Eggshell Thinning, Chlorinated Hydrocarbons, and Mercury in Inland Aquatic Bird Eggs, 1969 and 1970 [1]

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ABSTRACT

In the Upper Great Lakes States, 9 out of 13 species of fish-eating birds were found in 1969-70 to have sustained statistically significant decreases in eggshell thickness since 1946. Maximum changes in a thickness index occurred in great blue herons (−25%), red-breasted mergansers (−23%), common mergansers (−15%), and double-crested cormorants (−13%). Heron eggs taken in Louisiana generally displayed a smaller post-'46 change than herons in the Middle West. On a lipid basis, mean PCB- and DDE-residue levels exceeded 100 ppm in 7 out of 13 species in the Great Lakes States, and in 2 of 7 species in Louisiana, the average DDE: PCB ratios in the two regions being 1.25:1 and 3.9:1, respectively. Individual dieldrin values tended to be higher in Louisiana (31.6 and 13.95 ppm in heron species from two different locations), although values reached 10.1 and 9.4 ppm in great blue and black-crowned night herons in Wisconsin. BHC averaged 3.01 and 0.39 ppm in the Lake States and Louisiana, respectively. Of eggs examined for mercury, 29% had levels greater than 0.5 ppm, and 9% greater than 1.0 ppm on a wet-weight basis. Mercury levels in a small sample of eggs from Louisiana were consistently low. The differences in mercury levels between the two regions thus were similar to those found for the chlorinated hydrocarbons. While DDE was a prominent factor for most groups, especially herons, in relation to the eggshell thinning observed, dieldrin was also important to two groups even though DDE was present in much higher amounts. PCB's were also important to mergansers, while mercury was positively correlated with thickness index in grebes and negatively correlated in mergansers.

Introduction

This report summarizes the levels of chlorinated hydrocarbons and mercury in 117 eggs of 19 species of aquatic-feeding birds collected in 1969 and 1970 in the Upper Great Lakes States (Wisconsin, Michigan, and Minnesota) and in Louisiana. Shell thickness and a thickness index (*24*) for these 117 eggs are compared to museum data for eggs collected before 1947. Of the 30 species of raptorial and fish-eating birds in which the eggshell-thinning phenomenon has now been found in great Britain (*25*) and North America (*1*), 6 species are known to have DDE associated with this phenomenon (*2, 4, 7, 9, 13, 16, 32*). Blus *et al.* (*4*) used multiple regression to obtain a predictive equation for shell thickness of brown pelican eggs (*Pelecanus occidentalis*) in which DDE was the most important factor. Laboratory confirmation of this DDE effect has been provided by Heath *et al.* (*14*). Bitman *et al.* (*3*), and Wiemeyer and Porter (*31*). The effect of dieldrin on eggshell thinning has also been reported by Lehner and Egbert (*22*) and Enderson and Berger (*9*).

Because of the combined presence of the chlorinated hydrocarbons in animal tissues, partial-correlation analysis (*28, 29*) was used in this study to determine the importance of each chemical, including mercury, to eggshell-thickness changes in the 6 families and 19 species in our samples. Chemical residues in birds' eggs, of course, only reflect levels in the females that laid the eggs and are not necessarily the cause of shell-thinning.

[1] From the Department of Wildlife Ecology, University of Wisconsin, Madison, Wis. 53706, under contract with the Bureau of Sport Fisheries and Wildlife, U. S. Department of the Interior, Patuxent Wildlife Research Center, Laurel, Md.

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Methods

FIELD COLLECTIONS

All eggs were collected in 1970 except for those of the redbreasted merganser which were collected in 1969. Sites for collection within the Great Lakes area and Louisiana were selected largely on the basis of availability rather than with regard to probably "polluted" areas, except in the case of the herring gull. Herring gull eggs came from upper Green Bay, Wis., where eggs in previous years have had high levels of DDE (*20*). Only one viable egg was collected from each active nest, but all eggs were collected from nests containing only addled or abandoned eggs. All eggs were wrapped in aluminum foil and frozen until analysis.

EGGSHELL MEASUREMENTS

Eggshell measurements of pre-1947 eggs were taken from specimens in the collections of five museums and one private collector. All eggshells were measured following the methods of Hickey and Anderson (*16*). In order to increase sample size, we first compared pre-1947 data from different regions; if no significant differences were found, we combined the samples to form a larger one for comparison with the 1970 data. These data are presented in Table 1. For some species, a few eggs collected after 1946 (usually 1958 or after) were found in these collections. In these cases, comparisons were made in two ways, i.e., pre-1947 with 1970 and pre-1947 with 1970 and the post-1946 museum eggs combined.

