### AN ABSTRACT OF THE THESIS OF

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en Abstract approved:

Adult big brown bats' (Eptesicus fuscus) were collected from the attic of a two-story brick house in Pittsburg, Kansas, during the evenings of 30 and 31 March 1982. Non-lethal organochlorine residue concentrations were found in the brain tissues. Pesticides identified included p,p'-DDT, heptachlor, DDE, and dieldrin. These results appear to reflect the presence of pesticides, in the brain tissue of the big brown bat, from those being applied currently for pest control. They may constitute a hazard to this colony and other colonies of bats in the area. Observations by Dr. Horace Hays and his students at Pittsburg State University have shown that both the big brown bat and the endangered gray bat populations are declining in the Pittsburg area.

# ORGANOCHLORINE RESIDUES IN THE BIG BROWN BAT, EPTESICUS FUSCUS

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> by Thomas M. Klein May 1984

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#### INTRODUCTION

Bats are the only true flying mammals. Due to this unique characteristic, and to their size and shape, bats have become known as "flying mice". Only in the last 50 years has man learned much about the natural history of bats. Their nocturnal habits, affinity for eerie places like caves, and silent, darting flight have made them subjects of a great deal of folklore, superstition, and misconceptions (LaVall and LaVall, 1980). Unfortunately, a bat's appearance does not improve its maligned reputation. Evolutionary adaptations for flying, feeding, and hearing have provided bats with huge ears, grotesque facial features, and shrill voices. One of the major misconceptions about bats is their reputation for carrying rabies. Bats can and do carry rabies; however, compared to the skunk and fox, the frequency of rabies in bats is low (Tipton and Tipton, 1980).

Active at a time when most people prefer to be indoors, and able to function when and where man's most important sense, sight, is denied him, it is no wonder that bats seem supernatural. In actuality, bats are superbly adapted creatures that have evolved to exploit resources such as night-flying insects and dark caverns that are usually unavailable to diurnal and sight-dependent animals (LaVall and LaVall, 1980).

Bats belong to the order Chiroptera. The order Chiroptera is divided into two suborders: the Megachiroptera, and the Microchiroptera, which includes most of the families of bats. Included in the suborder Microchiroptera are the insectivorous bats. These bats obtain most of their insect food while in flight. They are relatively small in size and comprise the majority of the bat populations (Walker, 1975). Of the 16 living families of bats, three occur in the United States. The Phyllostomatidae (leaf nosed bats), Vespertillionidae (mostly hibernating species), and Molossidae (free-tailed bats) occur in varying concentrations across the North American continent (Barbour and Davis, 1969).

The big brown bat, <u>Eptesicus fuscus</u>, is a vespertillionid bat. The habitat of <u>E</u>. <u>fuscus</u> is closely related to man and the species is familiar to more people in the United States than any other species of bat. Favored roosts are in seldom-used buildings (Barbour and Davis, 1969).

This genus (Eptesicus), of about 30 species, is nearly world-wide in distribution. They are not strictly cave bats but often winter in buildings and storm sewers. There are usually movements of varying distances from winter to summer roosts (Walker, 1975). Factors such as birth place, weather, and acceptable hibernation sites all play a part in the site selection for winter hibernation. Distances travelled range from a few miles to 500 miles (Geluso et al., 1976). Females usually form maternity colonies. Breeding takes place in the fall with one or two young born from April to July (Walker, 1975).

<u>Eptesicus fuscus</u>, commonly known as the house bat or big brown bat, is distributed from Canada to South America. <u>Eptesicus</u> is from the Greek word, ptetikos, meaning "able to fly", or petomai, meaning "a house flier". The second part, <u>fuscus</u>, is the Latin word for "brown" and describes the color (Schwartz and Schwartz, 1981).

The body form and structure of the big brown bat can be confused with the mouse-eared bats. Characteristics for identification include general coloration, size, attachment of wing membrane, footsize, and

calcar (Table 1) (Walker, 1975).

Bats are an important part of the natural system. They help control nocturnal insects, some of which are agricultural pests or annoying to man. Many forms of cave life (i.e., salamanders, insects, etc.) depend on nutrients brought in by bats and released from their guano (feces). Bats have also contributed much to man's knowledge through studies of bat echolocation abilities and certain aspects of their physiology. Guano has become an important constituent in some commercial fertilizers due to its high nutritional value (LaVall and LaVall, 1980).

