RESIDUES IN FISH, WILDLIFE, AND ESTUARIES

Residues of Organochlorine Pesticides, Polychlorinated Biphenyls, and Mercury and Autopsy Data for Bald Eagles, 1969 and 1970 [1]

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ABSTRACT

Thirty-nine bald eagles found sick or dead in 13 States during 1969 and 1970 were analyzed for pesticide residues. Residues of DDE, dieldrin, polychlorinated biphenyls (PCB's), and mercury were detected in all bald eagle carcasses; DDD residues were detected in 38; DDT, heptachlor epoxide, and dichlorobenzophenone (DCBP) were detected less frequently. Six eagles contained possible lethal levels of dieldrin in the brain, and one contained a lethal concentration of DDE (385 ppm) in the brain together with 235 ppm of PCB's. Autopsy revealed that 18 bald eagles were illegally shot; other causes of death were impact injuries, electrocution, emaciation, and infectious diseases.

Introduction

Reichel *et al.* (*4*) presented pesticide residue data from bald eagles (*Haliaeetus leucocephalus*) collected in 1964 and 1965; a more recent report by Mulhern *et al.* (*2*) presented residue and autopsy data on bald eagles for 1966 through 1968. The purpose of this paper is to report the organochlorine pesticides, polychlorinated biphenyls (PCB's), and mercury residues and autopsy data for eagles collected in 1969 and 1970.

Sampling and Autopsy Procedures

As mentioned in previous report (*2, 4*), a systematic sampling procedure could not be used because of the relatively low populations of these birds and their protected status. Bald eagles found dead or moribund in the field are collected by Federal, State, and private cooperators, packed in dry ice, shipped air express to this laboratory, and stored intact in plastic bags at −25° C. The 13 States from which the 39 samples included in this report were collected are shown in Table 1; 28 birds were collected in 1969 and 11 in 1970. Only field specimens that were not decomposed were analyzed.

Autopsies were performed on all specimens to determine possible causes of death. Tissues for histological study were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with either hematoxylin and eosin, Ziehl-Neelsen acid-fast, periodic-acid Schiff (PAS), Giesmsa, Perl's Prussian blue, or the Van Kossa stain. The entire brain was removed and placed in an acetone-rinsed glass jar, and the remaining carcass (except for skin, feet, wings, liver, and gastrointestinal tract) was

TABLE 1.—*Distribution of eagles collected, by State and year of death, 1969 and 1970*

State	Number of Eagles Collected	
	1969	1970
Florida		1
Illinois	2	1
Iowa	1	
Maine	1	
Maryland		1
Michigan	4	3
Minnesota	6	1
Missouri	5	
North Dakota	3	
Ohio	2	
South Carolina		1
Wisconsin	4	2
Virginia		1
Total	28	11

[1] From the Bureau of Sport Fisheries and Wildlife, Patuxent Wildlife Research Center, Laurel, Md. 20810.

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wrapped in aluminum foil. The samples were usually processed for residue analysis within 1 week after dissection.

Analytical Procedures

The remaining carcass was ground and homogenized in a Hobart food cutter.

ORGANOCHLORINE PESTICIDES

The entire brain and a 20-g aliquot of the carcass were then extracted and cleaned up separately, using the procedure described by Mulhern *et al.* (*2*). The method consisted of Soxhlet extraction and cleanup by acetonitrile partitioning and Florisil column procedures. The eluate was concentrated and divided in half. One half was analyzed for pesticides, and the other half was set aside for PCB analysis.

The portion to be analyzed for organochlorine pesticides was streaked on a thin layer (TL) plate, and the pesticides were separated into four fractions and extracted with hot benzene (*1*). The pesticides present would be found in the following fractions: Fraction I—dieldrin, lindane, heptachlor epoxide, endrin, dichlorobenzophenone (DCBP), methoxychlor, and dicofol; Fraction II—*p,p'*- and *o,p'*-DDD; Fraction III—*p,p-'* and *o,p'*-DDT and *o,p'*-DDE; Fraction IV—*p,p'*-DDE heptachlor, aldrin, and mirex. Each fraction was analyzed by gas chromatography (GC) on a 4% SE-30/6% QF-1 column and confirmed on a 3% QF-1 or a 3% XE-60 column; instruments and operating parameters are described in Table 2.

