nated in a brief unavoidable shock, was then presented. This led to a reduction in the lick rate from which the suppression ratio could be calculated. Behavioral auditory thresholds were determined by plotting suppression ratios for frequencies of 1, 4, 8, 10, 12, and 16 kHz. This procedure was carried out in a specially designed conditioning box (*4*) placed in a sound-attenuated room; the acoustic field in the conditioning box was measured for the test frequencies at 60 dB (with reference to 0.0002 dyne/cm^2). Frequency discrimination was also determined by plotting suppression ratios for various changes in frequency ($\Delta f/f$) at 1, 4, 8, 10, 12, and 16 kHz, at an intensity of 60 dB.

In presenting the stimuli, the intensity was randomized by the computer over a 10-dB range to prevent the intensity maxima and minima in the auditory field being used as false clues. The cats were then given a series of intramuscular injections of kanamycin (200 mg per kilogram of body weight per day) for 10 days to selectively destroy the outer hair cells, and behavioral thresholds were determined shortly afterward to assess the extent of the hearing loss. Frequency discrimination at 60 dB was then measured, at least 14 days after the cessation of the ototoxic drug, at all the previous frequencies except 16 kHz, where the auditory behavioral threshold was too high. Behavioral thresholds were again determined to ensure that no progressive deterioration in thresholds had occurred. Immediately afterward, the animals were anesthetized, first with pentobarbital sodium (40 mg/kg, injected intraperitoneally) and then with supplementary injections to maintain a satisfactory anesthetic level. The animals were perfused, the cochleas were prepared for surface-preparation histology (*5*), and the inner and outer hair cells were photographed and counted along the length of the cochleas through the use of a differential interference phase contrast microscope. As a result, cochleograms showing the inner and outer hair-cell losses for different frequencies were constructed on the basis of the frequency-to-distance relationship determined from the studies of Schuknecht (*6*) and Kiang *et al.* (*7*).

Table 1 shows the changes in hearing thresholds in decibels following the administration of kanamycin and the percentage of inner and outer hair-cell losses for the test frequencies. Changes in frequency discrimination were evaluated with a two-way analysis of variance. The interaction of the $\Delta f/f$ effect and the predrug and postdrug effect was considered appropriate for assessing changes in frequency discrimination, and their F values are shown (Table 1). The hearing thresholds were increased by from 21 to 51 dB at 8, 10, and 12 kHz. Examination of the surface preparations showed the outer hair-cell loss was complete at the cochlear locations corresponding to 10 and 12 kHz, complete in one animal and partial for two at 8 kHz, and partial for two animals at 4 kHz. On the other hand, inner hair-cell loss was considerably less. Furthermore, at frequencies of 8 and 10 kHz where there was a marked loss of outer but not inner hair cells, there was no significant difference between the frequency discrimination result before and after the ototoxic antibiotic. A significant difference occurred only with the loss of inner hair cells in excess of 50 percent, at a frequency of 12 kHz. The significant result for cat 6 at 1 kHz can be explained from the raw data, which showed an improvement in frequency discrimination after the drug rather than a decrease; this was thought to be a practice effect.

The evidence indicates that frequency discrimination is not affected by the loss of outer hair cells. It is, however, reduced when the loss of inner hair cells is greater than 50 percent. Thus, the inner hair cells are important for frequency discrimination, and they can function normally without needing intact outer hair cells.

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Dieldrin-Induced Mortality in an Endangered Species, the Gray Bat (*Myotis grisescens*)

Abstract. *Brains of juvenile gray bats,* Myotis grisescens, *found dead beneath maternity roosts in two Missouri caves contained lethal concentrations of dieldrin. One colony appeared to be abnormally small, and more dead bats were found a year after the juvenile bats had been collected. This is the first report to link the field mortality of bats directly to insecticide residues acquired through the food chain.*

Insecticides have long been blamed for the decline of bat populations (*1*), but compelling data were lacking. Recently, strong, although indirect, data showed that DDE in the food chain probably has been an important cause of the decline of free-tailed bats (*Tadarida brasiliensis*) at Carlsbad Caverns, New Mexico (*2*). Our study documents mortality and probable population decline in the gray bat (*Myotis grisescens*) resulting from routine insecticide usage.

In August 1973 the U.S. Army Corps of Engineers completed its Environmental Impact Statement for the proposed Meramec Park Lake (MPL), Missouri. Because this statement predicted that the lake would have adverse effects on the habitat of the endangered Indiana bat (*Myotis sodalis*), a survey of all *Myotis* species in the park area was undertaken, sponsored jointly by the Corps of Engineers, the U.S. Fish and Wildlife Service (USFWS), and the Missouri Department of Conservation. This 18-month study began in July 1975. The gray bat, which is abundant in the MPL area, was added to the USFWS's list of endangered species in 1976. Dead gray bats found in three caves during the survey were sent to the Patuxent Wildlife Research Center (PWRC) in an effort to determine whether organochlorine pollutants were the cause of death (*3*).

