Effect of Dieldrin on Egg Hatchability, Chick Survival and Eggshell Thickness in Purple and Common Gallinules

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Since their introduction in the 1940's, organochlorine insecticides have become virtually universal contaminants of the world's environments, often including ecosystems in remote areas. Much concern has been expressed over the effect of these chemicals on bird populations, but relatively little data are available to justify many of the conclusions (1).

An excellent opportunity to study nesting populations of game birds associated with insecticide use was available in the relationship of the purple gallinule, Porphyrula martinica L., and the common gallinule, Gallinula chloropus L., to aldrin-treated rice fields of South Louisiana. In this area, seed rice was treated prior to sowing at the rate of 0.25 lb. aldrin/100 lb. seed. When sown, the treated seed presented an available food source (contaminated with 2,500 ppm aldrin) to these birds during the early part of the nesting season. Since aldrin is known to be readily oxidized to its epoxide, dieldrin, exposure of gallinules to aldrin-contaminated food should result in the appearance of dieldrin residues in the body tissues and eggs (2).

Causey et al. (3) demonstrated that eggs and tissues of both species of gallinules nesting in treated rice fields did indeed contain high levels of dieldrin. Although egg contamination averaged 6.51 and 9.37 ppm in the purple and common gallinule, respectively, there was no effect on clutch size or egg hatchability.

The significance of organochlorine insecticide residues on avian reproduction may be related more with chick survival than with egg production, fertility or hatchability (4). Absorption of the yolk after hatching may cause poisoning of young chicks by insecticide residues present in the egg. Other researchers have correlated reproductive problems in birds with changes in calcium metabolism and decreases in egg shell thickness (5-9).

The purpose of this study was to determine the effect of dieldrin on egg hatchability, chick survival and egg shell thickness in purple and common gallinules.

Methods and Materials

Collection and Incubation of Eggs - Entire clutches of eggs were taken from 37 purple gallinule nests (225 eggs) and 8 common gallinule nests (66 eggs) found in rice fields near Crowley,

Bulletin of Environmental Contamination & Toxicology,
Vol. 6, No. 6, 1971, published by Springer-Verlag New York Inc.

Louisiana, during the summers of 1968-69. During the same time, eggs were also collected from 30 purple gallinule nests (169 eggs) and 33 common gallinule nests (170 eggs) located in marsh areas of Louisiana which included Pass-A-Loutre Wildlife Refuge in Plaquemines Parish, Pecan Island in Vermilion Parish, Sabine Wildlife Refuge in Calcasieu Parish, and Rockefeller Wildlife Refuge in Cameron Parish. These eggs were used as controls since these areas are isolated and removed from areas treated with agricultural insecticides.

Clutches were coded, placed in game bird egg flats and immediately stored in a styrofoam ice chest for transport to Louisiana State University, Baton Rouge. A bottle of warm water was placed in the chest during excessively long collecting trips to maintain a high ambient temperature similar to field conditions.

Eggs were held in an electric incubator maintained at 37.7°C. and approximately 90% RH. Eggs were hand-turned and sprinkled with warm water 3 times daily. All eggs which did not hatch after 4 weeks were recorded as not hatching and discarded.

From each clutch containing more than 5 eggs, 2 or 3 eggs were taken for pesticide analysis. One egg was taken from all other clutches for analysis. Since gallinules begin incubation after the first egg is laid, clutches of eggs were candled to determine degree of embryonic development before incubation. When more than one egg was taken for analysis, the eggs showing the most advanced and least advanced embryonic development were selected. When only 1 egg was taken for analysis, the egg with the most advanced embryonic development was chosen. These eggs were coded, placed in plastic bags and frozen until prepared for analysis. Eggs used for analysis were disregarded when computing per cent hatch.

<u>Rearing of Chicks</u> - Newly hatched chicks were isolated in pint ice cream cartons to maintain their identity and allowed to remain in the incubator 24 hours. Chicks were then placed by clutches in cardboard boxes (approximately 30 cm high x 30 cm wide x 60 cm long) and hand-fed a ration of ground beef and "Little Friskies"® cat food soaked in warm water. Chicks were fed by hand every 2 hours, 12 hours a day for 14 days. The 14-day period was arbitrarily selected as a sufficient length of time for complete yolk absorption. Numbers of surviving and dead chicks were recorded by clutch during this period.

<u>Measurements of Eggshell Thickness</u> - The eggshells remaining after the 1969 hatch were coded, placed in paper bags, and stored in herbarium cabinets until determination of shell thickness was completed.

