PESTICIDE RESIDUES IN SELECTED TISSUES OF THE WHITE-TAILED DEER, *ODOCOILEUS VIRGINIANUS,* IN CALHOUN COUNTY, SOUTH CAROLINA[1]

By MARTIN J. BARRIER[2], JOHN K. REED, and LLOYD G. WEBB
Department of Entomology and Economic Zoology
Clemson University
Clemson, South Carolina

ABSTRACT

Pesticide residues were measured in selected tissues of the white-tailed deer, *Odocoileus virginianus,* from a leading cotton and soybean producing area in Calhoun County, South Carolina. A minimum of four deer per month were collected from March, 1968, to February, 1969. Nine additional deer were collected from the same area in August and November, 1969. Five deer from an area where pesticides had not been used were included as a control. Samples of fat, brain, liver, kidney, loin muscle, rump muscle, feces and rumen contents were lyophilized, extracted with hexane, then analyzed by gas-liquid chromatography.

Only DDT and metabolites were detected. Two residue peaks were evident, one in early spring, and another corresponding to the spray season in late summer. The highest mean residues (1.76 ppm) were found in fat, followed by brain, feces, rumen contents, liver, loin muscle, kidney, and rump muscle in that order. The highest total DDT residue (6.67 ppm) recovered in the fat of a deer collected at the height of the spray season did not exceed the maximum tolerance limit (7.0 ppm) presently established for beef by the FDA.

The p p′ DDT isomer was the most common residue detected in all tissues. Substantial p p′ DDD residues were recovered in the liver, feces, and rumen contents, but low levels of DDE and o p′ DDT were found in all tissues. There was no evidence suggesting that bioaccumulation of DDT occurred from one year to the next. Higher residues were found with increasing age and weight of the deer, but no difference in residues was detected between males and females.

Organochlorine pesticide residues, particularly DDT and metabolites are ubiquitous contaminants of our environment. The agricultural use of pesticides has contributed to the overall level of contamination; however, the production of many agricultural commodities would be seriously impaired without the use of pesticides. Effective control of cotton insects with DDT in combination with other insecticides has been a major factor in the success of cotton in the south. In many sections large populations of white-tailed deer, *Odocoileus virginianus*, are found within major cotton producing areas. Since deer often feed on crops or in areas sprayed with pesticides a knowledge of the effects of pesticide residues to deer is essential for proper management. This study was undertaken to measure and evaluate the levels of organochlorine pesticide residues in white-tailed deer in Calhoun County, South Carolina, a leading cotton and soybean producing area which also maintains a substantial deer population.

METHODS

The study was conducted from March, 1968, through February, 1969. A minimum of four deer each month for the one period was obtained for residue

Sponsored jointly by the Department of Entomology and Economic Zoology, Clemson University, and the South Carolina Wildlife Resources Department, Columbia, South Carolina.

[2] Present address: Department of Biology, Baptist College at Charleston, Charleston, South Carolina 29411.

analysis. Nine additional deer were taken in August and November, 1969, for comparisons with the preceding year. Samples of fat, brain, liver, kidney, loin muscle, rump muscle, rumen contents, and feces were obtained from each deer collected. These samples were frozen and maintained in this condition until extractions were made for organochlorine residue analysis.

Each sample was lyophilized, weighed, ground with a mortar and pestle or an electric mixer, and extracted with hexane according to the procedure given by de Faubert Maunder et al. (1964). Each sample extract was passed through a column containing Florisil® to remove impurities. Fat and brain samples were cleaned by an additional procedure using acetonitrile — hexane partitions to remove fats and oils.

