CONTAMINANT STUDIES

ON

ENDANGERED BATS IN NORTHEASTERN OKLAHOMA

BY

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INTRODUCTION

Three federally listed endangered bat species are known to inhabit Oklahoma. The gray bat (Mvotis qrisescens) is probably the most abundant, and is presently known to occur in Adair, Cherokee, Delaware, and Ottawa Counties. Gray bats are almost unknown outside of caves; not even mines have been found harboring this species. Major maternity colonies are nearly always in rather large caves, containing substantial streams, and are usually accessible only by boat or by wading deep water. Hibernation for almost the entire known population of gray bats occurs in half a dozen or so major caves which are characteristically deep and accessible only by use of the elaborate gear needed for vertical cave work. Summer and winter ranges are identical, and are concentrated in the cave region of Arkansas, Missouri, Kentucky, Tennessee, and Alabama (Figure 1). Northeastern Oklahoma is situated on the extreme western edge of the known range of the gray bat, and historically this species was probably limited to the limestone region of the state as delineated by the Arkansas River. No hibernacula are known to occur in Oklahoma, but maternity caves have been found and several are presently owned or are being managed by the U.S. Fish and Wildlife Service (FWS). Gray bats are very intolerant of disturbance and will either move to more remote areas of a cave or abandon it when disturbed. Gray bat colonies were long unknown because they were inaccessible; however, in the past few years, human disturbance has threatened their existence because of the popularity of spelunking. Other important caves have been commercialized or flooded. LaVal and LaVal (1980) estimate that gray bat populations declined 70-80 percent between 1930 and 1980.

The Indiana bat (Mvotis sodalis) is rare in Oklahoma, but is considered to be present in Adair, Delaware, LeFlore, and Pushmataha Counties. Throughout its range, this species is known primarily from the relatively few caves and mines in which it hibernates. Summer records are scarce and no breeding or maternity colonies are known. Speculation is that Indiana bats spend the summer singly or in small groups in hollow trees, beneath loose bark, under bridges, or in old buildings. Eastern Oklahoma is situated on the extreme western edge of the known range of the Indiana bat (Figure 2). The most recent record of the Indiana bat in Oklahoma is that of about half a dozen individuals hibernating in a cave in LeFlore County. The Indiana bat has decreased drastically or disappeared completely throughout its range in recent years. Commercialization, destruction, and disturbance of the hibernation caves are the most probable causes of the species demise. It has been suggested that unless proper protection can be afforded hibernacula, the Indiana bat will probably disappear within the near future. The FWS is not presently involved in the management of any areas in Oklahoma for the Indiana bat.

The Ozark big-eared bat (Plecotus townsendii inqens) is probably a relict population which has always been small in number. Its historic distribution was probably restricted to northwestern Arkansas, northeastern Oklahoma, and southwestern Missouri. In Oklahoma it is presently known to occur in Adair, Cherokee, and Delaware Counties. It is a subspecies or geographic race of the P. townsendii group, which although seldom abundant, is widespread in the western United States (Figure 3) and occupies a variety of habitats. No major colonies of Ozark big-eared bats are known; rather small numbers of bats have been recorded from several scattered caves in the region. P. townsendii inqens is primarily a cave dwelling species in both summer and winter and is seldom found outside or in buildings during the day. This species is intolerant of human

Figure 1. Distribution of the gray bat (Myotis grisescens) in the United States (Barbour and Davis 1969).

