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Dieldrin Residues In Eggs and Fat of Penned Pheasant Hens

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DIELDRIN RESIDUES IN EGGS AND FAT OF PENNED PHEASANT HENS

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

At present, there is considerable interest in the effects of insecticides upon our wildlife populations. It is well known that chlorinated hydrocarbon insecticides are present in tissues and eggs of birds that receive these chemicals in their diets. Some workers, for example Genelly and Rudd (1956) and DeWitt (1956), studied the effects of different levels of ingested insecticides on the reproduction of game birds. Additional studies are needed to relate the amount of insecticide in the diet to the level in the egg and that in turn to reproduction and effects on young birds.

Work on pheasants is of special interest at South Dakota Experiment Station because of the economic importance of this game bird to the state. Since dieldrin and aldrin have been used to control corn root worm on much of the prime pheasant range, and since dieldrin is a metabolite of aldrin (Bann et al. 1956), the experiment reported herein was carried on to study the residues of dieldrin in pheasants. The objectives were to determine and compare: (1) the trend of dieldrin deposition in yolks of eggs from hen pheasants fed different levels of the insecticide, (2) the trend of dieldrin deposition in the egg yolks after termination of treatment, and (3) the level of dieldrin in the fat of the birds after the egg laying period. This work is one phase of an extensive study on the relationship of insecticides to pheasants.

MATERIALS AND METHODS

Nine hen pheasants approximately one year of age, induced to laying by artificial light, were kept in individual cages (Fig. 1) and fed a basal pheasant breeding ration (Zip Feed Mills, Sioux Falls, S. Dak.) throughout the experiment. The birds were randomly assigned to treatments of 0, 2, and 4 milligrams of dieldrin mixed with lactose and given in gelatin capsules (Fig. 2) every seventh day. The treatments were administered for thirteen consecutive weeks.

For this study one egg from each hen was collected at about weekly intervals for the first twelve weeks and all eggs were collected for two weeks following the thirteenth treatment. Each egg was labelled and frozen for later analysis of the yolk. An egg yolk leaves the ovary approximately 24 hours before the egg is laid. Eggs that were laid during one week after a 24 hour period following each treatment were assigned to that particular treatment. These seven days were designated as a treatment week. Only yolks were analyzed for dieldrin. Ware and Naber (1961) and Azevedo, Hunt and Woods (1965) reported that the residues of other chlorinated hydrocarbons (lindane and DDT) were concentrated in the yolk with none being found in the albumen. Fourteen days after final treatment the hens were sacrificed and the fat from the breast region was analyzed for dieldrin.

Each sample of fat and egg yolk was analyzed using florisil sample cleanup and electron capture gas chromatographic (ECGC) analysis (Stemp et al. 1964). For the sample cleanup (Fig. 3),

Fig. 1. Individual cages used to hold pheasant hens during the study.

Fig. 2. Method used to administer gelatin capsules containing dieldrin to pheasant hens.

one g of egg yolk or fat was ground with 25-30 g of florisil until a free-flowing powder was obtained. An additional 30 g of florisil were placed in a 20 mm x 600 mm pyrex chromatographic column with the sample florisil mixture being added as the top layer. Approximately 750 ml of a mixture of 20% methylene chloride in petroleum ether (v/v) were used as the eluant. After elution the sample was evaporated to dryness with a rotating vacuum evaporator and transferred to a calibrated test tube using hexane as a solvent.

For the ECGC analysis, a two to four microliter portion of the sample was injected into a Wilkins Aerograph Hy-Fi Model 600-D chromatograph. A 250-millicurie tritium source Kovar cell detector and a model S-R 1 mv Sargent recorder were used with the chromatograph (Fig. 4). The column used was a 1/8" OD x 5' pyrex glass column packed with 5.0% Dow 11 Silicone 60/80 mesh (HMDS) treated Chromosorb W and it was operated at 190°C with a nitrogen gas flow rate of 75 ml/min. To further verify the identification of dieldrin, occasional samples were run on a second 1/8" OD x 5' pyrex glass chromatographic column packed with 2% (Fluoro) QF-1 Silicone on 60/80 mesh (HMDS) treated Chromosorb W which was operated at 120°C with a 50 ml/min nitrogen carrier gas flow rate.