The precise locations of the caves are confidential; therefore, we will refer to them by numbers assigned by the USFWS. Two of the three caves, caves 048 and 054, are in Franklin County; the third, cave 036, is in Dent County (*4*). These three caves contain the known maternity roosts of the gray bat in the MPL area. All bats found dead were juveniles. Data from another population

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(*5*) indicated that gray bats with forearm lengths of 39.5 mm or less cannot fly. Therefore, most of our bats (Table 1) probably had consumed only milk. Thirteen dead bats were collected at cave 048 on 3 July 1976, six at cave 054 on 18 June and 1 July 1976, and 20 at cave 036 on 15 June 1976. Because several bats were decomposed, only 28 were analyzed. The relative sizes of these maternity colonies are indicated by the maximum estimated number of nonflying young observed in each cave during June through July 1976: 1800 at cave 048, 3600 at cave 054, and 9000 at cave 036.

Dead bats were frozen, shipped to the PWRC, dissected into brain and carcass components as described in (*6*), and analyzed for organochlorine pollutants (*7*). Milk found in the stomachs of two bats from cave 048 was analyzed as one pooled sample.

Tissue residues of dieldrin were generally high at caves 048 and 054 but low at cave 036 (Table 1). Earlier studies (*6*) with other bat species and other organochlorine pollutants showed that residue concentrations in brains [parts per million (ppm), fresh weight basis] were linear functions of a balance between lipid and the same residue in carcasses (ppm, lipid weight basis). The data in Table 1 exhibit a similar relationship (correlation coefficient $r = .63$, $.01 > P > .001$, $N = 22$) (*8*). However, residues are occasionally "missed" by the analytical procedures in samples as small as these brains. Because dieldrin concentrations in the brains of bats 14 and 17 are minute relative to those that would be predicted from concentrations in their carcass fat, we believe that these two brain concentrations are erroneous. When these two data points are eliminated, $r = .83$ ($P < .001$, $N = 20$). The concentrations of other toxic compounds in tissues were small relative to concentrations that are known or suspected to be toxic (*9*). Even though the stomachs of all bats except bats 15 and 18 were empty—this milk contained 36 ppm of dieldrin (*10*)—fat reserves had not been exhausted (*11*).

In a controlled experiment with dogs (*Canis familiaris*), brain concentrations of dieldrin that were diagnostic of death ranged from 2.4 to 9.4 ppm (*12*). Brains of laboratory rats (*Rattus norvegicus*) killed by dieldrin contained 2.1 to 10.8 ppm of dieldrin (*13*). Dieldrin concentrations of 5.6 to 11.1 ppm were found in brains of cotton rats (*Sigmodon hispidus*) and 8.4 to 19.1 ppm in brains of cottontail rabbits (*Sylvilagus floridanus*) found dead in a dieldrin-treated area (*14*). On the basis of these results, we believe that some of the dead gray bats died of dieldrin poisoning at caves 048 and 054 (Table 1). At cave 048 bats 19 and 20 probably died of other causes, bats 14 and 17 were killed by dieldrin but brain concentrations were underestimated, and the remaining eight bats had lethal brain concentrations of dieldrin (Table 1). At cave 054 bats 22, 23, and 25 also had lethal concentrations of dieldrin (Table 1), but bat 26 died of unknown causes. The mortality at cave 036 was not related to dieldrin (Table 1); perhaps this roost was disturbed by a person or by some other potential predator when the adult bats were present, and the young were dropped by their escaping parents. Mortality caused by such a series of events has been observed at other roosts (*5*).

The route that dieldrin followed through the food chain to the pregnant or lactating adult gray bats is unknown. However, in June 1967 dieldrin in ground beetles (*Poecilus chalcites*) from two Missouri cornfields treated with dieldrin's parent compound, aldrin, reached an average 36.2 ppm. [Aldrin had been applied to control cutworms which are the larvae of several moth species, family Noctuidae (*15*).] We believe that a similar accumulation must have occurred in small moths (perhaps adult cutworms) or other small flying insects that develop in the soil and that one or more such species is an important prey item of the gray bats. A few parts per million of dieldrin in such prey could, after concentration through lactation, cause the observed mortality. Because pregnant bats arrive at these caves only days or hours before parturition, some of the dieldrin transferred to their young may have come from other localities. However, we believe that most of the dieldrin was acquired locally because of the apparent correspondence between mortality and nearby agriculture and because all foraging by lactating females occurred within several kilometers up- and downstream of these caves.

