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ORGANOCHLORINE RESIDUES AND EGGSHELL THINNING IN WOOD STORKS AND ANHINGAS

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Wood Storks (*Mycteria americana*) are somewhat less widely distributed today in the United States than they were in the early 1940s, and their numbers in Florida have declined to a fraction of those occurring there earlier (Ogden 1975, 1978, Palmer 1962). Populations of the Anhinga (*Anhinga anhinga*) have apparently remained generally stable. However, data on organochlorine residues in this species are of interest because of its close phylogenetic relationship with the Double-crested Cormorant (*Phalacrocorax auritus*) and Brown Pelican (*Pelecanus occidentalis*), 2 species in which eggshell thinning has been correlated with organochlorine residues, particularly DDE (Anderson and Hickey 1972, Blus 1970, Blus et al. 1971, 1972a, 1972b, Risebrough et al. 1971). Similar correlations have been reported in other fish-eating birds (Fox 1976, Vermeer and Reynolds 1970, Vermeer and Risebrough 1972), and Wood Storks and Anhingas are primarily fish eaters.

To determine whether either of these species might be adversely affected by environmental pollutants, we collected eggs and analyzed them for residues of organochlorines. We compared eggshell thickness of these eggs and others collected since 1946 (and now located in museum collections) with shell thickness of eggs collected before the widespread use of organochlorine pesticides.

The results reported here are part of a larger study to determine (1) geographic differences in the occurrence of environmental pollutants in Anhingas and wading birds (including herons, bitterns, ibises, and Wood Storks) in the eastern United States; (2) differences in environmental pollutant levels among those species nesting at the same localities; and (3) whether eggshell thickness had changed since the widespread use of organochlorine pesticides began in the mid-1940s.

METHODS

Wood Stork eggs were collected at the Merritt Island National Wildlife Refuge (NWR), Brevard County, Florida, in 1973. Anhinga eggs were collected at Merritt Island NWR and at 6 additional localities in 1972 and 1973: Lacassine NWR (Cameron Parish) and Atchafalaya Basin (St. Martin Parish) Louisiana; Yazoo NWR (Washington County) Mississippi; J. N. "Ding" Darling NWR (Lee County) and Payne's Prairie (Alachua County) Florida; and Okefenokee NWR (Ware County) Georgia.

Entire clutches were collected; when the clutches consisted of 2 or more eggs, 2 eggs from each clutch were wrapped in aluminum foil and placed in plastic containers to retard moisture loss. These eggs were refrigerated until they could be processed. Contents were then removed, placed into chemically clean jars, and then frozen pending analysis. Only 1 egg per clutch was analyzed, but shells of all eggs were saved for comparisons of eggshell thickness.

Egg volumes were measured to the nearest 1.0 ml by water displacement before the contents were removed. Residues were adjusted to fresh wet weight, assuming specific gravity of 1.0 as suggested by Stickel et al. (1973).

After the egg contents were homogenized in a mixer, a 10-g subsample was blended with sodium sulfate and extracted 7 to 8 hours with hexane in a Soxhlet apparatus. Cleanup of the extract, and separation and quantitation of pesticides and polychlorinated biphenyls (PCBs) were similar to the procedure used for the analysis of eagle carcasses (Cromartie et al. 1975). In summary, an aliquot of hexane extract equivalent to 5 g of subsample was passed through a Florisil column to remove lipids. An aliquot of this eluate was column chromatographed on silicic acid to separate the pesticides and PCBs. The organochlorines separated into 3 silicic-acid eluates were identified and quantitated by gas chromatography on a 1.83-m glass column packed with 4% SE-30/6% QF-1 on 100–120 mesh Supelcoport. PCBs were quantitated by comparing total peak area, measured by computing integrator, with that of Aroclor 1254 or 1260, whichever most closely resembled the gas chromatographic profile of the sample. Residues in 10% of the samples were confirmed with a combined gas chromatograph-mass spectrometer.

Samples were analyzed for DDE, DDD, DDT, dieldrin, mirex, heptachlor epoxide, oxychlordane, *cis*-chlordane (and/or *trans*-nonachlor), *cis*-nonachlor, HCB, toxaphene, endrin, and PCBs.

Recoveries of pesticides and PCBs from spiked egg tissue range from 83% to 104%. Residues in this report were not adjusted on the basis of these recoveries. Sensitivity of detection for the gas chromatograph was 0.1 ppm for pesticides and 0.5 ppm for PCBs. When PCBs were detected in trace amounts (< 0.5 ppm), they were considered as 0.25 ppm for purposes of this report.

