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Organochlorine Pesticide Residues in Guano of Brazilian Free-tailed Bats, *Tadarida brasiliensis* Saint-Hilaire, from East Texas

Brad S. Bennett · Monte L. Thies

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During the summer months, an estimated 100 million Brazilian free-tailed bats (*Tadarida brasiliensis*; Chiroptera: Molossidae) roost in natural and man-made structures across Texas (Schmidly, 1991), with two subspecies currently recognized in the United States. *T. b. cynocephala* is a nonmigratory resident of the eastern one-fourth of Texas across the southeast into Florida. *T. b. mexicana* is a migratory subspecies found throughout the remainder of Texas and the southwestern US. *T. b. mexicana* spends much of the winter in Mexico and Central America, but returns to roost sites in the US in the spring and summer months. Separation between the subspecies has been mapped along the Balcones Escarpment of east-central Texas and is based primarily on differences in migratory behavior and skull characteristics (Schmidly, 1991).

Although large, these population estimates fail to take into account declines in many free-tailed bat populations across their geographic range at sites. At Carlsbad Caverns, New Mexico, 8.7 million individuals were estimated to inhabit the cave in 1936. By 1973, only 200,000 bats remained (Altenbach et al., 1979). Since 1973, the population has demonstrated an increase, with approximately 1 million bats using the cave in the early 1990s (Thies and Gregory, 1994); however, the causes for these observed declines is still not fully understood.

The observable accumulation of organochlorine (OC) pesticide residues in body tissues of insectivorous bats has been well documented. These residues have been blamed at least in part for declines seen in a number of species (see Clark, 1981, 1988a for reviews), with specific studies focused on pesticide levels and their possible effects on *T. brasiliensis* in New Mexico and Oklahoma (Geluso et al., 1976, 1981; Thies and McBee, 1994; Thies et al., 1996; Thies and Thies, 1997). Elevated body burdens in controlled experiments by Geluso et al., (1976) clearly demonstrated the adverse effects of releasing sequestered pesticide loads faced by young bats when body fats are burned during their first fall migration. Additional studies have also examined metal accumulation in livers (Thies and Gregory, 1994) and guano deposited by free-tailed bats in their roosts (Petit and Altenbach, 1973). Although metal residues were found, levels were not considered high enough to be harmful.

Studies have shown bats to be no more physiologically sensitive to OC pesticides than other mammals (Clark, 1988b); however, insectivorous diets of many bats may lead them to acquire substantial pesticide loads. It has been estimated that free-tailed bats consume 6–8,000 metric tons of insects annually in Texas alone (Schmidly, 1991). Being fat-soluble, OC pesticides accumulate in stored body fat from these insect diets. During times such as migration when this fat is metabolized, OC exposure may reach lethal levels, particularly in the brain. These fat soluble pesticides are readily passed in milk from mother to pup, so pups may have substantial pesticide residues in the fats they utilize during the critical, demanding period of weaning and first migration (McCracken, 1986).

The movement of nutrients and energy from one feeding group to the next represents bioaccumulation through the food chain. As a result, OC pesticides should be detectable throughout the food chain and should contribute some quantifiable portion to the bat's accumulated pesticide residues. An individual bat may accumulate a number of

B. S. Bennett · M. L. Thies (✉)
Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77341, USA
e-mail: woodrat@shsu.edu

pesticides from the insects it ingests that may then be excreted to some degree. As a result, bat guano may serve as an indirect and nondestructive reference to the exposure and accumulated pesticide loads occurring in a population of bats. A positive correlation has been demonstrated between the levels of OC in guano and those present in bat carcasses from the same colony (Clark and Prouty, 1976). Insects foraging within the guano deposits may then accumulate the residual OC pesticides in the guano and continue the food chain cycle. Although a number of studies have demonstrated accumulation and movement of OC pesticide residues in the food chain (Geluso et al., 1976; Clark and Prouty, 1976; Clark et al., 1981), effects of chronic exposure have never been adequately examined.