Several samples of each tissue were fortified prior to processing with 1.0 ppm each of p p′ DDT, o p′ DDT, p p′DDD, and p p′ DDE. Recovery of the added pesticides in the non-fatty tissues were as follows:

	Mean	Std. Error
p p′ DDT	82%	4.43
o p′ DDT	81%	3.87
p p′ DDD	78%	5.22
p p′ DDE	89%	3.32

Recovery of the added pesticides in the fatty tissues (fat and brain samples) were as follows:

	Mean	Std. Error
p p′ DDT	73%	3.29
o p′ DDT	72%	3.03
p p′ DDD	67%	4.82
p p′ DDE	81%	3.62

Following clean-up procedures each sample extract was stored in a clean glass container for analysis by gas-liquid chromatography. A Micro-Tek model 2000 MF gas chromatograph equipped with a Ni-63 electron capture detector was utilized. The operating parameters of the instrument were as follows:

1. Detector Temperature: 275 C
2. Injection Port Temperature: 250 C
3. Column Temperature: 220 C
4. Columns: 6 feet by ¼-inch glass and 4 feet by ¼-inch glass
5. Column Packing: 10% DC-200 on 80/100 mesh Gas Chrom Q, 1.5% OV-17 and 1.95% QF-1 on Gas Chrom Q, and 2.0% OV-101 and 3.0% QF-1 on Gas Chrom Q.
6. Carrier Gas: High Purity Nitrogen

Two aliquots of each sample extract were injected into the gas chromatograph. All finite values were averages of the two injections.

Water samples from two streams, and soil samples representing a variety of

croplands were collected during the year and analyzed for pesticide residues. Water samples were collected each month and soil samples were collected twice during the study.

A method of least-squares analysis of variance with unequal subclass numbers (Harvey, 1960) was employed in the development of the empirical model used to evaluate the data. A multiple covariance full model was developed as follows:

$$y_{ij} = u + a_i + b_1 (X_{1j}-\overline{x1}) + \ldots b_k (X_{kj} - \overline{x_k}) + e_{ij}$$

where: y_{ij} = the j^{th} observation in the i^{th} tissue,

u = the overall mean for the y_{ij} values,

a_i = the effect of the i^{th} tissue,

$b(1...k)$ = the partial regression of the dependent variable (y) on the independent variable (X), when the discrete variable (a_i) is held constant,

X = the continuous independent variable for the y_{ij}

$\overline{x}$ = the arithmetic mean of the X values,

e_{ij} = the random errors, which are assumed to be independent and normally distributed.

The independent variables considered in the full model include the following: sex, age, weight, condition, food habits of the deer collected, month, mean monthly temperature, mean monthly rainfall, mean monthly residues in two streams located in the study area, and the season of pesticide application. An indication of the effect of each variable was found by using the "step-down" regression method with correlation matrices. At each stage of the step-down procedure variables whose contributions to the least-squares equation were not significant, were examined. The variable with the least contribution, as determined by a partial F-test, was removed from the equation. This sequential procedure was terminated when all remaining sources of variation in the empirical equation were found to be statistically significant. The final model was a one-way classification equation with one covariate as follows:

$$y_{ij} = u + a_i + b_1 (X_{1j}-\overline{x_1}) + e_{ij}$$

where: y_{ij} = the j^{th} observation in the i^{th} tissue,

u = the overall mean for the y_{ij} values,

a_1 = the effect of the i^{th} tissue,

b_1 = the partial regression of the dependent variable (y) on the independent variable (X),

X_{1j} = the continuous independent variable for the corresponding y_{ij} observation (X_{1j} 1 = weight covariate),

$\overline{x}$ = the arithmetic mean of the X values,

e_{ij} = the random errors, which are assumed to be independent and normally distributed.

Duncan's multiple range test was used to evaluate significant differences between tissues and between months for DDT residues. (Duncan, 1955).

RESULTS

A total of 37 male and 17 female deer were collected from March, 1968, through February, 1969. The sex ratio was biased since predominately males were collected during the hunting season (August through December). An estimated age of each deer was based on the sequence of eruption and wear of teeth (Mosby, 1963). The age of deer obtained in this study ranged from 4.0 months to 6.5 years, averaging approximately two years. Approximately 50 per cent of the deer were adults (over 18 months), 45 per cent yearlings (6-18 months) and 5.0 per cent were fawns (less than 6 months). Food habit analyses of the rumen contents indicated a substantial proportion of the total diet was composed of farm crops. Identified were spring wheat (February and March), soybean leaves (spring and summer) and soybean beans (fall and winter).