Mean organochlorine concentrations in the samples were computed on individual sample values (the residue concentration + 1) transformed to common logarithms. (After computing these values, we took their antilogs and then subtracted 1 from that value. This returned our measurements to the original units. The addition of 1 facilitated the transformation of zero values to logs.) The data were analyzed on a CDC 6400 computer using packaged subroutines from the Statistical Package for the Social Sciences (Nie et al. 1975). In some instances the presence of many zero values prevented transforming to the normal distribution, but we also calculated means of these log-transformed data (see Ohlendorf et al. 1978 for further explanation). We performed a one-way analysis of variance on the log-transformed data for DDE and PCBs to detect significant differences ($P < 0.05$) among the mean levels of these chemicals at the different localities for Anhingas, and among species at Merritt Island. We used the Scheffé procedure (Scheffé 1959) of multiple comparison of means to group the localities or species by mean chemical concentration into homogeneous subsets.

Eggshell thickness was measured to the nearest 0.1 mm with a modified Starrett micrometer after the shells had dried at room temperature for a least 1 month. Three measurements were taken at the "equator" of each egg and included the shell and shell membranes. Measurements were averaged to yield a single value for each egg in the clutch. Statistical testing (2-way, non-random model, analysis of variance) of eggshell thickness was based on clutch mean thickness.

For each species, eggshell thickness data were first grouped into two time periods,

TABLE 1

ORGANOCHLORINE RESIDUES IN WOOD STORK EGGS FROM MERRITT ISLAND NATIONAL WILDLIFE REFUGE, FLORIDA, 1973*

		Residues in ppm (Wet Weight)		
	Number with Residues	Geometric Mean	95% C.I.	Range[2]
DDE	10	4.0	2.0–7.3	1.2–19
DDD	2	0.026	0–0.07	ND–0.13
DDT	3	0.24	0–0.65	ND–1.9
Dieldrin	2	0.053	0–1.55	ND–0.50
Mirex	7	0.30	0.07–0.56	ND–1.4
Oxychlordane	2	0.030	0–0.08	ND–0.20
Cis-chlordane[1]	2	0.032	0–0.08	ND–0.24
Cis-nonachlor	1	0.063	0–0.22	ND–0.84
HCB	1	0.0046	0–0.01	ND–0.047
Toxaphene	3	0.059	0–0.15	ND–0.41
PCBs	10	1.2	0.7–1.9	0.43–3.3

* One egg from each of 10 clutches. Average lipid content = 5.5%. Heptachlor epoxide and endrin were not found in the samples.
[1] And/or *trans*-nonachlor.
[2] ND = not detected.

pre-1947 and 1947–1973. Data from various individual localities within time periods were subjected to analysis of variance and multiple comparisons tests before pooling into 1 region for storks and 3 for Anhingas. Localities with significant differences in mean eggshell thickness ($P < 0.05$) were not pooled. Differences in mean thickness between time periods were tested by individual t-tests within each region.

ORGANOCHLORINE RESIDUES

Wood Stork.—DDE and PCBs occurred in all 10 eggs of the Wood Stork; mirex occurred in 7 (Table 1). Eight other organochlorines were found, but they were present in fewer than half of the samples. Heptachlor epoxide and endrin were not detected.

Residue levels of DDE in Wood Stork eggs were significantly higher ($P < 0.05$) than residues in eggs of 9 other species sampled at Merritt Island NWR (Table 2) but not significantly different from residues in eggs of Great Blue Herons (*Ardea herodias*), Black-crowned Night Herons (*Nycticorax nycticorax*), and Cattle Egrets (*Bubulcus ibis*). In contrast, PCB residues in Wood Stork eggs were considerably lower than the DDE level (PCB/DDE ratio = 0.30), and there were no significant differences among species means.

Anhinga.—DDE and PCBs were found in 45 and 24 of the 46 Anhinga eggs analyzed; other residues occurred less frequently (Table 3). Residues of DDD, DDT, mirex, and HCB were found more frequently in samples from

TABLE 2

COMPARISON OF DDE AND PCB RESIDUES (PPM, WET WEIGHT) IN EGGS OF 13 AVIAN SPECIES COLLECTED AT MERRITT ISLAND NATIONAL WILDLIFE REFUGE, 1972 AND 1973