Bat populations have been declining at an alarming rate in recent years (USDI, 1981). Some factors contributing to this decline are destruction of habitat, pesticides, and physical disturbance. Loss of foraging habitat has resulted from reservoir construction, watershed development, forest conversion, urbanization, and cave commercialization. Lethal levels of pesticides have been found in dead bats in several studies (LaVall and LaVall, 1980).

The first bat deaths directly attributed to chemical contaminants in the environment occurred in 1949-1950 when DDT was applied to bats or their roosts for the purpose of extermination. During the two year period, it was first suggested that pesticides might be a cause of observed population declines in bats (USDI, 1981).

Sixteen mass die-offs of bats due to unknown causes have been described in published accounts. Since 1954, many studies have demonstrated that application of organochlorine pesticides to bats or bat roosts are effective means of bat eradication (USDI, 1981).

The most common organochlorine found in bats is DDE, the longlived

Parameter	Range
Total length	95-127 mm
Tail length	34-50 mm
Hind foot length	9-12 mm
Ear length	15-19 mm
Skull length	19 mm
Skull width	12 mm
Weight	14-21 grams

Table 1. Body measurements commonly used in the identification of the big brown bat, Eptesicus fuscus (Walker, 1975).

breakdown product of DDT (USDI, 1981). Polychlorinated biphenyls (PCB's) are industrial plasticizers that are often found in abundance, especially in urban and industrial areas. Less common but frequently recovered residues include dieldrin, DDT, DDD, heptachlor epoxide, oxychlordane, fractions of chlordane such as trans-nonachlor, cis-nonachlor, and cis-chlordane. Rare compounds include endrin, toxaphene, hexachlorobenzene (HCB), lindane, and mirex (USDI, 1981).

All of these compounds are highly fat soluble and are quickly taken up by adipose tissue after they enter the bloodstream. When fat reserves are large, they may absorb residues rapidly enough to prevent death if the rate of intake is not too high. When fat reserves are metabolized and decreased in total amount, residues concentrate in remaining fatty tissue. The principle site of toxic action is the brain. Fat composition of the brain remains nearly constant regardless of the level of fat reserves in the rest of the animal. Thus, organochlorines may become lethal to the organism when fat reserves are metabolized, since this causes residues to concentrate in the brain tissues. The affinity for fat also causes heavy excretion of residues in the milk of lactating mammals.

Even though these organochlorine chemicals share these features, they differ widely in toxicity, which in turn varies with the species or group of species being considered. There are also differences in how these chemicals are metabolized. For example, PCB's can cause death by either the typical neurotoxic mode or by a hemorrhagic mode depending on the dosage rate (Stickel, 1975).

Of the 16 known mass bat mortalities, 11 occurred in Texas, New Mexico (or Mexico within 370 miles of the United States border), one in Missouri and four in southwestern Victoria, Australia. Nine die-offs involved the Mexican free-tailed bat (<u>Tadarida brasiliensis</u>). In summary, these die-offs have involved bats of seven species, both sexes, and various ages, and they have occurred at different times of the year.

Laboratory experiments and examinations of tissues from bats sampled in the wild began in the early 1970's after experiments conducted at the University of Kentucky showed bats to be extraordinarily sensitive to pesticides (Luckens and Davis, 1964).

Young Mexican free-tailed bats collected from New Mexico, Texas, Colorado, Arizona, and California revealed the presence of DDT, DDE, DDD, Dieldrin, endrin, toxaphene, and Arocolor (Geluso et al., 1981). Lethal dose levels for many types of pesticides and their correlation to different species have been determined (Clark, 1981). Also studied were the effects of organochlorines on female little brown bats to discover their lethal dosage and to compare this with various avian life forms studied earlier. Of particular interest has been the effect of organochlorines on the nationally endangered gray bat (Clark et al.,

1980). Studies of effects of organochlorines on specific species, sexes, stress situations, and transfer to young have continued for the past few decades (Booth, 1965; Clark and Lamont, 1976; Clark and Krynitsky, 1978; Clark, 1979; Cockrum, 1970; Davis, 1966; Davis, 1967; Esher et al., 1980; Geluso et al., 1976; Geluso et al., 1981; Lucken and Davis, 1965; Luckens, 1973; Stickel, 1973; Tuttle, 1979).

Currently, populations of both the gray bat and the big brown bat in southeast Kansas have been declining since their discovery (personal communication, Dr. H. Hays). Due to past and present industrialization of the area, results of a study on these species for organochlorine levels could prove helpful not only in this years management but also for future management and protection of other wildlife in the area.

