# **DDT Residues In Snakes Decline Since DDT Ban**

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The use of DDT in the U. S. peaked in the late 1950s and declined thereafter, due both to its properties as an environmental contaminant and to the development of resistance by insect pests. Its use in domestic agriculture was banned in 1972. There is considerable interest in the persistence of DDT in the ecosystem. We report here on the decline in residues in a cotton agroecosystem 2-3 years after the DDT ban. Aquatic snakes were the organisms assayed.

Snakes are good indicators of ecosystem contamination by environmentally persistent insecticides. In addition to being ubiquitous, individuals have very limited home ranges, habitat preferences are either terrestrial or aquatic, and feeding habits permit ready determination of relative trophic levels.

Recently snakes were used as indicators in studies of radiocesium contamination from a nuclear reaction reactor (BRISBIN et al., 1974) and measurements of lead and insecticide loads in a relatively uncontaminated ecosystem (BAUERLE et al., 1975). A previous study from this laboratory (FLEET et al., 1972) utilized snakes to compare insecticide contamination of two Texas agro-ecosystems; one a cotton growing area where insecticides were used heavily in the 1960's, and the other a grazing area where little insecticide had been used. In the fat of five snake species, total residues of DDT and dieldrin were 14 to 386 times greater in snakes from the area of heavy insecticide use. At similar trophic levels, water feeding snakes had higher residues than terrestrial feeders. Species of higher trophic levels had higher residues than species of lower trophic levels. Numbers were greater, but there were fewer species in the area of heavy insecticide use. Oviparous species, once present in the area as shown by specimens in the University Collection, were almost totally absent.

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In an attempt to find a biochemical basis for the differences in species diversity, STAFFORD et al. (1977) measured levels of NADPH-dependent microsomal oxidases in two species of Agkistrodon (cottonmouths and copperheads) and three species of Natrix (water snakes). Microsomal oxidases are important in animals in regulating steroid levels and in detoxifying xenobiotics (CONNEY & BURNS 1975). Oxidase activity was highest in cottonmouths, the top level predator in aquatic environments and lower in Natrix and the more terrestrial copperhead. N. rhombifera, the one species absent from the area of heavy insecticide use had the lowest level of oxidase activity. It was concluded that snake species with high levels of oxidative detoxifying enzymes are best able to survive in contaminated ecosystems.

### MATERIALS AND METHODS

# Collection of specimens

Snakes were collected from the cotton agro-ecosystem previously studied by FLEET et al. (1972). The study area is riparian woodland within 50 m of former river channels and sloughs on the flood plain of the Brazos River situated 24 Km SW of College Station, Texas in Burleson Co. It is adjacent to cropland, which, from the late 1940's through the 1960's, was sprayed repeatedly with DDT and other insecticides to control cotton insects. According to local entomologists, the major insecticide treatment used in 1971 was a 4:2:1 combination of toxaphene:DDT:methyl parathion. In 1974-5, toxaphene:methyl parathion at a 2:1 ratio was most heavily used.

Between 31 March 1974 and 18 April 1975, 39 man-hours were spent collecting and observing snakes. Observation-collection techniques were the same as those utilized previously. Snakes were frozen alive upon capture and autopsied later. Length, weight and sex of each individual was recorded, stomach contents identified, and fat bodies excised and weighed. All residue analyses were performed on fat body tissue except for shelled eggs from Coluber constrictor and mature ova from Storeria dekayi.

## Residue analysis

Residues were determined with a Micro-Tek Model 120 Gas Chromatograph using a Ni-63 detector, according to standard methods (FDA, 1968) with minor modifications. Confirmation of residue peaks was by comparison with retention times of known materials. All laboratory procedures were identical to those used previously. Residues in parts per million are based on wet weight of tissue samples.

#### RESULTS

# Collection of specimens

Data on the 76 individual snakes of 11 species observed or collected in 1974-5 are presented in Table 1. For purposes of comparison, collection data from the 1971 study, prior to the DDT ban, are included. Six of 76 individuals (4 of 11 species) were oviparous in 1974-5 where only 1 of 71 individuals collected in 1971 was oviparous and that one was represented only by a shed skin. The Chi-square for the 2 ratios of egglayers to livebearers (1 and 71 vs. 6 and 70) yielded a value of 3.47 (.06 < PC .07).

TABLE 1

Numbers of snakes identified during 1971 and 1974-5 observation periods. Oviparous species indicated by asterisks.

Species	1971	1974-5
Natrix erythrogaster (Plain-bellied water snake	14	16
Natrix fasciata (Banded water snake)	9	22
Regina grahami (Graham's water snake)	2	1
Thamnophis proximus (Ribbon snake)	7	1
Storeria dekayi (Brown snake)	7	1
Agkistrodon contortrix (Copperhead)	3	5
Agkistrodon piscivorus (Cottonmouth)	29	24
Lampropeltis getulus (Common king snake*)	_	1
Elaphe obsoleta (Rat snake*)	1	1
Opheodrys aestivus (Rough green snake*)	_	2
Coluber constrictor (Racer*)	_	2

#### Insecticide residues

Tissues of 24 individuals of 10 species collected in 1974-5 were analyzed for insecticide residues (Table 2). Organochlorines, primarily p,p' DDT and its major metabolite, p,p' DDE, were found in every sample. In 1971 all samples contained dieldrin residues, the maximum concentration being 13.8 ppm. In 1974-5 only 1 sample contained as much as a trace of dieldrin. Approximately 65 percent of 1971 samples contained p,p' DDD residues in concentrations up to 7.3 ppm; in 1974-5 this DDT metabolite was absent.

