residues as indicated by samples analyzed). Five litters included at least one dead young, and these five litters contained significantly more of the polychlorinated biphenyl (PCB), Aroclor® 1260, than did the 21 litters with only living young.

In the present study, I dosed half of a similar group of pregnant big brown bats with additional PCB to verify that this pollutant could cause stillbirths as was suggested by the earlier study.

MATERIALS AND METHODS

On 21, 22, and 23 May 1975, I captured 36 pregnant big brown bats at Montpelier Barn and two private residences in Laurel, Prince Georges County, Maryland. Upon capture, bats were taken to Patuxent Wildlife Research Center where they were caged individually in stainless steel wire mesh cages that measured 18 X 22 X 37 cm. Laboratory temperature averaged 30.8°C. Subdued sky light entered through two draped windows. Water was provided from rodent watering bottles attached to cage fronts.

Before I caged them, I anesthetized each bat with the inhalant anesthetic Metofane (Pittman-Moore, Inc., Fort Washington, Pennsylvania) and measured the occlusal tip width of the upper canine (canine tip width, CTW) with an ocular micrometer in a 30X dissecting microscope. This measurement is an indicator of relative age (CHRISTIAN 1956).

Half of the bats collected from each site on each of the three dates were randomly selected to receive mealworms reared in wheat bran containing 10 parts per million (ppm) of Aroclor 1260. Samples of these mealworms taken during the feeding period (23 May through 19 June) averaged 6.36 ppm Aroclor 1260. The other

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bats received mealworms reared in untreated wheat bran, and samples of these mealworms averaged 0.08 ppm Aroclor 1260. Bats were fed 15 mealworms/bat/day from 1 to 2 days after capture until young were born. Mean weights of mealworm samples taken for chemical analysis from PCB and control cultures did not differ significantly.

Parturition began 5 June and the last litter was born 19 June. One bat gave birth to three young; all others had two. After parturition, each female and her young were killed by freezing.

All bats were thawed and weighed prior to dissection. In dissection of the adult females, wings, feet, skin, and gastrointestinal tract were discarded. The head was severed at the base of the skull and the major masses of musculature were removed. This musculature was included with the body portion, which was analyzed as "carcass." Neonates were analyzed in their entirety except for removal of the gastrointestinal tract. This procedure was used because several births went undetected until after some nursing had occurred. Therefore, the stomach contents, if any, (as much as 0.088 g) were most appropriately placed with the carcass of the female parent for analysis. Female carcasses and neonates were placed individually into preweighed clean glass jars, weighed, and refrozen until just before being ground.

I collected guano from the catch pans of all cages on 2 and 6 June. Approximately 8.5 g (after desiccation with calcium carbonate at room temperature) was obtained for chemical analysis from both control and dosed bats.

Preparatory for residue analysis, all samples were ground with anhydrous sodium sulfate. The dried mixture was extracted with hexane in a paper extraction thimble on a Soxhlet apparatus for approximately 7 hr. The extracts were blown dry, frozen, and shipped to WARF Institute, Inc. of Madison, Wisconsin for chemical quantification of PCB. Percent lipid was determined from the weight of the dry extract. At WARF, extracts were dissolved with petroleum ether. A 5 to 15 ml aliquot was put through previously standardized Florisil. The resulting solutions were concentrated on a steam bath and made to 25 ml with iso octane. Ten µl or less was injected into a Hewlett-Packard 5713 gas chromatograph equipped with an electron capture detector. The column was 1.5% SP-2250/1.95% SP-2401 on 100/120 supelcoport. Column, detector, and injector temperatures were 210°, 300°, and 250°C. Flow rate of nitrogen was 60 ml/min.

Average percentage recovery for Aroclor 1260 from spiked samples of corn oil was 102%. Residue data were not adjusted for recovery. The level of sensitivity was 0.05 ppm. Confirmation consisted of analyzing 18 samples in duplicate. Results are given as ppm of fresh (or "wet") weight unless stated otherwise. Geometric means are given for residues because of skewness in the data. Arithmetic means are given with standard errors; geometric means are given with 95% confidence intervals (CI). Significance levels: * = $0.05>P>0.01$; ** = $0.01>P>0.001$; *** = $P<0.001$.

RESULTS

Uptake of PCB. PCB increased in dosed bats and their litters through parturition (Table 1). The regression for these increases between the times the first and last bats gave birth are shown in Figure 1. PCB in control bats did not change during this time (Fig. 1).