In addition to shell thickness, we relied on a statistic—thickness index—initially used by Ratcliffe (*24*) and calculated as shell weight divided by the product of egg length times breadth. The thickness index was used to measure eggshell changes for two reasons: (1) it seems to be less subject to variation in the measuring technique when many species with very different eggshell char-

TABLE 1.—*Comparison of pre-1947 eggshell thickness and thickness index with 1970 data and with 1970 and post-1946 museum egg data combined*

Common and Scientific Name	Collection Region	Mean Thickness in mm ± 95% Confidence Level			Mean Thickness Index ± 95% Confidence Level		
		(N) Pre-1947	(N) 1970 *and* (N) 1970 and Post-1946 Museum Egg Data Combined	Percent Change	(N) Pre-1947	(N) 1970 *and* (N) 1970 and Post-1946 Museum Egg Data Combined	Percent Change
Red-necked grebe (*Podiceps grisegena*)	Wisconsin	(112) 0.357 ± .005	(2) 0.350 ± .00	− 2.0	(112) 1.84 ± .02	(3) 1.62 ± .21	− 13.0*
	Wisconsin and Ontario		*(6) 0.338 ± .015*	*− 5.3*		*(7) 1.68 ± .10*	*− 8.7***
Pied-billed grebe (*Podilymbus podiceps*)	Wisconsin	(71) 0.286 ± .005	(9) 0.288 ± .021	+ 0.7	(71) 1.47 ± .02	(9) 1.45 ± .12	− 1.4
	Wisconsin					*(17) 1.42 ± .06*	*− 3.4*
White pelican (*Pelecanus erythrorhynchos*)	Minnesota	[a] (92) 0.683 ± .010	(10) 0.633 ± .040	− 7.3*	[a] (94) 3.33 ± .05	(10) 3.12 ± .22	− 6.3
Double-crested cormorant (*Phalacrocorax auritus*)	Minnesota	[a] (350) 0.430 ± .003	(19) 0.370 ± .014	− 14.0*	[a] (370) 2.12 ± .02	(19) 1.80 ± .08	− 15.1*
Great blue heron (*Ardea herodias*)	Wisconsin	[a] (170) 0.393 ± .004	(5) 0.330 ± .029	− 16.0*	[a] (288) 2.05 ± .02	(5) 1.54 ± .18	− 24.9**
Common egret (*Casmerodius albus*)	Wisconsin	(235) 0.295 ± .003	(4) 0.275 ± .062	− 6.8	(235) 1.49 ± .01	(4) 1.38 ± .34	− 7.4*
Black-crowned night heron (*Nycticorax nycticorax*)	Wisconsin	[a] (134) 0.287 ± .003	(6) 0.272 ± .023	− 5.2	[a] (158) 1.44 ± .02	(6) 1.30 ± .14	− 9.7
American bittern (*Botaurus lentiginosus*)	Wisconsin	(68) 0.243 ± .004	(1) 0.24	0	(72) 1.22 ± .02	(1) 1.18	− 3.3
Hooded merganser (*Lophodytes cucullatus*)	Wisconsin	(44) 0.614 ± .019	(11) 0.599 ± .035	− 2.4	(44) 4.00 ± .11	(11) 3.82 ± .24	− 4.5
Common merganser (*Mergus merganser*)	Wisconsin and Michigan	(45) 0.437 ± .014	(13) 0.368 ± .012	− 15.8**	(62) 2.49 ± .06	(13) 2.11 ± .08	− 15.3*

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acteristics are considered; and (2) there seems to be a change in density of the shell in very thin-shelled eggs in some species (*10*).

CHEMICAL ANALYSIS

All eggs were analyzed for chlorinated hydrocarbons by WARF Institute, Inc., Madison, Wis., using gas chromatography. Methods of analysis followed those given by Anderson *et al.* (*2*) with the exception described here. A Barber-Coleman Pesticide Analyzer Model 5360 with two columns was used: one column was packed with 5% DC-200 on 60/70-mesh Chromiport XXX, with a column temperature of 200°C and a nitrogen flow rate such that *p,p'*-DDT had a retention time of 6-8 minutes. The other column was packed with 3% OV-17 on 100/120-mesh Gas Chrom Q, with a column temperature of 195° C, and a flow rate such that lindane had a retention time of 1 minute. The estimated polychlorinated biphenyl (PCB) values were determined from the heights of the peak between TDE and DDT and from the peak at DDT after saponification. Recovery rates were 80-90%.