TABLE 2.—*Gas chromatographic operating parameters using electron capture detection*

	Columns, Glass 6' × ¼" O.D.		
Instrument	Hewlett-Packard 5750	Packard	Packard
Detector	nickel 63	tritium	tritium
Liquid phase	4% SE-30/6% QF-1	3% QF-1	3% XE-60
Support	Supelcoport	Gas Chrom Q	Gas Chrom Q
Mesh size	100/120	100/120	60/80
Flow rate	argon/methane at 60 ml/min	N_2 at 40 ml/min	N_2 at 100 ml/min
Temperature	190° C	175° C	175° C

In addition to the TL zonal separations and dual-column gas chromatographic confirmation, the residues in 10% of the samples were semiquantitatively confirmed by TLC (silica gel plate—2% ethyl ether in hexane developing solvent).

Positive identification of dieldrin residues in brains of certain eagles was accomplished with the use of an LKB gas chromatograph-mass spectrometer (GC-MS) equipped with a 2% SE-30, 80/100 mesh column. Operating conditions were: flow rate—35 ml/min of helium; oven temperature—200° C; flash heater—220° C; separator—240° C; and ion source—290° C. The ionization potential was 70 ev, and accelerating voltage 3.5 kv. The sensitivity was approximately 50 ng. The mass spectra obtained from the samples were compared with spectra obtained from a dieldrin standard in respect to parent peak, Cl^{37} multiplet peaks, base peak, and fragmentation pattern.

Polychlorinated biphenyls (PCB's) with GC patterns most closely resembling Aroclor 1254® were found in all samples in Fraction IV and, in smaller amounts, in Fraction III. A previous study (*2*) showed that PCB compounds (Aroclor 1254® as reference) did not interfere in the quantitative determination of DDE unless the ratio of PCB's to DDE exceeded 2:1; samples were run on a 3% OV-17 column and quantities were measured by a disk integrator. Thus, a separate study was made to determine whether PCB compounds interfered with DDE when the DDE was quantitated by peak height using the 4% SE-30/6% QF-1 column. Standards of Aroclor 1248®, 1254®, and 1260® were each mixed with *p,p'*-DDE to obtain the follwing ratios of PCB's to DDE—1:1, 3:1, 5:1, 10:1, 15:1, and 20:1. The GC instrument parameters were the same as those used to measure DDE in the eagle samples. The results are given in Table 3.

TABLE 3.—*The interference from PCB compounds in the determination of p,p'-DDE*

p,p'-DDE [1]	Ratio of PCB to DDE					
	1:1	3:1	5:1	10:1	15:1	20:1
	AROCLOR 1248®					
% Recovered	100	102	100	105	105	105
% Error	—	+2	—	+5	+5	+5
	AROCLOR 1254®					
% Recovered	100	100	100	103	110	122
% Error	—	—	—	+3	+10	+22
	AROCLOR 1260®					
% Recovered	100	100	103	95	92	90
% Error	—	—	+3	−5	−8	−10

[1] Quantitation by peak height.

Using peak height, the amount of DDE recovered remained within ± 5% of true value with all three Aroclors up to a 10:1 PCB's to DDE ratio; the error increased to +10% and −8% at a 15:1 ratio of PCB's to DDE using Aroclor 1254® and 1260®, respectively.

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To determine the efficiency of the analytical procedure, a pesticide recovery study was run with duck wings; 20-g aliquots of duck wing homogenates, containing trace quantities of pesticides, were spiked to contain the following: 5.0 ppm DDE, 0.2 ppm DDD, 0.1 ppm DDT, 0.2 ppm dieldrin, and 5.0 ppm Aroclor 1254®. The average recoveries from three determinations were 77% DDE, 95% DDD, 79% DDT, 85% dieldrin, and 90% PCB's.

In reporting residues in eagle samples, no corrections were made for recoveries. The lower limit of detection was arbitrarily set at 0.05 ppm; quantities <0.05 ppm were reported as trace.

PCB's

The second half of the eluate was analyzed for PCB's using the semiquantitative determination by thin layer chromatography (TLC) as described by Mulhern *et al.* (*3*) with the following modification: the sample eluate was evaporated to dryness, followed by the addition of 2 ml of oxidizing agent (1.5 g of CrO_3 in 59 ml of HOAc and 1 ml of H_2O). This agent was prepared by saturating 59 ml HOAc with a portion of the 1.5 g CrO_3; the remaining CrO_3 crystals were dissolved with 1 ml H_2O. The lower detection limit was set at 1.0 ppm; a trace value for PCB's was considered to be <1.0 ppm.

