ORGANOCHLORINE RESIDUES IN BROWN PELICAN EGGS: RELATION TO REPRODUCTIVE SUCCESS

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ABSTRACT

This study was conducted to determine the influence of residues of organochlorine pollutants on reproductive success in the brown pelican. A sample egg was taken from each of 93 *marked nests in the nesting colony in South Carolina. Periodic visits were made to determine the fate of marked nests. Each sample egg was analysed for residues of organochlorine pollutants. Nest success in the brown pelican was related to residues of DDE and dieldrin in sample eggs. DDE seemed primarily responsible for nest failure; however, deleterious effects of this pollutant on nest success were not satisfactorily separated from those induced by dieldrin. Significant intercorrelation of all five organochlorine residues identified in the eggs complicated the relationship of residues to nest success. Maximum DDE residues in an egg from a successful nest were* 2·37 *µg/g and* 8·48 *µg/g in an egg from an unsuccessful nest. Comparable maximum residues for dieldrin in sample eggs were* 0·54 *µg/g (successful) and* 0·99 *µg/g (unsuccessful). Nineteen of the* 26 *eggs from unsuccessful nests contained DDE residues that exceeded* 2·37 *µg/g, and* 14 *of these* 26 *eggs contained dieldrin residues that exceeded* 0·54 *µg/g. Residues of DDD, DDT or PCBs in sample eggs were not significantly related to nest success. Reproductive success in the brown pelican colony was subnormal in the years of study—*1971 *and* 1972*—but reproductive success was normal in those nests in which the sample egg contained either* $\leq 2{\cdot}5$ *µg/g of DDE or* $\leq 0{\cdot}54$ *µg/g of dieldrin.*

Environ. Pollut. (7) (1974)—
Printed in Great Britain

INTRODUCTION

The eggshell thinning phenomenon has been well documented in wild birds (Ratcliffe, 1967*a*; Hickey & Anderson, 1968; Ratcliffe, 1970; Anderson & Hickey, 1972). DDE has been shown to be the principal agent inducing eggshell thinning in the brown pelican (*Pelecanus occidentalis*) (Blus *et al.*, 1971, 1972*a*, 1972*b*); and captive birds of several species fed DDE have exhibited eggshell thinning (Heath *et al.*, 1969; Wiemeyer & Porter, 1970; Longcore *et al.*, 1971; McLane & Hall, 1972).

Eggshell thinning of 15 to 20% over a period of years is generally considered to result in declining populations of birds (Anderson & Hickey, 1972). This is somewhat corroborated in short-term studies with captive birds fed DDE in that deleterious effects on reproductive success occur when eggshell thinning is below 18% (Heath *et al.*, 1969; Longcore *et al.*, 1971). It is assumed that eggshell thinning in wild birds is related to lowered reproductive success and this assumption is supported by results obtained with experimental birds. However, the degree of response of wild birds to a pollutant may be different from that of captive birds. The brown pelican seems much more susceptible to DDE-induced eggshell thinning than any species used experimentally. It is possible to calculate the level of DDE that induces 15% eggshell thinning (between 4 and 5 μg/g in the brown pelican (Blus *et al.*, 1972*a*)), and extrapolation from experiments with captive birds suggests that this level would result in a declining population if maintained over a period of years. However, a more direct measure of the association of residues with reproductive success is desirable because eggshell thinning is only one of the factors that may influence productivity.

MATERIALS AND METHODS

We obtained a more direct measurement by comparing residues in sample eggs to nest success on Marsh Island, Cape Romain National Wildlife Refuge (South Carolina), in 1971 and 1972. Nests were selected for sampling over as wide an area as possible at different times during the nesting season. Numbered aluminium stakes with attached flagging or plastic tape were used to mark 93 nests; the stakes were driven into the ground near the nests. We visited the nesting colony on seven occasions in 1971 and on 16 occasions in 1972. Our visits were short—usually not much more than an hour in duration. Prolonged observations were never localised in a particular segment of the colony except on two visits in 1972 when a blind (hide) was employed. One egg was collected from each of the marked nests. The sample egg was collected from a complete clutch in most cases, but an egg was sometimes taken from an incomplete clutch. A nest was considered successful if one or more downy young (3 weeks of age) were produced and unsuccessful if no downy young were produced. Young in two nests disappeared soon after hatching, and these were classified as unsuccessful. There were 50 nests whose fates were unknown; four which were visited too infrequently to determine nest success, 35 where the nest markers could not be located and 11 where no additional eggs were produced after a single egg was collected. Six nests were flooded by saltwater; these were excluded from the analysis. A freshly laid egg was taken from each of 19 nests, and an incubated egg, the stage of incubation varying from several days to about 30 days (near hatching), was collected from each of 18 nests.

