Effect of Dieldrin and Aroclor 1242 on Japanese Quail Eggshell Thickness

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Eggshell thinning in wild birds has been correlated with certain chlorinated pesticide residues (HICKEY and ANDERSON, 1968; ANDERSON et al., 1969; RATCLIFFE, 1970; BLUS et al., 1971; GRESS et al., 1971; KEITH et al., 1971). Thinning has been produced experimentally by feeding DDT[2], DDE[3], and dieldrin[4] to captive mallards (Anas platyrhynchos) (HEATH et al., 1969; LEHNER and EGBERT, 1969; HAEGELE and TUCKER, 1974), black ducks (A. rubripes) (LONGCORE et al., 1971), Japanese quail (Coturnix c. japonica) (HAEGELE and TUCKER, 1974; BITMAN et al., 1969; CALL and HARRELL, 1974; STICKEL and RHODES, 1970), American kestrels (Falco sparverius) (PORTER and WEIMEYER, 1969; WEIMEYER and PORTER, 1970), screech owls (Otus asio) (MCLANE and HALL, 1972), and ring doves (Streptopelia risoria) (HAEGELE and HUDSON, 1973). Several mechanisms have been postulated as contributory to thinning: inhibition of the enzymes carbonic anhydrase (BITMAN et al., 1970; PEAKALL, 1970) and Ca-ATPase (MILLER et al., 1975) in the shell gland; interference with medullary bone formation (OESTREICHER et al., 1971); hypothyroidism (JEFFERIES and FRENCH, 1971); and hepatic microsomal enzyme induction (PEAKALL, 1967).

The objective of this study was to test eggshell thinning with a species which is easily maintained in a laboratory. Japanese quail were selected because they lay prolifically for long periods under controlled lighting; however, they proved to resemble other gallinaceous species in not being especially susceptible to eggshell thinning. This paper presents the results of prototype tests with Aroclor 1242 (polychlorinated biphenyl, 42% Cl) and dieldrin (87% HEOD) and an evaluation of the methodology.

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[2]1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane.

[3]1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene.

[4]1,2,3,4,10,10-hexachlor-exo-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene.

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Procedures

Two experiments were conducted with 6-month-old Japanese quail hens which had been laying continuously since puberty. The first experiment was a 45-day presentation of Aroclor 1242 at the dietary concentration of 10 ppm. The second experiment lasted 75 days, and dieldrin was used at the dietary concentrations of 1, 3, and 9 ppm. Concurrent controls accompanied each experiment. The experiments were basically similar, except the dieldrin protocol was modified slightly to accommodate procedural improvements indicated after analysis of the Aroclor experiment. Procedural differences are described as appropriate.

Ten or 11 hens per treatment were assigned to individual pens (measuring 31 x 51 x 25 cm high) in 4-tiered battery cages and were exposed to a light regimen of 14L:10D. After 2 to 3 weeks acclimation, the appropriate diets were presented ad libitum for the duration of the experiment. A completely randomized experimental design was used with treatments and birds assigned by random numbers. Diets were prepared by mixing a solution of Aroclor or dieldrin and corn oil into commercial turkey breeder mash in the ratio of 1:99, by weight. Control diets also contained corn oil in the ratio of 1:99.

Eggs were collected and dated daily. Eggshells, including membranes, were air-dried for 1 week before measurement. Measurement was at 3 or more points around the equator with a micrometer sensitive to 10μ. The mode was the statistic recorded for each egg. Comparisons of shell thickness were based on successive eggs laid by each hen after each 10-day interval.

In the Aroclor test, samples of 5 eggs were collected. From that collection comparisons were made on the basis of the first egg laid, the first 3 eggs, and all 5 eggs. For multiple-egg sequences the average thickness of successive eggs from each hen was the statistic used for all comparisons. In the dieldrin test, comparisons were restricted to 3-egg samples.