Residues Detected. Gas chromatographic detection of p p′ DDT, o p′ DDT, p p′ DDD, and p p′ DDE was accomplished. Traces of other organochlorines, including lindane, heptachlor, dieldrin and aldrin, were suspected in several samples but were not verified.

Significantly higher residues (.05 per cent level) were found in fat than in any of othe other tissues. (Table I). DDT residues in fat ranged from 0.22 to 6.67 ppm and averaged 1.76 ppm for the entire year. Residues in brain averaged 1.04 ppm and were significantly higher than in any other tissue except fat. Lowest residues averaging less than 0.4 ppm were found in muscle tissue and the kidney. Mean, minimum, and maximum DDT residues and the proportion of each component of total DDT are given for the eight tissues analyzed (Table II). On a percentage basis p p′ DDT was the highest residue detected ranging from 47 per cent in the liver to 82 per cent in the brain. DDD ranged from 8.0 per cent in the brain to 37 per cent in the liver, while DDE ranged from 8.0 per cent in the brain to 17 per cent in the rumen contents and feces. The o p′ DDT isomer made up less than 10 per cent of total DDT in all tissues examined.

Monthly Comparisons. Mean monthly variations in total DDT residues are graphically depicted for the eight tissues in Figures 1 - 3. Figures 4 and 5 illustrate the monthly variations in the different components of DDT in fat and liver, respectively. In general, DDT residues were higher during or immediately after the spray season which extends from July to mid-September in the study area. A secondary peak of residues was evident for the early spring months (Figures 1-3). The different components of total DDT generally varied directly with the p p′ DDT isomer. An exception was DDD in the liver which was the major form of DDT in the fall and winter (Figure 5).

Fat and liver samples from nine deer, two collected in August and seven in November, 1969, were analyzed and the results compared statistically to those of August and November of the previous year (Table III). No significant difference in DDT residues between months of consecutive years was found in these tissues.

Fat and kidney samples from five deer collected in December, 1968, from a wildlife management area in Pisgah National Forest in western North Carolina were analyzed for pesticide residues, and the results were compared to those of the study area for the same month (Table IV). There was no record of pesticide use in this area of the National Forest. Significantly more p p′ DDT, DDD, and DDE residues were recovered in fat of deer from the study area. However, no significant differences were evident between the two areas for residues in the kidney.

The correlations between individual or environmental variables and residues in the deer tissues were mostly insignificant. A small correlation was evident between higher residues in deer as age or weight increased. The levels of DDT

Table I. Significant differences at the 0.05 per cent level of pesticide residues in tissues of the white-tailed deer, as determined by Duncan's multiple range test.

Total DDT

Treatment	Tissue mean	Treatment mean				
fat	1.76	1.04	a*			
brain	1.04	0.34		b		
feces	0.72	0.01			c	
rumen contents	0.70	-0.01			c	
liver	0.40	-0.31				d
loin muscle	0.38	-0.33				d
kidney	0.35	-0.36				d
rump muscle	0.32	-0.39				d

p p' DDT

Treatment	Tissue mean	Treatment mean				
fat	1.15	0.69	a			
brain	0.84	0.38		b		
feces	0.43	-0.03			c	
rumen contents	0.39	-0.07			c	
loin muscle	-.26	-0.20			c	
kidney	0.23	-0.23			c	
rump muscle	0.20	-0.26			c	
liver	0.19	-0.28			c	

o p' DDT

Treatment	Tissue mean	Treatment mean				
rumen contents	0.08	0.04	a			
fat	0.08	0.04	a			
feces	0.05	0.03	a	b		
loin muscle	0.03	-0.01		b	c	
rump muscle	0.03	-0.01		b	c	
kidney	0.02	-0.02			c	
brain	0.02	-0.02			c	
liver	0.02	-0.02			c	

