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Gray Bats Killed by Dieldrin at Two Additional Missouri Caves: Aquatic Macroinvertebrates Found Dead

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We learned previously (CLARK et al. in manuscript) that mortality due to dieldrin poisoning occurred during 1976-78 in two maternity colonies of the endangered gray bat (*Myotis grisescens*) at Bat Cave No. 2-3 and Roaring Spring Cave, Franklin County, Missouri. Residues of heptachlor-related chemicals in bats increased markedly in 1977 to potentially dangerous concentrations and remained elevated in 1978. The colony that showed the greater mortality disappeared in 1979 and was not present when the roost caves were visited in 1982.

In 1980 and 1981, dead gray bats containing lethal levels of dieldrin and high concentrations of heptachlor-related compounds were found at Hunter Cave and Devil's Icebox Cave, Boone County, Missouri. The objectives of the present paper are to report this additional mortality and to note a concurrent die-off of aquatic macroinvertebrates at Devil's Icebox Cave.

MATERIALS AND METHODS

Hunter Cave is a transient gray bat cave (LAVAL and LAVAL 1980) about 15 km SSE of Columbia, Boone County, Missouri. On 23 July 1980, one of us (RLC) found 18 dead gray bats on the cave floor beneath a cluster estimated at 400-500 bats. Three of these dead bats showed no signs of decomposition and were analyzed. Unusual bat mortality was not observed in previous years during visits to the cave. The cave was visited in July 1981, but no mortality was observed.

Devil's Icebox Cave is about 10 km S of Columbia, Boone County, Missouri, or about 5 km NNW of Hunter Cave. Some gray bats bore young there in 1981 but the cave is categorized as transient (LAVAL and LAVAL 1980). On 14 July 1981, RLC counted about 24 dead gray bats. The colony was estimated at 500 bats. Another incapacitated bat was found alive on the cave floor. The bat found alive, plus three of the dead that had not decomposed, were analyzed. Twenty-five decomposed gray bats were found in the cave on 10 September 1980, but due to their state of decay none were collected for analysis. This cave had been visited frequently during the past 15 years by personnel of both the U.S. Fish and Wildlife Service, National Fisheries Research Laboratory, Columbia, Missouri, and of the State of Missouri, Rockbridge State

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Park, but unusual bat mortality was not reported.

All seven bats analyzed in this study had forearms of adult size (Table 1). However, only the bat found alive showed wear on the canine teeth, therefore it is possible the other bats were young-of-the-year and had just reached maturity.

TABLE 1

Dieldrin residues from seven gray bats found in two caves in Boone County, Missouri. Bat no. 200 was sick, the other bats were dead. ND, not detected.

Bat number	Sex	Forearm length (mm)	Dieldrin (ppm) Brain Fresh weight basis	Carcass Lipid weight basis	Carcass Fresh weight basis
			Devil's Icebox Cave		
200	M	43.5	ND	4.1	0.26
201	F	43.6	21	900	21
202	F	43.1	13	970	27
203[a]	M	43.3	11	--	--
			Hunter Cave		
204	F	43.2	5.6	430	19
205	F	43.2	11	730	18
206	F	43.1	14	410	10

[a]Extracted carcass sample was spilled.

Bats were recovered under authority of the Federal Endangered Species Permit PRT-8-31-C. Bats were frozen, shipped to the Patuxent Wildlife Research Center, and dissected into brain and carcass components as described previously (CLARK et al. 1978). Each brain and carcass, and a single sample of masticated insects from the stomach of bat no. 200 were analyzed for *p,p'*-DDT, *p,p'*-DDD, *p,p'*-DDE, dieldrin, heptachlor epoxide, oxychlordane, *cis*-chlordane, *trans*-nonachlor, endrin, hexachlorobenzene (HCB), toxaphene, mirex, and polychlorinated biphenyls (PCB). The recovered PCB resembled Aroclor 1260. Samples were homogenized and extracted as described by CROMARTIE et al. 1975. We cleaned extracts using Sep-Pak Florisil cartridges (Waters Associates, Inc.). We pushed each extract (10 ml volume) through a cartridge using a 10-ml syringe with a Luer-lok tip. Pesticides and PCB were eluted from the cartridge with 10 ml of 6% ethyl ether in hexane (v/v). We separated pesticides and PCB into four fractions by silica gel column chromatography (100-120 mesh, Davison Chemical Division, W. R. Grace and Co.) using procedures described by KAISER et al. 1980. Residues were quantitated by

electron-capture gas-liquid chromatography with a 1.5/1.95% SP-2250/2401 column. Average percentage recoveries of pesticides and PCB from spiked sample tissue ranged from 83-97%, except *trans*-nonachlor was 45%. Residue data were not adjusted on the basis of these recoveries. We confirmed residues in three samples using a Finnigan 4000 gas-liquid chromatograph-mass spectrometer.

Lipid levels were determined from weights of dried hexane extracts of carcasses (CROMARTIE et al. 1975). The lower limits of sensitivity for residues in carcasses were 0.1 ppm for pesticides and 0.5 ppm for PCB; in brains and stomach contents the limits were 0.5 ppm for pesticides and 2.5 ppm for PCB.

