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Organochlorine Residues in Bats from Eckert James River Cave, Texas

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Organochlorine (OC) pesticides have become a widespread component of our environment as attested to by the nearly universal presence of residues in the tissues of wild vertebrates (McBee and Bickham 1990). As such, a principle concern is the possibility that these pesticides may in part cause dwindling reproductive success and decline in populations of animals occupying relatively high trophic status. Insectivorous bats play an extremely important role in maintaining a balance in ecological systems by serving as biological controllers of insect populations. However, in recent years, severe declines in the numbers of bats have been observed. Much of this observed decline has been attributed to the destruction of habitat and lethal exposures to agricultural chemicals (see reviews by McBee and Bickham 1990 and Dustman and Stickel 1969).

The exposure and toxic effects of OC pesticides have been well documented for only a handful of bat species (Thies et al. 1996; Thies and McBee 1994; Clawson and Clark 1989; Clark and Krynitsky 1983; Clark et al. 1983; Clark 1981; Geluso et al. 1981, 1976), with much research focusing on the population of Brazilian free-tailed bats (Tadarida brasiliensis; Chiroptera Molossidae) at Carlsbad Caverns, New Mexico. The population has suffered a decline in numbers from an estimated 8.7 million in the 1930s to about 200,000 in 1973 (Geluso et al. 1976). This population also has demonstrated the highest levels of organochlorine pesticide contamination for any species of bat examined to date (Thies et al. 1996; Thies and McBee 1994 Geluso et al. 1981, 1976). These residues have been attributed to bats being exposed to pesticides while individuals were inhabiting the United States and areas of Mexico and Central America (Geluso et al. 1981; White and Krynitsky 1986).

Thirty-two species of bats are known to occur in Texas (Schmidly 1991). However, only the population of T. brasiliensis from Bracken Cave, Comal County, has been examined for exposure to pesticides (Clark et al. 1975). Colonies of 2 to 3 million Brazilian free-tailed bats and 20,000 cave myotis (Myotis velifer; Chiroptera: Vespetilionidae) also inhabit Eckert James River cave (EJRC), Mason County, Texas. M. velifer, which has never been examined for exposure to pesticides, is ecologically similar to T. brasiliensis in its diet, activity patterns, and roosting behavior. It is frequently found occupying many of the same maternity caves as T. brasiliensis throughout the southwestern United States (Hayward 1970; Fitch et al, 1981). Although not a long-range migrator like freetails, cave myotis may fly several hundred miles from one cave to another to hibernate through the winter. This seasonal movement is typically oriented in an east-to-west direction (Barbour and Davis 1969) and therefore does not result in animals moving into

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areas of Mexico and Central America where organochlorine insecticides have been and still are being used (Miller 1994).

This project identifies the presence of organochlorine pesticides in two species of bats, T. brasiliensis and M. velifer, from EJRC. Data obtained in this study provide critical information on differences in pesticide contamination between two ecologically similar species occupying the same geographic locality. Because one species migrates and the other does not, these data also provide valuable information on sources of residue accumulation and the potential effects of contamination on species abundance and biodiversity.

Specific objectives of this study were to: 1) determine body burdens of organochlorine residues in T. brasiliensis and M. velifer collected from EJRC; 2) examine seasonal patterns in pesticide contamination within each species; 3) examine differences between species: and 4) compare these data with historical databases available for other bat populations.

MATERIALS AND METHODS

Sixty-five specimens (31 T. brasiliensis and 34 M. velifer) were collected using hoop nets in the entrances of EJRC as colonies left for nightly feeding excursions. Bats were divided into relative age groups based on tooth wear following Anthony (1988). Ten “old” female T. brasiliensis and 12 “old” female M. velifer were collected in May 1993 and returned to Sam Houston State University (SHSU) for analysis. Because no known-age specimens of either species are available for tooth wear comparisons, an accurate age determination was not possible (Thies et al. 1996; Thies and McBee 1994). Therefore, “old” bats were defined as those individuals showing symmetrical wear of both canines approximately halfway to the gums. Due to extended delays in obtaining final approval from The Nature Conservancy of Texas to collect specimens at EJRC, the M. velifer already had given birth at the time of the May collection whereas all T. brasiliensis were still carrying embryos. A second collection of adult females and six juveniles of each sex for each species was made in August 1993 for post-reproductive comparisons. Juvenile T. brasiliensis approximately 12-14 weeks old were identified by size and selected by examination of cartilage bands in the joints of fingers: individuals were retained if two distinct bands were observed when using a flashlight to backlight the wing. Juvenile M. velifer (approximately 20 weeks old) were also identified by body size, weight and level of ossification in the finger joints.