TABLE 1

Comparisons of means for control and PCB-dosed big brown bats. Means are accompanied by 95% confidence limits or standard errors.

	Control Bats N = 18	PCB-dosed Bats N = 18	Statistical Significance
PCB in parent females (ppm)	2.23 1.94-2.57	20.34 18.33-22.58	t = 26.55***
PCB in liters (ppm)	0.47 0.38-0.59	4.38 3.63-5.28	t = 16.57***
Frequency of litters with dead young	3/18 16.7%	1/18 5.6%	χ^2 = 1.12NS
Litter weight			
Absolute (g)	5.37±0.21	5.38±0.13	t = 0.06NS
As % of female weight	35.35±1.10	34.80±0.93	t = 0.39NS
Adult female weight (g)			
At capture	20.47±0.54	21.24±0.52	t = 1.02NS
After birth	15.17±0.31	15.54±0.31	t = 0.85NS
% decrease	25.56±1.09	26.51±1.26	t = 0.56NS
Parturition date (June 1975)	13.78±0.86	12.89±0.63	t = 0.83NS
Days in captivity	22.83±0.82	22.00±0.62	t = 0.81NS
Tooth wear (% change)	133.37±28.80	97.51±18.66	t = 1.04NS
% fat			
Litters	1.77±0.06	1.76±0.04	t = 0.16NS
Adult female carcasses	4.73±0.34	5.18±0.48	t = 0.78NS

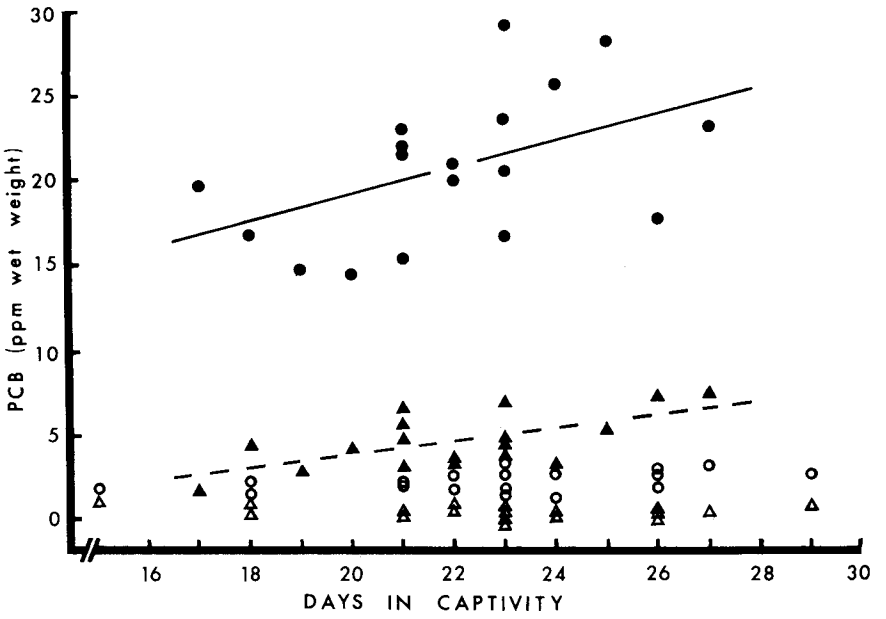

FIGURE 1. Accumulation of PCB (Aroclor 1260) in female big brown bats and their litters during the period of parturition. The relationships for PCB-dosed females (solid circles) and their litters (solid triangles) are Y = 3.280 + 0.795X, r = 0.480* and Y = -4.077 + 0.397X, r = 0.637**. Open circles represent control females; open triangles are control litters.

PCB in litters averaged 13.3% of PCB in carcasses of parent females for both dosed and control bats. Apparently, different dietary intake did not affect the amount that crossed the placenta.

I suspected that total micrograms of PCB in litters was related to amounts in their female parents. Separate correlations for controls (r = 0.193) and dosed bats (r = -0.363) were not significant; however, the relationship for the combined data was highly significant (r = 0.799***). This correlation was also found in the earlier study that did not involve dosing (CLARK and LAMONT 1976).

The accumulation of PCB is described in a general way by a comparison of residues in the diet with residues in the guano. First, the PCB for diets was converted to ppm of dry weight. To do this, a sample of mealworms was dried at 38°C in a drying oven for 2 days. It contained 71.8% water. With this value, I estimated that the control diet contained 0.28 ppm and the PCB diet 22.5 ppm of PCB by dry weight. Comparable data for the guano samples (collected during approximately the second week of dosage) are 0.17 ppm and 2.12 ppm. Apparently, accumulation was greater

among dosed bats. This picture is incomplete because some residue was probably lost with urine.