The 86 eggs analyzed for mercury included all eggs from the Great Lakes area (one herring gull sample was lost) and 1 egg per species, chosen randomly, from the 7 species of herons from Louisiana. Five black tern eggs were analyzed by the Gulf Radiation Technology Division, Gulf Energy and Environmental Systems, Inc., using neutron-activation analysis, the samples analyzed being mostly albumen mixed with a small amount of yolk. Weighed portions of each sample were sealed in vials and irradiated at a flux of 10^{12} thermal neutrons per cm^2 per second together with a mercury comparator standard. The irradiated samples were digested, in the presence of mercury carrier, in a mixture of HNO_3 and

TABLE 1.—*Comparison of pre-1947 eggshell thickness and thickness index with 1970 data and with 1970 and post-1946 museum egg data combined*—Continued

Common and Scientific Name	Collection Region	Mean Thickness in mm ± 95% Confidence Level			Mean Thickness Index ± 95% Confidence Level		
		(N) Pre-1947	(N) 1970[1]/ *(N) 1970 and Post-1946 Museum Egg Data Combined*	Percent Change	(N) Pre-1947	(N) 1970[1]/ *(N) 1970 and Post-1946 Museum Egg Data Combined*	Percent Change
Red-breasted merganser *(Mergus serrator)*	Wisconsin	(141) 0.365 ± .003	(11) 0.303 ± .013	− 17.0**	(148) 2.01 ± .02	(11) 1.55 ± .08	− 22.9**
Herring gull *(Larus argentatus)*	Wisconsin	[2] (369) 0.375 ± .002	(10) 0.328 ± .021	− 12.5*	[2] (456) 1.73 ± 0.01	(10) 1.49 ± .10	− 13.9*
Black tern *(Chlidonias nigra)*	Wisconsin	(91) 0.155 ± .002	(5) 0.132 ± .023	− 14.8**	(91) 0.69 ± .01	(5) 0.62 ± .08	− 10.1**
Green heron *(Butorides virescens)*	Louisiana	(100) 0.176 ± .002	(3) 0.163 ± .038	− 7.4	(105) 0.89 ± .01	(3) 0.81 ± .31	− 9.0*
	Louisiana and South Carolina		*(11) 0.166 ± .008*	*− 5.7**		*(11) 0.83 ± .05*	*− 6.7**
Little blue heron *(Florida caerulea)*	Louisiana	(18) 0.239 ± .007	(5) 0.222 ± .030	− 7.1	(18) 1.14 ± .04	(5) 1.09 ± .10	− 4.4
Cattle egret *(Bubulcus ibis)*	Louisiana	(7) 0.236 ± .007	(5) 0.228 ± .010	− 3.4	(7) 1.10 ± .05	(5) 1.06 ± .06	− 3.6
	Louisiana and South Carolina		*(20) 0.228 ± .007*	*− 3.4*		*(20) 1.07 ± 0.3*	*− 2.7*
Common egret *(Casmerodius albus)*	Louisiana	(235) 0.295 ± .003	(5) 0.292 ± .013	− 1.0	(235) 1.49 ± .01	(5) 1.44 ± .08	− 3.4
Snowy egret *(Leucophoyx thula)*	Louisiana	(85) 0.235 ± .004	(5) 0.222 ± 0.27	− 5.5	(85) 1.14 ± .02	(5) 1.08 ± .11	− 5.3
	Louisiana and South Carolina		*(9) 0.220 ± .014*	*− 6.4**		*(9) 1.07 ± .06*	*− 6.1**
Louisiana heron *(Hydranassa tricolor)*	Louisiana	(8) 0.240 ± .013	(5) 0.236 ± .030	− 1.7	(8) 1.19 ± .05	(5) 1.14 ± .14	− 4.2
Yellow-crowned night heron *(Nyctanassa violacea)*	Louisiana	(71) 0.287 ± .004	(5) 0.282 ± .027	− 1.7	(71) 1.45 ± .02	(5) 1.44 ± .16	− 0.7

[1] Red-breasted merganser collected in 1969.
[2] Pre-1974 data from Anderson and Hickey (*1*)

* Significant difference ($P<0.00$.) by Student's t-test.
** Highly significantly different ($P<0.005$)

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H_2SO_4 under reflux conditions. Radiochemical procedures were used to isolate mercury as the metal. Multichannel gamma-ray spectrometry was used to identify and quantitate mercury. The remaining eggs were analyzed for mercury by WARF, Inc., using the method in *The Analyst* (*19*), modified by atomic absorption spectrophotometry with cold vapor.

These two methods measure total mercury, both organic and inorganic. Because of the biological methylation of mercury (*18, 21*), and the biological route that mercury takes in reaching eggs, we feel it is safe to assume that most of the mercury was in the methylated form. Neither of the two methods gives consistently higher results than the other, but both apparently are not extremely precise (R. F. Christensen, *personal communication*). However, both methods give high rates of recovery of standards (approximately 91% for atomic absorption and 96% for neutron activation).