DDD

Treatment	Tissue mean	Treatment mean				
fat	0.31	0.19	a			
liver	0.15	0.03		b		
feces	0.12	0.00		b	c	
rumen contents	0.12	0.00		b	c	
brain	0.09	-0.03		b	c	
loin muscle	0.05	-0.06			c	
kidney	0.05	-0.07			c	
rump muscle	0.04	-0.08			c	

DDE

Treatment	Tissue mean	Treatment mean				
fat	0.22	0.13	a			
feces	0.12	0.02		b		
rumen contents	0.11	0.02		b		
brain	0.10	0.01		b	c	
rump muscle	0.05	-0.04			c	d
liver	0.05	-0.04			c	d
kidney	0.05	-0.04			c	d
loin muscle	0.04	-0.05				d

*means followed by the same lower case letters are not significantly different.

Table II. Mean, minimum, and maximum DDT residues (ppm) and the proportion of each component of total DDT recovered in selected tissues of the white-tailed deer collected over a one-year period from March, 1968, to February, 1969.

Tissue	mean	minimum	maximum	Proportion of p p′ DDT	Proportion of o p′ DDT	Proportion of DDD	Proportion of DDE
fat	1.76	0.22	6.67	0.67	0.04	0.17	0.12
brains	1.04	tr*	5.32	0.82	0.02	0.08	0.08
feces	0.72	tr	3.46	0.57	0.08	0.18	0.17
rumen contents	0.70	tr	2.06	0.55	0.10	0.18	0.17
liver	0.40	tr	2.19	0.47	0.04	0.37	0.12
loin muscle	0.38	tr	2.11	0.70	0.07	0.13	0.10
kidney	0.35	tr	1.31	0.67	0.05	0.14	0.14
rump muscle	0.32	tr	1.49	0.62	0.09	0.13	0.16

*tr = trace

residues were not significantly different between male and female deer.

No significant concentrations of residues were detected in the water samples from the two streams within the study area. The principle residue in the water samples was DDE. Analysis of the soil samples showed only insignificant concentrations of DDT, DDD, and DDE.

DISCUSSION

Pesticide residues represented in this study are in general agreement with studies in deer from other areas. Pillmore and Finley (1963) reported DDT residues up to 36.5 ppm and averaging 3.0 ppm in mule deer, *Odocoileus hemionus*, immediately after forested area was sprayed at the rate of one pound DDT per acre. Pillmore (1961) found DDT residues in mule deer up to 24 ppm after one month and up to 19 ppm two months after the area was sprayed with DDT. Finley (1960) detected DDT from 6.0 to 9.0 ppm in fat and an average of 1.0 ppm in lean meat in mule deer following the application of a pound of DDT per acre. Jewell (1966) and Stiekel (1968) found DDT residues in mule deer averaging less than 3.0 ppm during the year following application of DDT. The average of 0.14 ppm of DDT in the fat of deer collected in Pisgah National Forest is in agreement with Walker et. al. (1965) and Greenwood (1967) who found an average of 0.1 ppm and 0.2 ppm of DDT, respectively, in big game animals collected in areas not recently treated with DDT.

An increase in DDT residues during and soon after the spray season was evident. A gradual decrease in residues during the winter (except for December) should be expected as residues are metabolized and excreted. The high December residues was thought to be related to the high percentage of soybean seeds consumed by deer collected this month. Translocation of organochlorine pesticides from the soil into soybean seeds has been demonstrated (Newsom, 1967). A lower but consistent peak of DDT residues was evident in most tissues from deer collected in the early spring months (Figures 1-3). A possible explanation for the spring peak is translocation of DDT from the soil into new spring growth which may be consumed by deer. Translocation of organochlorine pesticides from soil into plants has been demonstrated (Newsom, 1967; Barrentine and Cain, 1969; Reed and Priester, 1969). The higher residues in the rumen contents (Figure 3) during the spring months indicated a greater consumption of DDT. A food habit study of deer at this time showed a consumption of spring wheat which may have been planted in fields previously sprayed with DDT. Another possible mechanism resulting in higher residues in the spring is utilization of fat reserves by deer at the time actual foods are limited. Residues formerly stored in fat would be released and enter other tissues as well as the remaining fat deposits, resulting in a concentration of residues.