TOTAL MERCURY

Total mercury was determined by cold vapor atomic absorption. A separate 2-g portion of the homogenized carcass was digested by refluxing with sulfuric and nitric acids and then oxidized with hydrogen peroxide. Hydroxylamine hydrochloride and stannous chloride were added to the digest to reduce mercury (II) ions to mercury metal. The sample was aerated, and the mercury in the air stream passing through a gas cell was measured by atomic absorption. The lower limit of detection was approximately 0.02 ppm in a 2-g sample.

Methyl mercuric hydroxide and mercuric chloride were added to eight 2-g portions of an eagle carcass, and from 90% to 107% of the mercury was recovered.

METHYL MERCURY

Methyl mercury in ground bald eagle carcasses was determined in duplicate by the method of Jensen (Naturvardsverkets Specialanalytiska Laboratorium Lanktbrukshogskolan Uppsala, Sweden. *Unpublished method sent to Organization for Economic Cooperation and Development members, Sept. 1968*). In this procedure the lipids were extracted from a 1-g sample, wet tissue, by shaking with 30 ml methanol:ethyl ether, 3:1 mixture. After centrifuging and decanting, the remaining tissue was dried *in vacuo*, and 1 ml each of 0.5 M HBr and 0.5 M $CuBr_2$ followed by 10 ml benzene:toluene (4:1) were added. The mixture was shaken thoroughly, centrifuged, and chilled until the water layer was frozen. The organic layer was then decanted into a tube for injection into a MicroTek MT220 gas chromatograph equipped with a Ni^{63} detector on pulsed voltage operation. When chopped mallard muscle tissues spiked with 2 or 6 μg of mercury as methyl mercury hydroxide were analyzed by this procedure, 81% recoveries were obtained. Two column packings were used; one consisted of 15% Carbowax 20 M on 60/80 mesh Chromosorb G, acid washed, DCMS treated, at 185° C. Under these conditions injection of 1 ng of Hg as methyl mercury bromide produced a peak height of 10% of full-scale deflection. Some fouling of the detector was encountered with this column, and the degree of sensitivity was only fair. Thus, a second column packing, 5% Hi-Eff-10B (phenyl diethanolamine succinate) on 60/80 mesh Gas Chrom Q, was used. Operating temperatures of this column ranged from 145° to 160° C. Injection of approximately 0.4 ng Hg as methyl mercury bromide produced a peak height of 10% of full-scale deflection. This was considered to be the lower limit of reliable detection for the instrument and these operating conditions. This column packing apparently did not foul the detector, although continual clogging of the long exit tube on the detector was observed. This problem was partially alleviated by wrapping the exit tube with heating tape.

Results and Discussion

RESIDUES

The data for chlorinated pesticides, PCB's and total mercury residues found in bald eagles collected in 1969 and 1970 are summarized in Table 4. Results for eagle carcasses and brains are reported on a wet-weight basis. Medians rather than arithmetic means were used because the residue levels were asymmetrically distributed. All 39 carcass samples contained DDE, dieldrin, PCB's, and mercury; 38 contained *p,p'*-DDD.

The median value of DDE in the 1970 carcass samples was 2.6 times larger than in 1969, but both values were close to or within the median range of 4.9-16.6 ppm found for 1964-1968 (*2, 4*). For 1970, the median concentration of DDE in the brain was over 14 times greater than any median value observed since 1964; however, no trend can be established from these data because of the small number of samples collected, the wide range in DDE levels, and the absence of a systematic sampling procedure. Only two States, Minnesota and Wisconsin, were represented continuously from 1964-1970, often by one or two birds.

Significant correlations between residues of PCB's and DDE in the brain were obtained for both 1969 and 1970 samples; the correlations indicate that in the 28

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samples statistically analyzed, there was an association between the levels of PCB's and DDE in the brain (Table 5). A more general inference may become possible with the analysis of future samples. The correlation between residues of PCB's and DDE in the brain could not be attributed to interference of PCB's with GC quantitation of DDE since the study of PCB interference in DDE quantitation showed only a 5% error in the amount of DDE recovered at the 10:1 level of PCB's to DDE and only one sample had a PCB's to DDE ratio greater than 10:1 (*viz* 11:1). The median value of PCB's to DDE in brains was 3.1 in 1969 and 2.2 in 1970.