Results and Discussion

EGGSHELL CHANGES

The major post-1946 eggshell changes encountered in this study involved great blue herons (−25%), red-breasted mergansers (−23%), common mergansers (−15%), and double-crested cormorants (−15%) in that order, all of these specimens collected in 1969 or 1970 from the Great Lakes States (Table 1). In this region, 9 of 13 species exhibited a statistically significant ($P<0.05$) change in shell thickness or thickness index. However, for those species in which addled eggs were included, the sample is biased because these eggs have been incubated and the thinnest eggs would have been broken (*11*).

The heron eggs taken farther south displayed a generally smaller post-1946 eggshell change. While this change was statistically significant in 2 out of 7 species, eggs of the 7 southern species shown in Table 1 involved a mean (unweighted for sample size) change in thickness index amounting to −4%, whereas eggs of the four herons in the Great Lakes States showed a mean change of −11%. In eggs of the common egret, the only species for which we had samples from both Louisiana and the Great Lakes States, the thickness index change in Wisconsin was −7% ($P<0.05$) and in Louisiana, −3% (not significant).

Eggs of each of the 19 species studied had a negative change in mean thickness index, although many of these changes were not statistically significant. Mean changes in measured thickness were negative in all but the pied-billed grebe. The mean thickness of one museum clutch of post-1946 Louisiana heron eggs from Texas was significantly greater than the pre-1947 mean. Changes in three museum clutches of Louisiana heron eggs from Mexico were −13% ($P<0.005$) in thickness and −12% ($P<0.005$) in thickness index. The probability of negative changes in the 19 thickness-index estimates due to chance alone is infinitesimal ($P< 19 \times 10^{-7}$) and for negative changes in 18 of the 19 thickness estimates is very small ($P< 36 \times 10^{-9}$) (R. G. Heath, *personal communication*).

The differences between percent decrease in thickness and thickness index for great blue herons (Table 1) illustrate the value of the index. Thin-shelled great blue heron eggs have very rough shells which are difficult to measure accurately with a thickness micrometer; apparently, these eggs are also less dense. The thickness index takes into account both this change and the actual thickness, and probably is better related to the decrease in breaking strength that accompanies eggshell thinning.

In general, the consistent pattern of eggshell change in these species strongly suggests that, with larger post-1946 samples, statistically significant decreases in shell thickness will be found in virtually all fish-eating species of birds in these latitudes of North America. We are uncertain about the biological significance of decreases in shell thickness below 10%. Certainly, widespread eggshell breakage does not occur with changes below this magnitude. Normal eggshells do exhibit wide variation in thickness. Ernst Mayr (*personal communication*) feels that natural selection would produce an eggshell somewhat thicker than that normally needed to prevent breakage. Under normal conditions, the selection pressure against a genotype with too thin an eggshell is probably greater than that against a genotype which uses excess metabolic energy in producing a thick eggshell.

RESIDUE LEVELS IN EGGS

In Table 2, eggshell changes are compared with mean residues of organochlorines, PCB's, and mercury in the eggs. Means influenced by one high value are indicated by footnotes.

Residues of DDE and PCB's varied greatly, both among and within species. The herring gull eggs, collected by D. W. Anderson, from Sister Island in Green Bay had by far the highest levels of any species, while red-breasted merganser eggs collected 1 year earlier from the same island had almost 10 times less (Table 2). The levels of DDE and PCB's found in eggs from Louisiana were much lower than those in eggs from the Great Lakes States, with one exception, the green heron eggs from near Monroe, La. where DDT has been used extensively on cotton in the past (L.D. Newsom, *personal communication*). Eggs from Louisiana generally had less PCB's in relation to DDE than those from the Great Lakes States (see DDE: PCB ratios in Table 2).

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TABLE 2.—*Eggshell thickness changes and mean residues of organochlorines, PCB's, and mercury by species*