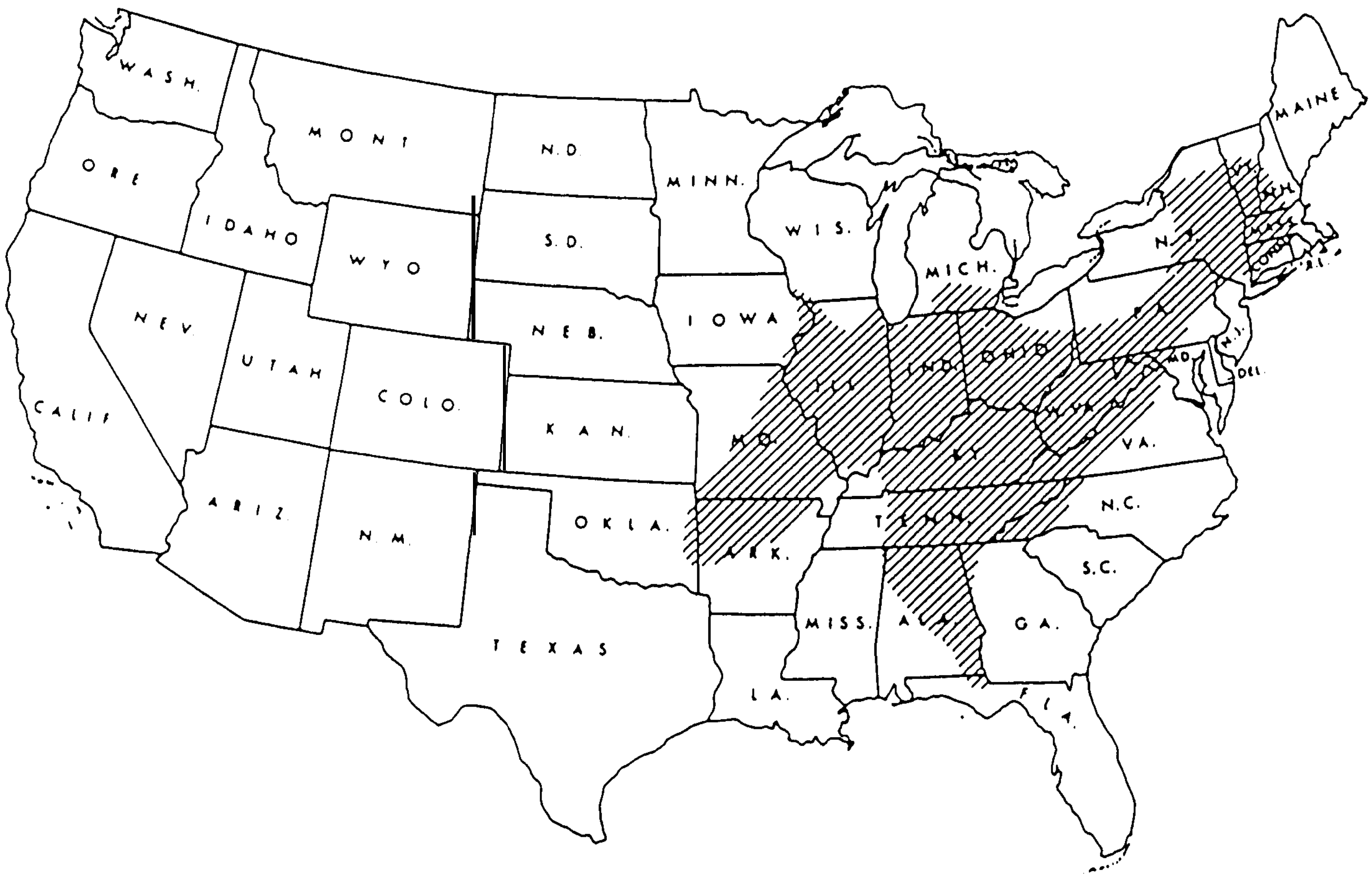

Figure 2. Distribution of the Indiana bat (Myotis sodalis) in the United States (Barbour and Davis 1969).