Although we are unable to estimate the overall impact of dieldrin on gray bat populations in the MPL area, 74 additional dead gray bats were found when cave 048 was checked on 8 July 1977. Furthermore, the colony at cave 048 was unusually small in 1976 but it was still smaller in 1977. These observations suggest heavy long-term and continuing

Table 1. Dieldrin residues from 28 juvenile gray bats found dead in three Missouri caves in 1976. ND, not detected.

Bat number	Sex	Forearm length (mm)	Dieldrin residues		
			Brain	Carcass	
			Fresh weight basis (ppm)	Lipid weight basis (ppm)	Fresh weight basis (ppm)
			Cave 048 (N = 12)		
8	M	41.1	10	948	69
15	M	38.2	9.8	738	77
11	M	36.3	9.4	950	41
13	M	32.4	8.4	239	14
18	F	28.0	7.2	378	13
12	M	21.4	5.9	800	18
10	M	32.3	5.9	578	33
9	M	33.8	5.0	580	52
20	M	33.3	0.76	200	13
17	M	38.7	0.48	800	86
14	F	41.9	0.40	1050	92
19	M	21.6	ND	200	5.1
			Cave 054 (N = 4)		
22	F	41.3	8.4	1379	100
23	F	38.7	5.1	300	4.3
25	M	31.4	2.7	167	2.3
26	M	29.0	0.63	18	0.36
			Cave 036 (N = 12)		
30	M	17.1	0.61	1.7	0.14
31	F	24.9	0.59	20	0.96
28	M	24.6	0.58	43	1.3
29	M	27.2	ND	9.6	0.26
32	M	19.1	ND	ND	ND
33	M	18.2	ND	ND	ND
34	M	20.1	ND	ND	ND
36	M	25.9	ND	17	0.67
37	M	17.1	ND	ND	ND
39	F	13.6	ND	ND	ND
41	F	16.0	ND	ND	ND
42	F	25.1	ND	11	0.45

exposure to dieldrin. Additional mortality may occur away from roosts under conditions that cause rapid fat utilization and, consequently, residue mobilization (*2*). Such conditions include migratory stress (*16*) and initiation of flight by juveniles (*17*). Dieldrin mortality may also be common in other parts of the species' range where row crop agriculture is extensive, but it may be undetected because many maternity roosts are located over water where dead bats probably disappear rapidly.

Populations of the Indiana bat in the MPL area also may have experienced mortality caused by dieldrin. However, because maternity roosts are probably small and located in trees (only two have ever been found) (*18*), the impact of dieldrin may never be known. Populations of the Indiana bat in the MPL area declined an estimated 48 percent between March 1975 and March 1976, but human disturbance of caves was thought responsible (*19*).

Even though aldrin and dieldrin were banned by the U.S. Environmental Protection Agency effective 1 October 1974, the sale and use of existing stocks remains legal. Therefore, we do not know when the use of aldrin will cease, nor do we know how long dieldrin will persist in prey insects once the use of aldrin has stopped. Soil residues of dieldrin showed no significant reduction 6 years after application of aldrin on Missouri cornfields (*20*). We urge that the potential threat to these bats posed by the chemicals now being substituted for aldrin (heptachlor and toxaphene in Missouri) be considered before their use becomes widespread.

