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Pesticide Contamination of Endangered Gray Bats and Their Food Base in Boone County, Missouri, 1982

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Missouri provides important summer habitat for endangered gray bats (*Myotis grisescens*). Most caves occupied by these bats are scattered throughout the Ozark Plateau south of the Missouri River. Only two populations are found in the northern half of Missouri; one in Ralls County in northeast Missouri and one in Boone County in the central part of the state (LaVal and LaVal 1980). These populations occupy largely agricultural areas (corn, soybeans).

The Boone County gray bat population uses four caves; two are maternity sites (Holton and Devil's Icebox Caves), and two are transient roosts (Hunter's and Lewis and Clark Caves). Seven gray bats, three found dead in Hunter's Cave in July 1981 and three found dead and one moribund in Devil's Icebox Cave in July 1981, were analyzed and the results reported (Clark et al. 1983a). Each of the six dead bats had lethal concentrations of dieldrin in their brains, and four of the six also had lethal concentrations of heptachlor epoxide. The brain of the moribund bat contained no detectable residues.

Dieldrin-induced mortality between 1976 and 1978 in a Franklin County, Missouri, gray bat population was documented previously (Clark et al. 1978, 1983b). Residues of heptachlor epoxide increased to lethal concentrations in 1977 and remained high in 1978 (Clark et al. 1983b). Dieldrin's parent compound, aldrin, was used extensively in the 1960s and 1970s to control cutworms (larvae of several moth species, Family Noctuidae). Although this use was cancelled in 1974, dieldrin is highly persistent in soils where aldrin was applied (Korschgen 1971). Hepatchlor was substituted after aldrin was cancelled. Heptachlor was cancelled in 1978, but Missouri was granted a 1-year extension for use on corn.

Our objectives were (1) to determine whether pesticide residues were involved in gray bat mortalities discovered at

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Holton and Hunter's Caves, and (2) to measure pesticide residues in other species of bats and in the bats' insect food base.

MATERIALS AND METHODS

Clawson visited the four Boone County gray bat caves twice weekly from July 6 to August 12, 1982. Visits were made when the bats were away feeding to avoid undue disturbance. Dead or dying gray bats were noted, fresh specimens were collected, and guano was sampled at least once in each cave. Guano was scraped from the surface of accumulations, so it represents bats in their 1982 activity period, and it was wrapped in aluminum foil to prevent subsequent contamination. All areas of Holton Cave and Lewis and Clark Cave were examined on each visit. In Hunter's and Devil's Icebox Caves, extensive passage lengths and water barriers prevented penetration beyond the first bat roosts on most visits, but each cave was surveyed completely at least once during the study period. Gray bats were recovered under authority of the Federal Endangered Species Permit PRT-8-31-C.

Mist nets and an ultrasonic bat detector were used to verify gray bat foraging areas. Insects were collected from July 7 to August 11, 1982, at six sites within the feeding range of the Boone County bats. Two sites were in the Missouri River bottomlands and four were along creeks. An ultraviolet light trap and suction trap powered by a gasoline generator were used to collect insects. Insects too small or large (<11 mm or >25 mm long) to be gray bat prey were discarded; all other Coleopterans (primarily rove beetles, Family Staphylinidae), Tricopterans and Lepidopterans were grouped in composite samples for chemical analysis.

Bat, guano and insect samples were frozen within 1 hr of collection. Samples were shipped by air freight to the Patuxent Wildlife Research Center, Laurel, Maryland, for analysis. Bats were dissected and their brains and carasses (i.e., whole bat minus skin, head, feet, wings and gastrointestinal tract) analyzed. Each brain, carcass, guano sample and insect sample was analyzed for *p,p'*-DDT, *p,p'*-TDE, *p,p'*-DDE, dieldrin, heptachlor epoxide, oxychlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor, endrin, hexachlorobenzene (HCB), toxaphene and polychlorinated biphenyls (PCBs). This paper discusses only dieldrin and heptachlor epoxide because other contaminants were present in amounts less than those of any known concern.

Methods of sample preparation, extraction, cleanup and separation of pesticides from PCBs were those of Cromartie et al. (1975). Silica gel (100-200 mesh, grade 923, Davison

Chemical Division, WR Grace & Co.) was substituted for the SilicAR. The silica gel separation was collected in four fractions rather than three to ensure separation of dieldrin and endrin (Kaiser et al. 1980). The samples were not analyzed for mirex, therefore, fractions I and II of the silica gel separation were combined. Pesticides were quantified by electron-capture, gas-liquid chromatography using a 1.83 m by 4 mm id glass column packed with 1.5/1.95% SP-2250/2401 on 100/200 mesh Supelcoport at 210°C. Residues in 10% of the samples were confirmed with a Finnigan model 4000 gas-liquid chromatograph/mass spectrometer.