Species or Egg	Collection Location	Number in Sample	Percent Change in Shell Thickness	Percent Change in Shell Thickness Index	Mean Residues in ppm							Ratio of DDE to PCB's
					Percent Lipid	DDE [1]	PCB's [2]	Dieldrin [1]	DDT + TDE [1]	BHC [1]	Mercury [3]	
GREAT LAKES												
Red-necked grebe	Rush Lake, Wis.	3	− 2.0	−13.0*	8.43	646.8	744.6	8.70	33.07	10.00	0.16	0.87
Pied-billed grebe	Rush and Mud Lakes, Wis.	9	+ 0.7	− 1.4	8.49	82.6	77.6	2.39	5.13	0.60	0.20	1.06
White pelican	W. Minnesota	5	− 7.3*	− 6.3	5.32	37.1	15.4	1.64	2.36	0.72	0.24	2.41
Double-crested cormorant	W. Minnesota	8	−14.0*	−15.1*	4.37	[4] 188.4	132.2	7.07	4.98	15.4	0.43	[4] 1.43
Great blue heron	Horicon Marsh, Wis.	5	−16.0*	−24.9*	4.83	472.1	175.3	10.10	44.27	0.79	0.29	2.69
Common egret	Horicon Marsh, Wis.	4	− 6.8	− 7.4*	6.24	[4] 306.0	108.1	4.28	23.42	0.94	0.26	[4] 2.83
Black-crowned night heron	Horicon Marsh, Wis.	6	− 5.2	− 9.7	6.68	81.6	70.2	9.37	6.07	0.76	0.48	1.16
American bittern	N. Wisconsin	1	0	− 3.3	7.27	6.1	12.2	0.21	0	0.14	0.15	0.50
Hooded merganser	N. Wisconsin	8	− 2.4	− 4.5	16.59	14.6	17.6	0.25	0.51	0.20	0.96	0.83
Common merganser	U.P., Mich., and N. Wis.	13	−15.8*	−15.3*	21.16	110.6	260.2	7.40	5.26	0.51	0.57	0.42
Red-breasted merganser	Green Bay, Lake Mich.	8	−17.0*	−22.9*	17.26	257.1	489.0	3.46	19.65	1.28	[4] 1.59	0.59
Herring gull	Green Bay, Lake Mich.	10	−12.5*	−13.9*	7.63	2160.4	2224.5	13.01	33.27	7.64	[4] 0.54	0.97
Black tern	Green Bay, Lake Mich.	5	−14.8*	−10.1*	9.65	50.7	89.7	2.56	2.98	0.20	0.49	0.57
Mean for Great Lakes			− 8.7	−11.4	9.53	339.5	339.7	5.50	13.92	3.01	0.49	1.25
LOUISIANA												
Green heron	Monroe	3	− 7.4	− 9.0*	6.69	209.7	11.0	0.88	31.63	0.12	*0.16	19.06
Light blue heron	Ferriday	5	− 7.1	− 4.4	6.13	39.2	13.5	4.56	5.51	0.10	*0.08	2.90
Cattle egret	Avery Island	5	− 3.4	− 3.6	6.50	26.0	28.0	5.66	1.86	0.17	*0.05	0.93
Common egret	Avery Island	5	− 1.0	− 3.4	4.77	46.3	[4] 120.9	8.67	6.20	[4] 1.73	*0.09	[4] 0.38
Snowy egret	Avery Island	5	[5] − 5.5	[5] − 5.3	6.49	24.6	24.7	1.06	1.02	0.53	*0.18	1.00
Louisiana heron	Avery Island and Ferriday	5	− 1.7	− 4.2	5.90	12.3	28.5	0.63	1.15	0.10	*0.09	0.43
Yellow-crowned night heron	Ferriday	5	− 1.7	− 0.7	3.85	52.5	18.6	0.18	13.95	0.36	*0.20	2.82
Mean for Louisiana			− 4.0	− 4.4	6.05	58.7	35.0	3.09	8.76	0.39	0.12	3.93

[1] Lipid-weight basis.
[2] Wet-weight basis.
[3] Influenced by one high value.
[4] N = 3.
[5] N = 9.
[6] N = 1.
† Significant difference when post-1946 museum data are included.
* Significant difference ($P<0.05$) by Student's t-test.

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Dieldrin residues were generally correlated with those of DDE ($r = 0.49$, $P<0.001$), although the amounts were much lower than DDE and PCB's. Residue concentrations in eggs from Louisiana, however, were similar to those from the Great Lakes States. This perhaps reflects agricultural practices, since dieldrin is widely used to dress rice seed in Louisiana (L. D. Newsom, *personal communication*). As shown below, it apparently was important in eggshell thinning, at least in eggs of most species from the Great Lakes region.

Residues of DDT + TDE were correlated with DDE similar to dieldrin ($r=0.44$, $P<0.001$). This is expected since the DDE and TDE are degradation products of DDT. The levels of DDT + TDE were of the same order of magnitude as dieldrin and those in eggs from Louisiana were again similar to those from the Great Lakes States.

BHC was present in higher concentrations in eggs from the Great Lakes States, just as DDE was. The reason for this difference is not readily apparent since BHC is used in agriculture in both areas.