The identification and quantitative analysis were accomplished by comparing the retention time and peak area of the sample with the retention time and peak area of a dieldrin standard. Parts per

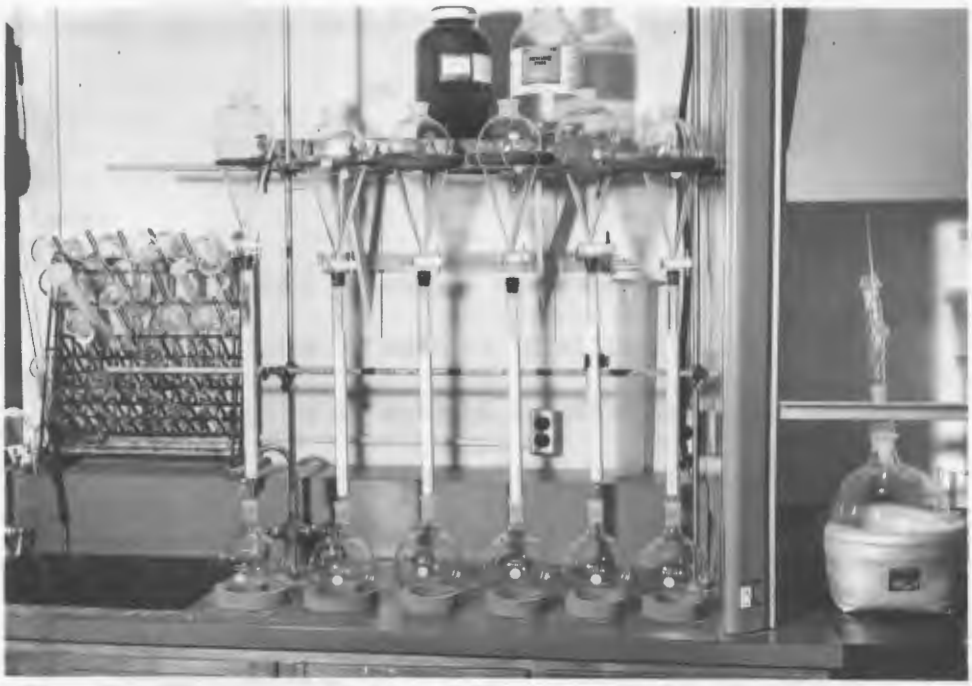

Fig. 3. The apparatus used for the sample cleanup procedure.