The data for methyl mercury residues in 29 of the 39 carcasses are presented in Table 6; the 10 carcasses containing <1.0 ppm total mercury were not analyzed. Seven samples, which were reanalyzed in duplicate, contained less than 65% methyl mercury, presumably due to the presence of significant amounts of inorganic mercury in the soft tissues or in the bone fragments mixed with the ground carcass. The equivalent ppm mercury present as methyl mercury ranged from 0.38 to 44.56 ppm Hg. Lethal levels of methyl mercury for bald eagles have not been established.

One finding of prime interest was that pesticide poisoning possibly accounted for the death of seven bald eagles. Residues in these birds were semiquantitatively confirmed by TLC. In addition, dieldrin residues in four of these birds were analyzed and confirmed by GC-MS. Six of the eagles contained possible lethal levels of dieldrin in the brain (Table 7); two of these birds were collected in 1969 and four, in 1970. The effects of the

TABLE 4.—*Pesticide residues in bald eagles, 1969 and 1970*

[T = <0.05 ppm] [1]

Compound	Year	Residues in PPM [1]					
		Carcass			Brain		
		Median	Range	N [2]	Median	Range	N [2]
p,p'-DDE	1969	6.9	0.16-30	28	0.68	T-62	28
	1970	18	1.5-78	11	26	0.22-385	11
p,p'-DDD	1969	1.0	0.06-0.27	27	0.20	T-2.7	18
	1970	1.5	0.28-11	11	1.5	0.05-7.2	11
p,p'-DDT	1969	0.22	0.07-0.75	12	0.06	0.06	1
	1970	0.10	0.09-1.1	6	0.34	0.23-0.69	4
Dieldrin	1969	0.41	T-6.5	28	0.27	T-8.0	18
	1970	0.74	<0.10-18	11	2.0	0.23-11	10
Heptachlor epoxide	1969	0.06	T-0.35	22	0.04	0.003-0.06	6
	1970	0.10	T-0.41	9	0.36	0.06-1.0	7
Dichlorobenzophenone	1969	0.42	0.08-0.77	7	0.31	0.07-0.81	4
	1970	T	T	1	0.30	T-1.1	4
PCB Compounds	1969	10	T-150	28	2.5	T-65	28
	1970	20	4-200	11	46	T-230	11
Mercury	1969	1.5	0.52-43	28			
	1970	2.5	0.47-9.4	11			

NOTE: A total of 28 birds were collected in 1969 and 11 birds in 1970.

[1] Calculated on a wet-weight basis.

[2] Number of specimens that contained residues; the median is based on this number.

TABLE 5.—*Association between PCB and DDE residues in bald eagles, 1969 and 1970*

Year	Carcass				Brain			
	PCB/DDE		N [1]	Correlation Coefficient	PCB/DDE		N [1]	Correlation Coefficient
	Median	Range			Median	Range		
1969	1.5	0.4-10	26	0.287	3.1	0.9-11	18	=0.908 [2]
1970	1.3	0.5-3.3	11	0.451	2.2	0.6-7.5	10	=0.752 [3]

[1] Number of specimens that contained more than trace amounts of both PCB compounds and DDE

[2] $P = <0.01$.

[3] $P = <0.05$.

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high levels of dieldrin in the brains of the two eagles that drowned may have caused the birds to fall into the water and drown. The dieldrin levels ranged from 4.6 to 11 ppm, wet-weight basis. Experimental and field data have shown that the lowest lethal brain residue for dieldrin is about 4 or 5 ppm (*5*). The seventh eagle, an adult female from Michigan, contained 385 ppm of DDE and 6 ppm DDD in the brain, together with 235 ppm of PCB's. The level of DDE alone is well within the lethal range (*6*), but the high level of PCB's suggests that these compounds may have also contributed to death. In this study and previous studies (*2, 4*) since 1964, with the exception of one eagle of unknown sex, all bald eagles found to be possibly poisoned by dieldrin or DDT metabolites have been females. Of the 153 eagles analyzed during 1964-1970, 15 (9.8%) possibly died of dieldrin poisoning.

AUTOPSY DATA

The autopsy results for the 39 bald eagles are summarized in Table 8. Illegal shooting remained the most frequent single cause of death among the bald eagles examined in this laboratory with 46% of the birds collected during 1969-1970 having been shot.

The four eagles dying from impact injuries, most frequently as the result of hitting a power line or tower, included one eagle from Virginia which had been struck by a private jet aircraft.

Three eagles were extremely emaciated; two of these were later found to have possible lethal levels of dieldrin in the brain (Table 7), and the third, a bird from Wisconsin, had multiple injuries from porcupine quills. Two quills were still embedded in the back of the eagle's oral cavity, suggesting that this eagle died from the effects of starvation and secondary bacterial infection (see below).

