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SIGNIFICANCE OF CHLORINATED HYDROCARBON RESIDUES TO BREEDING PELICANS AND CORMORANTS

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INTRODUCTION

This PAPER reports levels of chlorinated hydrocarbons present in eggs and spring food of Double-crested Cormorants (*Phalacrocorax auritus*) and White Pelicans (*Pelecanus erythrorhynchos*) and the effects the residues might have upon the reproductive physiology of these species. On the basis of research concerning fat-kinetics in certain migratory birds (Hanson 1962, Weise 1963, Brenner 1967, and others), there is good reason to believe that residues in the eggs provide a reliable index to fat-stored contamination in the female, especially in birds with small clutches. The precise relationships have yet to be studied in detail (Lockie 1967, Stickel 1968).

STUDY AREAS AND METHODS

Study Areas

Sampling was conducted in the summer of 1965 on 19 lakes and impoundments in 10 watersheds of the prairie-states and provinces (Figure 1). Eleven cormorant and five pelican colonies were involved. Pesticides used locally now or in the recent past in most of our study areas included most commonly DDT, Aldrin, Dieldrin, Sevin, Toxaphene, and mercury seed-dressings. Toxaphene used for fish control in the Dakotas was a potential contaminant (Henegar 1966, Needham 1966), although it would likely be distributed in a nonrandom pattern. Toxaphene has been shown to be an important local contaminant in fish-eating birds (Rudd 1964:259; and J. O. Keith, *personal communication*). Dieldrin use at a rate of 2 oz/acre in North Dakota and southern Manitoba and Saskatchewan has in the past been widespread during heavy grasshopper outbreaks. In the other Canadian areas we sampled, known insecticide use appeared to be very slight or negligible. Chlorinated hydrocarbons of pesticide or industrial origin have been detected as fallout in rainwater in England (Wheatley and Hardman 1965, Tarrant and Tatton 1968), in rainwater and dust in Ohio (Cohen and Pinkerton 1966), in airborne particles over the Atlantic Ocean (Risebrough et al. 1968*a*) and in resident wildlife of Antarctica (Tatton and Ruzicka 1967). Local minor usage or nonuse therefore does not

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necessarily mean that residues will not be detected. The industrial pollut include the polychlorinated biphenyls (PCB's) which, like the insecticides, now widely distributed in wildlife (Jensen 1966, Widmark 1967, Holmes 1967, Risebrough et al. 1968*b*, Koeman et al. 1969).

Sampling and Pooling

Fish were sampled by angling, gill-netting, and by picking up regurgita meals. To minimize the effects of digestion, only whole regurgitated fish w taken. We pooled 329 of these fish for chemical analysis by segregating of approximately the same age and weights from each area. Fish up to were occasionally seen as pelican meals, although both pelicans and cormor left materials at their colonies that were composed of minnows and fish up 1 or 2 lb. We observed no other cold-blooded vertebrates or invertebrates the regurgitated boluses at the colonies at the time of sampling. Wh differences in fish size were encountered, we used equal volumes of ground material to give each fish equal representation in the pool. The fish wer completely wrapped in aluminum foil and frozen (−23°C) until prepara for chemical analysis (1 to 3 months following collection).

A total of 89 cormorant and 54 pelican eggs were collected, one fr each nest sample, in a pattern distributed as evenly as possible across the l axis of each colony. These were combined into 59 pools for chemical analy The field-collected eggs were wrapped individually in aluminum foil and frozen until analysis as above with fish. No attempt was made to collect that we found on the ground outside of nests. We attempted to pool of roughly equivalent incubation stages for each colony and species. Disparity of breeding schedule in pelicans (Shaller 1964, Diem and Condon 1967) bias samples to a subcolony rather than truly represent the entire breeding population for a given location. This problem needs further research. Different breeding units in a given colony, i.e., groups with separate, distinct phenologies, may represent different wintering groups (Ward 1924, Diem Condon 1967), or groups with similar physiological conditioning for breed (Shaller 1964). They may also represent renesting or younger birds, especially if found on the periphery of their colony site.

Laboratory Analyses

ORIGINAL ANALYTICAL PROCEDURE. WARF Institute, Inc. (formerly the Wisconsin Alumni Research Foundation, referred to following as WARF) conducted the laboratory analyses. The original analytical procedure follows methods outlined by the U.S. Food and Drug Administration (USFDA 1965).

All samples were ground and then homogenized in a blender. Subsamples were then mixed with sodium sulphate. The latter were extracted for eight more hours in a Soxhlet apparatus with ether:petroleum ether (70:170). After the samples had been extracted in the Soxhlet apparatus, they were made up to 50-ml volumes and divided into two portions. One portion was cleaned by passing it through a florisil column (USFDA 1965). The solvent comprised 150 to 200 ml of ethyl ether:petroleum ether in the first elution. The proportion of ethyl ether was 3 to 5%, depending on the activation of the florisil

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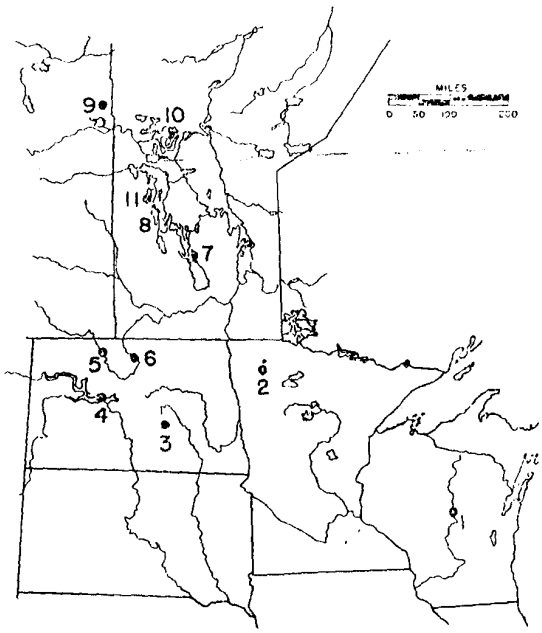

FIGURE 1. Map showing locations of pelican and cormorant breeding colonies used for pesticide studies in 1965. Locations are numbered as follows: 1. Lake DuBay, Wis.; 2. Agassiz National Wildlife Refuge (NWR), Minn.; 3. Chase Lake NWR, N.D.; 4. Lake Garrison, N.D.; 5. Upper Souris NWR, N.D.; 6. Sahcer (formerly Lower Souris) NWR, N.D.; 7. Dog Lake, Man.; 8. Lake Winnipegosis, Man.; 9. Suggi Lake, Sask.; 10. Moose Lake, Man.; 11. Pelican Lake, Man. Nesting pelicans were sampled at Chase Lake, Dog Lake, Suggi Lake, Moose Lake, and Pelican Lake.

