

# PESTICIDE RESIDUES IN BEAVER AND RIVER OTTER FROM ALABAMA<sup>1</sup>

by

EDWARD P. HILL

Alabama Cooperative Wildlife Research Unit  
Auburn University  
and

JOHNNY W. LOVETT

Alabama Cooperative Wildlife Research Unit  
Auburn University

## ABSTRACT

Tissue samples from beaver and river otter taken by trappers mostly from southeastern Alabama during January through March 1973 and 1974 were subjected to pesticide residue analyses. Residues found were Mirex, p,p' DDT, p,p'DDE, p,p'DDD, Heptachlor epoxide, Endrin, Dieldrin, Toxaphene and PCB as Aroclor 1260. The occurrence of Mirex in 14 of 19 river otter taken in 1974 may have resulted from large area aerial application of Mirex for fire ant control. Composite samples of beaver from the Chattahoochee River contained 31 times more total residues than composite samples from other areas.

## INTRODUCTION

Contamination of the environment with pesticides has become an increasingly important problem during the past three decades (Nicholson 1959; Carson 1962; Kuhr et al. 1974). The seriousness of the problem was brought into focus when replacement of traditional chemical analytical procedures by gas chromatography techniques greatly increased the research capabilities of agencies concerned with pesticide residue analysis. It was soon revealed that many nontarget native fauna contained pesticide residues of sufficient concentration to cause concern (Dustman 1967; Rudd and Genelly 1956; Kreitzer 1974).

Fish taken from the Chattahoochee and Alabama Rivers were found to contain 6 ppm DDT+15 ppm PCB and 1.2 ppm DDT+5.4 ppm PCB respectively (Staff, Alabama Pesticide Laboratory, 1971). The extent to which these and other compounds are present in other fauna throughout Alabama watersheds is unknown.

Beaver (*Castor canadensis*) meat is used as human food in Alabama. The beaver is vegetarian; thus, there should be relatively little biomagnification of organochlorine residues through its short food chain. No reports of pesticide residues in beaver were found in a search of the literature, and since tissue samples from a series of beaver were readily available, analyses were run to determine if pesticides were present. Studies were also conducted to see if pesticide residues existed in the river otter (*Lutra canadensis*), which normally has a longer and more complex animal food chain than the beaver.

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## METHODS

### *Beaver:*

Carcasses of beaver were collected daily from trappers during January through March 1973. Fat and upper hind leg muscle tissues were saved and frozen in plastic bags, samples of which were later analyzed for polychlorinated biphenyls.

Six composite (200 gm) samples of fat were prepared from equal amounts of tissue from individual beavers as follows: (1) 6 beaver from Uchee Creek in Russell County, (2) 7 from Cahaba River drainage in Bibb County, (3) 10 from upper Cowikee Creek in Bullock County, (4) 10 mostly from Cowikee Creek in Barbour County, (5) 4 from Sougahatchee Creek in Lee County, and (6) 8 from the Chattahoochee River in Russell County.

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Each composite sample was run through a meat grinder two times. The grinder was disassembled and washed thoroughly with hot water after each sample. A 100-gram portion of each sample was analyzed by the Alabama Pesticide Laboratory using methods outlined in Vol. 1 of the Pesticide Analytical Manual, USDHEW (1968). One known standard per each 10 tissue samples was processed through the entire analysis to monitor accuracy. The methods of fat extraction, acetonitrile partitioning and florisil clean-up, were as follows:

#### *Fat Extraction:*

1. 100 grams of each tissue sample were weighed into 250ml beaker and transferred to a blender.
2. The beaker was rinsed with 100ml ethyl alcohol, which was then added to the tissue sample and the mixture blended three minutes.
3. The beaker was washed again with 200ml hexane, which was added to the mixture, and blended three more minutes.
4. Each sample was poured into a centrifuge bottle and centrifuged at 1500 RPM for 10 minutes.
5. The supernatant was poured into a 1-L separatory funnel containing 500ml of 2% NaCl.
6. The separatory was up-ended three times and the contents allowed to separate. The lower layer (containing alcohol) was drained and discarded.
7. Each sample was washed twice with 100ml 2% NaCl, shaking each washing 30 seconds.
8. The hexane extracts were poured through 3.0cm of anhydrous Na<sub>2</sub>SO<sub>4</sub>.
9. Each sample was evaporated in a steam bath to obtain fat.
10. Three grams or less of the extracted fat were weighed out for acetonitrile partitioning.