Contents of individual eggs were analysed by electron capture gas chromatography; certain residues were confirmed by either thin layer chromatography or mass spectrophotometry. The residues found were p,p'-DDE, p,p'-DDT, p,p'-DDD, dieldrin and PCBs. The PCBs in these pelican eggs resembled Aroclor 1260 or those Aroclors that lie near 1260. Some of the eggs contained small quantities of mirex, heptachlor epoxide, and metabolites of chlordane. A full description of the residue analyses is presented by Blus *et al.* (in press) and Mulhern *et al.* (1970, 1971). Residue values were transformed to logarithms ($\log_{10}$) for statistical computations. A two-way analysis of variance (fixed model) was used to compare residues in eggs in the four treatment groups that consisted of freshly laid and incubated eggs further divided into eggs from successful and unsuccessful nests.

RESULTS AND DISCUSSION

Eleven nests produced downy young, 26 produced no downy young, and the fate of 50 nests was unknown. Residues of the five organochlorines tended to be lower in those eggs collected from successful nests when compared with those collected from unsuccessful nests (Table 1); but analysis of variance revealed that these differences were significant only for DDE, dieldrin, and DDD (Table 2). Analysis of variance revealed that DDT and DDD were the only residues altered by the stage of incubation of the sample egg—DDT in eggs decreased significantly ($P < 0{\cdot}025$) during incubation and DDD increased significantly ($P < 0{\cdot}05$). These changes were apparently due to metabolism of DDT by the developing embryo. This was previously reported by Abou-Donia & Menzel (1968). The DDT residues were so small that metabolism of DDT to DDE, also reported by Abou-Donia & Menzel (1968), would result in a very small increase in the DDE level. While we considered combining DDT with DDD for the statistical analysis, this may have tended to oversimplify a complicated problem. There was a positive, significant intercorrelation among each of the residues (Table 3). These intercorrelations among residues in brown pelican eggs were noted previously (Blus *et al.*, 1971). Determination of the relation of egg residues to nest success was made more difficult because of the intercorrelations.

TABLE 1
ORGANOCHLORINE RESIDUES IN 37 SINGLE EGGS FROM INDIVIDUAL PELICAN NESTS

	µg/g (fresh wet weight)									
	Successful nests					Unsuccessful nests				
	DDE	Dieldrin	PCBs	DDT	DDD	DDE	Dieldrin	PCBs	DDT	DDD
	Eggs freshly laid when collected									
	1·20	0·28	3·9	0·05	0·16	1·56	0·19	5·7	0·32	0·29
	1·90	0·52	6·0	0·12	0·15	2·30	0·37	3·5	0·15	0·29
	2·05	0·17	3·9	0·24	0·18	2·33	0·05	6·0	0·05	0·26
	2·26	0·29	3·9	0·42	0·61	2·46	0·43	36·5	0·52	0·47
						2·47	0·15	4·4	0·10	0·11
						2·66	0·64	6·5	0·18	0·30
						3·49	0·75	7·8	0·27	0·27
						3·50	0·74	6·8	0·28	0·50
						3·75	0·63	7·6	0·25	0·63
						3·92	0·44	9·4	0·15	0·33
						3·97	0·61	9·2	0·18	0·43
						4·14	0·71	9·8	0·25	0·36
						5·24	0·77	16·7	0·45	0·61
						6·05	0·92	9·9	0·79	1·04
						8·48	0·40	32·3	0·39	0·95
G*	1·80	0·29	4·34	0·16	0·23	3·43	0·43	9·06	0·24	0·39
	Eggs incubated when collected									
	1·30	0·33	5·3	0·05	0·48	1·77	0·36	3·4	0·05	0·25
	1·39	0·19	3·0	0·19	0·27	1·93	0·58	6·5	0·37	0·57
	1·58	0·19	3·9	0·05	0·19	1·96	0·52	1·9	0·05	0·54
	1·76	0·29	3·8	0·05	0·17	2·25	0·32	9·0	0·05	0·38
	1·90	0·54	17·3	0·18	0·71	2·88	0·50	8·6	0·05	0·60
	2·25	0·42	18·6	0·13	0·37	3·32	0·58	9·0	0·18	0·70
	2·37	0·35	5·1	0·13	0·61	3·97	0·84	5·4	0·47	0·77
						3·99	0·52	9·2	0·30	0·55
						3·99	0·83	9·2	0·05	1·04
						3·99	0·99	8·3	0·33	0·39
						4·37	0·64	10·4	0·05	0·83
G	1·75	0·31	6·28	0·10	0·35	2·97	0·58	6·67	0·12	0·56