Eggshell thickness was calculated by measuring with a micrometer pieces of shell taken from 4 points around the girth of each shell. These were then averaged to the nearest 0.001 in. for each egg. Thickness in each case represented the shell itself plus the dried egg membrane.

Cleanup and Extraction - Each egg was allowed to thaw, then cracked and the contents blended in a Sorvall Omni-Mixer® cup at 5000 rpm for 3-5 minutes. A 2 g sample was quickly weighed into a tared 1 oz. medicine vial and stored in a freezer at -10°C. until analyzed. Samples were cleaned and extracted using the method described by Cummings et al. (10) with the following modifications:

1) Two g samples from individual eggs were used.
2) Samples were ground in 250 ml beakers using small glass pestles.
3) Eluants were collected in 400 ml beakers and evaporated in a water bath.
4) The 15 per cent ethyl ether-petroleum ether eluting solvent was changed to 20 per cent.
5) The final volume of eluant was adjusted to a 1 g/ml concentration for injection into a gas chromatograph.

A florisil recovery and reagent blank was run with each series of samples. Recovery standards averaged 83.6 per cent with a range of 71-97 per cent. All residue analyses were completed by electron-capture gas chromatography using the same procedures and conditions as described by Causey et al. (3). The sensitivity level for all residues except DDT was 0.01 ppm. The level for DDT was 0.05 ppm.

Random samples were selected for qualitative verifications of residues by thin-layer chromatography using the technique described by Damaska (11). The combined 20 per cent eluants from all the egg samples were concentrated and scanned from 2 to 15 microns with a Perkin-Elmer Model 21 Infrared Spectrophotometer® using a 0.5 mm sealed micro-cell with NaCl windows.

Results and Discussion

Residue analysis - The results of the analyses of the egg samples collected in rice fields during the summer months of 1968-69 are presented in Table 1. The average dieldrin contamination of eggs of purple and common gallinules collected in 1968 was 9.36 and 17.48 ppm, respectively. Average dieldrin contamination of eggs collected in 1969 was 3.80 ppm for purples and 4.78 ppm for commons. DDE (p, p'-isomer) was present in all egg samples except 9 but at much lower levels than dieldrin.

Lower levels of dieldrin were detected in eggs collected in 1969 than in 1968. The recommended agricultural practice of pretreating rice seed with aldrin for rice water weevil (*Lissorhoptrus oryzophilus* Kuschel) control was discontinued after 1968 due to the development of high levels of resistance to the chemical by this insect (12). Although aldrin was not recommended in 1969, some seed companies sold treated seed since there was no alternative control for the rice water weevil. The reduction in the amount of aldrin-treated seed rice used in 1969 reduced the amount of contaminated rice available to birds arriving on the nesting grounds at this time. This probably accounts for the decrease in dieldrin contamination of eggs from 1968 to 1969.

TABLE 1

Organochlorine insecticide residues in gallinule eggs from rice fields, Crowley, Louisiana, 1968-1969.

		PPM			
		Dieldrin		p, p' - DDE	
Species	Samples	Average	Range	Average	Range
			1968		
Purple	26	9.36	3.23-16.43	0.19	Trace[a] -0.46
Common	6	17.48	4.69-28.07	0.70	0.11-1.84
			1969		
Purple	33	3.80	1.56-13.62	0.15	Trace[a] -0.38
Common	12	4.78	1.16-10.70	0.18	0.11-0.38

a/ Trace recorded when residues were below 0.05 ppm.

Forty-six egg samples of the purple gallinule and 41 of the common gallinule from marsh areas were analyzed and only 2 samples were found to contain a detectable amount of dieldrin (0.17 and 0.28 ppm). Of these egg samples, 46 contained p, p' -DDE at an average level of 0.22 ppm with a range of 0.11 - 1.21 ppm. Two eggs collected at Pass-A-Loutre Wildlife Refuge also contained a detectable amount of p, p' -DDT (1.54 and 1.03 ppm).

Polychlorinated biphenyls (PCB's) are often found in tissues of wild animals and interfere with pesticide residue analysis (13). PCB residues were detected in only 5 of the egg samples analyzed, and all of these came from the Sabine Wildlife Refuge.