#### *Acetonitrile Partitioning*

11. The fat was dissolved in 50ml hexane, transferred to a 125-ml separatory funnel and extracted three times with 50ml portions of hexane-saturated acetonitrile by shaking the separatory funnel vigorously one minute, 30 seconds, and 30 seconds respectively.
12. As each extraction separated into layers the lower acetonitrile layer was drained into a 1-L separatory funnel containing 100ml petroleum ether and 500ml of 2% NaCl.
13. The 1-L separatory funnel was shaken, the contents allowed to separate, and the lower level was drained off and discarded.
14. The sample was washed twice with 100ml 2% NaCl, each washing being shaken 30 seconds before being drained off.
15. Each sample was poured through anhydrous Na<sub>2</sub>SO<sub>4</sub>. The Na<sub>2</sub>SO<sub>4</sub> was rinsed with three portions (10ml each) of petroleum ether, and the entire elute was evaporated to 10ml in a steam bath.
16. Each 10ml sample concentrate was transferred to a column containing 28 grams of activated florisil<sup>2</sup> topped with ½ inch-thick layer of anhydrous Na<sub>2</sub>SO<sub>4</sub> pretreated with 35 to 40ml of hexane. The sample container was rinsed with hexane which was added to the column.
17. The column was eluted with 200ml of eluting mixture A containing 3% ethyl ether<sup>2</sup> in hexane.
18. After changing the receiving container the column was eluted with 200ml of eluting mixture B containing 15% ethyl ether<sup>2</sup> in hexane.
19. Each elutant was concentrated to 5ml for injection on gas chromatograph columns.

The gas chromatography procedures involved use of a Model 200 Varian Aerograph gas chromatograph with a tritium electron capture detector as described in the Pesticide Analytical Manual (1968). Different columns were used to confirm the identity of compounds reported. Columns were ½ inch in diameter and 5 feet long and were packed with Gas chrom Q, one coated with 3% OV 17 and another with 10% DC 200.

Pesticide residues were computed by laboratory staff from gas chromatography recorded graphs. Results were reported in parts per million of extracted fat (lower sensitivity limit 0.01 ppm). Where appropriate, results were converted to whole wet weights by the senior author.

#### *River Otter:*

River otter carcasses were collected from trappers daily during 1973 but at less regular intervals during 1974. Samples consisting of muscle tissue from the neck and upper hind leg from each of 13 otter were saved and frozen in plastic bags in 1973. Similarly, tissues were saved from 19 recently skinned otter taken in 1974. These tissues were frozen in aluminum foil. Otter tissue samples taken during both years were thawed, processed, and analyzed using the same laboratory techniques described above.

<sup>2</sup> The amount of florisil used and percent of ethyl ether hexane used in eluting mixtures varied depending on each new batch of florisil.

Confirmation analyses on five of the 1974 otter samples were run by the U. S. Fish and Wildlife Service Chemistry Laboratory, Patuxent Wildlife Research Center, Laurel, Maryland. These five samples were analyzed for organochlorine pesticides and polychlorinated biphenyls (PCB's) at a lower sensitivity limit of 0.10 ppm. The identity of residues in one sample was confirmed by gas chromatography-mass spectrometer.

## RESULTS

### *Beaver:*

Residues found in beaver were Dieldrin, p,p' DDT, p,p' DDE, p,p' DDD, and Heptachlor epoxide (Table 1). Only the composite sample from the Chattahoochee River near Cottonton contained more than 1.65 ppm total residues. Subsequent analyses of fat from the eight individuals comprising this composite revealed that one beaver, No. 522, contained 40.80 ppm p,p' DDE, 81.60 ppm p,p' DDT and 0.24 ppm Dieldrin. Total residues of DDT and metabolites reported in fat from the other seven beaver of the composite sample were 4.4, 6.8, 7.6, 10.3, 10.5, 11.5, and 14.6 ppm.