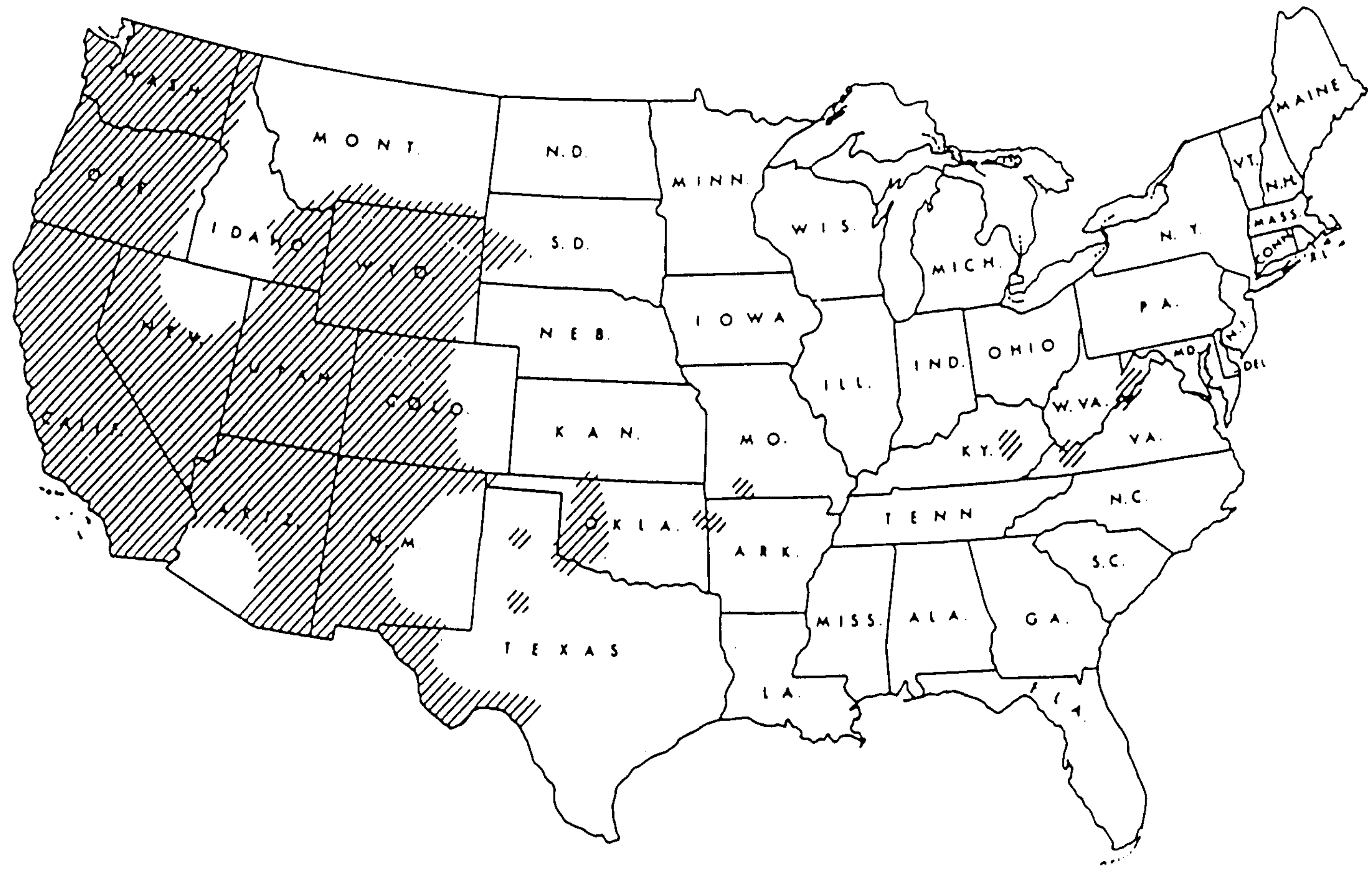

Figure 3. Distribution of Plecotus townsendii in the United States (Barbour and Davis 1969). The Ozark big-eared bat is a subspecies (P. townsendii ingens) restricted to the Ozarks.

disturbance and will abandon sites where they have been disturbed. The FWS currently manages several summer and winter Ozark big-eared bat caves in Oklahoma. Although a relatively large amount of information is available about the Western big-eared bat (P. townsendii), until recently little was known about the Ozark subspecies.