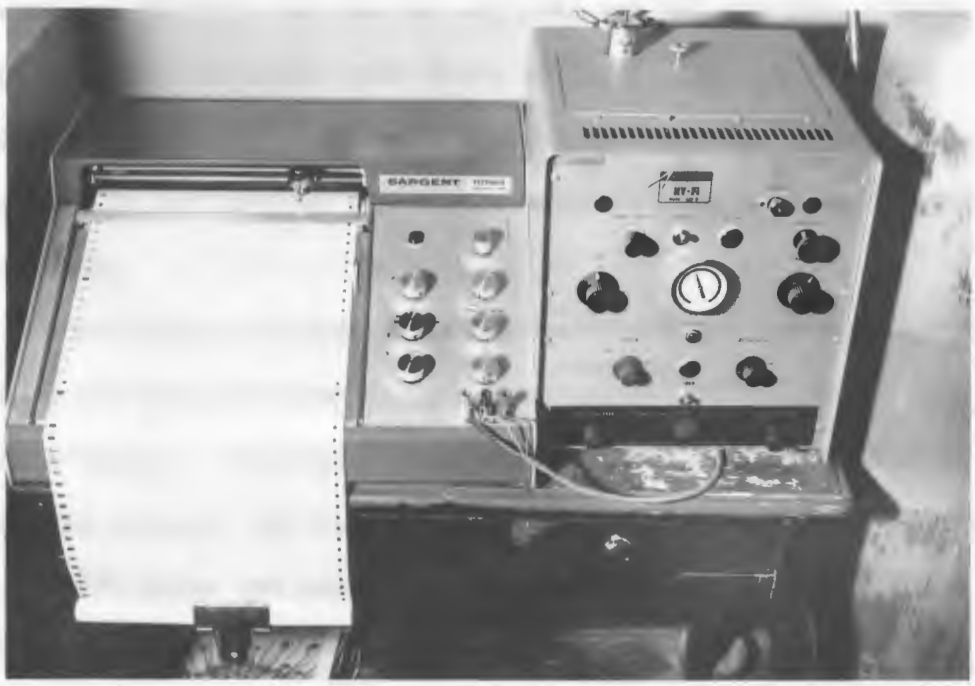

Fig. 4. The gas chromatograph and recorder used to analyze pheasant egg yolks and fat for dieldrin residues.

million were calculated using the following formula:

$$ppm = \frac{V \; w \; d_2}{W \; v \; d_1}$$

where:

W = weight of sample in grams

V = volume of extract in milliliters

v = volume of extract injected in microliters

w = weight of standard injected in nanograms

d_1 = recorder response for standard

d_2 = recorder response for sample

To determine the efficiency of the entire procedure, known amounts of dieldrin were added to control samples and the average recovery was found to be 96% for egg yolk and 91% for fat. These findings agreed favorably with Stemp et al. (1964) who reported recovery values of over 90% with a standard deviation of 3%. All values in the present study were corrected for the percentage of recovery.

The methylene chloride was spectroanalyzed (Fisher Scientific Company) and the petroleum ether was nanograde (Mallinkrodt Chemical Works). Florisil 60/100 mesh, activated at 65°C (Fisher Scientific Company) was heated at 140°C for 12-14 hours, mixed with 3% distilled water and held in an airtight container for 48 hours before use.

RESULTS AND DISCUSSION

Nine egg yolks from the hens in the control group (those not given dieldrin) were analyzed for dieldrin. These were the 1st, 3rd and 5th eggs laid by each hen in the group following the 13th and final week of treatment. Since no level of dieldrin greater than 0.1 ppm was found it was decided not to analyze additional eggs from these hens.

In egg yolks from hens receiving dieldrin in Treatment I (2 mg per week) and in Treatment II (4 mg per week), residues generally appeared within the first week after treatment and steadily built up as each capsule was administered weekly (Table 1 and Fig. 5). Hen 1 in Treatment II displayed a reverse in this trend as no eggs were laid during the first six weeks and the egg laid the seventh week contained the highest level of residue. This suggested that egg laying was a large factor in a hen's ability to pass dieldrin from the body. The dieldrin recovered in the egg yolk analyzed for each hen for each week was assumed to represent the average of all eggs laid during that particular week. The percentages of the total administered dieldrin deposited in the eggs laid during the experiment are shown in Table 2. Hens in Treatment I excreted 21.14, 13.97 and 37.47% and hens in Treatment II excreted 29.88, 22.68 and 20.71% via the egg yolk. Analysis of variance showed no significant difference (0.05) between treatment groups in this respect.

Table 1. Dieldrin (ppm) in the yolk of eggs laid during treatment period.

Week of treatment	Treatment I 2 mg/wk			Treatment II 4 mg/wk		
	Hen 1	Hen 2	Hen 3	Hen 1	Hen 2	Hen 3
1	0.6	0.7	1.1	---	5.3	---
2	2.6	---	2.3	---	9.3	---
3	4.8	---	6.0	---	10.3	11.7
4	5.0	5.2	6.0	---	12.4	13.0
5	5.7	6.1	6.8	---	11.9	15.4
6	5.7	7.9	8.2	---	12.7	13.5
7	6.3	9.0	9.8	40.1	15.8	17.3
8	5.9	9.2	10.5	35.9	15.0	15.5
9	6.6	8.7	15.2	40.1	18.8	18.6
10	6.5	8.2	13.2	35.6	18.9	19.9
11	7.6	8.8	22.1	32.7	20.4	19.2
12	7.8	7.6	26.5	27.5	18.0	---