Individual bats were sacrificed by cervical dislocation within 24 hr of capture and prepared for pesticide analyses following protocols described in Thies et al. (1996) and Thies and McBee (1994) as modified from Geluso et al. (1981) and Peterson et al. (1976). For sample preparation, specimens were skinned, wings were removed at the shoulder, and feet were removed at the ankle. Any fat adhering to the skin was removed and placed with the body. The stomach and intestines were removed to avoid analysis of insect remains or other dietary contaminants in the gastrointestinal tract. Embryos and placentae, when present, were removed from the uterus, which remained with the female, and examined for pesticide content independently (see Thies and McBee 1994). The head was removed at the first cervical vertebra and the brain carefully removed. Major cranial musculature was removed from the skull and placed with the carcass. Thus, the term carcass refers to the entire body minus skin, wings, feet, gastrointestinal tract, embryo, skull, and brain. Skins and skulls were prepared as museum teaching and research specimens and deposited in the SHSU Vertebrate Natural History Collection.

Immediately after removal, brains were placed in tared test tubes, weighed, and mixed with five times their weight of anhydrous sodium sulfate. Carcasses were also weighed, placed in individual glass jars with five times their weight of anhydrous sodium sulfate, frozen, homogenized in a blender, and transferred to flasks. A 20% acetone in isooctane (v/v) extraction solvent was added to each test tube and flask (brains 3 mL; carcasses 40 mL). Test tubes were sonicated in an ultrasonic cleaner for 15 min and flasks were shaken for 10 min. Both were agitated periodically for 24 hr and allowed to stand an additional 24 hr. One ml of brain extract and 3 mL of carcass extract were evaporated in tared vials to determine fat content. Remaining extracts were cleaned prior to analysis by adding activated Florisil and concentrated into a final volume of isooctane for gas chromatography. Liquid-liquid partitioning was omitted because it was found to be an unnecessary step (Geluso et al. 1981).

Prior to analysis, an aliquot of each sample was spiked with 100 pg/µl of methoxychlor standard. Samples were analyzed for identifiable organochlorine pesticides including o,p' - and p,p' -DDT, o,p'- and p,p' -DDD, p,p' -DDE, aldrin, lidane, heptachlor, heptachlor epoxide, dieldrin, endrin, and PCB congeners using a Hewlett Packard 5890 capillary column gas chromatography equipped with an electron capture detector. SPB-608 and PTE-5 (Supelco®) 30 m X 0.25 mm id X 0.25 µm film columns were used for identification and confirmation of pesticide residues.

Efficiency of tissue extraction techniques was quantified by spiking and extracting carcass and brain tissues from laboratory-raised mice (Mus musculus) with known quantities of pesticide standards. Extraction blanks were also prepared as a quality control by extracting pure anhydrous sodium sulfate with the solvent mixture. Results were reported in µg/g wet weight. Instrument detection limits were: 0.10 pg/g for aldrin and heptachlor epoxide; 0.25 pg/g for lindane, heptachlor, p,p' -DDE, o,p' -DDT, and dieldrin; 0.50 pg/g for endrin, p,p' -DDT, o,p' - and p,p' -DDD, and PCB congeners. Extraction efficiencies ranged from 84% for dieldrin and p,p' -DDE to 123% for o,p' -DDT. Recovery rates from brain tissues were consistently lower for all residues: recovery of p,p' -DDE from brain tissue was 71%.

Weights of carcasses and brains and fat content were determined for all 65 specimens and were analyzed using ANOVA. Differences in pesticide residues within and among species, sex, age ("old" adult versus juvenile), and collection periods were compared using ANOVA. Because residue quantities were positively skewed and non-normally distributed, data were log(x+l) transformed and geometric means and ranges were determined prior to statistical analyses. Simple correlations among pesticide residues and species, sex, and age also were examined.