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The second elution was comprised of 220 to 250 ml of the same solvents but with 15% ethyl ether. The extracts were analyzed with gas chromatographs (GC) (Barber Coleman, model GC 5000, and Jarrell-Ash, model 28-700) equipped with electron-capture detectors. The glass columns were ¼-inch by 4-ft, packed with 5% DC-200 (12,500) on Cromport XXX. The column temperatures were 210°C. The flow rate of nitrogen was 75 cc/minute.

The second portions of the subsamples were used for fat determinations. These portions were placed in preweighed beakers and dried first over a steam bath and then transferred to a 40°C oven for 2 to 4 hours. The beakers were then reweighed, weight of fat doubled, and the per cent fat calculated on the basis of the original "fresh" weight of the extracted sample.

Solvent and glassware blanks showed no interfering peaks which would interfere with the determination of DDT and PCB compounds. Recoveries, using this procedure, from egg and tissue samples spiked with DDE, TDE, DDT, or Dieldrin, with levels greater than 0.05 ppm, have been tested by WARF chemists and found to be 85 to 100 per cent (F. B. Coon, *personal communication*).

SAPONIFICATION, REANALYSIS, AND REINTERPRETATION OF ORIGINAL CHROMATOGRAMS. The recent discovery of polychlorinated biphenyls in British, Swedish, and North American wildlife samples and their possible interference with the determination of other compounds prompted us to reevaluate our original findings. Five extracts of eggs were randomly selected from the original series, reran again on the GC in 1969, then treated with alcoholic KOH (USFDA 1968; Risebrough et al., in press, 1969) and analyzed for the third time on the GC.

The polychlorinated biphenyls are mixtures of compounds which differ in the number and the position of the chlorine atoms on the biphenyl molecule. The commercial preparations are graded according to their average chlorine content. A chromatogram on a DC-200 column of Aroclor 1254, the average chlorine content of which is 54%, is shown in Figure 2*a*. The PCB peaks labelled 8, 9, and 10 have retention times on DC-200 columns, relative to *p,p'*-DDE, of 1.25, 1.48, and 1.75. They were usually the most conspicuous of the PCB peaks in our chromatograms obtained with electron capture detectors. These three compounds also constitute a large fraction of the total polychlorinated biphenyl in the Aroclor 1254 mixture.

Peak 8 with retention time 1.25 may thus interfere with the determination of *p,p'*-TDE, which has a retention time of 1.27 on DC-200 columns. Similarly, peak 10 may interfere with the determination of *p,p'*-DDT, which has a retention time of 1.68. On this column, however, peak 9 with retention time 1.48 does not interfere with the determination of any of the DDT group. Although PCB peak at 1.25 is selectively degraded by ultraviolet light (Risebrough et al., in press, 1969), most of our chromatograms of wildlife extracts indicated that peaks 8, 9, and 10 had approximately the same height. From

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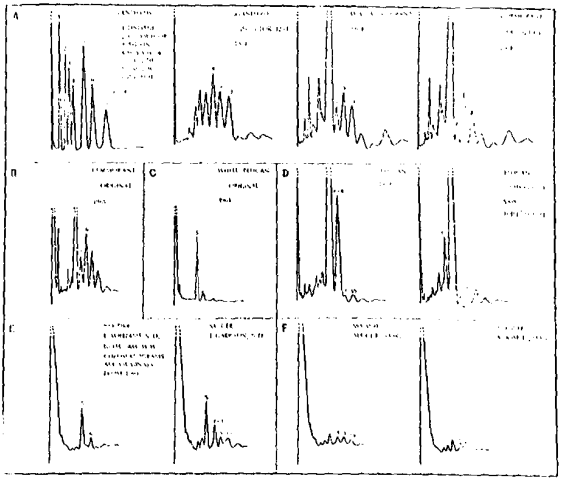

FIGURE 2. Examples of chromatograms of egg and fish extracts under different treatments, showing their different basic characteristics and responses. These chromatograms do not represent specially selected examples. The 1969 GC runs, the "original" chromatograms from 1965, and the fish chromatograms are each traced on their own scales and represent different recorders on the DC-200 column. A. Standards, the original, and saponified extracts of cormorant eggs. The standards represent an injection of 0.05 micrograms per ml each of *p, p'*-TDE and *p, p'*-DDT and 0.4 micrograms per ml of Aroclor 1254. The conversion factor of 10 (see text) was estimated from these differences as well as differences in peak responses. The cormorant extracts (1969) show little change in DDT-TDE interfering peaks upon saponification, suggesting little or no presence of DDT or TDE. The *p, p'*-DDE is off-scale on these cormorant chromatograms and is indicated by a dashed line, as are following example. B. Original chromatogram of cormorant egg extracts from Dog Lake, Manitoba. C. Original chromatogram of pelican egg extracts from Moose Lake, Manitoba. D. Original and saponified extracts of white pelican eggs from Chase Lake, North Dakota, showing conversion *p, p'*-TDE, revealing smaller peaks underneath. E. Original chromatograms of fish extracts (locations are given in diagram), suggesting prevalence of DDE, TDE, and DDT, in that order, superimposed on a background of PCBs. F. Same as E, but from our northernmost sampling sites, showing a general PCB pattern with possibly very small amounts of *p, p'*-DDE present.

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visual examination of the chromatograms of the cormorant egg extracts in Figures 2*a* and 2*b* it was therefore possible to conclude that there was relatively little or no *p,p'*-TDE or *p,p'*-DDT in the extract. In the chromatograms of the unsaponified extracts of the pelican egg in Figures 2*c* and 2*d*, however, the peak after DDE was much higher than the PCB peaks which followed. It would consist, therefore, mainly of *p,p'*-TDE. Since peak 10 was approximately as high as peak 9, it likely consisted mainly of PCB and not *p,p'* DDT.

Saponification of extracts converts *p,p'*-DDT to *p,p'*-DDE and *p,p'*-TDE to *p,p'*-DDMU but does not affect the PCB compounds (Risebrough et al., in press, 1969). The profile of the cormorant chromatogram was therefore unchanged with saponification, but the profile of the pelican chromatograms changed after saponification because of the conversion of *p,p'*-TDE to *p,p'*-DDMU.

In order to obtain correlation coefficients between PCB content and eggshell thickness, it was necessary to estimate the relative PCB concentrations. On a strictly empirical basis, it was found that Aroclor 1254 could be quantified by considering peak 10 as *p,p'*-DDT and multiplying that value by 10. Since peak 10 had originally, in most cases, been quantified as *p,p'*-DDT, and since many of the original extracts contained little or no *p,p'*-DDT, it was possible to obtain a crude estimate of PCB by visual examination of the chromatograms.

Five extracts originally prepared in 1965 were saponified in 1969 to remove interfering *p,p'*-DDT and *p,p'*-TDE. For these samples, PCB was quantified as Aroclor 1254 by relating peaks 9 and 10 to the corresponding peaks of chromatograms of standard Aroclor 1254. The results obtained by the two methods are presented in Table 1.