The highest total DDT residue for all deer analyzed was 6.7 ppm in fat. Thus, the tolerance limit of 7.0 ppm set on beef by the Food and Drug Administration (USDA, 1969) was not exceeded. It is unlikely that the low DDT residues found in deer of the study area represent potential hazards to deer or to man in the consumption of venison. Toxicity studies in deer and related animals indicated that much higher residues than are reported here are necessary to induce symptoms of DDT poisoning. Pillmore and Finley, (1963) reported that 500 ppm of DDT was acutely toxic to a mule deer. However, 15.2 grams of DDT given daily in the feed to another mule deer resulted in no visible symptoms of poisoning after 67 days. Orr and Mott (1945) and Spicer et. al. (1947) found considerable variation in susceptibility to DDT poisoning in domestic livestock, although none were visibly susceptible to doses below 100 ppm.

Sublethal doses may, however, result in reproductive impairment, alter behavior patterns, and disrupt species balance (Stickel, 1968). Korschgen and Murphy (1967) found evidence of a reduced reproductive rate in the

white-tailed deer when maintained on a diet containing 25 ppm of dieldrin per day. The toxicity of dieldrin, however, is much greater than DDT in mammals (O'Brien, 1967).

Reductive dechlorination of DDT to DDD apparently occurs in the rumen, and perhaps to some extent in the intestines since some DDD was found in the rumen contents and feces (Table II). Mendel and Walton (1968) confirmed conversion of DDT to DDD in the intestines of rats by bacterial action, although Finley and Pillmore (1963) suggested that the liver was the major site for DDD production in mule deer. Both the alimentary canal and liver are probably sites for DDD production in the white-tailed deer. Dehydrochlorination to DDE was not indicated as a major metabolic fate of DDT in deer in this study (Table II).

ACKNOWLEDGMENTS

W. E. Johnston, Assistant Professor of Experimental Statistics, Clemson University, provided guidance in statistical evaluation. O. H. Weinges, Sr. and O. H. Weinges, Jr. allowed deer to be collected from their land under the auspices of the South Carolina Wildlife Resources Department when the hunting season was closed.

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Table III. Comparisons between years by month and by tissue of total DDT residues in white-tailed deer collected in August and November, 1968 and 1969.

Tissue	Date collected	No. Samples	Total DDT (ppm)	F ratio	Significance
liver	Aug. 1968	5	0.11	0.039	ns
	Aug. 1969	2	0.21		
liver	Nov. 1968	4	0.26	0.858	ns
	Nov. 1969	6	0.32		
fat	Aug. 1968	5	1.28	0.016	ns
	Aug. 1969	2	1.19		
fat	Nov. 1968	5	2.21	3.107	ns
	Nov. 1969	7	0.87		

ns = not significant

Table IV. Comparisons of DDT residues in the fat and kidney between white-tailed deer collected from the study area in Calhoun County (Area 1) and Pisgah National Forest in western North Carolina (Area 2).

Tissue	Area	No. Samples	Residues (pp,)	F ratio	Significance
			Total DDT		
fat	1	4	3.19	13.073	P(.01)
	2	5	0.14		
kidney	1	5	0.16	0.388	ns
	2	5	0.11		
			p p' DDT		
fat	1	4	2.16	6.846	P(.05)
	2	5	0.14		
kidney	1	5	0.10	0.468	ns
	2	5	0.08		
			o p' DDT		
fat	1	4	0.12	1.310	ns
	2	5	0.00		
kidney	1	5	0.00	0.400	ns
	2	5	0.00		
			p p' DDD		
fat	1	4	0.69	16.302	P(.01)
	2	5	0.00		
kidney	1	5	0.03	0.109	ns
	2	5	0.01		
			p p' DDE		
fat	1	4	0.22	5.974	P(.05)
	2	5	0.00		
kidney	1	5	0.02	2.132	ns
	2	5	0.02		