In an effort to ascertain the effects of handling on rate of laying and shell thickness, all hens were weighed periodically during the Aroclor test and their pre- and post-weighing performances were compared. Feed consumption was measured during the dieldrin test.

All analyses were by one-way analysis of variance. Means were separated according to the method of Duncan (1955). The 5% level of significance was accepted, but values approaching significance ($0.05<P<0.10$) are also indicated.

Results

Aroclor 1242: Eggs from Japanese quail fed Aroclor 1242 at the dietary concentration of 10 ppm for 40 days had significantly

(P<0.05) thinner shells than eggs from controls (Table 1). Average thinning was 5.2% based on 3- and 5-egg samples and 4.7% for 1-egg samples. Results for all sample sizes were similar; the maximum differences among means within either treatment for the various sample sizes was only 3μ (≃1.5%). Nevertheless, only the multiple-egg samples were statistically separable at P<0.05.

Within 10 days after presentation of treated diets, birds fed Aroclor 1242 were forming slightly thinner (≃1.4%) eggshells than the controls. Whether this difference was induced or simply represents the pretreatment norm is unknown. Beyond 10 days the magnitude of difference in shell thickness between treatments increased linearly with time until the experiment's conclusion. After 40 days, 10 of 11 hens fed Aroclor 1242 were consistently forming thinner eggshells ($\bar{x}$ = 5.2%) than they had been after 10 days. At the same time, 7 of 11 control hens were also forming slightly thinner shells than they had been after 10 days, but the difference was less than 2%. Table 2 shows 10- to 40-day shell thickness comparisons for individual hens based on 3-egg samples. Shell thickness for successive eggs comprising multiple-egg samples for a given hen was quite uniform. For example, in Table 2, 39 of 44 sets of 3-egg samples had differences between extreme eggs of 10μ or less. Nine samples had identical measurements for all 3 eggs. As would be expected, extremes for 5-egg samples were more variable, but even they differed by 10μ or less about 65% of the time.

Average rate of laying during the 45-day experiment was 0.934 (SD=0.067) egg per day for controls and 0.941 (SD=0.077) egg per day for Aroclor-treated birds. All test hens laid at a rate of at least 0.77 egg per day. Four hens from each treatment laid an egg for every day.

Handling of hens during weekly weighings did not appear to affect laying, eggshell thickness, or egg weight. Numbers of eggs laid during the 2 days preceding and the 2 days following each weighing were similar. Hens on both treatments averaged 0.91 egg per hen-day before weighing. After weighings, controls and Aroclor-treated birds averaged 0.89 and 0.90 egg per hen-day, respectively. No statistically significant difference was detected for either shell thickness or egg weight between eggs laid on the day before and day after each hen weighing. Hen weights did not change appreciably during the experiment.

Dieldrin: Japanese quail eggshell thickness was not significantly changed by dietary dieldrin when presented at concentrations of 1, 3, or 9 ppm for 75 days (Table 3). Slight, but non-significant, time-related shell thinning occurred for all treatments. The relativity between average shell thickness for the treatments was essentially unchanged during the study, i.e., 1 ppm > 9 ppm > control > 3 ppm.

For 2 weeks preceding the dieldrin experiment, all birds were fed "control" feed (i.e., 1% corn oil added) for establishments of

TABLE 1

Comparison of mean shell thickness (μ) of Japanese quail eggs, estimated from periodic samples of 1, 3, and 5 successively laid eggs per hen, during 45 days of dietary exposure to Aroclor 1242.