One or more PCB compounds may emerge at approximately the same time as *p,p'*-DDE, but it is evident from the chromatograms of Figures 2*a*, *b*, *c*, and *d* that the DDE peak was always much higher than the PCB peaks and that there was no significant PCB interference in the determination of DDE. This seems generally true for extracts of North American wildlife (Risebrough et al. 1968*b*) but chromatograms of extracts of a European Kestrel (*Falco tinnunculus*) (Holmes et al. 1967) and a Common Eider (*Somateria mollissima*) (Koeman et al. 1969) suggest that PCB may be more abundant than DDE as an environmental pollutant in some areas of Europe. Moreover, some of the PCB compounds with lower molecular weights, including those interfering with *p,p'*-DDE on QF-1 columns may be selectively metabolized (Risebrough and Anderson, in preparation).

The eggs were measured as described by Anderson and Hickey (1969). Because they showed geographical variations in egg size, shell weight, and shell thickness, it became necessary to compare eggs from the same geographical areas.

The residues are presented as ppm wet weight ("fresh" weight) unless otherwise stated. Chlorinated hydrocarbon insecticides in general are pre-

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Sample No.-Species	Treatment	Per Cent Water	Per Cent Fat	Residue Determinations in ppm Wet-weight				
				Dieldrin	*p, p'*-DDE	*p, p'*-TDE	*p, p'*-DDT	Est. Aroclor
LSE-2-CORMORANT	1	84.7	3.2	0.10	2.8	0.20	0.30	—
(Salyer NWR, N.D.)	2	85.0	2.5	0.05	3.1	NE	NE	—
	3	—	—	NE	4.3	0.04	0.09	4
	4	—	—	NE	NE	0	0	3
DUE-1-CORMORANT	1	82.9	5.0	0.80	45.0	1.05	2.80	—
(Lake DuBay, Wisc.)	2	84.4	4.4	0.44	44.0	NE	NE	—
	3	—	—	NE	44.0	0.18	0.00	23
	4	—	—	NE	NE	T	0	28
WNE-11-CORMORANT	1	83.3	5.0	0.45	18.0	0.50	1.40	—
(L. Winnipegosis, Man.)	2	83.5	6.5	0.28	22.8	NE	NE	—
	3	—	—	NE	20.0	0.35	0.44	23
	4	—	—	NE	NE	T	0	14
CHE-7-WHITE PELICAN	1	82.1	4.5	0.10	2.5	0.60	0.20	—
(Chase Lake, N.D.)	2	83.6	3.8	0.05	2.5	NE	NE	—
	3	—	—	NE	2.6	0.47	0.00	1
	4	—	—	NE	NE	0.60	0.10	1
SUE-8-WHITE PELICAN	1	82.5	4.3	0.15	2.2	0.25	0.20	—
(Suggi Lake, Sask.)	2	85.2	4.1	0.07	2.4	NE	NE	—
	3	—	—	NE	3.8	0.18	0.05	1
	4	—	—	NE	NE	0.20	0.15	1
Mean Deviation Between Methods 1 and 2		+1.2	−0.1	−0.14	+0.82	—	—	—
Per Cent Difference from Original Value		+1.4	−4.5	−43.8	+5.8	—	—	—
Mean Deviation Between Methods 1 and 3		—	—	—	—	−0.30	−0.88	—
Per Cent Difference from Original Value		—	—	—	—	−57.7	−89.8	—

[1]Residue analysis methods are numbered as follows (see text):
1. Standard GC in 1968 (USFDA 1965).
2. Standard GC in 1969 (USFDA 1968), TDE and DDT not estimated (NE).
3. Standard GC in 1969, TDE and DDT estimated by change in peak heights with saponification.
4. Visual examination of chromatograms using criteria described in text. When PCB interference was considered negligible, as for the TDE value of CHE-7, the original value was retained. In others, per cent interference was estimated, recorded as a fraction of the original, present in trace amounts (T), or absent (0).

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Sample No.-Species	Treatment	Per Cent Water	Per Cent Fat	Residue Determinations in ppm Wet-weight				
				Dieldrin	*p, p'*-DDE	*p, p'*-TDE	*p, p'*-DDT	Est. Aroclor
LSE-2-CORMORANT	1	84.7	3.2	0.10	2.8	0.20	0.30	—
(Salyer NWR, N.D.)	2	85.0	2.5	0.05	3.1	NE	NE	—
	3	—	—	NE	4.3	0.04	0.00	4
	4	—	—	NE	NE	0	0	5
DUE-1-CORMORANT	1	82.9	5.0	0.80	45.0	1.05	2.80	—
(Lake DuBay, Wisc.)	2	84.4	4.4	0.44	44.0	NE	NE	—
	3	—	—	NE	41.0	0.18	0.00	23
	4	—	—	NE	NE	T	0	28
WNE-11-CORMORANT	1	83.3	5.0	0.45	18.0	0.50	1.40	—
(L. Winnipegosis, Man.)	2	83.5	6.5	0.28	22.8	NE	NE	—
	3	—	—	NE	20.0	0.35	0.44	23
	4	—	—	NE	NE	T	0	14
CHE-7-WHITE PELICAN	1	82.1	4.5	0.10	2.5	0.60	0.20	—
(Chase Lake, N.D.)	2	83.6	3.8	0.05	2.5	NE	NE	—
	3	—	—	NE	2.6	0.47	0.00	1
	4	—	—	NE	NE	0.60	0.10	1
SUE-8-WHITE PELICAN	1	82.5	4.3	0.15	2.2	0.25	0.20	—
(Suggi Lake, Sask.)	2	85.2	4.1	0.07	2.4	NE	NE	—
	3	—	—	NE	2.8	0.18	0.05	1
	4	—	—	NE	NE	0.20	0.15	1
Mean Deviation Between Methods 1 and 2		+1.2	−0.1	−0.14	+0.82	—	—	—
Per Cent Difference from Original Value		+1.4	−4.5	−43.8	+5.8	—	—	—
Mean Deviation Between Methods 1 and 3		—	—	—	—	−0.30	−0.88	—
Per Cent Difference from Original Value		—	—	—	—	−57.7	−89.8	

[illegible]

[illegible] Residue analysis methods are numbered as follows (see text):
1. Standard GC in 1965 (USFDA 1965).
2. Standard GC in 1969 (USFDA 1968). TDE and DDT not estimated (NE).
3. Standard GC in 1969. TDE and DDT estimated by change in peak heights with superification.
4. Visual examination of chromatograms using criteria described in text. When PCB interference was considered negligible for the TDE value of CHE-7, the original value was retained. In others, per cent interference was estimated, recorded as a fraction of the original, present in trace amounts (T), or absent (0).