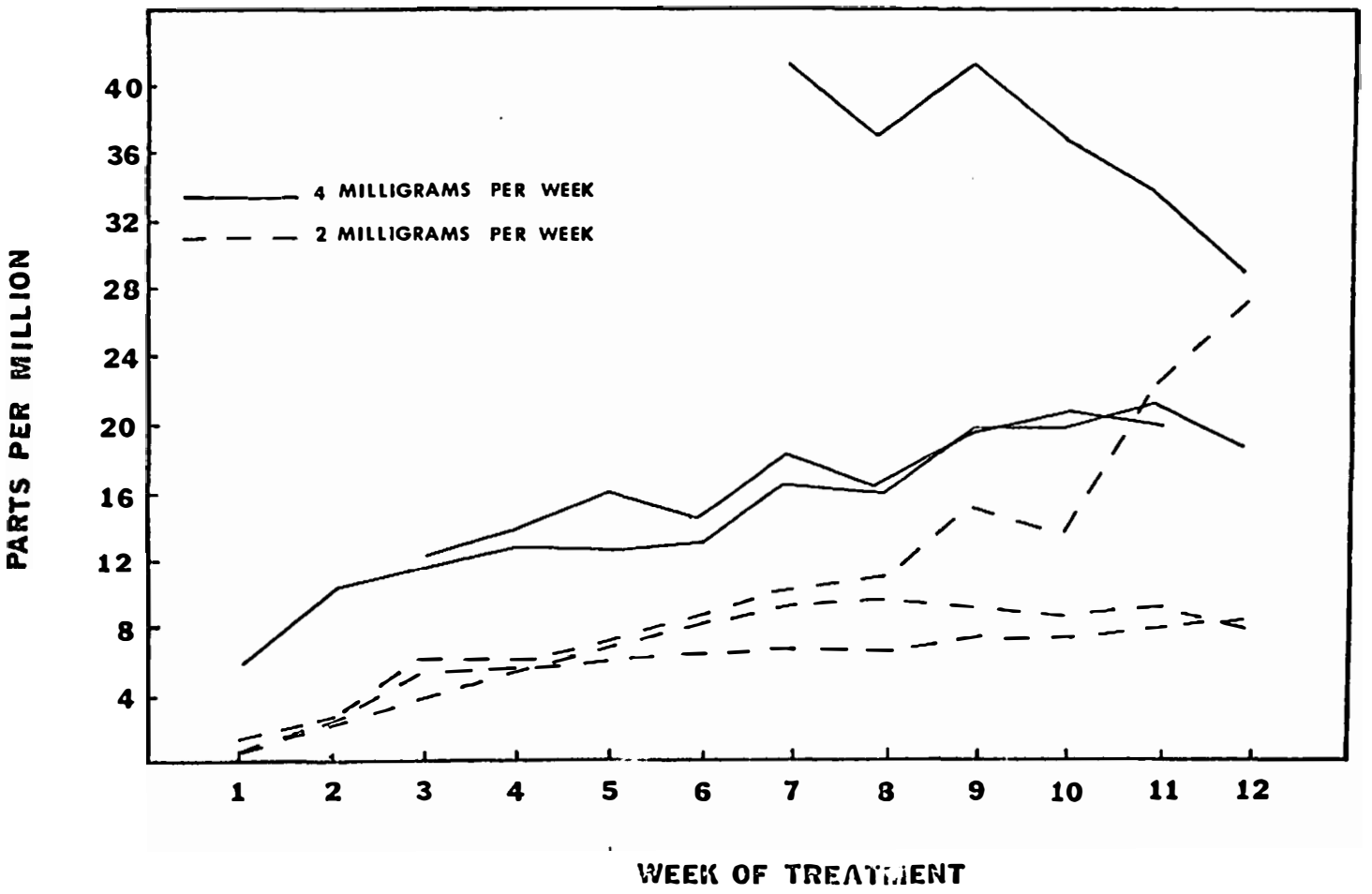

Fig. 5. Dieldrin in yolk of eggs laid during treatment period.