In addition to human disturbance and destruction of habitat, pesticides are often cited as one of the potential major causes in the decline of endangered bat species. Several cases of pesticide poisoning have been reported for gray bats (Clark et al 1978, Clark et al 1983, Clawson and Clark 1989). Endangered bat species in Oklahoma feed exclusively on insects, and in fact, food habits within a species may be relatively restricted. For example, it has been suggested that the Ozark big-eared bat may feed entirely on moths, while gray bats are known to feed almost exclusively over water. Contamination of localized food supplies by persistent pesticides could lead to significant quantities being ingested by bats, with subsequent hazardous bioaccumulation. The same process of bioaccumulation through the food chain could take place with other organic compounds and elemental contaminants as well.

The U.S. Fish and Wildlife Service established Oklahoma Bat Caves National Wildlife Refuge in 1985 to protect endangered bats by preserving and managing several of their known cave habitats. In an effort to better assess the overall status of the populations inhabiting these caves, samples of Ozark big-eared bat and gray bat guano were collected from caves and analyzed for contaminants. In addition, during the course of other, on-going studies related to the bats, dead individuals were encountered on the floor of the caves and were collected for chemical analysis. The purpose of this report is to present the results of contaminant analyses on these samples. The author would like to acknowledge William Puckett, Steve Hensley, and Keith Martin for their assistance in collecting the samples for this project.

METHODS

All samples were collected during summer (May-August) 1990 from caves in Adair and Delaware Counties, Oklahoma. Seven guano samples were collected from active roost sites in five caves (Table 1). The most recently deposited guano was removed from the cave floor with a stainless steel spoon, placed in glass jars, and frozen as soon as possible. Four dead gray bats were removed from the floor of two caves (Table 1), wrapped in aluminum foil and frozen. Whole carcass and guano samples were later packed in dry ice and shipped to U.S. Fish and Wildlife Service contract laboratories where they were ground, homogenized, and analyzed for organochlorine pesticides, total polychlorinated biphenyls, aliphatic hydrocarbons, polynuclear aromatic hydrocarbons, and selected elements (Table 2).

RESULTS AND DISCUSSION

Organochlorine Pesticides and PCBs

The occurrence and effects of several organochlorine pesticides and their metabolites (OCs) have been studied in various bat populations. Clark et al (1988) investigated the possible role of OCs in the death of gray bats inhabiting caves in the vicinity of a DDT manufacturing plant near Huntsville, Alabama. Although OCs (particularly DDE and DDD) were notably high in both

Table 1. Samples collected during summer 1990 from Oklahoma Bat Caves National Wildlife Refuge.

Sample No.[1]	Sample Type	Species	Location/ Replicate	Sample Weight[1] (g)	Moisture* (%)	Lipid[2] (%)
LRB-1	1 Whole Carcass	Gray bat	AD-8/a	7.6	69.9	2.68
LRB-2	1 Whole Carcass	Gray bat	DL-92/a	13.2	73.8	2.56
LRB-3	"	"	" /b	15.6	72.9	2.74
LRB-4	"	"	" /c	7.4	72.8	3.74
LRB-5	Guano	Gray bat	AD-8/a	391	78.5	0.82
LRB-6	"	"	" /b	372	77.0	1.12
LRB-7	Guano	Gray bat	DL-92/a	294	74.0	0.54
LRB-8	"	"	" /b	359	78.0	1.39
LRB-9	Guano	Ozark Big-eared bat	AD-10/a	165	67.5	0.18
LRB-10	Guano	Ozark Big-eared bat	AD-17/a	143	56.5	0.32
LRB- 11	Guano	Big brown bat	AD-15/a	313	58.5	0.58
LRB-5 (duplicate)				391	78.0	0.94

[1]Catalog No. 6461
[2]Determined by contract laboratory for organic analyses, Mississippi State Chemical Laboratory

Table 2. Analyses performed on samples collected in 1990 from Oklahoma Bat Caves National Wildlife Refuge.