DDE		PCBs	
Species[1]	Geometric Mean	Species	Geometric Mean
Wood Stork	4.0 A[2]	Great Blue Heron	2.4 A[2]
Great Blue Heron	2.1 AB	Black-crowned Night Heron	1.8 A
Black-crowned Night Heron	1.0 AB	Great Egret	1.5 A
Cattle Egret	0.93 AB	Wood Stork	1.2 A
Great Egret	0.66 B	Anhinga	1.1 A
Snowy Egret	0.54 B	Louisiana Heron	0.81 A
Green Heron	0.49 B	Little Blue Heron	0.54 A
Louisiana Heron	0.49 B	Snowy Egret	0.53 A
Little Blue Heron	0.41 B	Cattle Egret	0.53 A
Anhinga	0.39 B	Green Heron	0.44 A
Glossy Ibis	0.34 B	White Ibis	0.21 A
Least Bittern	0.29 B	Least Bittern	0.17 A
White Ibis	0.27 B	Glossy Ibis	ND A

[1] Scientific names for species not mentioned in the text are: Great Egret (*Casmerodius albus*), Snowy Egret (*Egretta thula*), Green Heron (*Butorides striatus*), Louisiana Heron (*Hydranassa tricolor*), Little Blue Heron (*Florida caerulea*), Glossy Ibis (*Plegadis falcinellus*), Least Bittern (*Ixobrychus exilis*), and White Ibis (*Eudocimus albus*).
[2] Within each chemical, means that share the same letter are not significantly different ($P > 0.05$) from each other. ND = not detected.

inland localities than in eggs from other areas. PCBs were found more commonly in the eggs from Merritt Island NWR than in those from other areas. The overall frequency of occurrence of residues (see frequency index, Table 3) was highest in the samples from inland localities. Heptachlor epoxide, *cis*-chlordane, *cis*-nonachlor, toxaphene, and endrin were not detected in the Anhinga eggs.

Among the localities, mean DDE residues were much higher in samples from Yazoo NWR and the Atchafalaya Basin than in those from other localities, and the highest DDE residue in an individual sample (15 ppm) was in an egg from the Atchafalaya Basin (Table 4). The lowest mean DDE residues were in eggs from Merritt Island, "Ding" Darling, and Okefenokee National Wildlife Refuges.

There were no differences among mean PCB residues for the various localities; means for other chemicals were not tested because the chemicals were found in less than half of the eggs. PCB/DDE ratios were below 1.0 at all localities except at Merritt Island NWR; there the PCBs were 2.8 times the level of DDE (Table 4).

TABLE 3

FREQUENCIES OF ORGANOCHLORINE RESIDUES IN ANHINGA EGGS, 1972 AND 1973*

	Number (%) with Residues			
	Inland[1] ($N = 21$)	Gulf Coast[2] ($N = 15$)	Atlantic Coast[3] ($N = 10$)	Total ($N = 46$)
DDE	21 (100)	15 (100)	9 (90)	45 (97.8)
DDD	2 (9.5)			2 (4.3)
DDT	5 (23.8)			5 (10.9)
Dieldrin	1 (4.8)	1 (6.7)		2 (4.3)
Mirex	2 (9.5)			2 (4.3)
Oxychlordane	1 (4.8)	1 (6.7)		2 (4.3)
HCB	2 (9.5)			2 (4.3)
PCBs	11 (52.4)	6 (40)	7 (70)	24 (52.2)
Total Occurrences	45	23	16	84
Frequency Index[4]	0.165	0.118	0.123	0.140

* Heptachlor epoxide, *cis*-chlordane, *cis*-nonachlor, toxaphene, and endrin were not found in the samples.
[1] Includes Atchafalaya Basin (LA), Yazoo NWR (MS), Payne's Prairie (FL), and Okefenokee NWR (GA).
[2] Includes Lacassine NWR (LA) and Darling NWR (FL).
[3] Includes Merritt Island NWR (FL).
[4] Computed as: $\frac{\text{Total occurrences}}{\text{Possible occurrences}}$; Possible occurrences = No. of clutches from that habitat (i.e. Inland, etc.) $\times$ 13 chemicals.

DDD was found only in 2 eggs from Yazoo NWR, where eggs also had the highest mean DDT level (Table 4). Mirex was found in 2 eggs from Payne's Prairie, and HCB in 2 eggs from the Atchafalaya Basin.

EGGSHELL THICKNESS

Wood Stork.—Although mean shell thickness of eggs collected since 1946 was significantly less (–8.9%; $P < 0.001$) than the mean for eggs collected in Florida prior to 1947 (Table 5), eggshell thickness was not significantly correlated ($P > 0.05$) with any of the organochlorine residues (Table 6). However, the sample size was small, and the negative correlation of DDE and eggshell thickness approached significance ($P = 0.115$), meaning that eggs with higher DDE levels tended to have thinner eggshells. Correlations of eggshell thickness with most organochlorines were not tested because only DDE, PCBs, and mirex occurred in more than half of the samples.