Table 2. Dieldrin deposited in the yolk of eggs laid by Hen 1 in Treatment I (2 milligrams per week).

Week of treatment	Eggs laid during week	Average wt. of yolk in grams	Total grams of yolk	ppm of dieldrin	Total milligrams of dieldrin	Cumulative milligrams of dieldrin	Cumulative % of dieldrin fed
1	6	11.5162	69.0972	.57	.0394	.0394	1.97
2	6	11.5162	69.0972	2.63	.1817	.2211	5.53
3	5	11.5162	57.5810	4.83	.2781	.4992	8.32
4	5	11.5162	57.5810	4.97	.2862	.7854	9.82
5	7	11.5162	80.6134	5.73	.4619	1.2473	12.47
6	6	11.5162	69.0972	5.65	.3904	1.6377	13.65
7	6	11.5162	69.0972	6.28	.4339	2.0717	14.80
8	7	11.5162	80.6134	5.85	.4716	2.5432	15.90
9	7	11.5162	80.6134	6.58	.5304	3.0737	17.08
10	4	11.5162	46.0648	6.54	.3013	3.3749	16.88
11	7	11.5162	80.6134	7.58	.6111	3.9860	18.19
12	4	11.5162	46.0648	7.79	.3586	4.3448	18.10
13*	12	11.5162	138.1944	8.33	1.1512	5.4960	21.14

* Following the 13th week of treatment eggs were collected for 14 days.

Table 2. (continued) Dieldrin deposited in the yolk of eggs laid by Hen 2 in Treatment I (2 milligrams per week).

Week of treatment	Eggs laid during week	Average wt. of yolk in grams	Total grams of yolk	ppm of dieldrin	Total milligrams of dieldrin	Cumulative milligrams of dieldrin	Cumulative % of dieldrin fed
1	1	11.5162	11.5162	.73	.0084	.0084	.42
2	---	---	---	---	---	.0084	.21
3	---	---	---	---	---	.0084	.14
4	1	11.5162	11.5162	5.21	.0600	.0684	.86
5	4	11.5162	46.0648	6.05	.2787	.3471	3.47
6	5	11.5162	57.5810	7.90	.4549	.8020	6.68
7	4	11.5162	46.0648	8.95	.4123	1.2143	8.67
8	6	11.5162	69.0972	9.16	.6329	1.8472	11.55
9	5	11.5162	57.5810	8.73	.5027	2.3499	13.06
10	5	11.5162	57.5810	8.17	.4785	2.8284	14.14
11	5	11.5162	57.5810	8.77	.5050	3.3334	15.15
12	5	11.5162	57.5810	7.58	.4365	3.7698	15.71
13*	11	11.5162	126.6782	9.17	1.1614	4.9312	18.97

* Following the 13th week of treatment eggs were collected for 14 days.

Table 2. (continued) Dieldrin deposited in the yolk of eggs laid by Hen 3 in Treatment I (2 milligrams per week).

Week of treatment	Eggs laid during week	Average wt. of yolk in grams	Total grams of yolk	ppm of dieldrin	Total milligrams of dieldrin	Cumulative milligrams of dieldrin	Cumulative % of dieldrin fed
1	4	11.5162	46.0648	1.08	.0498	.0498	2.49
2	5	11.5162	57.5810	2.33	.1342	.1839	4.59
3	5	11.5162	57.5810	5.97	.3438	.5277	8.80
4	6	11.5162	69.0972	5.97	.4125	.9402	11.75
5	5	11.5162	57.5810	6.81	.3921	1.3329	13.33
6	4	11.5162	46.0648	8.17	.3763	1.7093	14.24
7	5	11.5162	57.5810	9.83	.5660	2.2753	16.25
8	2	11.5162	23.0324	10.45	.2407	2.5160	15.73
9	5	11.5162	57.5810	15.15	.8724	3.3834	18.80
10	5	11.5162	57.5810	13.17	.7583	4.1418	20.71
11	5	11.5162	57.5810	22.08	1.2714	5.4132	24.61
12	6	11.5162	69.0972	26.52	1.8325	7.2457	30.19
13*	11	11.5162	126.6782	19.70	2.4956	9.7412	37.47

* Following the 13th week of treatment eggs were collected for 14 days.