Recoveries of pesticides and PCBs from fortified samples of chicken eggs and mallard carcasses run concurrently with bat samples ranged from 76% to 104%. Residue concentrations were not corrected for percent recovery. The lower limits of reportable residues were 0.1 ppm for pesticides and 0.5 ppm for PCBs in carcasses and insects and 0.5 ppm for pesticides and 2.5 ppm for PCBs in brains. Means are geometric because of positive skewness in the residue data. A value equal to one-half the detection limit was assigned to "not detected" values to allow computation of geometric means.

RESULTS AND DISCUSSION

Eight dead gray bats were collected at Holton Cave. Four of these, all juvenile males, were suitable for analysis. Forty-three dead gray bats were found at Devil's Icebox Cave, but none was fresh enough for analysis. None of 25 dead gray bats found at Hunter's Cave was suitable for analysis, but a juvenile male found near death on the ground was collected. No dead or moribund bats were found at Lewis and Clark Cave. Nine thousand gray bats, including young, were estimated in the Boone County population that occupied Holton, Devil's Icebox, Hunter's and Lewis and Clark Caves during our study.

Two adult female gray bats that became entangled in mist nets and drowned before they could be freed were kept for pesticide analysis. Seven red bats (*Lasiurus borealis*) and two eastern pipistrelles (*Pipistrellus subflavus*) collected in gray bat feeding areas appeared healthy at capture but were analyzed for comparison with gray bats.

Dieldrin concentrations in guano from Hunter's (0.45 ppm dry weight) and Devil's Icebox (0.61 ppm) Caves (Table 1), were high enough ($\geq$0.38 ppm) to be associated with dieldrin-induced mortality (Clark et al. 1982). The 0.31 ppm dieldrin at Lewis and Clark Cave indicated substantial exposure to the pesticide.

Two of four juvenile gray bats found dead in Holton Cave had died of dieldrin poisoning (Table 2). This case suggests

Table 1. Dieldrin and heptachlor residues (ppm dry weight) in guano samples from four Boone County gray bat caves, 1982.

Cave	Site in cave	Dieldrin	Heptachlor epoxide
Devil's Icebox	Devil's Graveyard	0.61[a]	0.15
Devil's Icebox	Maternity Roost	0.20	ND[b]
Holton	Bat Room	ND	ND
Holton	Maternity Roost	ND	ND
Hunter's	Bat Room	0.45[a]	ND
Lewis and Clark	Primary Roost	0.31	ND

[a] Concentration associated with mortality in other colonies, see Clark et al. (1982).

[b] Residue was "not detected" in the chemical analysis.

that some mortality may occur even when dieldrin concentrations in guano are below detection. Both pipistrelles and five of seven red bats had measurable concentrations of dieldrin and heptachlor epoxide in carcass or brain or both.

Twenty-five of 29 (86%) insect samples contained measurable dieldrin, heptachlor epoxide, or both (Table 3). The mean dieldrin concentration for all Coleopteran samples was 0.41 ppm wet weight (95% CI=0.17-0.99 ppm), for Tricopterans 0.27 ppm (0.15-0.51 ppm) and for Lepidopterans 0.07 ppm (0.04-0.12 ppm). Nine of ten Coleoptera and Tricoptera samples contained measurable dieldrin, whereas only two of nine Lepidoptera samples were contaminated at measurable levels. Dieldrin in all insect samples averaged 0.21 ppm (0.13-0.33 ppm), and heptachlor epoxide averaged 0.18 ppm (0.13-0.25 ppm).

Insect samples from the feeding range of the Boone County gray bats show widespread pesticide contamination of this endangered species' food, with both terrestrial insects (Coleoptera and Lepidoptera) and aquatic insects (Tricoptera) contaminated. Gray bats are opportunistic feeders and take insects as they become available through the summer (Clawson, unpublished data). Coleopterans, with their high contaminant levels, are eaten extensively.

No experimental evidence exists as to whether diets with these levels of dieldrin and heptachlor epoxide (Table 3) are lethal to bats. A diet with 20 ppm (dry weight) dieldrin was lethal to laboratory rats (*Rattus norvegicus*) within 75 days (Harr et al. 1970); short-tailed shrews (*Blarina brevicauda*) survived 17 days on a diet containing 50 ppm(dry

Table 2. Dieldrin and heptachlor residues (ppm wet weight) in gray, eastern pipistrelle and red bats from Boone County, 1982.