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3. Bats were recovered under authority of the Federal Endangered Species Permit PRT-8-31-C.
4. Cave 048, situated in a bluff just above the Bourbeuse River floodplain, was dry; its mouth was less than 100 m from a field, and most of the nearby floodplain was cultivated to row crops. Riparian forest was narrow or absent. The bats foraged up- and downstream over the river adjacent to the cultivated fields. Cave 054 was adjacent to the Meramec River and contained a large spring which emerged from the cave but did not pass beneath the bat roost. Fields of row crops were directly across the river from the cave. On 1 July 1976, bats from cave 054 were seen foraging over the river as far as 13 km downstream near cultivated fields and as far as 17 km upstream near forest [R. K. LaVal, R. L. Clawson, M. L. LaVal, W. Caire, *J. Mammal.* **58**, 592 (1977)]. Cave 036 was a dry cave situated in a bluff above the Meramec River. Nearby was a large pasture but no row crops. Most of the narrow floodplain both up- and downstream from the cave was densely forested, although some row crops were present.
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7. The brain and carcass of each individual 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's). The PCB that was recovered resembled Aroclor 1260 in all cases. Hexane extracts of samples were cleaned by Florisil column chromatography, and the eluate containing the pesticides and PCB's was fractionated by Silicar column chromatography [E. Cromartie, W. L. Reichel, L. N. Locke, A. A. Belisle, T. E. Kaiser, T. G. Lamont, B. M. Mulhern, R. M. Prouty, D. M. Swineford, *Pestic. Monit. J.* **9**, 11 (1975)]. Fractions were analyzed with a gas-liquid chromatograph (Hewlett-Packard 5753) equipped with a ^{63}Ni detector in a column containing OV-17 with 1.95 percent QF-1 at 195°C. The gas flow rate in the column was 60 ml/min; 5 percent methane in argon was used, with a purge rate of 40 ml/min. Average percentage recoveries from spiked tissues ranged from 89 to 104 except for HCB, which was 80. Residue data were not adjusted on the basis of these recoveries. All residues are reported as parts per million by weight of fresh (wet) tissue unless noted otherwise. The lower limit of sensitivity was 0.1 ppm for carcasses and 0.5 ppm for brains and milk. Residues in 10 percent of the samples were confirmed on a gas chromatograph–mass spectrometer (LKB 9000) with operating conditions as described in Cromartie *et al.*
8. The six bats without measurable dieldrin in their carcasses were excluded from this analysis.
9. Brains of bats from cave 048 contained as much as 4.9 ppm DDE, 1.5 ppm heptachlor epoxide, and 1.4 ppm oxychlordane. Brains of bats from cave 054 contained up to 3.0 ppm DDE, 1.8 ppm heptachlor epoxide, 2.7 ppm oxychlordane, 3.9 ppm *trans*-nonachlor, and 13 ppm PCB's. Brains of bats from cave 036 contained up to 2.9 ppm DDE, 4.8 ppm heptachlor epoxide, and 3.3 ppm oxychlordane.
10. This milk also contained 4.1 ppm heptachlor epoxide, 0.58 ppm oxychlordane, 0.90 ppm *cis*-chlordane, and 1.4 ppm *trans*-nonachlor.
11. Means and standard errors for the percentages of carcass as lipid were cave 048, 6.22 ± 0.82; cave 054, 3.02 ± 1.43; and cave 036, 4.34 ± 0.44.
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Dual Actions of Substance P on Nociception: Possible Role of Endogenous Opioids

Abstract. *Substance P produces analgesia when administered to mice in very small doses by the intraventricular route (1.25 to 5 nanograms per mouse). The analgesic effect can be blocked by naloxone. At higher doses (greater than 50 nanograms per mouse), this activity is lost. At these higher doses, however, substance P produced hyperalgesia when combined with naloxone and analgesia when combined with baclofen [β-(4-chlorophenyl)-gamma-aminobutyric acid]. Substance P may have dual actions in brain, releasing endorphins at very low doses and directly exciting neuronal activity in nociceptive pathways at higher doses.*

Substance P (SP), the undecapeptide which was discovered in brain and intestine by Von Euler and Gaddum (*1*) almost five decades ago, has been suggested to be a transmitter utilized by primary sensory afferents (*2–4*). Otsuka and colleagues (*4*) reported that the hexapeptide fragment of SP, pyro-Glu-Phe-Phe-Gly-Leu-Met-NH_2 (SP^{6-11}; < Glu, pyrrolidone carboxylic acid; Phe, phenylalanine; Gly, glycine; Leu, leucine; Met, methionine) is more active in spinal cord than SP itself. Hughes (*5*) originally reported a tentative amino acid content of the endogenous opioid peptides which was very similar to the amino acid composition of SP^{6-11}, and this prompted us to question whether this fragment of SP might be Hughes' peptide or a very closely related substance. Indeed, we observed that SP administration into the lateral ventricles of mouse had an analgesic effect antagonizable by naloxone as has been reported also by Stewart and colleagues (*6*). Our studies with the mouse vas deferens preparation, however, showed that neither SP nor its hexapeptide fragment, SP^{6-11}, had any direct action on opiate receptors. We describe these experiments here and provide evidence that SP has dual effects on pain perception and may interact with enkephalins in controlling nociceptive processes.

Substance P was tested for analgesic activity by the mouse hot-plate procedure (*7*), with the plate maintained at 52°C. Cox standard mice (20 to 23 g) were used in these studies. The test utilized an apparatus with an electrically heated, thermostatically controlled metal plate (Technilab Instruments, model 475 analgesiometer). A Plexiglas cylinder, 12 inches (1 inch = 2.54 cm) high, 4.75 inches in inner diameter, and open

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