* G = Geometric Mean.

TABLE 2
GEOMETRIC MEANS (WEIGHTED) OF ORGANOCHLORINE RESIDUES IN 37 EGGS FROM SUCCESSFUL AND UNSUCCESSFUL NESTS OF THE BROWN PELICAN

Nest status	Geometric Mean (µg/g, fresh wet weight)				
	DDE	Dieldrin	PCBs	DDT	DDD
Successful	1·77	0·30	5·50	0·11	0·30
Unsuccessful	3·23†	0·49*	7·94	0·17	0·46*
Error Mean Square (Log Residue)	0·0250	0·0671	0·0700	0·1214	0·0532

* $P < 0{\cdot}025$.
† $P < 0{\cdot}005$.

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TABLE 3

ASSOCIATION OF FIVE ORGANOCHLORINE RESIDUES FOUND IN 37 BROWN PELICAN EGGS

Chemical	Simple correlation coefficient (r)			
	Dieldrin	*PCBs*	*DDT*	*DDD*
DDE	0·564**	0·585**	0·480‡	0·599**
Dieldrin		0·351*	0·400†	0·563**
PCBs			0·407†	0·478‡
DDT				0·337*

* $P < 0·05$.
† $P < 0·02$.
‡ $P < 0·01$.
** $P < 0·001$.

The maximum DDE residue in an egg from a successful nest was 2·37 μg/g. The maximum DDE residue in an egg from an unsuccessful nest was 8·48 μg/g and 19 of 26 eggs from unsuccessful nests (73·1 %) contained more than 2·37 μg/g. Use of binomial tables (Mainland *et al.*, 1956) to test these data further revealed that production of downy young seemed related primarily to DDE in the egg (Fig. 1). Of 20 nests in which the sample egg contained ≤2·5 μg/g of DDE, 11 produced at least one downy young (probability of a successful nest = 0·550;

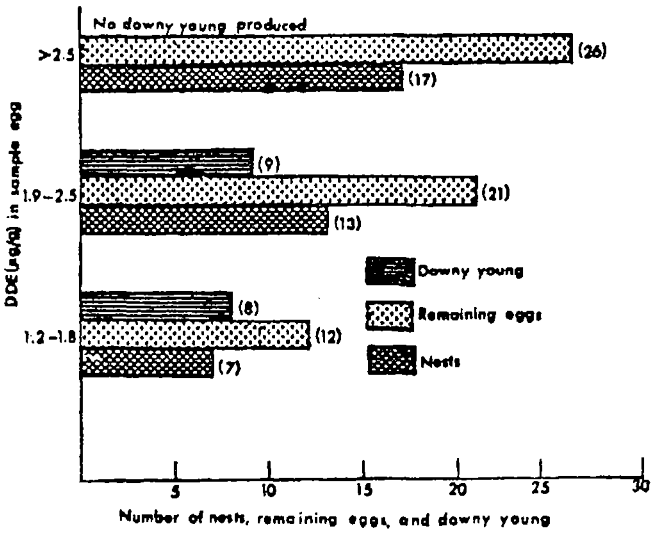

Fig. 1. Nests, remaining eggs, and downy young related to DDE content of sample eggs.