Possible effects of PCB. The added PCB caused no additional stillbirths (Table 1). Among 18 dosed females, only the triplet litter contained a single dead young. Among 18 control females, one litter included one dead young and both young were dead in two other litters. Ten additional comparisons involving means of litter weight, adult female weight, parturition date, days in captivity, tooth wear, and percentage fat also failed to show any effect of the PCB (Table 1).

Litter weight (as % of female weight) was not related to ppm PCB in the litter ($r = -0.210$ for controls, $r = 0.115$ for dosed bats, $r = -0.035$ for combined data). Litter weights were also independent of days in captivity (r values = 0.111, 0.000, 0.077). Weight of the female parent was the only factor found that appears to have influenced litter weight (absolute weights recorded at parturition). This relationship was significant for controls ($r = 0.630^{**}$) and for combined data ($r = 0.465^{**}$), but not for dosed litters ($r = 0.247$).

Weights of parent females (at parturition), while not affected by PCB, did show a slight but significant decrease that was correlated with days in captivity for the combined data ($r = -0.344^{*}$, controls $r = -0.317$, dosed bats $r = -0.356$). The food ration may not have been sufficient for maintenance.

Parturition date was related to weight at capture; heavier bats gave birth sooner (combined data $r = -0.665^{***}$, controls $r = -0.578^{*}$, dosed $r = -0.786^{***}$). Neither parturition date nor weight at capture were related to age (as indicated by CTW).

Cause of stillbirths. If elevated PCB levels do not cause stillbirths, what, then, produced the significant association between elevated PCB residues and stillbirths found earlier (CLARK and LAMONT 1976)? Apparently, it resulted because both high PCB levels and stillbirths occur more often, albeit independently, in younger parent female bats. The earlier study showed a relationship between CTW and total micrograms of Aroclor 1260 in the parent female plus her litter; younger females contained more residue than older ones. This correlation was significant even though the CTWs were measured after parturition, and therefore, after considerable cage wear. The toothwear that occurred in captivity was described elsewhere (CLARK 1976). Present data, based on CTWs measured at capture, show a similar decline in PCB (among control bats) with increasing age (CTW) of the parent female (Fig. 2, $r = -0.635^{**}$). The association between low CTW and stillbirths was indicated when I compared CTW for females with dead young (mean = 0.2530±0.0094 mm, N = 4) and females with only live young (mean = 0.4586±0.0353 mm, N = 32, $t = 5.631^{***}$; variances differed and were not pooled as per SNEDECOR and COCHRAN 1967). This association was not evident in the earlier study, probably because of the extensive tooth wear.

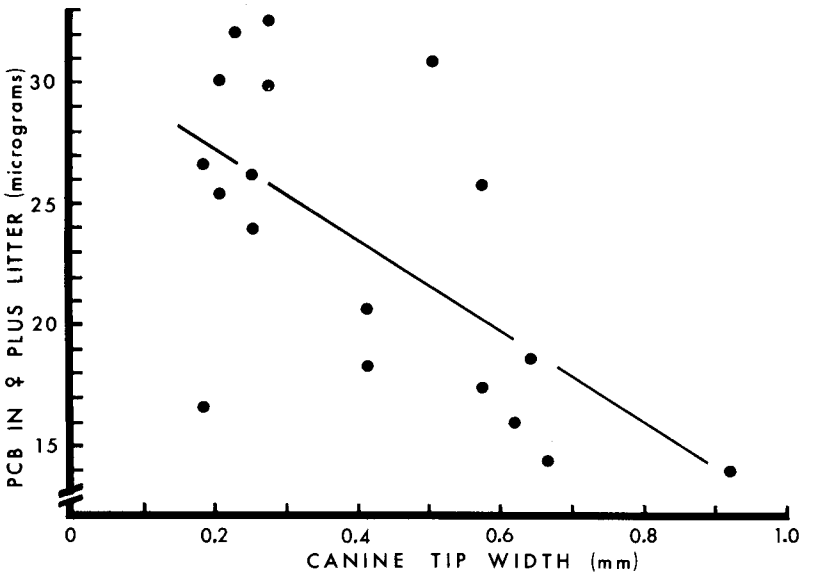

FIGURE 2. Decline of PCB (Aroclor 1260) with increasing age (as indicated by CTW) of control female big brown bats. Y = 31.0 - 18.8X, r = -0.635**.