Mercury values for the various species (Table 3) are expressed in ppm on a wet-weight rather than lipid-weight basis because mercury is distributed more or less evenly throughout the egg and is apparently not lipophilic as are the chlorinated hydrocarbons. For those species with all eggs containing less than 0.25 ppm of mercury (American bittern, pied-billed grebe, white pelican, red-necked grebe, and all seven species of herons from Louisiana), the residues may represent background levels.

Of all eggs analyzed for mercury in this study, 29% had at least 0.5 ppm and 9% at least 1.0 ppm. Nine of the 13 species from the Great Lakes States had one or more values above the 0.5-ppm level. Fimreite (*12*) fed grain treated with a mercurial fungicide to pheasants; these birds, when sacrificed, had 3-13 ppm mercury in their livers, and their eggs with 0.5 to 1.5 ppm suffered greater embryonic mortality than controls. Vermeer (*31*), however, sampled one egg in each of 28 successful herring gull nests and found 24 to contain mercury levels between 0.5 and 2.0 ppm. The significance of our mercury data is thus unknown at this time.

Eggs of the three merganser species had relatively high levels of mercury, with one clutch of inviable hooded merganser eggs having >2 ppm. These were also the only species for which abandoned clutches, dump nests, or un-

TABLE 3.—*Distribution of mercury residues in eggs*

Species of Eggs	Area of Collection	Number of Eggs	Number of Eggs per Residue Level (ppm, wet-weight basis)				
			<0.05	0.05-0.24	0.25-0.49	0.50-0.99	>1.0
Red-necked grebe	Rush Lake, Wis.	3		3			
Pied-billed grebe	Mud and Rush Lakes, Wis.	9		7	2		
White pelican	Milan, Minn. (western)	5		2	3		
Double-crested cormorant	Milan, Minn. (western)	8		1	3	4	
Great blue heron	Horicon Marsh, Wis.	5		3	1	1	
Common egret	Horicon Marsh, Wis.	4	1	1	1	1	
Black-crowned night heron	Horicon Marsh, Wis.	6		3	1	1	1
American bittern	Ladysmith, Wis.	1		1			
Hooded merganser	Chippewa Co., Wis.	6		2	2	2	
Hooded merganser	Price Co., Wis.	2					2
Common merganser	Brule & Pine Rivers, Wis.	2			1		1
Common merganser	Seney, Mich. (U.P.)	11			8	2	1
Red-breasted merganser	Sister Island, Green Bay, Wis.	3					3
Herring gull	Sister Island, Green Bay, Wis.	9			5	4	
Black tern	Oconto, Wis. (on Green Bay)	5			3	2	
Seven species of herons and egrets	Louisiana	7		7			
TOTAL		86	1	30	30	17	8

[1] Four of these were reported as >1.0 ppm, but had to be adjusted for desiccation.

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hatched eggs were found in the course of our searching for active nests. Of 16 nests located for the three species of mergansers, 11 were in one or another of these categories. However, some eggs with more than 1.0 ppm came from active nests and were in an advanced incubation stage when taken. The addled red-breasted merganser eggs (Table 4) showed high mercury levels (1.1-1.9 ppm) and also high dieldrin levels (6.44-9.73 ppm, lipid basis.), while viable eggs collected from the same nests had much lower dieldrin levels (0.15-2.37 ppm). Viable eggs were not analyzed for mercury. The comparison of means of dieldrin did not show a significant difference. The sum of DDT and TDE was also higher in addled than in viable eggs. However, the combination of aldrin (the parent compound of dieldrin) and mercury is apparently more toxic to birds than is mercury alone (*5, 6, 8*). These data suggest a need for research into the relationship between dieldrin and other chemicals and embryonic mortality in this species.

Some of our highest mercury values were from eggs collected in the Chequamegon National Forest and Nicolet National Forest in northern Wisconsin, and from the Seney National Wildlife Refuge in Michigan. Mercury values in eggs of herring gulls and black terns collected from Green Bay, Lake Michigan, were also somewhat higher than in most eggs from other areas. Eggs from other areas showed no consistent differences, except that levels in eggs from Louisiana were consistently low.

CONTRIBUTIONS OF RESIDUES TO EGGSHELL THINNING

Both DDE and dieldrin have been associated with eggshell thinning (*2, 7, 22*). However, as our data show, an egg with high levels of one type of chlorinated hydrocarbon will often have high amounts of the others as well. Therefore, spurious correlation is a possibility, and in our data, this was apparently the case. In simple correlation, all of the residues had significantly negative coefficients with percent of pre-1947 thickness index in one category or another when we combined the data into family groups. We, therefore, used partial correlation (*29*) to segregate the spurious correlations and determine the residues which were significantly related to eggshell thinning. In order for the analysis to be valid, it was necessary to assume that the samples were collected in random fashion and that the transformed data were normally distributed. Our data essentially satisfied both requirements. Since the sample of herring gull eggs was not a random one and also because the distribution of residue values was very different from those of any of the other species, these data were excluded from all analyses of combined groups. All residue data were transformed to their logarithms for the correlations because this gave a more normal distribution of residue values. Virtually all correlation coefficients were increased when the log transformation was used. Partial correlation coefficients between percent change in pre-1947 thickness index and residues were calculated for all of the chemicals which were significantly correlated with percent thickness index in the simple correlations.