ORGANOCHLORINES’	ALIPHATIC HYDROCARBONS’	F’OLYNUCLEAR AROMATIC HYDROCARBONS’
HCB	n-dodecane	napthalene
alpha-BHC	n-tridecane	fluorene
gamma-BHC	n-tetradecane	phenanthrene
beta-BHC	octylcyclohexane	anthracene
delta-BHC	n-pentadecane	fluoranthrene
Oxychlordane	nonylcyclohexane	pyrene
Heptachlor Epoxide	n-hexadecane	1 ,2-benzanthracene
gamma-Chlordane	n-heptadecane	chrysene
trans-Nonachlor	pristane	benzo (b) fluoranthrene
cis-Nonachlor	n-octadecane	benzo (k) fluoranthrene
Endrin	phytane	benzo (e) pyrene
Dieldrin	n-nonadecane	benzo (a) pryene
alpha-Chlordane	n-eicosane	1,2,5,6-dibenz-anthracene
Toxaphene		benzo (g,h,i)-perylene
p,p’-DDE		
o,p’-DDE		
p,p’-DDD		
o,p’-DDD		
p,p’-DDT		
o,p’-DDT		
Mirex		
PCBs (total)		

ELEMENTS*

Arsenic
Aluminum
Berrylium
Cadmium
Chromium
Copper
Iron
Manganese
Mercury
Nickel
Lead
Selenium
Zinc

‘Analyzed by Mississippi State Chemical Laboratory, Mississippi State University.
*Analyzed by Environmental Trace Substances Research Center, University of Missouri.

carcass and guano samples, concentrations in the brains of dead bats were below that which is known to be lethal. Dieldrin, a highly persistent breakdown product of aldrin, is known to have killed gray bats in several localities in Missouri in the 1970s (Clark et al 1978, Clawson and Clark 1989). Following the cancellation of aldrin in 1974, heptachlor was used extensively in its place. Almost immediately, heptachlor epoxide began to occur in dangerously high concentrations in these same Missouri gray bat populations (Clark et al 1983).

In the present study, a total of nine OCs were detected in gray bat carcasses (Table 3). Five of the compounds, DDE, dieldrin, oxychlordane, heptachlor epoxide, and trans-nonachlor were found in all of the specimens, suggesting that contamination is probably widespread. Concentrations of each compound varied among individuals, indicating perhaps sexual or age differences in the distribution of contaminants within the population.

In spite of the array of compounds found in carcasses, only DDE was detected in guano (Table 3). Concentrations were fairly consistent regardless of species or location, ranging from 0.05 to 0.14 ppm dry-weight.

The concentrations of OCs, found in carcasses and guano in this study, are less that those that have been associated with direct, pesticide-induced mortality in previous investigations. Thus, it may be concluded that OCs were probably not the direct cause of death for these particular bats. However, this study does demonstrate that a variety of OCs are present in the Oklahoma population, and the significance of this presence is not well understood. The suceptability of gray bats to OCs is dependent on factors such as life stage and overall body condition. If one assumes that concentrations of OCs in the environment will continue to decrease over time (since they have been discontinued), then OCs should not pose a threat to the continued existence of gray bats in northeastern Oklahoma.

Aliphatic Hydrocarbons

Aliphatic hydrocarbons (alkanes), ranging in molecular size from n-dodecane to n-eicosane, were present in all of the carcass and guano samples (Table 4). There appeared to be marked differences in the concentration of some specific compounds and in total alkanes with respect to individuals, species, and location. For example, n-tridecane ranged from 0.05 and 0.06 ppm in Ozark big-eared bat guano to 3.9 ppm in guano from the big brown bat. Odd-numbered carbon molecules were predominant in all samples, comprising between 64 and 84 percent of the total in the gray bat carcass samples, and from 62 to 93 percent of the total in guano samples. According to some authors (Farrington 1973; Giger et al 1974), the predominance of odd-numbered carbon compounds indicates hydrocarbons of recent biological origin. Petroleum hydrocarbons supposedly have approximately equal amounts of even and odd-numbered carbon compounds. Some authors have used the pristane/N-C17 and phytane/N-C18 ratios in birds to indicate chronic exosure to petroleum pollutants (Hall and Coon 1988). In samples from the present study, both the pristane/N-C17 and phytane/N-C18 ratios were consistently less than 1 .O, suggesting little bioaccumulation of pristane or phytane in tissues.