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TABLE 2. Chemical names of compounds discussed in the text

p, p'-DDE	1, 1-dichloro-2, 2-bis (p-chlorophenyl) ethylene
p, p'-DDT	1, 1, 1-trichloro-2, 2-bis (p-chlorophenyl) ethane
p, p'-TDE (DDD)	1, 1-dichloro-2, 2-bis (p-chlorophenyl) ethane
p, p' DDMU	1-chloro-2, 2-bis (p-chlorophenyl) ethylene
Aldrin	Not less than 95% of 1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 8, 8a-hexahydro-1, 4-*endo-exo* 5, 8-dimethanophthalene
Sevin	1-naphthyl methylcarbamate
Dieldrin	Not less than 85% of 1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-*endo-exo*-5, 8-dimethano-naphthalene
Endrin	1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4,-*endo-endo*-5, 8-dimethanonaphthalene
Heptachlor epoxide	1, 4, 5, 6, 7, 8, 8-heptachloro-2, 3-epoxy-4a, 4, 7, 7a-tetrahydro-4, 7-methanoindan
Toxaphene	chlorinated camphene containing 67% to 69% chlorine
PCB	chlorinated biphenyl
Lindane	1, 2, 3, 4, 5, 6-hexachlorocyclohexane, 99% or more gamma isomer

sented as "CH" in further discussion. *p,p'*-DDT plus its various metabolites are referred to collectively as "DDT-family" residues. The technical names of the compounds discussed in this text are listed in Table 2.

Statistical Analyses

Statistical analyses used in this study followed Steel and Torrie (1960). Most data were analyzed with an IBM 1620 computer.

RESULTS AND DISCUSSION

Residue Levels

No fish pools (Table 3) from our sample approached levels greater than 0.1 ppm (parts per million) of *p,p'*-DDE (< 0.08 ppm from a Lake Gimlet N.D., sauger pool). No Dieldrin values for fish exceeded 0.015 ppm. Heptachlor epoxide (HE), Endrin, Aldrin, and Toxaphene were not detected in any of the fish pools. PCB residues were detected in all fish samples, but were usually present in trace amounts (Table 3 and Figures 2*e, f*). Since laboratory solvent and glassware blanks showed no evidence of PCB contamination, it would appear that the presence of PCB in fish from remote areas suggests widespread aerial fallout. Although there is substantial evidence for aerial fallout of both DDT and PCB in pelagic areas (Risebrough et al. 1968*b*) we believe that further sampling is necessary to show that this PCB was derived from aerial fallout.

The mean residues for cormorant and pelican eggs were expectedly higher than those for fish (Table 3). Dieldrin, *p,p'*-DDE, and PCB's were universal in all the egg pools, whereas HE (0.3 to 0.5 ppm associated with higher levels of all residues) was detected in only three cormorant egg pools, one from Dog Lake, Manitoba and two from Pelican Lake, Manitoba. The highest cormorant egg residues were 45.0 ppm of *p,p'*-DDE and 28 ppm of estimated PCB's. These were from Lake DuBay, Wisconsin. The highest DDE residues from pelican eggs were from Pelican Lake, Manitoba and ran 4.8 ppm. PCB residues in this same sample were present in trace amounts. Our highest estimate for PCB's in pelicans was 1.2 ppm from two egg pools, one from Moose Lake.

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Species Grouping	No. Areas or Colonies	No. Pools[2]	Residues Detected:[1]							
			p, p'-DDE		Est. PCB		*p, p*-TDE		*p, p*-DDT	
			PPM[3]	%F[3]	PPM[3]	%F	PPM	%F	PPM	%F
Mean residues ± Standard Error										
Interior cormorant										
First nest	9	29	11.0±1.73	100	9	100	T	3	0.2±0.03	10
Renest	2	6	7.3±3.02	100	5	100	ND[4]	0	ND	0
Live eggs	8	26	11.1±1.90	100	8	100	ND	0	0.1±0.03	8
Dead eggs	8	9	8.2±2.33	100	8	100	T	11	T	11
All eggs	11	35	10.4±1.53	100	8	100	T	3	0.2±0.03	9
Interior pelican										
First nest	4	16	1.7±0.26	100	0.5	100	0.4±0.06	88	0.1±0.02	50
Renesters	1	4	1.6±0.35	100	1.1	100	0.3±0.08	100	0.1±0.01	100
Live eggs	5	11	1.9±0.32	100	0.7	100	0.4±0.07	100	0.1±0.02	73
Dead eggs	5	9	1.4±0.28	100	0.6	100	0.3±0.05	78	0.1±0.01	33
All eggs	5	20	1.7±0.22	100	0.6	100	0.4±0.05	90	0.1±0.01	60
Residues in range ppm										
Fish[5]	11	32	T–0.08	70	T–0.5[6]	100	T–0.01	13	T–0.01	13

[1]HE was also detected in 3 cormorant egg pools. Dieldrin was detected in all egg pools but no levels exceeded 0.4 ppm.
[2]Pools consisted of 2 and 3 eggs and occassionally single cormorant eggs. Variances in DDE were not significantly different when single eggs and pools 2 and 3 were compared in both species (Steel and Torrie 1960:83; comparison of *F*-values, where *F* = larger s²/smaller s²). No standard errors are reported for PCB's at this time, due to the nature of their estimates. T (eggs) ≈ < 0.1, T (fish) ≈ < 0.01.
[3]F = % frequency in total sample.
[4]ND = not detected after saponification.
[5]Fish sampled were as follows: Northern Pike (*Esox lucius*), Sauger (*Stizostedion canadense*), Walleye (*S. vitreum*), Yellow Perch (*Perca flavescens*), Black Bullhead (*Ictalurus melas*), Common Whitefish (*Coregonus clupeaformis*), White Sucker (*Catostomus commersoni*), and various minnows (Brook Stickleback, *Eucalia inconstans* and Shiners, *Nototropis* sp.). Probably due to the general low levels reported here in relation to the sensitivity of our analytical techniques, there were no apparent differences between the residues of the various species of fish. There were no differences between fish of the same species from lake to lake, probably for the same reasons.
[6]In 87% of the fish extracts, estimated levels of PCB were below 0.1 ppm (trace amounts in PCB estimates).

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[illegible]

Species Grouping	No. Areas or Colonies	No. Pools[2]	Residues Detected:[1]							
			p, p'-DDE		Est. PCB		*p, p*-TDE		*p, p*-DDT	
			PPM[2]	%F[3]	PPM[2]	%F	PPM	%F	PPM	%F
Mean residues ± Standard Error										
Interior cormorant										
First nest	9	29	11.0±1.73	100	9	100	T	3	0.2±0.03	10
Renest	2	6	7.3±3.02	100	5	100	ND[4]	0	ND	0
Live eggs	8	26	11.1±1.90	100	8	100	ND	0	0.1±0.03	8
Dead eggs	8	9	8.2±2.33	100	8	100	T	11	T	11
All eggs	11	35	10.4±1.53	100	8	100	T	3	0.2±0.03	9
Interior pelican										
First nest	4	16	1.7±0.26	100	0.5	100	0.4±0.06	88	0.1±0.02	50
Renesters	1	4	1.6±0.35	100	1.1	100	0.3±0.08	100	0.1±0.01	100
Live eggs	5	11	1.9±0.32	100	0.7	100	0.4±0.07	100	0.1±0.02	73
Dead eggs	5	9	1.4±0.28	100	0.6	100	0.3±0.08	78	0.1±0.01	44
All eggs	5	20	1.7±0.22	100	0.6	100	0.4±0.05	90	0.1±0.01	60
Residues in range ppm										
Fish[5]	11	32	T–0.08	70	T–0.5[6]	100	T–0.04	13	T–0.01	13