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95% confidence limits = 0·315 to 0·770). Using the lower confidence limit, the probability that by chance all of the 17 nests containing >2·5 μg/g of DDE would be unsuccessful is 0·000001 or approximately once in 1,000,000 times. The maximum dieldrin residue in a sample egg from a successful nest was 0·54 μg/g. The maximum dieldrin residue in a sample egg from an unsuccessful nest was 0·99 μg/g, and 14 of 26 sample eggs (53·8%) from unsuccessful nests contained >0·54 μg/g of dieldrin. Use of the binomial tables also revealed that the level of dieldrin in the sample egg was related to nest success (Fig. 2). Of 23 nests in which the sample egg contained

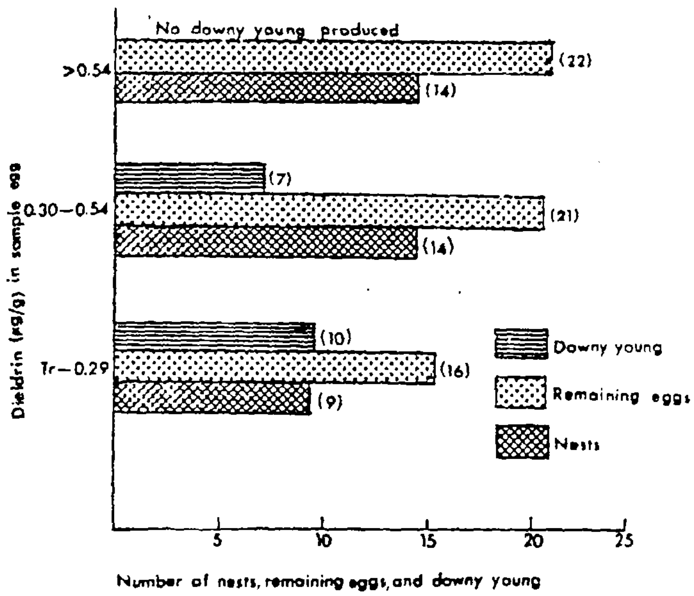

Fig. 2. Nests, remaining eggs, and downy young related to dieldrin content of sample eggs.

≤0·54 μg/g of dieldrin, 11 produced at least one downy young (probability of a successful nest = 0·478; 95% confidence limits = 0·306 to 0·732). Using the lower confidence limit, the probability that by chance all of the 14 nests containing sample eggs with >0·54 μg/g of dieldrin would be unsuccessful is 0·000034 to 34 times in a million. Use of the binomial tables indicated that DDT, DDD, or PCB residues had little influence ($P > 0{\cdot}10$) on nest success.

In the 20 nests in which the sample egg contained ≤2·5 μg/g of DDE (Fig. 1), 17 downy young were produced. Assuming that sample eggs would have produced

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downy young at the same rate as the remaining eggs (17 downy young from 33 eggs = 51·5%), production can be calculated to be 27 young or 1·36 young per nest, which is considered a normal rate of productivity for the brown pelican (Henny, 1972). Using the same analysis for dieldrin, 17 young were produced in 23 nests in which the sample egg contained $\leq 0{\cdot}54$ µg/g of dieldrin. Assuming that sample eggs would have produced downy young at the same rate as the remaining eggs (17 downy young from 37 eggs = 45·9%), production can be calculated to be 28 young or 1·22 young per nest, which is also considered a normal rate of productivity.

The relationship of DDE or dieldrin residues to nest success followed the same trend in each of the 2 years. Student's *t*-test was used to compare residue means; there were insufficient eggs to consider residues in relation to stage of incubation for each year. DDE or dieldrin residues in eggs from successful nests in each year were not significantly different ($P > 0{\cdot}05$) from each other nor were DDE or dieldrin residues in eggs collected each year from unsuccessful nests significantly different ($P > 0{\cdot}05$) from each other. DDE or dieldrin residues in eggs collected from unsuccessful nests in each year were significantly higher ($P < 0{\cdot}05$) than those residues in successful nests in either year. Residues of each organochlorine in eggs from nests where fates were unknown were lower than those found in eggs from unsuccessful nests, but they were higher than in those sample eggs from unsuccessful nests. Because of the similiarity of the relationship of egg residues to nest success in each year, data from both years were combined for statistical analysis.