Table 2. (continued) Dieldrin deposited in the yolk of eggs laid by Hen 1 in Treatment II (4 milligrams per week).

Week of treatment	Eggs laid during week	Average wt. of yolk in grams	Total grams of yolk	ppm of dieldrin	Total milligrams of dieldrin	Cumulative milligrams of dieldrin	Cumulative % of dieldrin fed
1	---	---	---	---	---	---	---
2	---	---	---	---	---	---	---
3	---	---	---	---	---	---	---
4	---	---	---	---	---	---	---
5	---	---	---	---	---	---	---
6	---	---	---	---	---	---	---
7	4	11.5529	46.2116	40.13	1.8545	1.8545	6.62
8	5	11.5529	57.7645	35.85	2.0709	3.9253	12.27
9	5	11.5529	57.7645	40.13	2.3181	6.2434	17.34
10	5	11.5529	57.7645	35.58	2.0552	8.2986	20.75
11	6	11.5529	69.3174	32.71	2.2674	10.5660	24.01
12	5	11.5529	57.7645	27.52	1.5897	12.1557	25.32
13*	11	11.5529	127.0819	26.60	3.3804	15.5361	29.88

* Following the 13th week of treatment eggs were collected for 14 days.

Table 2. (continued) Dieldrin deposited in the yolk of eggs laid by Hen 2 in Treatment II (4 milligrams per week).

Week of treatment	Eggs laid during week	Average wt. of yolk in grams	Total grams of yolk	ppm of dieldrin	Total milligrams of dieldrin	Cumulative milligrams of dieldrin	Cumulative % of dieldrin fed
1	3	11.5529	34.6587	5.33	.1847	.1847	4.62
2	4	11.5529	46.2116	9.28	.4288	.6136	7.67
3	6	11.5529	69.3174	10.31	.7147	1.3282	11.07
4	5	11.5529	57.7645	12.41	.7169	2.0451	12.78
5	3	11.5529	34.6587	11.93	.4135	2.4586	12.29
6	5	11.5529	57.7645	12.72	.7347	3.1933	13.31
7	5	11.5529	57.7645	15.77	.9109	4.1042	14.66
8	5	11.5529	57.7645	15.01	.8670	4.9713	15.53
9	5	11.5529	57.7645	18.75	1.0831	6.0544	16.82
10	5	11.5529	57.7645	18.91	1.0923	7.1467	17.87
11	6	11.5529	69.3174	20.40	1.4141	8.5608	19.46
12	5	11.5529	57.7645	18.02	1.0409	9.6017	20.00
13*	9	11.5529	103.9761	21.08	2.1918	11.7935	22.68

* Following the 13th week of treatment eggs were collected for 14 days.

Table 2. (continued) Dieldrin deposited in the yolk of eggs laid by Hen 3 in Treatment II (4 milligrams per week).

Week of treatment	Eggs laid during week	Average wt. of yolk in grams	Total grams of yolk	ppm of dieldrin	Total milligrams of dieldrin	Cumulative milligrams of dieldrin	Cumulative % of dieldrin fed
1	---	---	---	---	---	---	---
2	---	---	---	---	---	---	---
3	3	11.5529	34.6587	11.65	.4038	.4038	3.37
4	4	11.5529	46.2116	12.98	.5998	1.0036	6.27
5	6	11.5529	69.3174	15.39	1.0668	2.0704	10.35
6	5	11.5529	57.7645	13.51	.7804	2.8508	11.88
7	4	11.5529	46.2116	17.29	.7990	3.6498	13.04
8	5	11.5529	57.7645	15.45	.8925	4.5423	14.20
9	5	11.5529	57.7645	18.64	1.0767	5.6190	15.61
10	4	11.5529	46.2116	19.07	.8813	6.5003	16.25
11	3	11.5529	34.6587	19.16	.6641	7.1643	16.28
12	4	11.5529	46.2116	19.16	.8854	8.0497	16.77
13*	11	11.5529	127.0819	21.38	2.7170	10.7667	20.71

* Following the 13th week of treatment eggs were collected for 14 days.