[1]HE was also detected in 3 cormorant egg pools. Dieldrin was detected in all egg pools but no levels exceeded 0.4 ppm.
[2]Pools consisted of 2 and 3 eggs and occasionally single cormorant eggs. Variances in DDE were not significantly different when single eggs and pools 2 and 3 were compared in both species (Steel and Torrie 1960:83; comparison of *F*-values, where *F* = larger s^2 smaller s^2). No standard errors are reported for PCB's at this time, due to the nature of their estimates. T (eggs) = < 0.1, T (fish) = < 0.01.
[3]%F = % frequency in total sample.
[4]ND = not detected after saponification.
[5]Fish sampled were as follows: Northern Pike (*Esox lucius*), Sauger (*Stizostedium canadense*), Walleye (*S. vitreum*), Yellow Perch (*Perca flavescens*), Black Bullhead (*Ictalurus melas*), Common Whitefish (*Coregonus clupeaformis*), White Sucker (*Catostomus commersoni*), and various minnows (Brook Stickleback, *Eucalia inconstans* and Shiners, *Nototropis* sp.). Probably due to the general low levels reported here in relation to the sensitivity of our analytical techniques, there were no apparent differences between the residues of the various species of fish. There were no differences between fish of the same species from lake to lake, probably for the same reasons.
[6]In 87% of the fish extracts, estimated levels of PCB were below 0.1 ppm (trace amounts of PCB estimates).

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Manitoba and the other from Suggi Lake, Saskatchewan. The lowest cormorant egg residues of *p,p'*-DDE (1.4 ppm) were from Upper Souris, North Dakota and the lowest for pelicans (0.6 to 0.7 ppm) were from Dog Lake, Manitoba and Chase Lake, North Dakota. The lowest estimated PCB residues for pelicans were trace amounts found in egg pools from Chase Lake, North Dakota, and Dog and Pelican lakes, Manitoba. Plots of frequency distributions of the total residues suggested normal distributions, except for some slight skewness in the cormorants due to the single pool from Lake DuBay, Wisconsin.

The residues seem low enough to cause no alarm from an acute-toxicity point of view. As a source of food for fish-eating birds, the fish contained levels which were far below those required to induce poisoning in White Pelicans (J. O. Keith 1964) and in Bald Eagles, *Haliaeetus leucocephalus* (Stickel et al. 1966).

There were no significant residue differences between "live" and dead eggs of either species (*t*-test), as well as between their moisture percentages. Surviving Herring Gull (*Larus argentatus*) eggs have been shown to contain much higher levels (J. A. Keith 1966); and levels of chlorinated hydrocarbons that average above those we found in all pelicans and most cormorants were not believed to prevent the hatching of Bald Eagle eggs (Stickel et al. 1966). Extremely high levels in the yolks of Pheasant (*Phasianus colchicus*) eggs apparently did not affect their hatchabilities in incubators (Azevedo et al. 1965). In domestic chickens, Weihe (1967) showed that dietary levels as high as 200 ppm DDT only slightly reduced chick survival. Maximum yolk residues resulting from this diet were 300 ppm DDT and 80 ppm DDE.

Interspecific Differences

We estimated the 1965 dates of earliest egg-laying for each colony and for each species by backdating from the oldest young observed at the colony. Long-term arrival dates were estimated from the following sources: Bent (1922), Lewis (1929), Mendall (1936), and personal communications with refuge personnel. Apparently, pelicans arrive early on the breeding grounds (mean for five colonies = 19 April) and thus spend more time there before egg-laying (20 to 39 days) than cormorants (3 to 20 days), (95% C.L.). Cormorants arrive later (mean for seven general geographic areas = 3 May). Since pelicans probably winter farther south, they must have a more rapid migration than cormorants, or start north sooner. R. B. Klopman (*personal communication*) has observed pelicans arriving during cold and snowy weather at Dog Lake, and J. C. Bartonek (*personal communication*) has seen them on the ice at Pelican Lake. They are reported to arrive at Lake Yellowstone, Wyoming, very often while the lake is still frozen over (Diem and Condon 1967). Pelicans from more southern and western regions may not be under such "stresses," although they have been observed subject to early-season cold weather upon arrival at Nevada and California breeding grounds (J. O. Keith, *personal communication*). Cormorants, on the other hand, are known to migrate leisurely and usually do not arrive until the ice is out (Lewis 1929), although they generally winter farther north than pelicans. Interior cormorants most likely winter on the Gulf Coast and southern Mississippi River

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(Lewis 1929:5-14) and Rio Grande valleys (Palmer 1962:367). White Pelicans from the interior most likely winter on the Gulf Coast and more commonly farther south into Mexico (Palmer 1962:331 and Ann Gammell, *personal communication*).

The studies of Wesley et al. (1965) and Stadelman et al. (1965) in demonstrating the diminution of fat and egg residues with a "clean" diet, however, suggest that the length of time spent in relatively uncontaminated areas might be a possible bias concerning the dates on which the cormorant and pelican eggs were laid in relation to the time they had already spent on the breeding grounds. There were no apparent differences in the percentages of egg fat (pelican = 4.42 ± 0.32%, cormorant = 4.26 ± 0.34%; 95% C.L.). There were no intraspecific relationships among the following factors and residues of DDE, PCB, or Dieldrin: (1) number of days between mean colony egg-laying and laying of the samples we took, and (2) the number of days the birds were on the breeding grounds before laying the sample we took.

Lamb et al. (1967) suggested that egg-laying is a large factor in the ability of a female pheasant to excrete Dieldrin, and Hunt et al. (in press, 1969) suggested a similar phenomenon with DDT. Decreasing amounts of Dieldrin were present in the eggs after cessation of Dieldrin in the diet (it was still present in the eggs after 14 days). Other studies with various insecticides have shown that residues are deposited in the eggs for long periods of time following termination of experimental treatments (Ware and Naber 1961—Lindane, Azevedo et al. 1965—DDT, and Stadelman et al. 1965—Dieldrin). DDT-residue accumulation in the eggs and fat of domestic poultry and pheasants has been shown to be related to dietary levels (Draper et al. 1952; Azevedo et al., 1965; Hunt et al., in press, 1969). Ratio differences of DDE over estimated PCB show they are greater in pelicans (2.9:1) than in cormorants (1.3:1) suggesting that, if local food was of prime importance, ratios in eggs would be similar. There was no evidence of different contamination levels of food between pelicans and cormorants at the time of sampling as suggested by (1) lack of marked residue-level differences between all species of fish sampled (Table 3), and (2) the observations at many of the feeding areas of both species feeding on, and even competing for, the same abundant food sources. Possible differences in residue-storage physiology between pelicans and cormorants on similar diets remain unknown. Resident species, representing different food-web levels, in the Gulf of California have been shown (Risebrough et al. 1968*b*) to have a characteristic DDE/PCB ratio. Migratory species, which breed in the area, but winter elsewhere, had different DDE/PCB ratios. Although egg residue levels are ultimately related to dietary levels, they probably represent stored residues rather than direct, local dietary influence in the two migratory species we have studied here. Since there were no relationships between time of egg-collecting or laying and the amounts of residues present, and since it has already been suggested that egg residues are often acquired prior to arrival on the breeding grounds (Sheldon et al. 1963, Henriksson et al. 1966, Anderson 1967, and others) we conclude that the major