The primary purpose of this study was to examine a much neglected portion of food chain accumulation by determining OC pesticide loads within guano samples and dermestid beetles (Coleoptera: Tenebrionidae) feeding on the guano and bat carcasses. Although we could not effectively distinguish between subspecies of bats and the guano they produce, we hypothesized that pesticide residues in the guano would be lower in winter months when the nonmigratory *T. b. cynocephala* represents the bulk of the population. We expected to see an increase in pesticide residues in the guano after the return of *T. b. mexicana* from Mexico and Central America, where many of these OC compounds are still actively used in pest control. We also expected to see an increase in OC pesticide loads carried by dermestids associated with the beetles' consumption of mortalities within the adult members of the population and infant bats falling to the floor of the roost.

Materials and Methods

Data for this study were collected as a part of a larger project monitoring population composition and pesticide loads in *T. brasiliensis* inhabiting a warehouse belonging to the Texas Department of Criminal Justice in downtown Huntsville, Walker County, Texas. This colony, which occurs in an area of overlap between the two subspecies, ranges seasonally in size from several dozen to 2,000 individuals in the winter to over 15,000 by late summer. During the winter months (October–February), roosting bats occupy a juncture between the brick wall and roof on the north side of the building. Although food resources become limited during the winter months, resident individuals do not hibernate but remain active. During the spring and summer months (March–September), bats also occupy an elevated concrete mezzanine on the south wall. We assume that this seasonal disparity in numbers results from a winter population dominated by nonmigratory *T. b. cynocephala*, while the increased summer population also includes a large number of *T. b. mexicana* that over-wintered in Mexico and Central America.

From August 1998 through June 1999, discrete samples of guano and associated invertebrates were obtained by spreading a 75 cm x 1.5 m sheet of butcher paper below active areas of the roost. Guano was allowed to accumulate for 2–3 weeks before the paper was picked up and samples were collected. Samples were labeled, wrapped in aluminum foil, sealed in a zip-loc bag, and placed in a -20°C freezer to kill any invertebrates present in the samples. For pesticide analyses, subsamples consisting of fecal pellets and adult dermestid beetles were manually separated from each sample lot and processed in random order following protocols described in Thies and Thies (1997). For general sample preparation, subsamples were dried to a constant weight at 30°C, weighed, mixed with five times their weight of anhydrous sodium sulfate, homogenized with a mortar and pestle, and transferred to individual labeled flasks. An extraction solvent of 20% acetone in isooctane (v/v) was added to each flask and samples were agitated periodically for 24 hr and then allowed to stand an additional 24 hr. Samples were filtered through solvent-washed filter paper to remove any microscopic fragments that might interfere with chromatography. Aliquots of sample extracts, evaporated in tared vials to determine fat content, were then resuspended in isooctane and cleaned prior to analysis by adding activated Florisil.

Efficiency of tissue extraction techniques was determined by spiking and extracting carcasses of laboratory-reared mice (*Mus musculus*) with known quantities of pesticide standards. Extraction and procedural blanks were also prepared as a quality control by extracting pure anhydrous sodium sulfate with the solvent mixture.

Prior to analysis, each sample was spiked with 5 ng/µl of methoxychlor (2,2-bis(*p*-methoxyphenyl)-1,1,1-trichloroethane) as an internal 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, lindane, heptachlor, heptachlor epoxide, dieldrin, and endrin using a Varian 3600CX capillary column gas chromatograph equipped with dual electron capture detectors at 300°C. A split column (DB-5/DB-17) 30 m x 0.25 mm id x 0.25 µm film was used for identification and confirmation of pesticide residues. Each sample run was 32 min long with an injector temperature of 180°C and initial oven T of 190°C (held for 1 min) ramped to 270°C at 2.4°/min. Results were reported in µg/g dry weight.

Data acquisition was conducted using a Varian Workstation v. 4.5 computer interface system. Peaks for identifiable OC residues were quantified using the observed peak areas for the methoxychlor spike and a series of standards obtained prior to tissue analysis. Pesticide loads were compared among guano and beetle samples using ANOVA.

However, because residue quantities were positively skewed and non-normally distributed, data were log ($x + 1$) transformed and geometric means and ranges were determined prior to statistical analyses (Thies and Thies, 1997).