RESULTS AND DISCUSSION

We found a number of significant differences among species, collection dates, age, and sex. Early summer T. brasiliensis carcass and brain weights and carcass fat content were greater than their respective M. velifer samples (Table 1). Both adult and juvenile T. brasiliensis demonstrated significant positive correlations between carcass and brain weights and fat content ($r = 0.617$, $P = 0.0040$; $r = 0.702$, $P = 0.0089$, respectively). Other correlations between brain weight and fat content were not significant. A comparison between May and August adults also

Table 1. Means and standard deviations for carcass and brain weight (g) and fat content (mg/g) for Tadarida brasiliensis and Myotis velifer collected from Eckerd James River Cave, Mason County, Texas.

	Age	Sex	N	Weight (g)	Fat Content (mg/g)
Tadarida brasiliensis					
Carcass					
May	A	F	10	6.955 ± 0.598^{a}	107 ± 29^{c}
August	A	F	9	5.782 ± 0.422^{d}	84 ± 21
	J	F	6	$3.460 \pm 1.074^{*}$	$71 \pm 38^{**}$
	J	M	6	$3.335 \pm 0.639^{*}$	$77 \pm 30^{**}$
Brain					
May	A	F	10	0.195 ± 0.012^{e}	80 ± 20
August	A	F	9	0.199 ± 0.016	87 ± 10
	J	F	6	0.169 ± 0.021	$64 \pm 5^{***}$
	J	M	6	0.178 ± 0.010	$68 \pm 7^{***}$
Myotis velifer					
carcass					
May	A	F	12	$6.061 \pm 0.373^{a,b}$	73 ± 10^{c}
August	A	F	12	$5.227 \pm 0.205^{b,d}$	66 ± 33
	J	F	6	$4.669 \pm 0.223^{*}$	$43 \pm 6^{**}$
	J	M	6	$4.688 \pm 0.254^{*}$	$47 \pm 10^{**}$
Brain					
May	A	F	12	0.182 ± 0.011^{e}	71 ± 10
August	A	F	10	0.190 ± 0.008	80 ± 8
	J	F	6	0.180 ± 0.010	$89 \pm 8^{***}$
	J	M	6	0.185 ± 0.010	$79 \pm 5^{***}$

A = Adult; J = Juvenile; F = Female; M = Male; N = sample size; pairs of letters indicate comparisons between adult groups which showed significant differences; asterisks indicate comparisons between juvenile groups in which significant differences were found.

demonstrated a significant decrease in carcass weight through the summer for both species. Because differences between sexes within species were not significant for juveniles, samples were pooled for further analyses. Juvenile T. brasiliensis carcasses were significantly smaller but contained a higher mean fat content in both brain and carcass tissues as compared to juvenile M. velifer.

DDE residues were observed in all specimens examined. Statistical comparisons for adult females collected in May showed T. brasiliensis to carry significantly more p,p' -DDE in their carcasses than female M. velifer also collected in May (Table 2, P = 0.0001) or T. brasiliensis collected in August (Table 2, P = 0.0058). Juvenile T.

brasiliensis collected in August also contained significantly higher p,p' -DDE residue levels in both carcass (P = 0.0016) and brain (P = 0.0066) as compared to the August adult female T. brasiliensis. Juvenile T. brasiliensis also carried significantly higher p,p' -DDE residue levels in both carcass (P = 0.0001) and brain (P = 0.0009) as compared to juvenile M. velifer. All other comparisons were not significant.

Significant positive correlations were found between carcass fat content and p,p' -DDE concentration for both adult and juvenile M. velifer (r = 0.677, P = 0.0003; r = 0.658, P = 0.0180; respectively). Negative correlations were found for both brain weight and p,p' -DDE concentration (r = -0.592, P = 0.0039) and brain fat content and p,p' -DDE concentration (r= -0.400, P = 0.0202) for the adult female M. velifer, as well as adult brain weight and p,p' -DDE concentration (r= -0.582, P = 0.0128) and juvenile brain fat content and p,p' -DDE concentration (r= -0.772, P = 0.0021) in the T. brasiliensis examined.

Ten T. brasiliensis embryos also demonstrated the presence of p,p' -DDE, with levels ranging from below detection limits (< 0.10 pg/g) to 4650 pg/g wet weight. Embryonic weight ranged from 1.394 to 2.441 g (mean= 1.913 g) and fat content ranged from 1 to 31 mg/g (mean = 14 mg/g). Correlation analyses showed strong positive relationships for brain and embryo fat content (r= 0.955; P < 0.0001) and brain and embryo p,p' -DDE concentrations (r = 0.722; P = 0.0415).