TABLE 6.—*Mercury found in 29 bald eagle carcasses, 1969 and 1970*

Total ppm Hg [1]	Methyl Mercury Equivalent ppm Hg	% of Total Hg Found as Methyl Mercury
1.01	0.68	67.3
1.02	0.96	94.1
1.12	1.01	90.2
1.13	1.09	96.5
1.17	0.98	83.8
1.26	0.76	60.3
1.31	0.90	68.7
1.42	0.92	64.8
1.49	1.28	85.9
1.53	1.41	92.2
1.53	0.38	24.8
1.70	0.80	47.0
1.72	1.36	79.1
1.77	1.75	98.9
2.07	1.74	84.0
2.18	1.45	66.5
2.20	1.88	85.4
2.50	1.99	79.6
2.74	2.44	89.0
2.96	0.48	16.2
3.01	2.85	94.7
3.58	1.56	43.6
4.42	2.05	46.4
5.65	4.68	82.8
5.83	3.98	68.3
8.32	6.71	80.6
9.40	2.72	28.9
11.00	7.60	69.1
43.00	44.56	103.6
MEDIAN		
2.07	1.45	79.6

[1] Of the 39 samples collected, 10 contained <1.0 ppm total mercury and, thus, were not analyzed for methyl mercury.

TABLE 7.—*Data on six suspected cases of possible dieldrin poisoning and one suspected case of DDE poisoning, 1969 and 1970*

State	Year	Age [1]	Sex	Residue in Brain (ppm)	Autopsy Findings
DIELDRIN					
Missouri	1969	Ad	F	= 4.6	Drowning, emaciation
Wisconsin	1969	Ad	F	= 6.5	Open [2]
Illinois	1970	Im	F	4.6	Open
Michigan	1970	Ad	F	= 4.8	Drowning
Minnesota	1970	Im	F	= 5.9	Emaciated; shotgun pellets around tail
Maryland	1970	Ad	F	11	Open
DDE/PCB					
Michigan	1970	Ad	F	385/235	Parasitic enteritis

[1] Ad = adult, Im = immature.

[2] Dieldrin analyzed and confirmed by gas chromatography-mass spectrometry.

[3] Open = no diagnosis could be made on the basis of autopsy findings.

TABLE 8.—*Probable causes of bald eagle mortality, 1969 and 1970*

Cause of Death	Number of Eagles
Shot	18
Dieldrin [1]	6
DDE [1]	1
Impact	4
Electrocution	2
Emaciation	1
Nephrosis	1
Streptococcal infection	1
Avian cholera	1
Trapping injuries	1
Open	3
Total	39

[1] See Table 7 for details.

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One eagle from Michigan had an old trapping injury which had become secondarily infected and resulted in a fibrinous pericarditis. A pure culture of a gram-positive rod, subsequently identified as a *Lactobacillus*, was isolated from the pericardium. This isolate of *Lactobacillus* was noninfectious to laboratory mice and domestic pigeons and probably should be regarded as a postmortem contaminant.

Although *Pseudomonas aeruginosa* was isolated from the kidney and from an old infected bullet leg wound of an eagle from Wisconsin, the cause of death was a more recent gunshot wound which produced massive hemorrhage into the pericardial sac.

Pasteurella multocida, the causative organism of avian chlorea, was isolated from three eagles. The first isolation was from an adult male from Ohio and apparently represents a frank case of avian chlorea. The second isolation of *P. multocida* was from the heart of the emaciated eagle which had been injured by porcupine quills; the third isolation was from the liver of an eagle shot in Florida.

Several parasitic conditions were observed in these 39 eagles. Schizonts of *Leucocytozoon* were found in the hearts of an eagle from Maryland and one from Illinois; both eagles had possible lethal levels of dieldrin in the brain (Table 7). Another eagle from Michigan, subsequently found to have high levels of DDE and PCB's in the brain, had a severe enteritis caused by a large number of as yet unidentified flukes; although the enteritis was severe, it was not regarded as the cause of the eagle's death.

Conclusion

Since the bald eagle is located at the top of food chains and is the final recipient of environmental pollutants, the presence of PCB's, DDE, dieldrin, and mercury in all 39 samples from 13 States, demonstrates continued widespread environmental contamination by these compounds.

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See Appendix for chemical names of compounds discussed in this paper.

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