#### MATERIALS AND METHODS

Fifteen specimens of <u>Eptesicus fuscus</u> were collected from the attic of a two-story brick apartment house located at 1408 South Olive Street in Pittsburg, Kansas, on the evenings of 30 and 31 March 1982, between 1900 and 2300 hours. Specimens were mist netted as they exited the attic walls of the building through openings in the eaves of the roof.

All bats were immediately killed, wrapped in aluminum foil and placed in a cooler kept at an average temperature of 0°C. Sex and relative age were determined after specimens were thawed in the laboratory before analysis. Due to the time of collection, all specimens were considered adult, since young-of-the-year had not been born yet, and the yearlings were sufficiently developed enough to be indescernible from other adults.

The brain of each animal was removed and placed in a sealed foil packet. Both brain tissue and carcass were then returned to the freezer until further analysis. The brain of a laboratory rat was then obtained to be used as a spiked control. This sample was treated the same as experimental samples.

Analytical procedures were adapted from techniques described in the EPA Manual of Analytical Methods for the Analysis of Pesticides in Humans and Environmental Samples (EPA, 1980). All procedures and techniques were subjected to quality control measures as described in the EPA Manual for Analytical Quality Control for Pesticides (EPA, 1979).

For tissue preparation, brains were weighed and placed in a 22-23 mm ID dual tissue grinder and homogenized with 2.5 ml acetonitrile. At this time, 20 nanograms of aldrin in 0.1 ml hexane were added to the control tissue. Samples were transferred to centrifuge vials and centrifuged at 3000 rpm for 15 minutes. The supernatant was collected in a 50 ml round bottom test tube. The centrifugation procedure was repeated twice on each sample.

Twenty-five ml of 2 % aqueous sodium sulfate were added to the combined supernatants and mixed with a Vortex mixer for two minutes. The aqueous acetonitrile mixture was then extracted with one five ml and two two ml portions of hexane. These extracts were combined and concentrated to 500 microliters in a 10 ml evaporative concentrator fitted with a modified Micro-Snyder column (2-3 grains of  $Na_2SO_4$  were used as boiling chips). Acetic anhydride (0.3 ml) and pyridine (0.3 ml) were added to the concentrated solution and incubated in a water bath at  $60^\circ-65^\circ$ C for 30 minutes.

Nine ml of 2 % aqueous sodium sulfate were added to the incubated solution and extracted with two three ml portions of hexane. The extracts were combined and evaporated to 300  $\mu$ l. The remaining solution was stored in foil-lined containers at -18°C.

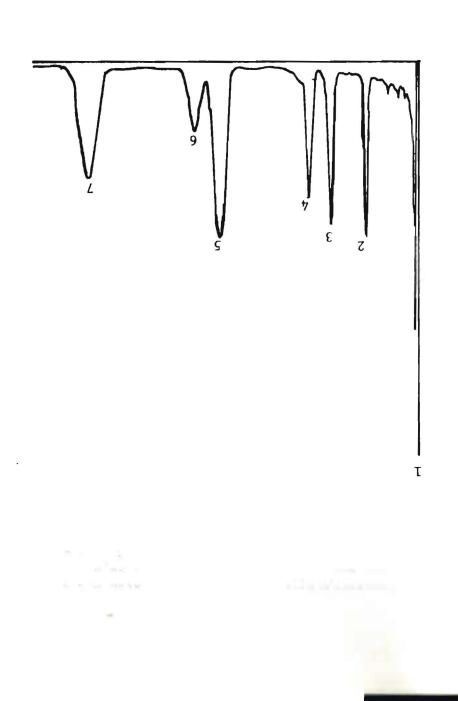
For Florisil chromatography, a size B Chromaflex column with a fritted disk was packed with 1.6 g of 60-100 mesh Florisil activated at 1200°F. Granular sodium sulfate (1.6 g) was added to the top of the column. The column was washed with 50 ml of pesticide grade hexane followed with 50 ml of pesticide grade methanol. The column was then dried in a drying oven at 130°C for at least 12 hours. After removal from the oven the column was allowed to cool to room temperature and then prewetted with 10 ml hexane before application of sample.

The 300  $\mu$ l extract was transferred to the column with a disposable glass pipet. Eluate was immediately collected in a 20 ml round bottom test tube. The foil-lined sample containers were rinsed twice with 0.25 ml hexane and the rinse transferred to the column. Fractionation was begun using a total of 12 ml hexane followed by 12 ml of 1 % methanol in hexane. The eluate was labeled fraction 1 and would contain the organochlorines heptachlor, aldrin, p,p'-DDE, o,p'-DDT, if present.