Anhinga.—Mean shell thickness of recent eggs from Louisiana and Mississippi was significantly less (–7.5%; $P < 0.05$) than the mean for pre-1947

TABLE 4

ORGANOCHLORINE RESIDUES IN ANHINGA EGGS, 1972 AND 1973*

	Number with residues	Residues in ppm (Wet Weight) Geometric mean[2]	95% C.I.	Range[3]	PCB/DDE ratio
LOUISIANA:					
Lacassine NWR (4)[1]					0
DDE	4	0.79 AB	0.12–1.8	0.31–1.6	
Atchafalaya Basin (10)					0.1
DDE	10	2.1 A	0.8–4.3	0.60–15	
DDT	2	0.03	0–0.06	ND–0.15	
Dieldrin	1	0.01	0–0.03	ND–0.09	
HCB	2	0.01	0–0.02	ND–0.05	
PCBs	3	0.23 A	0–0.56	ND–1.3	
MISSISSIPPI					
Yazoo NWR (3)					0
DDE	3	3.5 A	0.2–15	2.0–7.1	
DDD	2	0.07	0–0.24	ND–0.13	
DDT	3	0.30	0.07–0.59	0.19–0.38	
PCBs	1	0.11 A	0–0.72	ND–0.36	
FLORIDA:					
Darling NWR (1)					0.5
DDE	11	0.41 B	0.13–0.75	0.12–2.5	
Dieldrin	1	0.02	0–0.05	ND–0.18	
Oxychlordane	1	0.01	0–0.04	ND–0.14	
PCBs	6	0.21 A	0.06–0.39	ND–0.72	
Merritt Island NWR (10)					2.8
DDE	9	0.39 B	0.20–0.62	ND–0.93	
PCBs	7	1.1 A	0.18–2.6	ND–6.4	
Payne's Prairie (7)					0.8
DDE	7	0.76 AB	0.39–1.2	0.39–1.6	
Mirex	2	0.05	0–0.14	ND–0.24	
Oxychlordane	1	0.01	0–0.04	ND–0.08	
PCBs	6	0.58 A	0–2.1	ND–7.2	
GEORGIA:					
Okefenokee NWR (1)					0.6
DDE	1	0.42 AB	–	–	
PCBs	1	0.25 A	–	–	

* One egg from each of 46 clutches. Average lipid content = 5.6%. All eggs were analyzed for all chemicals listed in methods. Heptachlor epoxide, *cis*-chlordane, *cis*-nonachlor, toxaphene, and endrin were not found in any of the eggs.
[1] Number of samples collected and analyzed from that locality.
[2] For DDE and PCBs (considered separately), locality means that share the same letters are not significantly different from other locality means for that chemical.
[3] ND = not detected.

TABLE 5

COMPARISON OF WOOD STORK AND ANHINGA MEAN EGGSHELL THICKNESS (MM) IN 2 TIME PERIODS

	1865–1946		1947–1973				
Species State(s)	N[1]	Shell Thickness	N[1]	Shell Thickness	% Change	t	P
Wood Stork							
FL	93	0.530	20	0.483	−8.9	9.54	<0.001
Anhinga							
FL	104	0.343	45	0.345	+0.6	0.47	>0.05
GA, SC	10	0.340	1[2]	0.363			
LA, MS, TX	6	0.352	29	0.326	−7.5	2.47	<0.05

[1] N = number of clutches.
[2] Single clutch from this time period was inadequate sample for making comparison.

eggs, but there was no significant change ($P > 0.05$) in shell thickness of eggs from Florida (Table 5). We did not have an adequate sample of recent eggs from Georgia and South Carolina to compare with the pre-1947 eggs from those states.

The change in clutch mean eggshell thickness was significantly correlated ($P < 0.05$) with the concentration of DDE in the eggs, but not with the concentration of PCBs or total organochlorines (Table 7). We did not test correlations with other chemicals because they occurred in less than half of the eggs.

TABLE 6

CORRELATION MATRIX OF ORGANOCHLORINE RESIDUES IN WOOD STORK EGGS AND CHANGE IN THICKNESS OF THE EGGSHELLS FROM MERRITT ISLAND NATIONAL WILDLIFE REFUGE, FLORIDA, 1973*

	Spearman Rank Correlation Coefficient[1]			
	Mirex	PCBs	Total Organochlorines	Clutch Mean thickness[2]
DDE	0.288	−0.248	0.770***	−0.418*
Mirex		−0.055	0.546**	0.301
PCBs			0.079	−0.115
Total Organochlorines				−0.042

* N = 10 clutches.
[1] Probability of correlations this high or higher as follows: * P = 0.115, ** P = 0.052, *** P < 0.005.
[2] Thickness as a % of the pre-1947 mean shell thickness for Wood Stork eggs from Florida.