Generally, as each weekly treatment was administered, milligrams of dieldrin in the yolks increased (Table 2). However, if the number of eggs laid during a particular week decreased the total milligrams of dieldrin in the yolks also decreased as no large increases in ppm of dieldrin were found. The number of eggs laid per treatment week varied (Appendices A and B). If a hen did not lay for a number of days the next egg did not show an extreme increase or decrease in residue content. The pattern of egg laying did not determine the amount of dieldrin the birds were able to excrete in the eggs.

A slight rise then a slow decline of residue deposition occurred in the eggs laid by all hens during the fourteen days after termination of treatment (Table 3 and Fig. 6). Ware and Naber (1961) stated that 53 days following termination of lindane treatment low levels were still being found in the eggs of chickens. Azevedo et al. (1965) found pheasants passed DDT residues into their eggs for nine weeks even though the ingestion of DDT was terminated at the beginning of egg laying. Stadelman et al. (1965) demonstrated that the eggs and tissues of laying chickens contained residues of dieldrin 26 weeks after exposure to low levels of the insecticide.

During the two week period following the final treatment, Hen 3 in Treatment I (2 mg per week) deposited about 2.5 milligrams of dieldrin in 11 eggs (Table 2). This was more dieldrin than the amount of the final treatment. Either dieldrin was deposited in

Table 3. Dieldrin (ppm) in the yolk of eggs laid during a 14-day period following final treatment and in fat at end of the egg laying period.

Egg No.	Treatment I 2 mg/wk				Treatment II 4 mg/wk			
	Hen 1	Hen 2	Hen 3	Average	Hen 1	Hen 2	Hen 3	Average
1	8.9	9.1	25.8	14.6	31.1	20.4	22.9	24.8
2	8.8	11.9	27.5	16.1	32.9	25.5	22.7	27.0
3	10.3	11.8	25.6	15.9	32.1	23.0	24.2	26.4
4	9.0	10.0	24.1	14.4	28.6	22.1	24.6	25.1
5	8.5	10.1	19.1	12.6	33.1	23.7	25.9	27.6
7	8.1	9.0	17.7	11.6	25.6	18.4	19.4	21.1
9	7.3	7.3	14.5	9.7	18.5	19.9	17.5	18.6
11	---	7.7	15.5	10.3	19.2	---	18.4	18.8
12	6.9	---	---	---	---	---	---	---
Fat	18.9	18.5	23.9		45.3	42.6	35.6	