A second fraction (fraction 2) was collected by eluting the column with a second 12 ml portion of 1 % methanol in hexane. This fraction would contain dieldrin, heptachlor epoxide, endrin, beta-BHC, lindane, and p,p'-DDD, if present. Eluates from both fractions were evaporated to 500 µl and 300 µl, respectively.

Samples were analyzed for lindane, heptachlor, aldrin, DDE, dieldrin, endrin, and DDT using a Varian Aerograph Series 1400 gas chromatograph fitted with a 10 % OV-101 column and an electron capture detector. A column temperature of 200°C, detector temperature of 250°C, and injector temperature of 230°C were used with a nitrogen gas flow of approximately 34 ml/min. Results were recorded on a Fisher Recordall Series 500 recorder at a chart speed of 1.0 cm/min.

A standard mixture of organochlorine pesticides were run at varying times to check for instrument deviation (Figure 1, Table 2). A second run was performed by the same methods using a 4 % OV-101, 6 % OV-210 column with a nitrogen gas flow rate of 32 ml/min. Five microliters of each sample fraction were injected for analysis during testing on both columns (Figure 1, 2). Figure 1. Results of standard mixture chromatographed on a 10 %-OV 101 column. (1) solvent front, (2) Lindane, (3) Heptachlor, (4) Aldrin, (5) DDE and Dieldrin, (6) Endrin, (7) DDT (Not specifically identified, but believed to be p,p'-DDT). The column temperature was 200°C, detector temperature 250°C, and injector temperature 230°C. N<sub>2</sub> gas flow was 36 ml/min, and the chart speed 1.0 cm/min. A five µl sample was injected. The concentrations in the standard mixture are listed in Table 2.



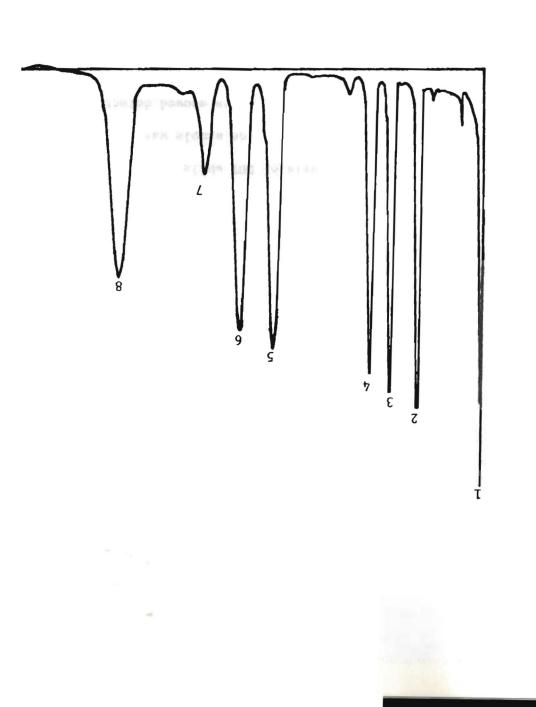
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Table 2. Concentrations of pesticides in standard mixture. Retention time was the time it took for the sample to pass through the 10 % OV-101 column using aldrin (1.00) as the basis for comparison.

	Distance from solvent front (cm)	Retention Time (min)	Concentration 50 pg/ml
Lindane	3.45	0.46	50
Heptachlor	5.95	0.79	60
Aldrin	7.55	1.00	60
Dieldrin	13.85	1.83	100
DDE	13.85	1.83	100
Endrin	15.60	2.07	100
DDT	23.30	3.09	200

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Figure 2. Results of standard mixture chromatographed on a 4 % OV-101, 6 % OV-210 column. (1) solvent front, (2) Lindane, (3) Heptachlor, (4) Aldrin, (5) DDE, (6) Dieldrin, (7) Endrin, (8) DDT (Not specifically identified but believed to be p,p'-DDT). The column temperature was 200°C, detector temperature 250°C, and injector temperature 235°C. N2 gas flow of 32 ml/min. and the chart speed 1.0 cm/min. A five µl sample was injected. The concentrations in the standard mixture are listed in Table 2.