Results and Discussion

Because many of the sampling periods were represented by insufficient guano or beetles for quantifiable chemical analyses, only the results from eight collection dates were compared. Most of these dates occurred in the late winter and spring when either colony size was low or adult beetles were not found. Although other compounds were found, *p*,*p*'-DDE was the only organochlorine compound found in all samples. Levels of *p*,*p*'-DDE were low, ranging between 0.001 and 0.015 μg/g for guano and 0.001 and 0.006 μg/g for the dermestid beetles (Fig. 1).

Concentrations of *p*,*p*'-DDE were found in all guano and beetle samples examined. Levels of *p*,*p*'-DDE within the guano may have some significance related to migratory versus nonmigratory behavior in the two subspecies of free-tailed bats. Levels of *p*,*p*'-DDE were high in the summer and beginning of fall; however, *p*,*p*'-DDE levels declined during the winter and rose sharply again towards the end of winter and beginning of spring. While the winter residents always have low levels of *p*,*p*'-DDE, the high levels during the summer may be explained by the substantial presence of *T. b. mexicana*. From winter, the *p*,*p*'-DDE residues rise in the spring due to the return of *T. b. mexicana*. Since *T. b. cynocephala* are the winter residents, their residues are low simply because they do not migrate to Central America where many of these chemicals are still used.

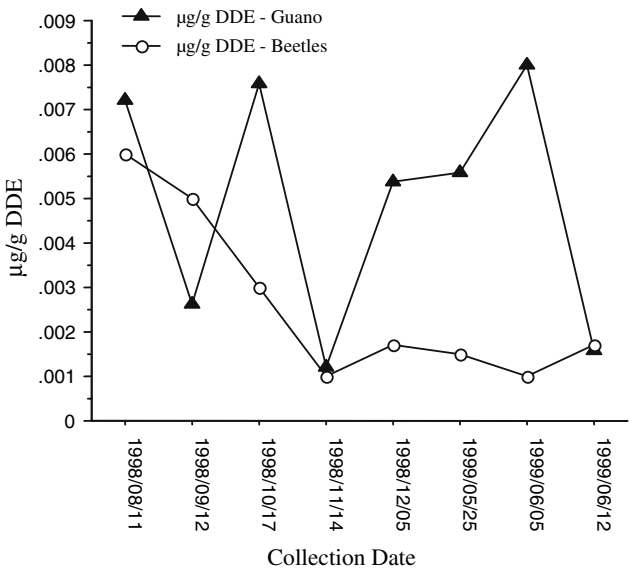

Fig. 1 Comparison of *p*,*p*'-DDE content (μg/g dry weight) in guano and dermestid beetle samples collected from the Huntsville Unit colony

By comparison, concentrations of DDE in the beetles found eating the guano were generally lower than for the guano itself. Clark and Prouty (1976) stated that there was a correspondence between levels of organochlorine residues in guano and in bat carcasses from the same colony. Clark et al. (1981) also gave reasons as to the advantages of sampling guano as opposed to the bats themselves. First, this is a more accurate, long-term measurement of the condition of the colony using a single guano sample that contains feces from more bats than could be analyzed individually. Secondly, it also allows for only one analysis, therefore guarding against analytical error. Thirdly, bats do not have to be collected and sacrificed.

Even with the use of DDT compounds being banned in the United States thirty years ago, metabolites are still being found. Recently, there have been more efforts trying to explain high *p*,*p*'-DDE levels in wildlife in parts of Texas, New Mexico, and Arizona (Clark, 1988a). However, it is reasonable to anticipate minuscule levels of highly persistent degradation products such as *p*,*p*'-DDE to be accumulated by animals. Food chain residues of OC pesticides have probably been involved in declines of some bat populations including the endangered gray bat in Missouri and Alabama and the free-tailed bats at Carlsbad Cavern, New Mexico. As a representation of the food chain, the beetles contained a concentration of around 0.003 μg/g *p*,*p*'-DDE. This amount of *p*,*p*'-DDE seems low, but it must be considered that this represents only one gram of beetles. Bats will generally consume one-half their weight in insects per night. With this in mind, it may be obvious that accumulation of OC pesticides readily occurs through the food chain. It shows a continuous loop from prey (insects), to digestion, and to guano, where the beetles examined in this study most likely received their pesticide exposure.

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