In the present study, these associations did not cause control litters with dead to contain more PCB than other control litters, perhaps because the sample of litters with dead was very small. Nevertheless, the basis for such a difference was present because the three control parent females with dead young contained more PCB (mean = 2.95 ppm, CI = 1.96-4.44 ppm) than did the 15 other females (mean = 2.11 ppm, CI = 1.82-2.46 ppm) ($t = 2.021$, $0.1>P>0.05$).

Comparison of results from different analytical laboratories. Fourteen samples analyzed by WARF Inc. were also analyzed by the Chemistry Section at the Patuxent Wildlife Research Center to determine whether the data given above could be compared directly with those of the earlier study (CLARK and LAMONT 1976). Significant differences were found and made rigorous comparisons impossible. Results for adult control bats reported by WARF Inc. averaged 1.8 times more Aroclor 1260 than those from Patuxent, whereas results for control litters reported by Patuxent averaged 4.8 times more Aroclor 1260 than those from WARF Inc. After adjustment for these differences, we found that levels reported here for controls are similar to those reported in CLARK and LAMONT (1976).

DISCUSSION

Dietary Aroclor 1254, at lower dosages than those of the present study, prevented reproduction or caused a high incidence of dead young in ranch mink (*Mustela vison*) (RINGER et al. 1972, PLATONOW and KARSTAD 1973). PLATONOW and KARSTAD (1973) reported that only one of 12 mink receiving a diet with 0.64 ppm of Aroclor 1254 reproduced, and her three kits all died the day after birth. Muscle tissue of these females averaged less than 1 ppm. Big brown bats must be much less sensitive to PCB than mink because bat carcasses averaged 20.34 ppm of Aroclor 1260 without reproductive effects.

Blubber of six female California sea lions (*Zalophus californianus*) that produced premature pups contained an average 112.4 ppm of Aroclor 1254 along with high residues of DDT (chiefly DDE) (DELONG et al. 1973). When expressed on a lipid weight basis, residues of Aroclor 1260 in the dosed big brown bats averaged 422.7 ppm (95% CI = 348.2-513.1 ppm). If the PCB should prove to be responsible for premature births in sea lions, I would again conclude that the big brown bat is less sensitive.

Although earlier results (CLARK and LAMONT 1976) seemed to indicate that PCB residues caused stillbirths in big brown bats, this is not the case. Elevated PCB residues and dead young in bat litters were actually independent of each other but were both associated with a third factor--age of the female parent bat. Stillborn young occur naturally in the colonies studied (9 of 62 litters born in the laboratory, or 14.5%, included at least one dead young) and must be the result of unknown reproductive difficulties experienced by young female bats. Statistics for the 1972 human population of the United States show that 35.3% of all fetal deaths were first births and that the percentage declined with increasing birth order (U.S. DEPT. OF HEALTH, EDUCATION AND WELFARE 1976). This pattern among big brown bats, rather than PCB residues, must be responsible for the stillbirths among these bats.

SUMMARY

In a previous study (CLARK and LAMONT 1976), 26 pregnant big brown bats were captured, caged, and fed uncontaminated mealworms until their litters were born. Immediately after parturition, female bats and litters were frozen. Five litters included at least one dead young, and these five litters contained significantly more of the PCB, Aroclor 1260, than did the 21 litters with only living young.

The present study attempted to verify that Aroclor 1260 could cause stillbirths. I fed 18 of 36 pregnant big brown bats mealworms containing 6.36 ppm of Aroclor 1260 prior to birth of their litters. Both carcasses and litters of dosed females contained approximately 10 times more PCB than their respective controls, but no additional stillbirths resulted. Three of 18 control litters included dead young, whereas the comparable ratio among litters from dosed females

was one of 18. Additional comparisons involving means of litter weight, adult female weight, parturition date, days in captivity, tooth wear, and percentage fat also failed to show any effect of the PCB.

The association found earlier between PCB and dead young (CLARK and LAMONT 1976) was not one of cause and effect. In both studies, bats that had not been dosed showed greater PCB residues among younger females. Among control bats in the present series, females that produced dead young were significantly younger (that is, showed significantly less tooth wear) than other females. In sum, whereas dead young seemed to have been caused by greater residues, these two factors were actually independent of each other but associated with a third factor--age of the female parent bat.

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