\*

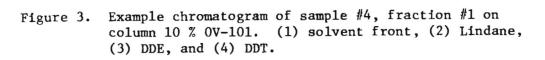
#### RESULTS

Identification and quantification of various organochlorine pesticides in brain tissues of big brown bats were the primary emphases of this study. All values for pesticides are expressed as micrograms per milliliter, which is the equivalent of parts per million. Pesticide peaks at 5.5 cm (#1), 7.1 cm (#2), 9.0 cm (#3), and 12.5 cm (#4), on both columns, were not identifiable since the reference mixture did not contain those specific compounds (Table 2). The pesticide peak at 19.0 cm (#5) was tentatively identified as o,p'-DDT from manufacturer reference charts for standard reagents. Examples of chromatograms from tissue samples are shown in figures 3, 4, 5, and 6.

The majority of pesticides identified in both fractions and columns range between 10-190 x  $10^{-3}$  ppm. Of the 11 tissue samples containing pesticides, all showed levels of DDT while only two samples showed any appreciable levels of dieldrin. One sample was found to have DDE, two samples had lindane, and three samples showed detectable levels of heptachlor (Table 3).

Three unknown peaks were shown in analysis. These peaks occurred at 5.5 cm, 9.0 cm, and 19.0 cm on both columns. The most common unknown found was at 5.5 cm. These unknowns were shown both in fractions one and two. Unknown #5 was only found with samples containing dieldrin.

On the column 4 % OV-101, 6 % OV-210, 14 samples exhibited pesticide levels ranging from 7.8-135.5 x  $10^{-3}$  ppm. Of the pesticides found, one sample showed DDT. The second column showed no relationship between unknown sample #5 and samples containing dieldrin. Six samples showed unknown #1, one with unknown #2, four with unknown #3, seven with unknown #4, and 14 with unknown #5 (Table 4).





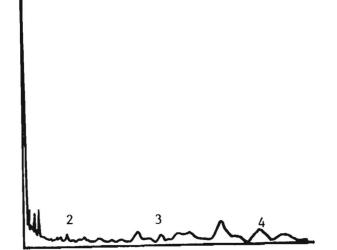


Figure 4. Example chromatogram of sample #3, fraction #2 on column 10 % OV-101. (1) solvent front, (2) DDE, and (3) DDT.

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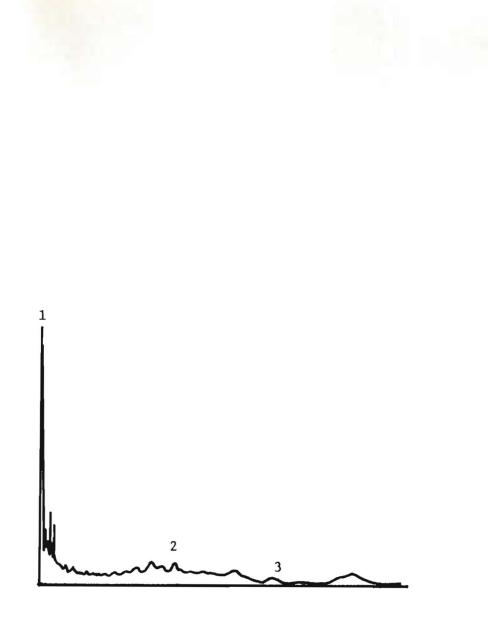
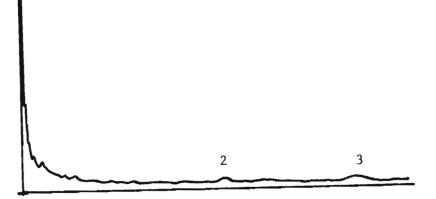
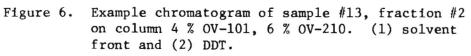




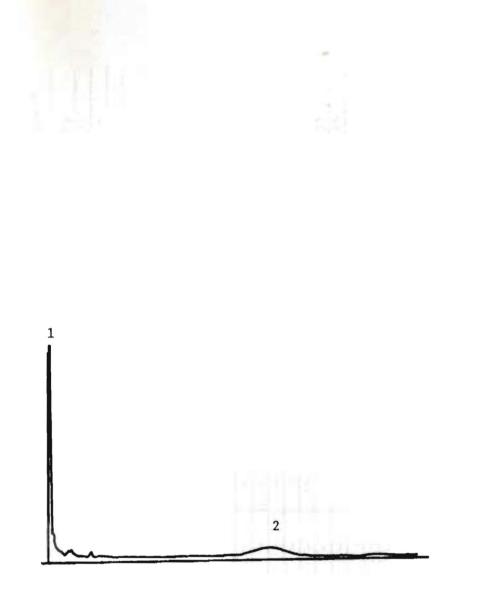
Figure 5. Example chromatogram of sample #14, fraction #1 on column 4 % OV-101, 6 % OV-210. (1) solvent (2) unknown #4, and (3) p,p'-DDT.