Upon examination of residues of DDE and dieldrin in individual eggs (Table 1), the close intercorrelation of these two chemicals is evident. There is only one egg from an unsuccessful nest in which dieldrin (0·58 µg/g) exceeds the maximum level found in an egg from a successful nest, and the DDE level (1·93 µg/g) falls below the maximum level found in an egg from a successful nest. Otherwise, eggs from unsuccessful nests contain levels of DDE and dieldrin that are both below the maximum levels found in an egg from a successful nest (6 eggs), both above the maximum levels (13 eggs), or with DDE above the maximum level and dieldrin below the maximum level (6 eggs). This relationship tends to indicate that nest success is primarily dependent upon the DDE level in the female that is reflected by the residue in the egg. However, the effects of dieldrin and DDE were not completely identified. All of the pollutants found in the pelican eggs are presumably toxic to the embryo or hatched young, but their effects on toxicity may not be strictly additive with regard to the quantitative effect induced by a given unit of chemical. Thus, little would be gained and much might be lost in assuming straightforward additivity without first demonstrating this relationship. There is also the problem of separating embryotoxic effects from eggshell thinning effects, although, in the final analysis, eggshell thinning may result in cracking or crushing of the eggshell that is toxic to the embryo. DDE appears to induce all or most of the eggshell thinning found in the brown pelican (Blus *et al.*, 1971, 1972*a*, 1972*b*); and this chemical would be expected to contribute to all of the deaths of embryos caused by crushing or cracking of the eggshell attributable to environmental pollution. There seemed to be some indication that DDE is embryotoxic in the intact egg at the low levels found in this study; but this is difficult to determine, particularly in the presence of dieldrin. The principal effect of dieldrin is probably embryotoxicity in those eggs with shells with sufficient strength to withstand the weight stresses associated with incubation. There is also the possibility that dieldrin may be associated with post-hatching mortality (Koeman *et al.*, 1967) or adverse effects on behaviour of young birds (Baxter *et al.*, 1969). Although influences of pollutants on the breeding behaviour of birds have been mentioned as one of the factors adversely affecting reproductive success (Snyder *et al.*, 1973), there is little experimental evidence to confirm this. Snyder *et al.* (1973) mention possible abnormal parental behaviour in American kestrels (*Falco sparverius*) given dietary DDE plus PCBs.

Although there was no significant ($P > 0{\cdot}10$) interaction between stage of incubation and nest success in relation to levels of five chemicals in pelican eggs, there seemed to be a trend for DDE levels to vary with stage of incubation (Table 1). Of the five highest DDE residues, four occurred in freshly laid eggs collected from unsuccessful nests. This trend was also reflected in two freshly laid crushed eggs that contained 3·61 and 7·04 μg/g of DDE; these nests were not included in the analysis because only one egg was known to be laid in each nest. Two other freshly laid eggs which had been crushed were collected from unsuccessful nests, each of which contained three eggs. These eggs contained 3·52 and 3·08 μg/g of DDE, but were excluded from the analysis due to subsequent flooding by salt water. The dieldrin levels in these four eggs ranged from 0·32 to 0·50 μg/g. Crushed eggs were observed in two of the 26 unsuccessful nests included in the analysis. Thus, there is a tendency (not statistically significant) for freshly laid eggs from unsuccessful nests to contain more DDE than incubated eggs from unsuccessful nests. This may be related to eggs with higher DDE levels having a shorter nest life than those with lower levels because of cracking and crushing of shells thinned by DDE.