It appears from these data that the bat populations represented in this study are probably not being chronically exposed to large quantities of petroleum hydrocarbons. However, the origin and significance of aliphatic hydrocarbon compounds in these samples is presently unknown. Studies with birds have indicated that adults tolerate oil in the diet reasonably well, but that a

Table 3. Concentrations of organochlorine pesticides found in gray bats and bat guano from Oklahoma Bat Caves National Wildlife Refuge.

		ppm (dry weight)								
Sample Type	Location/ Replicate	p,p'-DDE	p,p'-DDD	o,p'-DDD	Dieldrin	alpha- Chlordane	Oxychlordane	Heptachlor Epoxide	trans- Nonachlor	HCB
Gray bat	AD-8/a	1.3*	0.07	0.13	0.10	--[1]	0.40*[2]	0.20	0.13	--
Gray bat	DL-92/a	0.31	--	--	0.04	--	0.08	0.04	0.04	--
"	" /b	1.9*	0.26	0.18	0.26	0.33	2.1*	1.4*	1.6*	0.04
"	" /c	1.2*	0.07	0.18	0.15	--	0.48*	0.15	0.11	--
Guano	AD-8/a	0.05	--	--	--	--	--	--	--	--
"	" /b	0.09	--	--	--	--	--	--	--	--
"	DL-92/a	0.12	--	--	--	--	--	--	--	--
"	" /b	0.14	--	--	--	--	--	--	--	--
"	AD- 10/a	0.06	--	--	--	--	--	--	--	--
"	AD- 17/a	0.11	--	--	--	--	--	--	--	--
"	AD- 15/a	0.05	--	--	--	--	--	--	--	--

[1] -none detected
[2] * confirmed by GC/Mass Spectrometry

Table 4. Concentrations of aliphatic hydrocarbons found in gray bats and bat guano from Oklahoma Bat Caves National Wildlife Refuge.

Sample Type	Location/ Replicate	n-dodecane	n-tridecane	n-tetradecane	n-pentadecane	n-hexadecane	n-heptadecane	pristane	n-octadecane	phytane	n-nonadccane	n-eicosane	Total
Gray bat	AD-8/a	0.07	0.50	0.07	0.13	0.07	0.20	0.03	0.07	0.07	0.10	0.10	1.41
"	DL-92/a	0.08	0.15	0.11	1.5	0.19	1.8	0.04	0.08	--		0.19	4.29
"	" /b	0.07	0.15	0.07	0.33	0.11	0.30	0.04	0.07	0.04	0.15 0.07	0.07	1.32
"	" /c	0.07	0.22	0.11	0.29	0.22	3.6’	--	0.51*	0.07	0.92*	0.26	6.27
Guano	AD-8/a	0.19	0.47	0.14	0.93	0.19	4.6	0.14	0.37	0.42	0.98	0.28	8.71
"	"	0.18	0.45’	0.14	1.4*	0.18	4.5*	0.14	0.50*	0.45*	1.8*	0.64*	10.38
"	" /b	0.13	0.35	0.13	0.74*	0.17	4.8*	--	0.39	0.61*	1.1*	0.22	8.64
"	DL-92/a	0.04	0.23	0.08	0.65	0.15	2.3	0.08	0.15	0.12	0.35	1.5	5.65
"	" /b	0.14	0.36	0.14	0.59	0.14	2.4	0.09	0.18	0.14	0.45	0.23	4.86
"	AD-10/a	0.03	0.06	0.06	1.1	0.06	0.18	0.09	0.09	0.03	0.06	0.25	2.01
"	AD-17/a	0.05	0.05	0.07	0.85	0.11	0.55	0.05	0.07	--	0.09	0.11	2.00
"	AD-IS/a	0.12	3.9	0.05	0.07	--	0.07	--	0.02	0.02	0.05	0.10	4.40