		Lindane	Heptachlor	Aldrin	DDE	Dieldrin	Endrin	o,p'-DDT	Unknown*
Sample	Fx1	_	144.6	_	-	_	-	133.9	#1
#1	Fx2	_	128.6	-	-	-	-	187.5	#1
Sample	Fx1	_	42.5	-	-	-	-	-	-
#2	Fx2	_	34.4	_		-	_	56.9	
Sample	Fxl	-	_	-	-	-	_	_	
#3	Fx2	_	_	-	-	_	-	-	-
Sample	Fxl	_	-	_	-	-	-	-	-
#4	Fx2	_	13.2	-	-	-	-	62.7	#1
Sample	Fxl	-	-	-	-	-	-	-	-
#5	Fx2	-	-	-		-	-	24.5	#1
Sample	Fx1	_	-	-		-	-	-	
#6	Fx2	_	-	-	-	-	-	-	-
Sample	Fx1	Trace	-	-	-	-	-	-	#1
#7	Fx2	8.9	-	-		-	_	36.8	#1
Sample	Fx1	_	-	_	-		_	40.8	#1
#8	Fx2	Trace	-	-	16.5	8.7	_	36.7	#1
Sample	Fx1	-	-	_	-	_	-	-	#3
<b>#</b> 9	Fx2	-	-	-	-	-	-	25.8	#5
Sample	Fx1	17.2	-	-		21.4	-	_	#1-#5
#10	Fx2		_		-	-	-	53.8	11-11
Sample	Fx1		-	-	-	-	_	21.3	#1
#11	Fx2	-	-	-	-	-	-	-	-
Sample	Fx1	-	-	-	-	_	-	-	-
#12	Fx2	-		-	-	-	-	-	-
Sample	Fx1	-	_	-	-	-	-	Trace	#1
#13	Fx2	-	-	-	-	-	-	_	#1
Sample	Fx1	-	-	-	_	Trace	-	22.7	-
#14	Fx2	-	-	-	-	-	-	27.3	_
Sample	Fx1	-	-	-	-	Trace	-	16.2	_
#15	Fx2	-	-	-	_	Trace	-	-	#5

Table 3. Pesticide concentrations identified on 10 % OV-101 column for fractions #1 and #2. Concentrations are in 10<sup>-3</sup> parts per million. Unknown #5 has been tentatively identified as p,p'-DDT.

\* Numbers refer to peaks found at 5.5 cm, 7.1 cm, 9.0 cm, 12.5 cm, and 19.0 cm respectively.

		Lindane	Heptachlor	Aldrin	DDE	Dieldrin	Endrin	o,p'-DDT	Unknown*
Sample	Fxl	_	_	-	_	24.8	-	-	#1-#5
#1	Fx2	-	-	-	7.8	23.1		-	#1-#5
Sample	Fx1	-	-	_	-	-	-	104.0	100 - M.S.
#2	Fx2	11.0	-	-	-	-	-	-	#5
Sample	Fx1	-	_	-	17.4	-	-	63.3	
#3	Fx2	-	-		8.7	-	-	19.0	201-40
Sample	Fx1		-	-	10.9	-	-	59.5	
#4	Fx2	-	-	-	-	-	-	31.2	#3-#5
Sample	Fx1	-	-	- 3	-	-	-	34.8	#2-#4
#5	Fx2	-	-	-	-	_	-	-	#5
Sample	Fx1	-		-	-	-	-	36.1	#4-#5
#6	Fx2	0 <del>4</del> 1	-			-	-	- 0.01	1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 - 1997 -
Sample	Fx1	-		-	-	-	-	47.0	#4-#5
#7	Fx2	-	-	-	-	-	-	-2016	#5
Sample	Fxl	-				-	-	56.0	#4-#5
#8	Fx2		-			9.2	_	-2114	#1-#3-#5
Sample	Fx1				-	135.5	-	31.5	#5
#9	Fx2			-	- 7	-	-	-	#1-#3-#5
Sample	Fx1		-		-	-	-	107.0	#1-#4-#5
#10	Fx2	-			-	-	_		#1-#5
Sample	Fx1	-			_	-	-	-	#3-#5
#11	Fx2	-		-	-	_		10.7	#4-#5
Sample	Fx1			<u> 1997 - 1997 - 19</u>	-	-	43.55	-	
#12	Fx2	-	_	_	-	_	-	10.9	#4-#5
Sample	Fxl	-	-	-	31.0	-		33.1	#5
#13	Fx2	-	-	-	-	-	-	-	#5
Sample	Fx1	-	-	-	_	-	-		-
#14	Fx2	-	-	-	-	-	-	-	#1-#5
Sample	Fxl	-	-		-	-	-	10.4	#5
#15	Fx2	_	_	_	10.4	14.4	-	_	#1-#5