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TABLE 4. — Simple correlations between various egg-shell measurements of cormorants and pelicans and their various detected residues in ppm wet weight in individual pools

Species Grouping	No.	Measurement[1]	Correlation Coefficients:			
			p, p'-DDE	Est. PCB	p, p'-TDE	p, p'-DDT
Cormorant:						
First nesters	29	WT	−.560**	−.463*	−.058	.203
		TH	−.797***	−.430*	.079	−.095
		T1	−.523**	−.347+	−.015	.221
		T2	−.527**	−.393*	.002	.201
Renests	6	WT	−.587	−.855*	ND	ND
		TH	−.441	−.395	ND	ND
		T1	−.706+	−.956**	ND	ND
		T2	−.648	−.892*	ND	ND
All nests	35	WT	−.562***	−.478**	−.086	.103
		TH	−.753***	−.449**	.036	−.142
		T1	−.547***	−.385*	−.045	.139
		T2	−.544***	−.424*	−.029	.128
Pelican:						
First nests	16	WT	−.432+	.046	−.468+	.235
		TH	−.445+	−.037	−.335	.329
		T1	−.550*	−.129	−.527*	−.058
		T2	−.521*	.089	−.548*	.115
All nests	20	WT	−.360	.094	−.392+	.250
		TH	−.436+	.059	−.320	.317
		T1	−.420+	.010	−.417+	.022
		T2	−.496*	.118	−.515*	.133

+$P \leq 0.07$
*$P < 0.05$
**$P < 0.01$
***$P < 0.001$
[1] WT = shell weight, including membranes.
TH = shell thickness, including membranes.
T1 = shell weight/egg volume.
T2 = shell weight/(length × breadth), Ratcliffe (1967).
ND = not detected at the levels of sensitivity used.

interspecific differences between the egg residues in pelicans and cormorants are probably due to dissimilar nonbreeding-area exposures.

Significance of Residues

RELATIONSHIPS BETWEEN RESIDUES AND EGGSHELL DEPOSITION. Initially, we computed correlation coefficients, relating four eggshell measurements to the residue contents of each egg on both a total-micrograms-present basis and a ppm-wet-weight basis (Table 4). The correlations must be considered in relation to the amounts of residues present and their frequencies in our samples (Table 3). The residues should also be considered as an index to the concentrations of CH's in the female, not as the direct cause of shell changes. Both bases of comparison (micrograms *vs.* ppm) were in close agreement, but

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TABLE 5. — Thickness of pelican and cormorant eggs from 1965 compared to pre-1940 museum specimens from the same general areas[1]

Species	Period	No.	Mean (mm) Thickness	95% C.L.	% Change
Pelican[2]	pre-1940	58	0.686	0.673–0.699	—
Pelican	1965	19	0.655	0.643–0.667	−4.5%
Cormorant[3]	pre-1940	100	0.434	0.429–0.439	—
Cormorant	1965	35	0.398	0.386–0.410	−8.3%

[1]These eggs were measured and obtained as described in Hickey and Anderson (1968); samples for the pre-1940 eggs were randomly selected from a larger pool of data.
[2]These means represent eggs taken in Alta., Sask., Man., N.D., Mont., and N.W.T. by egg-collectors.
[3]These means represent eggs taken as above in Alta., Sask., Man., Ont., N.D., Minn., and Mich.

we chose here to present the ppm-based data because most pesticide data in the literature have in the past been presented on this basis. Had we used addled eggs in our samples (thus likely desiccated), the calculations could only have been validly performed on the microgram basis.

The correlation coefficients suggest that in cormorants, *p,p'*-DDE and PCB's were closely related to changes in our indices of eggshell weight and thickness, as well as the actual measurements (Table 4). The residues of *p,p'*-DDT and *p,p'*-TDE were not present in sufficient quantity and frequency in the eggs of interior cormorants to be of prime importance in eggshell changes. The correlation between DDE and PCB in cormorants only was highly significant ($r = 0.5422$ $P < 0.001$), suggesting similar contamination patterns for these two compounds in that species. Both would naturally, then, correlate to shell changes, even if only one were having an effect. The stronger relationship of *p,p'*-DDE to eggshell changes in first-nest cormorants and the correlation of *p,p'*-DDE but not PCB's to eggshell changes in pelicans attests to the importance of that compound. The potential importance of PCB's is suggested, however, by their increased relationship to eggshell changes in renesting cormorants (Table 4). Further research is needed, however, before PCB's can be definitely tied to shell changes.

Regressions of shell thickness on both DDE and PCB's on a colony-basis (the ecological unit related to population changes) (Figure 3) show the importance of DDE and the possible importance of PCB's in predicting the magnitudes of shell change each population might be subject to ($P < 0.001$ for DDE, $P < 0.01$ for PCB's). Renests (Figure 3) show a tendency for eggshell "recovery" in cormorants in relations to both DDE and PCB's. The slopes are nearly equal, however (Figure 3*a*). Ludwig and Tomoff (1966) reported a higher nesting success in renesting Herring Gulls nesting at Grand Traverse Bay, Lake Michigan. That population had been subject to severe egg losses due to breakage and associated phenomenon in first nests.