Hatchability and Survival of Chicks - Data on hatch of eggs and survival of gallinule chicks observed during 1968 and 1969 are listed in Table 2. Statistical analyses (least squares analysis of variance for unequal subclass numbers) of these data revealed that hatch of eggs and survival of chicks containing high levels of dieldrin from populations of common and purple gallinules in rice fields were not significantly different ($P > .05$) from that of control eggs collected in marsh areas. These laboratory results on egg hatchability confirm those reported by Causey et al. (3) who conducted field experiments to determine hatchability of purple and common gallinule eggs. In addition, researchers studying other species of game birds have reported comparable hatchability figures from controlled experiments (14, 15).

The survival success (60-81 per cent) of purple and common gallinules in this study was high considering success in rearing similar wild birds in captivity. Lynch (16) reported that his success in rearing gallinules, rails and mallard ducks was never greater than 50-75 per cent.

Graves et al. (17) found that 4.8 ppm dieldrin in eggs of chickens had no effect on egg hatch or chick survival during a 14-day period. Balasubramaniam (18) found that feeding 10 ppm dieldrin in the diet of chickens did not significantly reduce egg hatch or chick survival. De Witt (19), however, reported that 10 ppm dietary dieldrin adversely affected egg hatchability and chick survival in quail and pheasant.

TABLE 2

Hatchability of eggs and survival of chicks from eggs collected in marshes and rice fields, 1968-1969.

Species	Number of Clutches	Number Eggs Incubated	Eggs Hatched		Survival of Chicks (after 14 days)	
			No.	Percent	No.	Percent
				Marsh Areas		
Purple	30	123	97	78	64	66
Common	33	129	111	86	90	81
				Rice Fields		
Purple	37	166	124	75	74	60
Common	8	48	41	85	25	61

Eggshell Thickness - Statistical analysis of eggshell thickness data taken from 109 purple and 49 common gallinule eggs collected from rice fields and marsh areas indicated no correlation between shell thickness and dieldrin contamination in these species when considered separately or when combined (Table 3). A significant correlation between shell thickness and *p*, *p*' -DDE (+ *p*, *p*' -DDT) concentrations was obtained with common gallinules. This correlation was positive, however, indicating an increase in shell thickness with increases in concentration of these residues at the levels detected. A significant reduction in eggshell quality was recorded in prairie falcons fed dieldrin contaminated starlings (5). In addition, sparrow hawks fed a combination of dieldrin and DDT produced eggs with significantly thinner shells (8). One ppm dieldrin and 5 ppm DDT in the diet were sufficient

TABLE 3

Simple correlation of eggshell thickness and dieldrin or DDE contamination in eggs of purple and common gallinules (combined and individual species analyses).

Variables	No. Observations	Average	Correlation
		Combined Analysis	
Dieldrin	158	1.49 ± .19 ppm	-0.04
DDE	158	0.26 ± .03 ppm	0.05
Thickness	158	218 ± 10.2 microns	
		Purple Gallinules	
Dieldrin	109	1.82 ± .23 ppm	-0.02
DDE	109	0.19 ± .01 ppm	-0.08
Thickness	109	203 ± 12.7 microns	
		Common Gallinules	
Dieldrin	49	0.79 ± .27 ppm	0.22
DDE	49	0.41 ± .09 ppm	0.33[a/]
Thickness	49	254 ± 35.6 microns	

[a/] Significant at the 5 per cent significance level.

to produce this effect. Peakall (20), however, reported that dieldrin injected (20 ppm) prior to egg laying in the ringdove produced no significant thinning of the eggshell.

General Considerations

Stickel (21) noted that declines in avian reproductive success under insecticidal treatment are almost always partial, are usually small, and rarely are eliminative. This seems to be true even when toxicant levels are high enough to kill a significant percentage of parents. The effects on reproduction of sublethal dosages over a long period of time are complicated by natural variability and are extremely difficult to measure.

Considering the data obtained during this study, the reproductive potential of purple and common gallinules associated with aldrin-treated rice fields does not appear to be seriously threatened by the presence of rather high levels of dieldrin in their eggs. However, this does not mean that there may not be an adverse effect on reproduction from these residues. Variation was great enough in this study that rather small effects could not be measured with accuracy.

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Acknowledgements

Appreciation is extended to the personnel of the Louisiana Wildlife and Fisheries Commission and the Rockefeller Wildlife Refuge for assistance in work conducted in marsh areas. The authors are particularly indebted to Mr. Larry McNease of the Rockefeller Wildlife Refuge. Appreciation is also extended to Dr. Rouse Caffey, Associate Director, L. S. U. Agricultural Experiment Station, who was Superintendent of the Louisiana State University Rice Experiment Station, Crowley, during the time this research was conducted, for his cooperation in making available part of the study area.