Our data did not satisfy the theoretical requirements for multiple-regression analysis, but we did calculate the coefficient of determination, R^2, to show the amount of variation in percent pre-1947 thickness index which could be accounted for by the combined residues.

The extent of correlation of a given residue type with the thickness index varied from family to family (Table 5). Sample sizes for most species were too small to permit meaningful intraspecific analyses by partial correlation.

TABLE 4.—*Residues and eggshell parameters for three viable (V) and three addled (A) eggs from the same nests of red-breasted mergansers*

Nest	Type of Egg	Residues in ppm [1]						Percent Lipid	Eggshell Thickness	Eggshell Thickness Index
		DDE	PCB's	Dieldrin	DDT+TDE	BHC	Mercury			
1	V	187	224	2.37	10.5	0.69	[2]	15.6	0.33	1.74
	A	336	294	9.73	43.7	0.85	1.1	18.8	0.34	1.72
2	V	201	607	0.15	19.0	0.90	[2]	16.8	0.28	1.51
	A	330	348	8.31	21.6	0.60	1.9	16.6	0.30	1.48
3	V	351	1,375	1.98	0	1.37	[2]	12.6	0.28	1.43
	A	328	644	6.44	27.7	5.06	1.90	25.3	0.29	1.55
MEAN	V	246.0	735.6	[3] 1.50	9.86	0.99		15.0	0.30	1.56
	A	297.6	428.7	[3] 8.16	31.0	2.17	1.6	20.2	0.31	1.58

[1] Residues on lipid basis for organochlorines, on wet-weight basis for mercury.
[2] Not analyzed for.
[3] Means significantly different ($P<0.01$) when calculated on wet-weight basis.

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TABLE 5.—*Coefficients of determination (R^2) and partial correlation coefficients for correlation of percent changes in shell-thickness index with the logarithm of each residue*

	Number of Eggs	R^2	DDE	PCB's	Dieldrin	DDT + TDE	BHC	Mercury
Eggs in which mercury was measured:								
Heron eggs	23	.543***	−.448*	−.224	−.196	+.333	—	—
Merganser eggs	24	.646***	+.100	−.205	−.241	+.102	−.175	−.410
All eggs in Upper Great Lakes States (12 species)	69	.392***	−.170	−.019	−.222	+.032	—	−.299*
All addled eggs (7 species)	30	.204*	—	—	—	—	—	−.451*
All viable eggs (16 species)	46	.505***	−.115	−.188	−.369*	+.032	—	—
Both viable and addled eggs (18 species)	76	.367***	−.126	−.039	−.242*	+.040	—	−.253*
Eggs without mercury considered:								
Heron eggs	49	.494***	−.575***	−.199	+.159	+.359*	—	
Merganser eggs	29	.609***	+.048	−.410*	+.115	−.062	−.199	
All eggs in Upper Great Lakes States (12 species)	74	.352***	−.225	−.174	−.097	+.063	—	
All viable eggs (16 species)	77	.470***	−.384***	−.284*	−.034	+.131	+.078	
Both viable and addled eggs (18 species)	107	.378***	−.335***	−.251*	−.003	+.122	+.157	

* P = <0.05.
** P = <0.01.
*** P = <0.005.

It appears, however, that there may be very important differences between families in the effect of a given compound on eggshell thinning. For the herons, DDE was the only compound which was significantly correlated with percent of pre-1947 thickness index. DDT + TDE showed a significantly positive correlation with percent of pre-1947 thickness index in the herons; however, this may be an artifact of the number of correlations performed, a statistically significant relationship occurring by chance alone. Previous field studies have failed to show that DDT or TDE was a significant factor in eggshell thinning; Jefferies (*17*) reported that DDT apparently increased eggshell weight in Bengalese finches, but this increase was very small (less than 1%) and probably not significant. For mergansers, PCB was the only compound among DDE, PCB, dieldrin, DDT + TDE, and BHC which was significantly correlated with percent of pre-1947 thickness index. Although the sample size was small (N=11), the six residue types together accounted for 97.6% ($P < 0.005$) of the variation in percent thickness index in the grebes. However, none of the individual residues was significantly correlated with percent of pre-1947 thickness index in simple correlation, and so we did not calculate partial correlation coefficients. For the other families and individual species, sample sizes were small, and the coefficient of determination did not show a significant reduction in variance of the percent thickness index.