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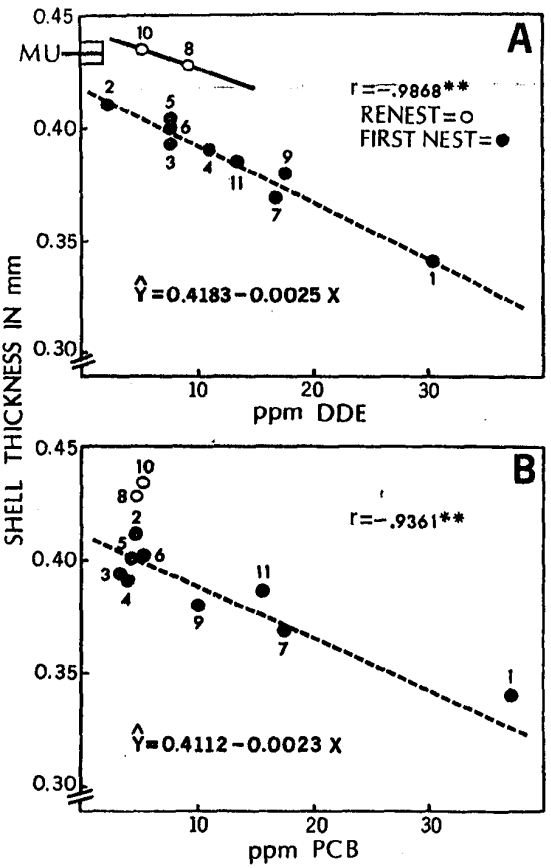
A
MU
r=-.9868**
RENEST=o
FIRST NEST=●
Ŷ=0.4183−0.0025 X
SHELL THICKNESS IN mm
ppm DDE
B
r=-.9361**
Ŷ=0.4112−0.0023 X
ppm PCB
0.45
0.40
0.35
0.30
10
20
30

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In pelicans, where all residues were present in much lower amounts than in cormorants (Table 3), *p,p'*-DDE and *p,p'*-TDE showed significant or nearly significant relationships with nearly all the eggshell parameters tested (Table 4). Unfortunately, only four first-nest colonies were represented, not enough points for a valid, predictive regression on the colony basis as above with cormorants. A regression of shell thickness and residues of DDE + TDE on an individual-pool basis (Figure 4) is nonetheless significant ($P < 0.05$).

It appears that in both species, measurable declines in shell weight and thickness can be related to the lowest residues of *p,p'*-DDE and PCB. Within the limits of our residue-detection ability, there does not appear to be a minimum effective level concerning changes in eggshell. Low levels of residues in the diet (Table 3) and the tendency for shell thickness recovery in renests suggests that breeding-season depletion of fat reserves is less important than the direct dietary effects on eggshell deposition in the two species we studied. It follows that residue-thickness relationships need not always relate, but may depend on the particular residue-exposure, ecological situation to which a given breeding bird (or group of breeding birds) is exposed prior to and during egg and shell formation.

Since the percentages of fat in cormorant eggs were essentially the same in first nests (4.24%) as in renests (4.37%) their residue differences might be explained by postulating some dilution in the fat of previously stored residues. Residues on a fat basis suggested this (262 ppm for first nests and 166 ppm for renests. The residues in the eggs of renests were lower, but not significantly so (Table 3), probably due to limited degrees of freedom in the small sample of renests. The differences in amount of residues in the fat of the eggs, then, could account for the lower mean residues in renests, but suggest that previously stored residues are still important in egg deposition.

To further test for changes in shell thickness, we independently compared our 1965 eggs with museum specimens from the same geographical areas, but from the pre-DDT period. The comparisons (Table 5) showed small, but significant ($P < 0.05$) changes in shell thicknesses of both pelicans and cormorants.

POPULATION STATUS. The populations of White Pelicans we studied seem stationary (Anderson 1967), except where they are locally subject to disturbance and molestation. Lies and Behle (1966) concluded that the White Pelican

FIGURE 3. Relationships between DDE residues (A), estimated PCB residues (B), and shell thickness in Double-crested Cormorants, plotted on a colony-basis. Individual points are numbered in accordance with Figure 1. Open circles represent renest colonies (the original colony of the season was destroyed or disturbed away from the first-nest site, therefore, phenologically behind other colonies from the same general latitude and longitude) and closed circles represent first-nest colonies. "MU" in the upper figure represents the museum mean thickness (Table 5), bounded by 95% Confidence Limits. Figure 3A, $P<0.001$; Figure 3B, $P<0.01$. The line-of-fit for renests in A. was fitted by eye but clearly resembled the calculated regression based on individual pools. A line-of-fit for renests in B., though significant on an individual-pool basis, was not obvious on a colony basis.

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has declined since the early 1900's due to its sensitivity to disturbances during breeding. The major reason for the long-term decline of the White Pelican seems to be related to increasing infringement upon its breeding habitat by advancing "civilization." In the United States, the refuge system has probably been responsible for the maintenance of essentially stable numbers. In Canada, the species is still threatened by disturbances of the nesting colonies by fishermen (Carson 1966), and by tourists and other factors (Houston 1962). One of the largest breeding colonies in Canada (at Primrose Lake, Saskatchewan) may be threatened by a bombing range.

The Lake DuBay, Wisconsin, breeding population of cormorants showing a 25% decline in shell thickness (Figure 3) has recently decreased to nearly zero (Anderson and Hamerstrom 1967). Since the report of Anderson and Hamerstrom (1967), these birds (about 15 adults in the area in 1967) occupied only one nest in 1967, which failed (C. R. Sindelar Jr., *personal communication*). J.J.H. and D.W.A. were unable to find any occupied nests on 31 May 1968 at Lake DuBay, although about 10 adults frequented the traditional nesting site, where a reasonably stationary population of Great Blue Herons (*Ardea herodias*) persists. A large flock of cormorants was observed in the rookery area on 23 June 1968 (Frances Hamerstrom, *personal communication*). Forty-two dark adults and eight light-coloured yearlings were seen. This flocking behavior is indeed unusual, during a period when the birds should have been exhibiting reproductive, not migratory, behavior. Traditionally, young of nearly banding age would be found at the colony by late June. Late nesting has characterized the cormorant rookery at Lake DuBay in recent years (Frances Hamerstrom, *personal communication*). In 1968, one cormorant nest was found by F. H. from which three abandoned eggs were taken. These eggs averaged 16 ppm *p,p'*-DDE, 46 ppm estimated PCB, and 1.5 ppm each of Dieldrin and HE. The mean thickness for these three eggs was 0.37 mm, predictable in Figure 3*a*.

Agassiz cormorants were known to be stationary in 1965, compared to the early 1940's (J. W. Ellis, *personal communication*). Upper Souris, Salyer, and Chase Lake cormorants (see Figure 1 for these locations) seemed stationary or not seriously declining in 1965 (Anderson 1967), although the Salyer colony seemed to be holding its numbers at a much lower level compared to the mid-1940's (M. C. Hammond, *personal communication*). Deterioration of nesting habitat and predation might have been important factors in the decline of the Salyer colony. Lake Garrison cormorants had increased up to the 1960's (G. Enyeart, *personal communication*) due to an opening of new habitat (flooded trees) and creation of abundant food supplies (Anderson 1967), although somewhat affected by shell changes in 1965 (Figure 3). The long-term status of the Canadian cormorant colonies we studied remains generally unknown.

In general, it appears that the status of a given colony can be related to the residues and shell thickness of the eggs from that colony, especially in areas such as Lake DuBay, where changes have been rather marked. Other ecological factors undoubtedly complicate the situations and probably modify them.

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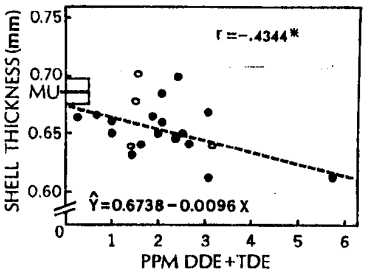

FIGURE 4. Relationship between DDE + TDE and eggshell thickness in White Pelicans, as described in Figure 3, except that these points represent individual pools rather than colonies. $P<0.05$.