We cannot completely rule out the possibility that some unidentified pollutant may have had significant adverse effects on nest success of the pelicans, but this possibility seems highly unlikely. Six of the eggs were analysed for residues of chromium, nickel, selenium, arsenic, mercury, lead, cadmium, copper, zinc, and magnesium. Concentrations of these metals did not seem unduly high—residues of Pb and Cd seemed unusually low—but little is known concerning the influence of low levels of these metals on reproductive success in birds.

It is also necessary to consider that some nests were unsuccessful for reasons other than residues in the eggs. Nest desertion is the most likely cause of lack of success because it may occur for various reasons such as inclement weather, food shortage, or human disturbance. Young may also die from diseases, inclement weather, and other factors. This might alter the relationship of residues to nest success by inclusion of eggs with low residues in the sample from unsuccessful nests and by possible desertion of some nests in which the sample egg contained >2·5 μg/g of DDE or >0·54 μg/g of dieldrin. This bias, if it occurred, was not sufficient to obscure the relationship between residue levels and nest success.

Our calculations assume that residues in one egg from a clutch of brown pelican eggs (mean clutch size is near three eggs) is representative of residues of other eggs in the clutch. We have residue data from two eggs from each of four clutches collected in Florida in 1969 that bear on this point. Analysis by a paired *t*-test (Simpson *et al.*, 1960) revealed no significant difference ($P > 0{\cdot}05$) in organochlorine residues relative to the order in which eggs were laid in the same clutch. Potts (1968) also found no significant differences in organochlorine residues in eggs of the same clutch of the shag (*Phalacrocorax aristotelis*). Vermeer & Reynolds (1970) reported highly significant correlations of residue levels between eggs of the same clutch of the California gull (*Larus californicus*). It appears valid to use residues in one egg of the clutch to represent residues in other eggs within the same clutch.

The evidence for influence of DDE and dieldrin residues on nest success is similar to evidence for the effects of DDE on eggshell thinning in wild birds; it is correlative evidence and should not be construed as exhibiting a cause-and-effect relationship. Cause-and-effect evidence may be drawn from experimental demonstrations that birds fed DDE experienced lowered reproductive success (Heath *et al.*, 1969; Longcore *et al.*, 1971). In the study of black ducks (*Anas rubripes*) fed DDE (Longcore *et al.*, 1971), lowered reproductive success occurred; however, DDE residues in the egg were much higher than those in the brown pelican eggs.

There have been few studies of effects of residues in eggs on success of marked nests. In several studies, dieldrin was reported to have a deleterious effect on nest success (Potts, 1968; Lockie *et al.*, 1969). In our study, dieldrin levels approximated those found in the Scottish golden eagle (*Aquila chrysaetos*); a mean level of 0·86 μg/g in the eagle eggs was associated with lowered reproductive success and reproductive success improved markedly when the average dieldrin level dropped to 0·34 μg/g (Lockie *et al.*, 1969). Snyder *et al.* (1973) found DDE related to nest success in Cooper's hawks (*Accipiter cooperi*), but there was little mention of residue levels in eggs from successful or unsuccessful nests. The average dieldrin level in Cooper's hawk eggs was low but the range was not given. Ratcliffe (1967*b*) found no significant difference in organochlorine residues in eggs from successful and unsuccessful nests of the peregrine (*Falco peregrinus*); however, residue analyses were made at a time when PCBs were not taken into account. PCBs may seriously interfere with analysis for certain other organochlorines unless suitable analytical procedures are followed.

Brown pelicans are reproducing subnormally in the colony involved in this study, and the number of young produced in each of the last four years is insufficient to maintain a stable population (Blus *et al.*, 1972*a*; Henny, 1972). The population in South Carolina declined from over 5000 breeding pairs in the 1950s to the current population of slightly over 1000 pairs (Beckett, 1966; Blus, 1970; Blus et al., 1972a).

The data presented in this study show a strong correlative relationship between DDE and dieldrin in eggs with reproductive success and decline of the brown pelican population in South Carolina.

ACKNOWLEDGEMENTS

We thank George A. Shegogue, Steve Joyner, Daniel Doshier, and others who helped with the field work. We thank Robert Heath and Lucille Stickel for their critical evaluation of the manuscript and Mr Heath for advice on statistical aspects.

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