The residue data from beaver taken from the Chattahoochee River near Cottonton indicate that collectively these beaver contained 31 times more total DDT and metabolites than each of the other composite samples. If it can be assumed that the residues in beaver from this watershed accumulated from direct feeding on vegetation, other aquatic organisms with more complex animal food chains could be expected to have even greater residues. This becomes more significant when one considers that the sample collection site was on the upper reaches of the nationally famous fishing and recreational area, Lake Eufaula. The source of these pesticides is unknown and further research is needed to provide additional information, particularly on residue levels in sport and commercial fish populations in Lake Eufaula.

The plastic bags (Glad, fold-lock top, sandwich bags, Union Carbide Corp. New York)<sup>3</sup> subjected to analysis for PCB's were reported to contain 0.175 ppm Aroclor 1242. Aroclor 1242 has a relatively short (low) retention time after the sample is injected into the chromatograph. It is indicated on the graph soon after the hexane solvent front. DDT and its metabolites have longer retention times. The indicated levels of these compounds reported in beaver should not have been altered by Aroclor 1242. No Aroclor 1260 or 1254 which may be interpreted as DDT or metabolites were found in the plastic bags.

### *River Otter:*

Residues found in muscle tissue from the 13 river otter collected in 1973 varied from 0.11 to 7.49 ppm (whole wet weight) DDT and metabolites (Table 2). Dieldrin and Heptachlor epoxide were also found. The primary source of pesticides from otter Nos. 98 and 99 is unknown. They may have come from agricultural applications made within the Watoola Creek watersheds in both Lee and Russell Counties or may have been associated with sources found in beaver from the Chattahoochee River drainage into which Watoola Creek eventually flows.

No Mirex was found in otter collected in 1973, but it occurred in 14 of the 19 otter from 1974 samples (Table 3). Six specimens from Bughall Creek near Fitzpatrick in Bullock County contained Mirex residues that may have resulted from spring 1973 treatment of 95,190 acres of land for fire ant control under cooperative efforts of the United States and Alabama Departments of Agriculture. Muscle from otter No. 87 taken from this stream in the winter of 1973 before Mirex treatment contained no Mirex residues.

Another otter, No. 300, taken from a branch of Halawakee Creek in Lee County contained 0.60 ppm Mirex. These residues may have resulted from aerial application of Mirex on 92,323 acres in Chambers County in the spring and fall of 1973, since portions of this watershed are located in Chambers County.

In the five otter tissue samples analyzed from 1974, the Patuxent Wildlife Research Center Chemistry Laboratory reported higher levels of p,p'DDE and p,p'DDD than was reported by the Alabama Pesticide Laboratory (Table 3). Also they reported PCB as Aroclor 1260, whereas it was not reported by the Alabama Pesticide Laboratory. Differences were also noted in the levels of Mirex reported by the two laboratories. A discussion of these differences with the Alabama Pesticide Laboratory director revealed that residues reported by his laboratory have consistently been in smaller amounts than reported by other laboratories.

<sup>3</sup> Use of name does not constitute recommendation.

Differences in residues reported by different laboratories have been discussed by Henderson et al. (1969) and is not the major consideration here. Of more importance is that the pesticides found have, through various pathways, reached the tissues of river otter and beaver and presumably, at least in the case of the river otter, fauna comprising their food chain. The immediate and long range effects that the residues have or may cause in populations of both of these mammals are unknown. Both mammals appear to be abundant at this time. Two trappers, for example, working in the region where most of the samples were taken caught over 400 beaver and 49 otter during a period of approximately two months.

Table 1. Pesticide residues reported in six composite samples of fat tissues from beaver taken in Alabama in 1973.

Composite No.	County	<i>p,p'</i> DDE	<i>p,p'</i> DDD	<i>p,p'</i> DDT	Total D's	Dieldrin	Heptachlor epoxide
1	Russell	.17		.34	.51	.011	.05
2	Bibb	.17		.28	.45	.01	
3	Bullock	.33		1.20	1.53	.012	
4	Barbour	.23		.34	.57		
5	Lee	.14		.20	.34	.014	.04
6 <sup>a</sup>	Russell	33.00	3.60	11.2	47.80	.084	

<sup>a</sup> Eight specimens taken from the Chattahoochee River near Cottonton.

Table 2. Pesticide residues (ppm wet weight) found in muscle tissue of 13 river otter taken in Southeastern Alabama in January and February, 1973.