variety of biochemical and physiological changes may occur. The types of changes noted in these feeding experiments such as depression of growth, impaired avoidance behavior, liver hypertrophy, splenic atrophy, kidney degeneration, hyperphagia, hemolytic anemic and depressed egg production are the kinds of effects that could have serious consequences in free-living populations (Hall and Coon 1988). These conditions, when combined with other forms of stress, could be lethal. Hall and Coon (1988) have suggested that any animal with demonstrated petroleum hydrocarbon residues in the tissues has suffered effects of the pollutant, but studies conducted to date have not related such sublethal effects to the status of wildlife populations. Research is needed to determine the effects of petroleum ingestion on bats and to aid management biologists in the interpretation of residue concentrations in bat carcass and guano samples.

Polvnuclear Aromatic Hydrocarbons

Polynuclear aromatic hydrocarbons (PAHs) are derived from a variety of sources, including new and used petroleum products, industrial activities, and combustion processes. Used motor oil is a common source of PAHs which often make their way into aquatic habitats via storm water runoff. The heavier PAHs, such as benzo(a)pyrene, are potent carcinogens; while some of the lighter compounds, such as naphthalene are more acutely toxic.

Three PAHs were found in carcass and guano samples (Table 5). Phenanthrene was present in all of the samples. Napthalene was present in all of the carcasses, but was found sparingly in guano. Fluorene was found sparingly in both carcass and guano samples. PAHs may be readily metabolized by mammals, and thus, may not bioaccumulate. Indeed, in some animals, it is the metabolites that are potent carcinogens and these are not readily detected during routine chemical analysis. Although it can be stated that PAHs should not be found at all in clean tissues, the real significance of these residues is unknown.

Elements

The elemental composition of gray bat carcasses is shown in Table 6. No real differences between carcasses from the two locations can be noted, and in general, concentrations of each element are relatively uniform. Since elemental contaminants tend to accumulate in specific organs or tissues of mammals (e.g. liver, kidney, or bone) it would have been better had these components been analyzed separately rather than whole carcasses. Unfortunately, there was not enough material available for this approach.

The elemental composition of bat guano probably reflects the residues present in the undigested portion of ingested prey species, and as such may provide some clues as to the location of contaminants in the environment. For example, arsenic concentrations in guano from Ozark big-eared bats at AD-17 appeared to be an order of magnitude higher than other species and other locations. This suggests that terrestrial insects (perhaps more specifically, moths) from the feeding area of this particular group of bats may contain elevated concentrations of arsenic. It can be noted that Ozark big-eared bat guano is also higher in chromium and lead than that of the other species. These differences may be a function of physiological attributes of the prey species, or they may reflect differing levels of environmental concentrations in the areas where the insects are produced. These relationships (i.e. the elemental composition of bat guano, the elemental composition of the prey, and the elemental composition of the environment where the

Table 5. Concentrations of polynuclear aromatic hydrocarbons found in gray bats and bat guano from Oklahoma Bat Caves National Wildlife Refuge.

		ppm (dry weight)			
Sample Type	Location/Replicate	Napthalene	Fluorene	Phenanthrene	Total
Gray bat	AD-8/a	0.20	0.03	0.07	0.30
Gray bat	DL-92/a	0.04	--	0.08	0.12
"	" /b	0.04	--	0.04	0.08
"	" /c	0.26	0.04	0.07	0.37
Guano	AD-8/a	0.05	--	0.16	0.21
"	" lb	--	--	0.13	0.13
Guano	DL-92/a	--	--	0.15	0.15
"	" /b	--	0.05	0.09	0.14
Guano	AD-10/a	--	0.03	0.03	0.06
Guano	AD- 17/a	--	--	0.05	0.05
Guano	AD- 15/a			0.05	0.05

Table 6. Concentrations of selected elements found in gray bats and bat guano from Oklahoma Bat Caves National Wildlife Refuge.