For 3 species, all aquatic, 3 or more individuals were sampled in both years. In these, ratios of DDE/DDT in  $\underline{N}$ . erythrogaster and  $\underline{A}$ . piscivorus more than doubled from  $\underline{1971}$  to  $\underline{1974-5}$ ; in  $\underline{N}$ . fasciata the ratio decreased slightly (Table 2).

TABLE 2.

Insecticide residues in snakes collected in 1974-5, and comparative DDE/DDT ratios in 1971 and 1974-5. Analyses were made on fat bodies unless otherwise noted and ppm are based on wet weight of tissue samples. Trace (+) equals residues of less than 0.1 ppm. Oviparous species indicated by asterisks.

				, 	
				Rat	
		DDE	DDT		'DDT
Species	Sex	(ppm)	(ppm)	1971	1974-5
Natrix erythrogaster	М	204.6	4.6		
	F	73.9	1.3		
	F	75.4	1.2		
	F	361.6	4.2		
	Mean	178.9	2.8	25	63
Natrix fasciata	M	172.5	6.1		
	M	364.0	8.2		
	F	148.1	5.4		
	F	161.7	8.2		
	Mean	211.6	7.0	38	29
Regina grahami	F	271.3	2.8	$170^{\mathrm{a}}_{\mathtt{h}}$	97
Thamnophis proximus	M	129.5	4.1	43 <sup>b</sup>	32
Storeria dekayi (ova)	F	6.2	0.8	6 <sup>a</sup>	8
Agkistrodon contortrix	M	35.9	1.2		
	М	271.7	1.3		
	F	108.7	0.2		
	F	39.4	0.3		
	Mean	113.9	0.8	81 <sup>c</sup>	229
Agkistrodon piscivorus	М	388.8	1.8		-
<u> </u>	F	162.3	0.8		
	F	158.1	0.6		
	F	155.5	0.7		
	Mean	216.2	1.0	98	217
Lampropeltis getulus*	F	596.6	14.6		41
Opheodrys aestivus*	M	51.2	+		
	F	6.9	0.5		14
	Mean	29.0			<del>-</del>
Coluber constrictor*	M	192.8	11.7		
	F	130.0	7.4		
	Mean	161.4	9.6		17
(eggs)	iicaii	24.0	1.0		24

a 1 individual. b 3 individuals. c 2 individuals.

An increase in DDE/DDT ratio would logically result from cessation of DDT input into the ecosystem. Mean 1974-5 residues of DDTs for these species declined 51.5, 64.4, and 62.2 percent for  $\underline{\mathbf{N}}$ . erythrogaster, A. piscivorus, and N. fasciata, respectively.

Average 1974-5 residues for the three species were 182, 217, and 219 ppm as compared with 368, 575, and 614 ppm in 1971.

Changes in total DDT residues from 1971 to 1974-5 were inconclusive in the four species for which fewer samples were available at one or both collection dates. In  $\underline{\mathbf{T}}$ ,  $\underline{\mathbf{proximus}}$  and  $\underline{\mathbf{S}}$ .  $\underline{\mathbf{dekayi}}$  residues declined from 1971 to 1974-5, while in  $\underline{\mathbf{R}}$ .  $\underline{\mathbf{grahami}}$  and  $\underline{\mathbf{A}}$ .  $\underline{\mathbf{contortrix}}$  they increased.

Residue levels were usually lower in females than in males. Females may eliminate significant insecticide residues by transfer to their offspring (see <u>C</u>. <u>constrictor</u> in Table 2 and STAFFORD et al., 1977).

Generally the pattern of residues in the 1974-5 samples was similar to that found in 1971. Livebearing species living near or taking their food from the water typically had higher residues than more terrestrial species. The high position of  $\underline{A}$ . piscivorus within the food chain (two stomachs contained other snakes) was confirmed by their high DDE/DDT ratio.

Residue levels in 4 of 5 oviparous individuals analyzed were higher than would have been expected on a basis of habitat or trophic level (WRIGHT & WRIGHT, 1957). The exception, a female <u>Opheodrys</u> <u>aestivus</u>, may have reduced her body burden via an egg clutch as did the female <u>Coluber constrictor</u> (Table 2). The generally higher levels of residues in egg layers suggests that as a group they may have less efficient mechanisms (lower microsomal oxidase levels) for eliminating toxicants than viviparous snakes.

### DISCUSSION

The overall decline in residues from 1971 to 1974-5 and the increase in numbers and species of egglaying snakes in the area adjacent to the cotton ecosystem are the major findings of this study. While changes in environmental conditions or other factors may be involved, it is probable that the cessation of DDT use is the major change that occurred between 1971 and 1974-5. The 50% decline in residues in 3 years represents evidence for fairly rapid ecosystem recovery and is in agreement with the results of other investigators (JOHNSON, 1974; NICKERSON & BARBEHEN, 1975; ANDERSON et al., 1975) who studied DDT residues in birds over roughly the same time span. On the other hand, the high residue levels still present in snakes indicate that complete cleaning of the ecosystem will not occur for many years.

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