Table 4. Pesticide concentrations identified on 4 % OV-101, 6 % OV-210 column for fractions #1 and #2. Concentrations are in 10<sup>-3</sup> parts per million. Unknown #5 has been tentatively identified as p,p'-DDT.

\* Numbers refer to peaks appearing at 5.5 cm, 7.1 cm, 9.0 cm, 12.5 cm, and 19.0 cm respectively.

Since this second column was more sensitive to organochlorine pesticides, more samples were found to have detectable levels of pesticides. To gauge the hazardousness of brain concentrations of pesticides, a range of 500-600 ppm was used as the level of lethality. This range was noted as lethal from studies on <u>Myotis lucifigus</u> (Clark and Stafford, 1981).

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#### DISCUSSION

The level of sensitivity of bats to organochlorine pesticides is unclear although lethal brain concentrations have been suggested to be 500-600 ppm (Clark and Stafford, 1981). Previous studies have suggested that due to seasonal variability of fat reserves in bats and abundance of insects for food, the susceptibility of bats to pesticides must also be variable (Davis, 1966; Geluso et al., 1981; Luckens, 1973). During the active months, March to November, when the food supply is abundant and fat reserves are building, susceptibility of bats to levels of organochlorine pesticides should decrease due to an increasing amount of solvent which can serve to sequester the pesticides away from nervous tissue. Juvenile bats probably have a relatively high susceptibility to pesticides since their fat reserves are low and metabolic rates high due to growth (Clark et al., 1978). Since body lipids can contain relatively high levels of pesticides during summer months, the stress period for bats concerning pesticide levels in the brain tissue would be during winter months when bats are least active. This stress period would, or could, be due to the metabolism of body fat during hibernation. As long as pesticides are contained in the body lipids there should be less than normal stress on the animal. The stress should occur when the pesticides are concentrated in the brain tissues during metabolism.

But during winter months the metabolic rate is reduced for hibernation. So more specifically, the highest stress period would be during the months of February to April when fat reserves from hibernation are low and body reserves from feeding have not been replenished.

The sampling period in March was chosen because body levels of lipids should be low, thus increasing the concentrations of pesticides in the brain tissues.

Unfortunately, samples of dead and dying bats were unobtainable because most bats that died fell inside the house walls and were out of reach. If there would have been a feasible method for obtaining some of these specimens, an analysis of the pesticide levels in the brain tissue might have shown higher levels if pesticide susceptibility actually was a contributing factor.

Levels above 100 x  $10^{-3}$  ppm were recorded. These results were substantially higher than the other sample values. The abnormally high levels were primarily during the first few runs.

In summary, results show pesticide levels in the range of 10 to  $190 \times 10^{-3}$  ppm in reasonably 'healthy' specimens. These pesticides appear to be primarily DDT residues. If these bats were healthy and still absorbing pesticides, then there is a possibility that the environmental levels of pesticides are high enough to be concerned about even though they do not seem to be lethal. Further analysis of dead and dying bats, pesticide levels in insects, and physiological analysis of pesticides metabolism are needed to better understand the effects of pesticides on bat populations and to ascertain what needs to be done to protect the species.

### SUMMARY

Previous studies with bat colonies have shown that critical illnesses result from poisonings with organochlorine pesticides. By actual contact, or through the food chain, these pesticides enter the body system and are concentrated primarily in lipid tissues. Once these lipids are metabolized, the pesticide contaminants appear to concentrate in the brain tissues. Tests for organochlorine pesticides on a colony of big brown bats in Pittsburg, Kansas, during the spring of 1982, showed low levels of p,p'-DDT, heptachlor, and DDE in the range of 10-190 x  $10^{-3}$ ppm. Even though these levels of pesticides are not lethal, continued exposure and biological magnification could increase levels of pesticides to levels which would warrant concern.