TABLE 7

CORRELATION MATRIX OF ORGANOCHLORINE RESIDUES IN ANHINGA EGGS AND CHANGE IN THICKNESS OF EGGSHELLS, 1972 AND 1973*

	Spearman Rank Correlation Coefficient[1]		
	PCBs	Total Organochlorines	Clutch Mean Thickness[2]
DDE	0.009	0.835***	–0.324*
PCBs		0.457***	0.169
Total Organochlorines			–0.129

* N = 46 clutches.
[1] Levels of significance indicated as follows: * $P < 0.05$ *** $P < 0.001$.
[2] Thickness as a % of the pre-1947 mean shell thickness for the region (see Table 5) in which the egg was collected.

DISCUSSION AND CONCLUSIONS

Because we collected Wood Stork eggs at only 1 locality, we could not determine geographic patterns in this species. However, in Anhingas organochlorine residues occurred more often in eggs from inland localities than in those from coastal localities. This pattern, plus the greater frequency of PCB residues in the Anhinga eggs from Merritt Island NWR, is generally consistent with our findings in other species (Ohlendorf et al. 1974, 1978, and unpublished data).

The residues found in the Wood Stork and Anhinga eggs may not directly reflect the levels found in the nesting locality, but they probably are representative for the general area of the nesting colonies. However, Wood Storks feed as far as 125 km from their colonies while nesting and they disperse from these areas after the nesting season; birds marked in Florida have been seen in Mississippi, Alabama, Georgia, and South Carolina (J. C. Ogden, pers. comm.).

Differences in residue frequency and levels among species nesting at Merritt Island NWR (or any other particular locality) might be due to differences in diet, feeding location, or physiology of the birds, or other factors. Diets of the species we studied vary with time and place, but Great Blue Herons, Great Egrets, and night herons generally feed on larger fish of different kinds than do the other birds (Bent 1922, 1926, Palmer 1962). Night herons are particularly active at dawn and dusk, whereas the other species feed more actively during the day. Cattle Egrets and ibises feed more extensively on invertebrates. Cattle Egrets feed almost altogether in terrestrial sites whereas ibises feed largely in mud flats. Other species feed primarily in aquatic areas, eating a variety of organisms, including fish of various sizes.

Wood Storks nesting at Merritt Island feed primarily in freshwater marshes along the St. Johns River when they are nesting (J. L. Baker and J. C. Ogden, pers. comm.), but feeding locations for the other species are not known, and the various species may be exposed to different arrays of contaminants. The Wood Storks may also tend to live longer, thereby having a longer time of exposure. Physiological differences among these species are not known.

Although the differences among locality means were not statistically significant, PCB residues in eggs of most species nesting at Merritt Island NWR (including Anhingas) were usually higher than in eggs from other localities in the South (Ohlendorf et al. 1974, 1978, and unpublished data).

In our more comprehensive survey of organochlorine residues in eggs of Black-crowned Night Herons, we found mean DDE residue levels similar to those of Wood Storks only in eggs from the northeastern Atlantic coastal localities (New Jersey to Massachusetts) and from Michigan (Ohlendorf et al. 1978).

Although we found that shell thickness of Wood Stork eggs collected in Florida since 1946 was significantly less than the historical mean, there was no indication of thin-shelled egg loss or reduction in clutch size in several Florida nesting colonies that were closely studied (J. C. Ogden, pers. comm.). After the first year of our study we had found no significant change in shell thickness of Anhinga eggs from Florida (Ohlendorf et al. 1974); our final results confirm this conclusion.

SUMMARY

All 10 Wood Stork eggs collected at Merritt Island National Wildlife Refuge in 1973 contained residues of DDE (geometric mean 4.0 ppm wet weight) and PCBs (1.2 ppm). Nine other organochlorines were found at lower frequencies in the eggs. Eggshells from the recent period were 8.9% thinner ($P < 0.001$) than pre-1947 samples; decrease in eggshell thickness was more closely correlated with DDE than other organochlorines and correlation of DDE and eggshell thickness approached significance ($P = 0.115$).

Anhinga eggs were collected at 7 localities; 45 of the 46 eggs analyzed contained DDE residues and 24 contained PCBs. Residues of other organochlorines were found less frequently. Shell thickness of recent eggs from Louisiana and Mississippi was significantly less (–7.5%; $P < 0.05$) than the mean for pre-1947 eggs, but there was no significant change in shell thickness of eggs from Florida. The change in clutch mean eggshell thickness was significantly negatively correlated ($P < 0.05$) with the concentration of DDE in the eggs.

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