Residues in two bald eagles suspected of pesticide poisoning

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Bald eagles (Haliaeetus leucocephalus) found dead or moribund in United States and Canada and reported to the Bureau of Sport Fisheries and Wildlife are submitted to this laboratory for analysis to determine the accumulation of chlorinated pesticides. This paper reports the results of such analysis of two eagles observed dying in the field.

Previous analyses of eagles have revealed many unidentified chlorine-containing compounds that seriously interfere with the proper identification of the chlorinated pesticides. The method devised to cope with this analytical problem is also presented.

Field and Autopsy Data

One eagle, an adult female, was observed falling out of the sky near Belleview, Florida on January 26, 1968. The bird died in three hours, with alternating periods of convulsions and tremors. The other specimen, an immature female, was found in tremors on the ground near Newton, Connecticut on April 30, 1967. The bird died in convulsions shortly after being captured.

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Autopsy revealed no injuries or gun shot wounds in either specimen. The birds were in good flesh, but without subcutaneous, abdominal or coronary fat. Although large numbers of coccidia were present in the small intestine of the Connecticut eagle, this infection is not believed to have been the cause of death. In a case of coccidiosis in a golden eagle (Aquila chrysaetos) reported by Mathey (1) the eagle was described as emaciated.

Bacterial cultures of the heart, liver, and lung tissues from the Florida specimen showed that fowl cholera organisms were not present. This was investigated because of a recent outbreak of fowl cholera in Florida.

Sample Preparation and Analysis

The entire brain, one half of the liver, and a 20 gm aliquot of the remaining carcass (except for skin, wings, feet, and gastrointestinal tract) were analyzed. The carcass was ground and mixed in a Hobart Food Cutter. The samples were mixed with sodium sulfate, extracted in a Soxhlet, cleaned up by acetonitrile partitioning, and florisil column as previously described (2). The residues in the clean extract were separated and removed in four fractions from a thin layer (TL) plate by the method described by Mulhern (3). The TL fractions were then analyzed by gas chromatography (GLC) using three columns; the chromatographic conditions are outlined in Table 1. All four fractions were analyzed on the

OV-17 column and the residues confirmed on either of the other two columns as shown in Table 2. The average recovery for the common chlorinated pesticides ranged from 85 to 96 percent from fortified eagle carcass tissue.

TABLE 1

Chromatographic Operating Conditions Using ^{3}H Electron Capture Detector

	Columns, glass 6" x ¼" OD		
	A	B	C
Liquid phase	3% OV-17	3% XE-60	10% QF-1
Mesh size, Gas Chrom Q	100/120	60/80	80/100
N_2 Flow rate, ml/min.	100	100	125
Temp. ^{o} C.	190	170	160
Retention time of Dieldrin, min.	14.0	16.3	19.0

The residues were further confirmed by TLC on MN Silica Gel G HR plates developed with hexane-ethyl ether (98:2) mixture and visualized with silver nitrate and UV light (2). The compounds p,p'-DDD olefin and p,p'-DDE cannot be fully resolved by TLC. Dieldrin residues in the Florida specimen also were confirmed by

the characteristic degradation compound produced by UV light treatment, as described by Banks and Bills (4). The p,p'-DDT detected in the Connecticut specimen was confirmed by the conversion to DDE with alkali (5).

TABLE 2

Relative Retention Times on Three GLC Columns of Pesticides Previously Separated into Four Fractions by TLC

Dieldrin = 1.00

Compound	TLC Fraction	Columns A	B	C
Dieldrin	1	1.00	-	1.00
Lindane	1	0.24	-	0.24
Heptachlor epoxide	1	0.61	-	0.61
4,4-dichloro-benzophenone	1	0.61	-	0.69
Endrin	1	1.25	-	1.17
o,p'-DDD	2	1.25	-	-
p,p'-DDD	2	1.65	-	-
o,p'-DDE	3	0.83	0.61	-
o,p'-DDT	3	1.54	1.21	-
p,p'-DDT	3	2.05	2.28	-
p,p'-DDD olefin	4	0.83	0.70	-
p,p'-DDE	4	1.04	0.86	-

Results and Discussion

Results of analyses are shown in Table 3.

The residues of dieldrin in the brain of the Florida specimen (7.0 ppm) and of DDT plus DDD in the brain of the Connecticut specimen (34.7 ppm) are of particular concern. Experimental studies have shown that residues of dieldrin and DDT in the brain are suitable for diagnosing the cause of death.

Stickel et al. (6) concluded from an experimental study with Japanese quail (Coturnix c. japonica) and from residues in animals found dead in the field following heavy dieldrin treatments that a concentration of 4.0 to 5.0 ppm of dieldrin in the brain indicated that the animal was in the danger zone. Robinson et al. (7), from experimental dosage of Japanese quail and domestic pigeons (Columba livia), estimated a critical level of 10 ppm dieldrin in the brain. This value may be slightly high because his analytical procedure did not separate dieldrin from DDE, which normally is present as background material.

Stickel et al. (8) estimated from experimental and field studies that a concentration of 30 ppm of DDT plus DDD in the brain indicated that the animal was in the danger zone.

Weight loss and depletion of fat occurred in all birds killed by DDT or dieldrin dosage (6,7,8).

TABLE 3

Pesticide Residues in Tissues of Bald Eagles
(ppm wet weight)

Compound	Florida Eagle			Connecticut Eagle		
	Carcass	Liver	Brain	Carcass	Liver	Brain
p,p'-DDE	28.9	73.8	27.1	263.0	170.0	88.6
p,p'-DDD	5.9	10.2	3.8	79.2	10.2	14.4
p,p'-DDT	0.5	ND	0.7	14.1	0.8	20.3
o,p'-DDT	ND	ND	ND	<0.1*	ND	<0.1*
Dieldrin	6.5	15.7	7.0	5.3	5.2	2.3
Heptachlor epoxide	0.07*	0.1*	0.07*	ND	ND	ND
p,p'-DDD olefin	2.9*	3.6*	2.8*	11.6*	7.1*	2.7*
Endrin	<0.1*	0.1*	<0.1*	ND	ND	ND
4,4'-dichloro-benzophenone	0.5	0.8	0.3	3.5	2.6	ND

ND = not detected * = not confirmed by TLC

The combination of death with tremors or convulsions, depletion of fat reserves and critical residues of dieldrin or DDT plus DDD in the brains leads to the conclusion that these eagles probably died of pesticide poisoning.

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