Sample Type	Location/Replicate	As	Cd	Cr	Cu	Hg	Pb	Se	Zn
Gray bat	AD-8/a	0.8	0.40	5.0	13	0.14	<2.0	2.3	105
Gray bat	DL-92/a	3.0	0.54	15	19	0.35	24	4.5	166
"	" /b	1.0	0.27	6.3	15	0.11	8.3	5.2	111
"	" /c	0.3	<0.20	3.9	9.1	0.24	<2.0	8.3	60
Guano	AD-8/a	3.4	2.4	1.9	142	0.52	2.4	7.4	762
"	" /b	8.6	2.4	1.7	149	0.54	2.0	4.4	774
Guano	DL-92/a	2.3	2.6	3.0	131	0.34	8.1	9.8	774
"	" /b	2.6	2.6	3.0	131	0.35	7.5	4.0	791
Guano	AD-10/a	2.5	3.9	34	98	0.14	23	0.9	612
Guano	AD- 17/a	66	3.8	57	119	0.08	23	1.2	1070
Guano	AD-15/a	5.8	1.2	0.6	114	0.22	1.0	1.5	528

insects are produced) would appear to be a fruitfull area for future research, and one that would have direct application for resource managers.

SUMMARY AND CONCLUSIONS

Our study shows that endangered bats in Oklahoma are being exposed to a variety of environmental contaminants. The questions that remain are whether or not these present exposures are having any detrimental impact on bat populations and whether or not exposures are increasing or decreasing. Our data suggest some ideas for furthur consideration.

It appears as though guano is not a particularly good medium to sample for organochlorines (OCs). There is little qualitative correlation between compounds detected in guano and those found in bat carcasses. This suggests that either whole carcasses or specific organs should be analyzed to effectively determine the kinds and amounts of OCs that bats are being exposed to. Since systematic collection of carcasses and organs, in numbers necessary for valid statistical analysis, is impossible for endangered species, it would appear that the only solution would be to find suitable surrogate species.

In contrast to OCs, it would appear that guano is a good indicator of aliphatic hydrobarbon residues in whole bats, and the real question here concerns the significance of these compounds to the well being of the individuals. This appears to be an area that only additional research can shed more light on.

Polycyclic aromatic hydrocarbons (PAHs) are serious contaminants, and a method should be sought to properly evaluate exposures and effects of these compounds to endangered bat populations. Perhaps some biomarker, such as the formation of liver tumors or the induction of specific enzymes, will have to be employed instead of chemical analysis. If this is the case, then perhaps the same surrogate species that are used for OCs could be used for PAHs.

The potential for using the elemental content of bat guano for evaluation of evnironmental contamination has been mentioned previously. Systematic sampling of guano deposits from various areas, coupled with the known food habits of individual species, should be a good way of monitoring trends and exposures of bat populations to trace elements and be a good indicator of both aquatic and terrestrial environmental quality as well.

The following actions will be taken by the Tulsa ES Field Office to implement a contaminants monitoring and evaluation program to protect endangered bay species in northeastern Oklahoma:

(1) Careful consideration will be given to selecting a surrogate species for the gray bat and the Ozark big-eared bat to be used to monitor exposure to OCs and effects of PAHs. If reliable surrogates can be identified, they will be used in a monitoring program. Continued opportunistic analysis of dead endangered bats will continue as they are encountered.

(2) An in-depth literature survey will be conducted to determine the significance of aliphatic hydrocarbon residues in bats. If it can be determined that there is need for information regarding these residues, guano from both species will be monitored at various locations.

(3) Trace elements will be monitored in guano from both species at various locations. Results will be correlated with known prey species and feeding areas and can be used to identify “hot spots” and temporal trends.

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