No.	Stream and County	Dieldrin	Heptachlor epoxide	<i>p,p'</i> DDD	<i>p,p'</i> DDE	<i>p,p'</i> DDT	Total D's
11	Cowikey Cr.-Barbour	T <sup>a</sup>		0.17	0.46	0.17	0.79
101	Cowikey Cr.-Barbour	T		0.05	0.12	0.01	0.19
68	Cowikey Cr.-Barbour		0.01	0.07	0.62	0.25	0.95
27	Loblockee Cr.-Lee	0.01		0.23	0.41	0.29	0.93
53 <sup>b</sup>	Opintlocco Cr.-Macon	0.02		0.24	0.99	0.63	1.86
78	Tallapoosa River-Elmore	T			0.12	0.22	0.34
82	Buckhorn Cr.-Pike	T			0.12		0.12
84	Conecuh River-Pike	T	T	0.02	0.06	0.02	0.11
86	Bear Cr.-Barbour			0.21	0.60	0.51	1.32
87	Bughall Cr.-Bullock	T	0.02	0.30	0.35	0.30	0.95
98 <sup>b</sup>	Watoola Cr.-Russell	0.04	0.12	0.60	1.98	0.60	3.18
99	Watoola Cr.-Russell			2.88	3.60	1.01	7.49
102	Watoola Cr.-Russell			0.12	0.40	0.12	0.64

<sup>a</sup> Trace amounts

<sup>b</sup> Contained less than .01 ppm Endrin.

Table 3. Pesticide residues (ppm whole wet weight) reported in muscle tissues of 19 river otter taken in Alabama in January and February 1974.

No.	Stream	Heptachlor			p,p' DDD	p,p' DDE	p,p' DDT	Total D's	PCB <sup>d</sup>
		Dieldrin	epoxide	Mirex					
123 <sup>a</sup>	Bughall Cr.	T <sup>b</sup>	0.01	1.07(5.8)	— (0.15)	0.25(1.3)	—	0.25(1.45)	(1.1)
124	Bughall Cr.	T	0.16	0.20(13.0)	—	T (1.1)	—	T	(2.1)
125 <sup>a</sup>	Bughall Cr.	T	T	1.62	0.02	0.29	0.71	1.02	—
137 <sup>a</sup>	Bughall Cr.	—	0.01	0.60	—	0.32	0.08	0.40	—
147	Bughall Cr.	T	—	0.53	—	0.08	—	0.08	—
149 <sup>a</sup>	Bughall Cr.	T	T	0.77(2.8)	—	0.34(1.3)	0.16	0.50	(0.58)
139 <sup>a</sup>	Martin Cr.	—	0.01	0.13	0.04	0.44	—	0.48	—
139 <sup>a</sup>	Martin Cr.	0.02	0.02	—	—	0.68	0.16	0.84	—
140	Martin Cr.	—	—	—	—	0.02	0.02	0.04	—
217	Piney Wood Cr.	T	—	T	—	0.01	—	0.01	—
218	Three Run Cr.	T	T	T	—	T	T	T	—
227	Cowikee Cr.	0.01	0.02	0.08	—	0.32	0.64	0.96	—
103 <sup>ac</sup>	Cowikee Cr.	T	—	—	—	0.21	—	0.21	—
250	Poor Cr.	T	T	—	T	0.16	T	0.17	—
300	Halawakee Cr.	T	0.04	0.60(3.0)	—	0.05(0.95)	—	0.05	(2.5)
500	Tombigbee River	T	—	0.06	—	0.01	0.64	0.65	—
501 <sup>a</sup>	Webb Cr.	0.04	0.02	0.60	—	0.36	0.79	1.15	—
502 <sup>a</sup>	Webb Cr.	0.02	0.02	—	—	0.36	0.05	0.41	—
503 <sup>a</sup>	Bird Cr.	T	T	0.09(0.63)	T(0.14)	0.12(1.4)	—	0.13(1.54)	(0.60)

<sup>a</sup> Contained trace amounts of Endrin.

<sup>b</sup> Trace amounts.

<sup>c</sup> Contained 16 ppm Toxaphene.

<sup>d</sup> PCB as Aroclor 1260.

Numbers in parentheses are residue levels reported by the U. S. Fish and Wildlife Service, Patuxent Wildlife Research Center Chemistry Laboratory.

The occurrence of Mirex in aquatic mammals following its widespread use in fire ant control is particularly significant. The occurrence of other pesticides in beaver from the Chattahoochee River is also noteworthy.

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