DECLINING POPULATIONS, REPRODUCTIVE SUCCESS, AND SUBLETHAL EFFECTS OF CHLORINATED HYDROCARBONS. Cramp et al. (1964) hypothesized that declines of raptors in Great Britain were due to "sub-lethal effects in reducing fertility." Their ranges of residue levels in four raptors were from 1.8 to 12.1 ppm. Lockie and Ratcliffe (1964) reported in great detail on Golden Eagle (*Aquila chrysaëtos*) egg breakage in Scotland. Egg residues on the order of 0.25 to 10.29 ppm were detected. Eyries with histories of 1.44 to 6.90 ppm showed definite egg breakage, whereas eyries with residues of 1.1 or less bred successfully or resulted in occasionally addled, but not broken eggs. A certain percentage of addled eggs was considered normal, as usually these eagles lay two eggs but raise only one young. These authors concluded that toxic chemicals were to blame as affecting behavior of adults. Lockie (1967) later stressing Dieldrin residues alone stated: ". . . the critical level at which behavior became upset as evidenced by egg-breaking, was no higher than 1 ppm (part per million) of Dieldrin in the egg contents. However, this is a very rough figure since other chemicals, for example DDE (a metabolite of DDT), BHC, and Heptachlor were present in varying amounts . . ." Wurster and Wingate (1968) associated residues of 3.61 to 11.04 ppm in the eggs of Bermuda Petrels (*Pterodroma cahow*) with their declining reproductive success. Ratcliffe (1967*a*) reported on egg breakage in British Peregrines (*Falco peregrinus*) and on residues associated with unsuccessful eyries (17.4 mean ppm CH residues) and eyries with improved success since 1963 (12.7 ppm). The general Peregrine population in Britain has not shown recovery, however, and Ratcliffe concludes that persistent organo-chlorine pesticides have been the causal factor in the decline and failure of recovery of these birds. In a pioneering study, Ratcliffe (1967*b*) showed that three declining species of British raptors (Peregrines, Golden Eagles, and European Sparrow Hawks [*Ac-*

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cipiter nisus]) had been laying thin-shelled eggs. This phenomenon was related to an environmental change which had occurred in 1947. Hickey and Anderson (1968) subsequently showed that some North American populations of Peregrine Falcons began to lay thin-shelled eggs in 1947, prior to their extirpation over large areas of the United States and southern Canada (summarized in Hickey 1969). Declining populations of Bald Eagles, Ospreys (*Pandion haliaetus*), and Peregrines displayed this shell-thinning phenomenon, but stationary populations of Ospreys, Red-tailed Hawks (*Buteo jamaicensis*), Great Horned Owls (*Bubo virginianus*), and Golden Eagles showed no evidence of shell-thinning (Hickey and Anderson 1968).

PHYSIOLOGICAL EFFECTS OF CHLORINATED HYDROCARBONS RELATING TO EGGSHELL FORMATION. The data presented in this paper suggest that DDE has a greater effect on shell thickness than PCB. The limited work so far carried out concerning the induction of steroid hydroxylating enzymes in birds by PCB and Dieldrin (Peakall 1967, Risebrough et al. 1968*b*) indicate, however, that both PCB and Dieldrin are more potent enzyme inducers than *p,p'*-DDE or *p,p'*-DDT. Elevated levels of steroid metabolism resulting from the induction of nonspecific oxidase enzymes in the microsomal fraction could affect calcium metabolism in several ways (Ratcliffe 1967*b*; Peakall 1967; Risebrough et al. 1968*b*; Wurster, in press, 1969). Estrogen and androgen are essential for the deposition of medullary bone, which is a major source of eggshell calcium (reviewed by Simkiss 1967:160-185). Vitamin D is essential for calcium absorption from the small intestine (reviewed by Simkiss 1967:62-71). The active form of Vitamin D is a derivative of Vitamin D_3 and is hydroxylated at the 25 position (Blunt et al. 1968). Nonspecific hydroxylation of the Vitamin D_3 molecule at different positions by the induced enzymes might therefore create an artificial Vitamin D deficiency.

Neither estrogen nor Vitamin D degradation, however, can adequately explain the absence of a no-effect range of concentrations of DDE upon shell thickness and the observed linear relationships between DDE concentration and shell thickness. This relationship, moreover, is linear to zero concentration of DDE and the shell thickness at zero concentration is equivalent to, or slightly less, than the shell thickness of eggs collected before 1940 (Figures 3 and 4). In addition to the data here presented for White Pelicans and Double-crested Cormorants, a linear relationship has also been found between DDE concentrations in the eggs and the thickness of the shells from Herring Gulls ($P < 0.001$) (Hickey and Anderson 1968). Feedback mechanisms would be expected to maintain essential estrogen levels at lower DDE concentrations. Similarly, gross abnormalities of Vitamin D metabolism would be expected only when induced steroid hydroxylase activities are significantly higher than normal, especially in fish-eating birds which receive adequate Vitamin D in their diets.

The enzyme carbonic anhydase is found in the cells of the shell gland where it catalyses the formation of the carbonate ions which combine with calcium to form the calcium carbonate of the eggshell (Common 1941,

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Gutowska and Mitchell 1945, Bernstein et al. 1968). Inhibition of this enzyme by sulfanilamide and other unsubstituted sulfonamides such as acetazolamide results in the production of thin-shelled eggs (Gutowska and Mitchell 1945, Benesch et al. 1945). Carbonic anhydrase inhibition in the shell gland by acetazolamide has been correlated with decreases in eggshell weight (Bernstein et al. 1968). Microgram amounts of DDT also inhibit carbonic anhydrase prepared from bovine erythrocytes (Keller 1952, 1963). Preparations of the mammalian enzyme are also inhibited *in vitro* by *p,p'*-DDE at physiological concentrations (R. W. Risebrough, in preparation). Inhibition *in vivo* of carbonic anhydrase in birds by DDE could therefore account for the linear relationships plotted in Figures 3 and 4. It would also explain how the effect of DDE upon shell changes could be greater than the effects of other chlorinated hydrocarbons which are more potent enzyme inducers.

CONCLUDING COMMENTS

The present study was largely accomplished before the nature of PCB interference in the determination of the DDT-compounds was known. The initial conclusions, however, were not greatly changed when corrections were made for this interference. DDE appears to be the environmental pollutant most responsible for the thin eggshell phenomenon. The effects upon avian reproduction of the polychlorinated biphenyls and other environmental pollutants, of elevated levels of steroid metabolism, of possible abnormal vitamin metabolism caused by environmental pollutants, and of the possible inhibition of carbonic anhydrase and other enzyme systems remain to be determined. We hope this will be accomplished before the fish-eating birds have disappeared.

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