ns = not significant

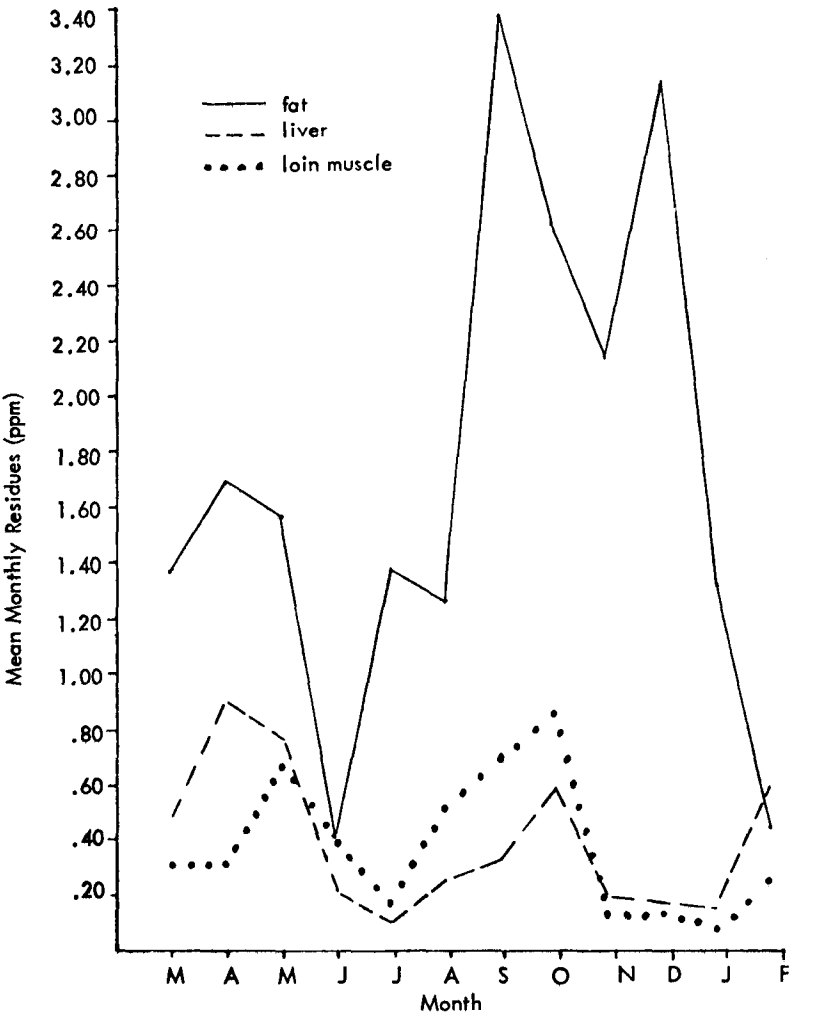

FIGURE 1. Total DDT residues in fat, liver, and loin muscle of white-tailed deer collected over a 12 month period from March 1968 to February 1969.