There are several theories concerning the physiological mechanism of eggshell thinning due to pesticides (*17, 26*). All have a common problem: they do not fully explain why there should be interfamily or interspecific differences in susceptibility to DDE-caused eggshell thinning. However, there do seem to be real differences, at least between such species as Japanese quail (*Coturnix coturnix*) and mallards (*Anas platyrhynchos*) (*15*). Since the species we studied are related in their food habits (all except the cattle egret feed on aquatic organisms, and most feed mainly on fish), we performed partial correlation analyses on the combined data, but with the herring gull data excluded. The results of these analyses (Table 5) should be viewed with caution, since they represent combined samples. The simple correlation of mercury with percent thickness index for addled eggs apparently carries over to the larger groups in which both addled and viable eggs were combined. When only viable eggs were considered, DDE, dieldrin, or PCB was the most important, and mercury was not correlated at all. The results do not mean that any of the residues which had significant partial correlation coefficients are importantly related

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to eggshell thinning in all of the species in the sample. Indeed, it is evident that this was not the case. However, the results do indicate that these residues were important to many of the species in the sample. Thus, we consider that both DDE and dieldrin are important to many species, especially herons, and PCB is important to mergansers and perhaps some other species as well in respect to eggshell thinning. It appears that DDT, TDE, and BHC have very little relation to shell thinning. The importance of mercury is puzzling, however, since its significance occurred in addled eggs but not in viable eggs. Larger sample sizes are needed for individual partial correlation analysis of each of the species studied here.

We noted consistently high correlations ($P<0.001$) between DDE and PCB's in all of our samples. This may be the result of similar storage characteristics in fat, similar distribution patterns in ecosystems, or causal relationships in fat storage. With regard to causal relationships in fat storage, Lichtenstein (*23*) reported increased mortality of houseflies when both DDT and PCB's were present as opposed to DDT alone, and Street (*30*) demonstrated a negative effect of DDT fed to rats on storage of dieldrin. However, in our samples there was a significantly positive correlation between dieldrin and DDT + TDE, suggesting little or no negative effect of DDT + TDE on dieldrin levels. Heath *et al.* (*15*) found no synergism between DDE and PCB's in toxicity tests on Japanese quail, but there remains a great deal to be learned of the relationships between PCB's and the other organochlorines. It is entirely possible that no causal relationships are involved, and that the high correlations observed are due solely to similar distribution patterns of the organochlorines in ecosystems.

All but 3 of the 34 simple correlation coefficients which we determined for DDE were negative, while only 24 were negative for dieldrin. We therefore feel that DDE significantly affects many species, but that the residue concentrations needed to cause a given degree of eggshell thinning vary greatly among species. For example, herring gull eggs had approximately 40 times as much DDE as black tern eggs, yet the mean change in thickness index was only 4% greater for herring gulls. Differences such as those, even when log transformations are used, reduce the magnitude of the correlation coefficient for the combined data, although DDE may cause eggshell thinning in both species.

In most cases, the R^2 values (Table 5) were rather low, although many were significant by the *F*-test. These chemicals are by no means the only ones to which birds are subjected in greater amounts today than in former times. Many natural environmental and physiological factors such as heat, humidity, and nutrition can affect eggshell thickness (*27*). Variation due to these other factors may mask the effects which we attempted to measure. However, it is clear that DDE and dieldrin are importantly related to eggshell thinning in many species, and that PCB's are similarly related to eggshell thinning in mergansers.

When we compared the mean change in thickness index to the mean of the sum of all organochlorines for each species, a trend toward a linear relationship on a log-log basis was evident (Fig. 1). However, the yellow-crowned night heron and pied-billed grebe data did not appear to fit the line. No statistical analysis of this relationship was possible because of differences in sample sizes.

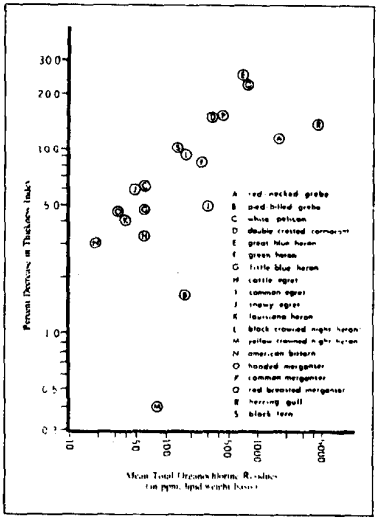


FIGURE 1.—*Mean shell-thickness index changes and mean residue levels for each species (log-log basis)*

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

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