Data analyses verified the presence of p,p' -DDE in both populations of T. brasiliensis and M. velifer at EJRC. However, the levels observed in all T. brasiliensis, with the exception of one adult female collected in May, were well below what would be expected to cause symptoms of pesticide poisoning for this species as presented by Clark (1981): no brain concentrations approached toxic levels. Observed p,p' -DDE levels also were comparable to adult female free-tails from Vickery Cave, Oklahoma (Thies et al. 1996; Thies and McBee 1994).

Patterns within this study's correlation analyses follow those of previous reports: pesticide loads appear to be directly related to both brain and carcass fat content. Higher pesticide loads in the carcass fat of adults were mirrored in the higher fat content within the carcass. These higher amounts of fat in the carcasses appear to provide a sequestering mechanism which helps to prevent transfer of OC pesticides into the nervous system and the subsequent demonstration of toxic effects.

Toxicity data for M. velifer are not available but, if this species responds in a manner similar to M. lucifugus, this species also appeared to be in no danger. Maximum brain concentrations were approximately 1000 times lower than lethal limits listed by Clark (1981) for M. lucifugus. Embryo concentrations were, however, comparable to those reported by Thies et al, (1996) and Thies and McBee (1994) for Vickery Cave and Carlsbad Caverns from 1990 and 1991.

Although it has been 20 years since use of DDT compounds has ceased in the United States, it is still reasonable to expect minute levels of highly persistent degradation products such as p,p' -DDE to be accumulated by animals with high trophic status. Hunt et al. (1986) speculated that DDT used in nearby Mexico was a potential source of observed DDE residues observed in killdeers, western kingbirds, red-winged blackbirds, and great-tailed grackles from the lower Rio Grande of Texas. However, comparatively low levels of parent compound in their samples did not support their hypothesis that DDE residues were recently derived from DDT use in Mexico. They instead attributed their results to DDE being

Table 2. Geometric mean and range in p,p' -DDE concentrations of carcass and brain tissues (µg/g wet weight parts per million) for Tadarida brasiliensis and Myotis velifer collected from Eckerd James River Cave, Mason County, Texas.

	Age	Sex	N	µg/g p,p'-DDE
Tadarida brasiliensis				
Carcass				
May	A	F	10	2.312 (0.963 - 87.355)[a, b]
August	A	F	9	0.386 (0.088 - 4.498)[a, c]
	J	F	6	4.025 (1.519 - 28.531)[c, *]
	J	M	6	7.756 (1.896 - 30.326)[c, *]
Brain				
May	A	F	10	0.186 (0.067 - 5.172)
August	A	F	9	0.150 (0.026 - 1.080)**
	J	F	6	0.765 (0.140 - 9.146)**, ***
	J	M	6	0.837 (0.043 - 4.122)**, ***
Myotis velifer				
Carcass				
May	A	F	12	0.193 (0.096 - 4.071)[b]
August	A	F	10	0.282 (0.135 - 0.622)
	J	F	6	0.428 (0.185 - 4.599)*
	J	M	6	0.230 (0.110 - 0.542)*
Brain				
May	A	F	12	0.098 (0.045 - 0.549)
August	A	F	10	0.064 (0.035 - 0.178)
	J	F	6	0.098 (0.036 - 0.406)***
	J	M	6	0.094 (0.012 - 0.357)***

A = Adult; J = Juvenile; F = Female; M = Male; N = sample size; pairs of letters and asterisks indicate comparisons between adult and juvenile groups which showed significant differences.

metabolically derived from one or more components of Kelthane®, a widely used miticide (Risebrough et al, 1985), and from other pesticides containing dicofol. DDE from this source is not associated with p,p' -DDT (Risebrough et al. 1985).

By comparing residue levels between these two species with their different migratory patterns, we expected M. velifer to demonstrate significantly lower residue levels as compared to T. brasiliensis. Our results do indicate that M. velifer are indeed picking up OC residues through their diet which must, due to their restricted range as year-round residents of the United States, be coming from agricultural areas in central and west Texas. These residues may have resulted from the breakdown of DDT or from recent applications of pesticides containing DDE in low levels or as a contaminant. The absence of DDT in our samples also supports the conclusion of Hunt et al. (1986) that the illegal use of DDT or transport of DDT

compounds from Latin America by migratory species cannot account for all of the DDE in our samples. The higher levels in T.brasiliensis may, however, be indicative of residue accumulation throughout their range.

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