Series size[1]	Statistic[2]	Days of treated diet[3] 10	20	30	40
		Control			
1	Mean (SD)	215(15.1)	214(15.7)	214(15.0)	211(15.8)
	Extreme eggs	190-240	190-240	180-230	190-240
3	Mean (SD)	214(13.4)	214(13.5)	214(15.6)	210(14.6)
	Extreme means	197-237	193-233	183-233	190-237
5	Mean (SD)	214(13.5)	214(11.2)	215(13.1)	211(11.9)
	Extreme means	194-230	194-230	186-232	196-234
		Aroclor 1242 (10 ppm)			
1	Mean (SD)	212(16.0)	207(10.0)	206(15.7)	201+(10.4)
	Extreme eggs	170-230	190-220	180-220	180-220
3	Mean (SD)	211(13.5)	210(10.9)	206(9.6)	199*(11.5)
	Extreme means	180-227	187-223	187-217	177-223
5	Mean (SD)	211(11.6)	209(11.9)	207(10.5)	200*(11.8)
	Extreme means	186-226	184-224	184-218	178-224

[1]The first, first 3, or all 5 eggs from each 5-egg series of successively laid eggs.
[2]All statistics are based on 11 hens per treatment. For 3- and 5-egg sets, means are computed from the means for the series from each hen.
[3]First day of each sample collection.
+Difference from control approaches significant (0.05<P<0.10).
*Difference from control statistically significant (P<0.05).

TABLE 2

Eggshell thickness (μ), by individual hen, for Japanese quail after 10 and 40 days exposure to control feed and Aroclor 1242[1].

Control						Aroclor 1242 (10 ppm)					
	10 days		40 days		Percentage		10 days		40 days		Percentage
Hen	Mean	Extreme eggs	Mean	Extreme eggs	change[2]	Hen	Mean	Extreme eggs	Mean	Extreme eggs	change[2]
1	203	200-210	200	200	-1.5	2	207	200-210	200	200	-3.4
3	197	190-200	213	210-220	+8.1	4	213	210-220	203	200-210	-4.7
5	237	230-240	217	200-230	-8.4	7	217	210-220	193	190-200	-11.0
6	197	190-210	190	190	-3.6	9	203	200-210	193	190-200	-4.9
10	227	220-230	237	230-240	+4.4	11	220	220	207	200-210	-5.9
13	203	200-210	200	200	-1.5	12	197	190-200	197	190-200	0.0
16	213	210-220	210	210	-1.4	15	220	220	203	200-210	-7.7
18	217	210-220	203	200-210	-6.4	17	220	220	190	180-200	-13.6
19	230	230	233	230-240	+1.3	20	217	210-220	203	200-210	-6.4
23	217	210-220	197	180-210	-9.2	21	227	220-230	223	220-230	-1.8
24	213	210-220	213	210-220	0.0	22	180	170-190	177	170-180	-1.7

[1]All means are for the first 3 eggs laid after 10 or 40 days.
[2]Percentage difference between collection means.

TABLE 3

Comparison of mean shell thickness (μ) of Japanese quail eggs, estimated from periodic samples of 3 successively laid eggs per hen, during 75 days of dietary exposure to dieldrin.

Statistic[1]	Treatment	Days of treated diet[2] 10	20	30	40	50	60	70
Mean (SD)	Control	192(15.6)	194(14.2)	199(13.2)	197(11.6)	194(14.0)	194(15.1)	194(12.8)
Extreme Means		163-210	167-213	170-213	170-210	170-213	170-217	167-203
Mean (SD)	1 ppm	204(11.4)	202(8.3)	202(9.7)	200(8.7)	192(13.7)	200(5.2)	198(8.6)
Extreme means		190-220	190-217	190-220	183-213	165-210	193-207	183-213
Mean (SD)	3 ppm	195(13.8)	195(15.9)	196(13.8)	190(14.1)	189(12.4)	189(16.8)	192(14.2)
Extreme means		173-220	173-227	173-220	170-217	173-217	157-217	177-213
Mean (SD)	9 ppm	202(16.9)	197(16.3)	200(14.0)	201(16.5)	195(18.5)	198(17.8)	194(17.8)
Extreme means		170-227	177-220	183-230	173-227	167-220	173-223	163-220

[1]All statistics are based on 10 hens per treatment. Means are computed from the means for the 3-egg series from each hen.
[2]First day of each sample collection.

baseline eggshell thickness and rate of laying. This was to ascertain whether any thickness difference seen early in the study was induced or simply a function of chance during randomization. During this pretreatment period the relationship between shell thickness for the various groups was 1 ppm > 9 ppm > control > 3 ppm. The average shell thickness during pretreatment was similar to that seen after 10 days of treatment (Table 3).