#### LITERATURE CITED

Barbour, T.W. and W.H. Davis. 1969. Bats of America. University Press of Kentucky. pp. 63-66.

Booth, E.S. 1965. A bat die off in Mexico. J. Mammal. 46(2):333-334.

Clark, D.R., Jr. 1979. Lead concentrations: Bats vs. terrestrial small mammals collected near a major highway. Environ. Sci. Technol. 13(3):338-341.

. 1981. Death in bats from DDE, DDT, or Dieldrin: diagnosis via residues in carcass fat. Bull. Environ. Contam. Toxicol. 26(3):367-374.

and A. Krynitsky. 1978. Organochlorine residues and reproduction in the Little Brown Bat, Laurel, Maryland - 1976. Pest. Monit. J. 12(3):113-116.

, T.H. Kunz and T.E. Kaiser. 1978. Insecticides applied to a nursery colony of Little Brown Bats (Myotis lucifigus): Lethal concentration in the brain tissues. J. Mammal. 59(1):84-91.

and T.G. Lamont. 1976. Organochlorine residues and reproduction in the Big Brown Bat. J. Wild. Mgt. 40(2):249-254.

, R.K. LaVall and A.J. Krynitsky. 1980. Dieldrin and Heptachlor residues in Dead Gray Bats, Franklin County, Missouri -1976 versus 1977. Pest. Monit. J. 13(4):137-140.

and C.J. Stafford. 1981. Effects of DDE and PCB (Aroclor 1260) on experimentally poisoned female Little Brown Bats (Myotis lucifigus): Lethal brain concentrations. J. Toxicol. Environ. Health 7:925-934.

- Cockrum, E.L. 1970. Insecticides and Guano Bats. Ecology. 51(5): 761-762.
- Davis, W.H. 1966. Pesticides and bats. Bat Res. News. 8(4):32.

1967. Toxicity of DDT to bats. Bat Res. News. 8(4):32.

- Environmental Protection Agency. 1979. Manual for analytical quality control for pesticides and related compounds in human and environmental samples. EPA-600/1-79-008.
- Environmental Protection Agency. 1980. Manual of analytical methods for the analysis of pesticides in humans and environmental samples. EPA-600/8-80-038.
- Esher, T.J., J.L. Wolfe and R.B. Koch. 1980. DDT and DDE inhibition of bat brain ATPase activities. Comp. Biochem. Physiol. 65(1): 43-45.

Geluso, K.N., J.S. Altenbach, and D.E. Wilson. 1976. Bat mortality: Pesticide poisoning and migratory stress. Science. 194(4261): 184-186.

. 1981. Variation in organochlorine residues of Young Mexican Free-Tailed Bats. Am. Midl. Nat. 105(2):249-257.

- LaVall, R.K., and L. LaVall. 1980. Ecological studies and management of Missouri Bats, with emphasis on cave-dwelling species. Missouri Dept. Conserv. Terrestrial Series #8.
- Luckens, M.M. 1973. Seasonal changes in the sensitivity of bats to DDT. Intercontinental Medical Book Corp., New York. pp. 63-75.

and W.H. Davis. 1964. Bats: Sensitivity to DDT. Science 146(3646):948.

. 1965. Toxicity of dieldrin and endrin to bats. Nature 207(4999):878-880.

- Schwartz, C.W. and E.R. Schwartz. 1981. Wild mammals of Missouri. University of Missouri Press. pp. 54-56.
- Stickel, W.H. 1973. Pesticide residues in birds and mammals. In: Environmental pollution by pesticides, C.A. Edwards, ed. Plenum Publishing Corp., New York. pp. 254-312.

. 1975. Some effects of pollutants in terrestrial ecosystems. In: Ecological Toxicology Research. S.D. McIntyre and C.F. Mills, eds. Plenum Publishing Corp., New York. pp. 25-74.

- Tipton, V.M. and A.R. Tipton. 1980. Winged Fingers, seeing ears. . . Nature Conservancy Magazine. March/April. pp. 16-19.
- Tuttle, M.D. 1979. Status, causes of decline, and management of endangered Gray Bats. J. Wild. Mgt. 43(1):1-17.
- United States Department of the Interior Fish and Wildlife Service. 1981. Bats and environmental contaminants: A review. Special Scientific Report-Wildlife #235.
- Walker, E.P. 1975. Mammals of the world. 3rd ed. Johns Hopkins University Press. Vol. I. p. 337.