RESULTS

Dieldrin was the principal organochlorine residue found in the gray bats (Table 1). The brains of the six dead bats contained heptachlor epoxide at 8.2, 4.0, 4.2, 3.1, 1.1, and 9.6 ppm and oxychlordane at 1.6, 0.76, 0.70, 0.69, 0.67, and 2.3 ppm. Two brains contained *cis*-chlordane up to 1.0 ppm, three contained *trans*-nonachlor up to 2.2 ppm, and one contained *cis*-nonachlor at 0.82 ppm, DDD at 0.70 ppm, and DDT at 0.82 ppm. Three brains contained DDE up to 4.1 ppm. The brain of the bat collected alive contained no residues.

The masticated insects found in the stomach of the live bat contained no residues except 0.83 ppm of *cis*-chlordane. Stomachs of the other bats were empty.

DISCUSSION AND CONCLUSIONS

The lowest brain concentration of dieldrin (5.6 ppm) among the six bats found dead (Table 1) is greater than the lowest lethal level observed in experimentally poisoned laboratory rats, *Rattus norvegicus* (HAYES 1974), dogs, *Canis familiaris* (HARRISON et al. 1963), and shrews, *Blarina brevicauda* (BLUS 1978). The 5.6 ppm also equals or exceeds the lowest level found in brains of cotton rats (*Sigmodon hispidus*) and cottontail rabbits (*Sylvilagus floridanus*) found dead in a dieldrin-treated area (STICKEL et al. 1969). Therefore, we believe the six bats found dead (Table 1) died of dieldrin poisoning.

Four of the six dead gray bats contained brain levels of heptachlor epoxide above 3.4 ppm. This concentration was the minimum lethal level (lethal range, 3.4-8.3 ppm) measured experimentally in birds when chlordane was fed and mortality resulted from the combined effects of heptachlor epoxide, oxychlordane, *trans*-nonachlor, and compounds C and E (STICKEL et al. 1979). Because the brains of these bats also contained oxychlordane and *trans*-nonachlor, the heptachlor epoxide levels above 3.4 ppm may be near lethality.

The contaminants at Hunter and Devil's Icebox caves are the same as those in dead bats recovered at the Franklin County caves (CLARK et al. in manuscript). Aldrin (dieldrin's parent compound) was applied to cornfields to control cutworms (larvae of several moth species, family Noctuidae) until it was banned in 1974. Then heptachlor was recommended as a substitute by the State of Missouri and was used through 1981. We assume aldrin and heptachlor are the sources of the observed residues even though heptachlor epoxide, oxychlordane, *cis*-chlordane, *trans*-nonachlor, and *cis*-nonachlor may result from the use of commercial chlordane.

It is not known why insecticide-induced mortality appeared during these years at Hunter and Devil's Icebox caves. We hypothesize that the bats changed feeding sites such that they were exposed to a heavily contaminated food source. It seems unlikely that applications of aldrin, or aldrin and heptachlor, were recently initiated within the feeding areas of bats from these caves. Devil's Icebox Cave is about 120 km WNW of Bat Cave No. 2, and movements of bats between these areas are extremely rare (LAVAL and LAVAL 1980). Therefore, we believe the sources of contamination for the Boone and Franklin County bats are geographically different and that the problem is of greater extent than previously recognized.

Bat mortality in July 1981 at Devil's Icebox Cave was concurrent with a die-off of macroinvertebrates in the outlet stream. In mid-June the stream appeared normal, but on 9 July 1981 dead invertebrates including amphipods (*Gammarus sp.*), snails (*Physa sp.*), and planaria (*Macrocotyla glandulosa*) were found. On 22 October 1981 (105 days after the die-off was discovered), amphipods were still absent from the outlet stream and could only be found 270 m and beyond within the cave. The planarian also was eliminated from the outlet stream and was severely reduced in the cave. As of May 1982 there was no improvement in these populations.

A sample of dead amphipods collected from the stream on 17 July 1981 and analyzed by the Missouri Department of Conservation contained dieldrin, heptachlor-related compounds, and DDE at concentrations greatly exceeding background levels which had rarely been above detection limits during the previous 15 years. Nevertheless, there is no proof that the pesticide residues killed the invertebrates and other causative agents are possible. Isopods (*Asellus sp.*) in the stream were not affected even though they are 128 times more sensitive to dieldrin than are the amphipods (JOHNSON and FINLEY 1980). It is possible, however, that one of the other residues caused the mortality. Also, because the amphipods were found feeding directly on dead bats, dieldrin may have been more available to them than to the isopods.

What killed the invertebrates remains an open question. However, the residues in the dead amphipods may have been carried into the cave by bats and then entered the aquatic environment when dying bats or bat guano fell or was washed into the stream.

Guano from the Bat Cave No. 2-3 colony in Franklin County where gray bats also died of dieldrin contained dieldrin at 1.1 ppm, heptachlor epoxide at 0.34 ppm, and DDE at 0.40 ppm (CLARK et al. 1982).

If the residues did kill the invertebrates, we have the ironical situation in which present environmental insecticide contamination may hamper future U.S. research efforts in aquatic toxicology.

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