Variability of extreme shell thickness measurements within the 3-egg samples for a given hen differed by 10μ or less 80% of the time. Changes in average shell thickness for 3-egg samples between successive collection periods were similar among treatments and were usually less than 2%.

Rate of laying averaged at least 0.91 egg per hen-day for all groups during the entire study; the median was 0.96 egg per hen-day. Six of the 40 hens laid 1.0 egg per day. Control hens and those fed 9 ppm dieldrin tended to lay at the highest rate throughout the study, including the 2-week acclimation period.

Food consumption and hen weights were unaffected by any test treatment.

Discussion

Statistically significant eggshell thinning was induced in Japanese quail by 40-50 days of exposure to Aroclor 1242 at the dietary concentration of 10 ppm. Using the same procedures, 1, 3, and 9 ppm of dieldrin did not cause thinning during 75 days of exposure. In both studies, slight, but consistent, time-related thinning was observed for nearly all birds, including controls.

The 5.2% eggshell thinning observed for Japanese quail fed 10 ppm Aroclor 1242 is similar to the 5.0% thinning reported for white leghorns (Gallus gallus) fed the same concentration by KEPLINGER et al. (1972). In their study comparisons were based on pooled samples; thus, time-related effects could not be compared. They did not demonstrate shell thinning for hens fed 1 ppm Aroclor 1242. In a study by CECIL et al. (1972), leghorns were fed Aroclor 1242 at 2 and 20 ppm for 8 weeks. No differences in shell thickness occurred between treatments or in relation to time. Most other studies of eggshell characteristics associated with Aroclor 1242 used dietary concentrations considerably above levels of relevance to our experiment.

Results of our study of eggshell thickness with dieldrin conflict with results reported for other studies. CALL and HARRELL (1974) fed 3.1 ppm dieldrin to Japanese quail for only 21 days and report shell thinning of 8.0%. But in their study they reduced the light regimen to 10L:14D for the first 7 days, then increased it to 24L:0D, thereafter. Interaction of photoperiod manipulation with eggshell deposition is not clear, but during the period of reduced light the controls continued to lay while laying was interrupted for hens fed dieldrin. LEHNER and EGBERT (1969) fed mallards 1.6, 4, and 10 ppm dieldrin for 2 breeding seasons and report eggshell thinning averaging about 4% for all treatments. In agreement with our findings, DAVISON and SELL (1972) fed white leghorns 10 and 20 ppm dieldrin for 12 weeks without affecting shell thickness. Of the above studies, this last one most closely simulates our experimental procedures in that they also used multiple-egg measurements for individual hens.

Shell thickness based on the average of successively laid eggs from each hen appears to be more critical than single-egg measurements. Even though the mean values were similar for 1-, 3-, and 5-egg samples during the Aroclor 1242 study, only the multiple-egg samples were separable statistically. As it is well-known that physical characteristics of consecutive eggs within a clutch are quite similar (ROMANOFF and ROMANOFF, 1949); the smaller sample has obvious logistic advantages. Empirically, our shell thickness values for 3- and 5-egg samples were extremely uniform. Multiple-egg measurements also have the advantage of reducing any statistical bias due to random outliers.

We have presented evidence that relatively critical comparisons of eggshell thickness are possible with the procedures described herein. The procedural strengths include: individual housing of hens, use of multiple-egg measurements, provision for determination of pretreatment baselines, and simple continuous monitoring of effects. Although Japanese quail are excellent experimental subjects, they do not appear to be as susceptible to eggshell thinning as certain other species.

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