Sp.	Sex	Age	Site	Condition when collected	Dieldrin		Heptachlor epoxide	
					Carcass	Brain	Carcass	Brain
Gray	M	Juv.	Holton Cave	Dead	8.3	5.6[a]	2.6	2.8
Gray	M	Juv.	Holton Cave	Dead	2.0	1.5	0.6	ND[b]
Gray	M	Juv.	Holton Cave	Dead	2.5	1.6	0.7	ND
Gray	M	Juv.	Holton Cave	Dead	11	4.5[a]	2.8	1.7
Gray	M	Juv.	Hunter's Cave	Moribund	2.5	1.3	0.9	ND
Gray	F	Ad.	Rocky Fork Cr.	Drowned[c]	ND	ND	ND	ND
Gray	F	Ad.	Bonne Femme Cr.	Drowned	1.4	ND	0.4	ND
Pip.	M	Juv.	Bonne Femme Cr.	Alive[c]	4.3	0.6	1.7	ND
Pip.	M	Ad.	Rocky Fork Cr.	Alive	0.4	ND	0.3	ND
Red	F	Juv.	Bonne Femme Cr.	Alive	1.1	0.5	0.3	ND
Red	F	Juv.	Bonne Femme Cr.	Alive	0.1	ND	0.1	ND
Red	M	Juv.	Bonne Femme Cr.	Alive	1.2	0.7	0.4	ND
Red	F	Ad.	Bonne Femme Cr.	Alive	ND	ND	ND	ND
Red	F	Ad.	Rocky Fork Cr.	Alive	0.6	0.7	3.2	0.8
Red	F	Ad.	Rocky Fork Cr.	Alive	ND	ND	ND	ND
Red	M	Ad.	Rocky Fork Cr.	Alive	0.1	ND	0.2	ND

[a] Lethal brain concentration (≥2.7 ppm), see Clark et al. (1978).

[b] Residue was "not detected" in the chemical analysis.

[c] In mist net.

weight) dieldrin (Blus 1978). If the most contaminated beetle sample contains 2.2 times 3.6 or 7.9 ppm dieldrin by dry weight (assuming that beetles are about 72% water, Clark and Kroll 1977), and if the gray bat diet were to consist only of such beetles, the dietary concentration of dieldrin would be well below that which was toxic to laboratory rats or short-tailed shrews. However, because dietary toxicity is a function of body fat level, with thin bats susceptible to much lower dosages of organochlorine pesticides than fat bats (Luckens 1973), the question remains open.

The milk diet of young bats may be more critical in this contaminant situation. Data from Franklin County, Missouri, showed dieldrin residues as high as 89 ppm (wet weight) in milk samples taken from stomachs of nursing young gray bats found dead; field-collected beetle samples contained up to 3.1 ppm (wet weight) dieldrin (Clark and Prouty 1984). Also, juvenile gray bats are about 1.9 times more sensitive to dieldrin than are adults (Clark et al. 1983b). These

Table 3. Dieldrin and heptachlor residues (ppm wet weight) in gray bat prey samples in Boone County, 1982.

Location	Order	Dieldrin	Heptachlor epoxide
Missouri River	Cole.	2.2,0.6,1.8,ND[a]	1.1,0.3,0.7,ND
Missouri River	Lepid.	ND,ND,ND	ND,ND,0.3
Missouri River	Tric.	0.7,0.7,0.7,0.3	0.3,0.2,0.3,0.2
Rocky Fork Cr.	Cole.	0.2,0.1	0.2,0.2
Rocky Fork Cr.	Lepid.	0.3,ND	0.2,0.1
Rocky Fork Cr.	Tric.	0.2,0.4	0.1,0.4
Bonne Femme Cr.	Cole.	0.2,0.7	0.1,0.5
Bonne Femme Cr.	Lepid.	ND,ND	0.2,0.3
Bonne Femme Cr.	Tric.	0.2,ND	0.1,0.3
Perche Cr.	Cole.	1.0	0.5
Perche Cr.	Lepid.	ND	ND
Perche Cr.	Tric.	0.1	0.1
Little Bonne F. Cr.	Cole.	0.4	0.2
Little Bonne F. Cr.	Lepid.	0.2	0.1
Little Bonne F. Cr.	Tric.	0.3	0.1

[a] Residue was "not detected" in the chemical analysis.

factors probably cause mainly young bats to die in contaminant situations such as this.

The Boone County gray bat population depends on all four of the study caves for its existence. Both Holton and Devil's Icebox Caves support maternity colonies, whereas Hunter's and Lewis and Clark Caves serve as bachelor roosts during the maternity period and as transient roosts for mixed sexes and ages before and after this period. Pesticide contamination at all four sites is alarming because of the endangered status of the gray bat.

Earlier studies have shown pesticide residue-induced mortality in Franklin County, Missouri, gray bats at Bat Caves 2-3 and Roaring Spring Cave (Clark et al. 1978), and at Hunter's and Devil's Icebox Caves, Boone County, Missouri (Clark et al. 1983a). The present study adds Holton Cave to this list. Based on this study, we cannot say whether other bat species in Boone County are experiencing mortality due to pesticides.

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