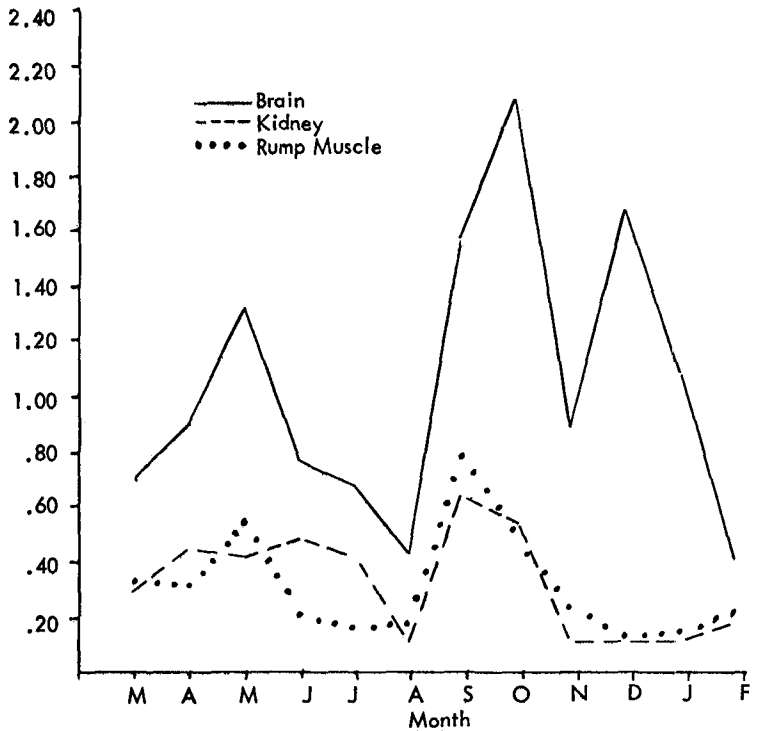

FIGURE 2. Total DDT residues in brain, kidney, and rump muscle of white-tailed deer collected over a 12 month period from March 1968 to February 1969.

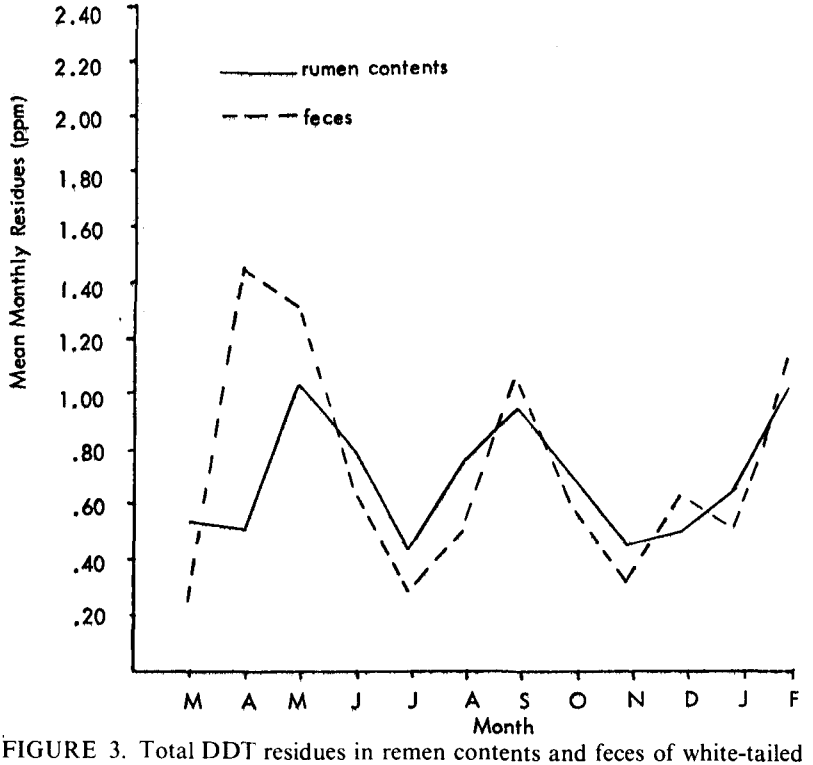

FIGURE 3. Total DDT residues in remen contents and feces of white-tailed deer collected over a 12 month period from March 1968 to February 1969.

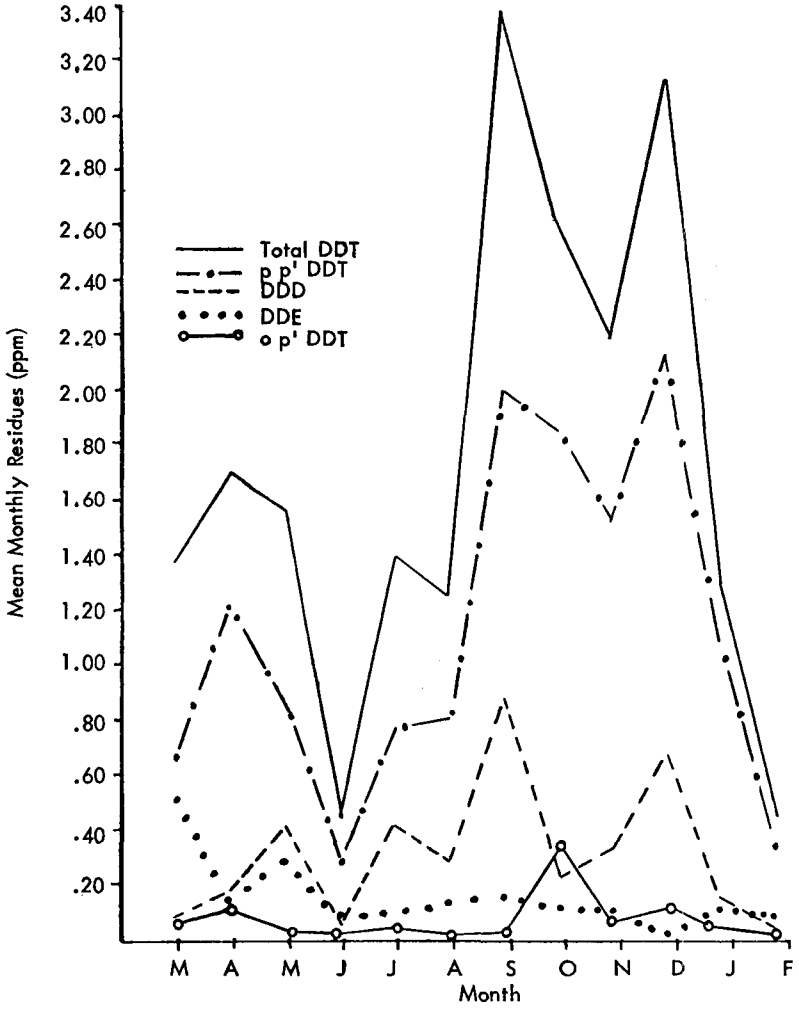

FIGURE 4. DDT, DDD, and DDE residues in the fat of white-tailed deer collected over a 12 month period from March 1968 to February 1969.

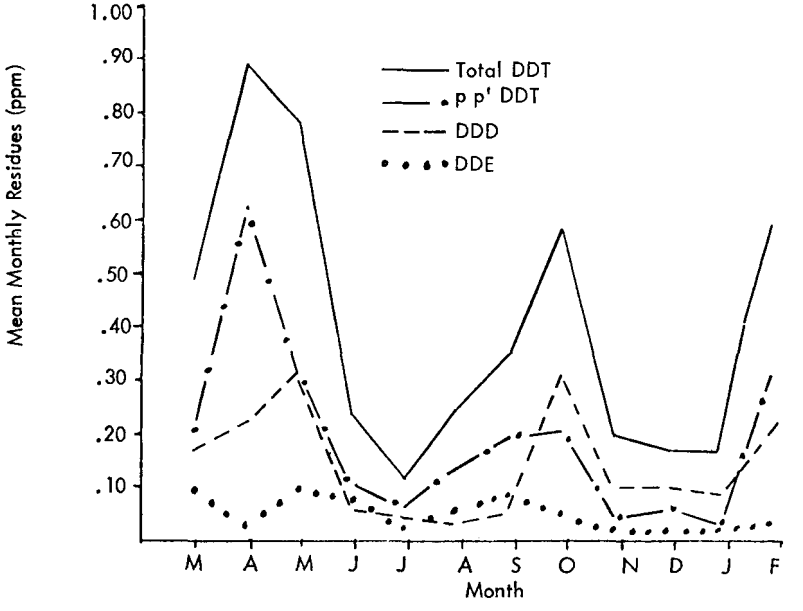

FIGURE 5. DDT, DDD, and DDE residues in the liver of white-tailed deer collected over a 12 month period from March 1968 to February 1969.