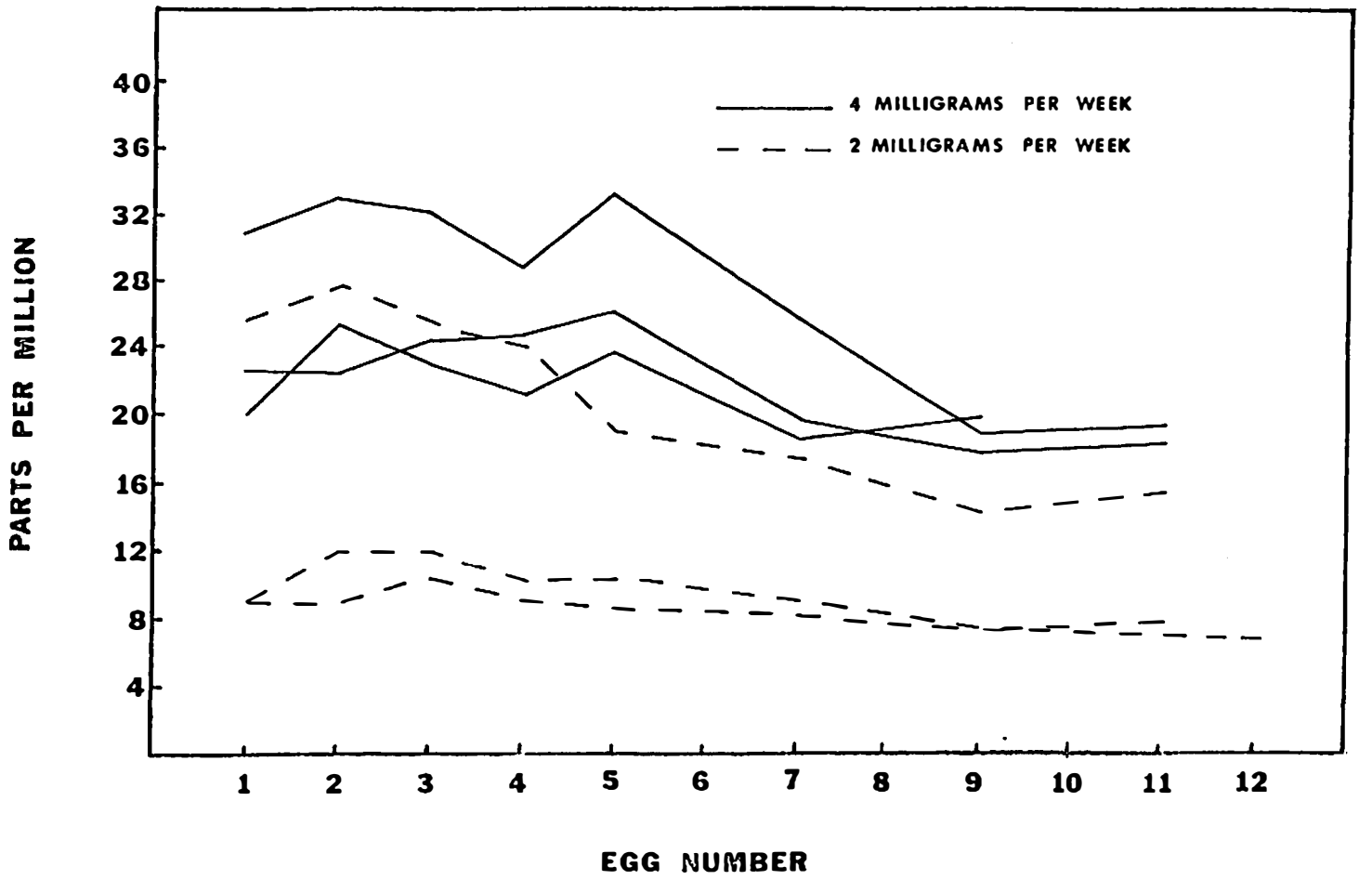

Fig. 6. Dieldrin in yolk of eggs laid during a 14-day period following final treatment.

the ovary as each treatment was ingested or residues from storage tissues in the body were being transferred to the ovary during the formation of the yolks.

Although the average ppm of dieldrin found in the egg yolks appeared to be greater for the higher treatment (Table 3 and Fig. 6), analysis of variance showed no significant difference (0.05) between treatment groups. This appeared to result from high variability between hens within treatments.

Liska et al. (1964) and Ware and Naber (1961) reported higher levels of residues of DDT and lindane in fat than in egg yolk of chickens. The results of the present study agree with their findings in that all but one hen deposited higher levels of dieldrin in the fat than in any one egg yolk. The difference in the level of residue in fat was found to be highly significant (0.01) between treatment groups (Table 3).

Stickel et al. (1965) concluded that methods other than encapsulated dosages would be needed to study the field effects of toxicants on woodcocks. This conclusion was based on the fact that when heptachlor was added to corn oil and presented in capsules, quantities of oil were passed in the feces in about 3 hours and as the authors stated (p 147), "...thus very likely ridding themselves of a large part of the heptachlor dose." The present study showed that from 18 to 37% of the total dieldrin which was presented in capsule form was deposited in the egg yolk. In addition, high quantities of residue were found in the fat. This would indicate

that high proportions of the dieldrin were absorbed when it was presented in gelatin capsules to pheasant hens.

This study showed that dieldrin fed in gelatin capsules occurred in its original form in pheasant eggs and fat for at least 14 days after termination of treatment. Thus, in the wild, if a hen is exposed to dieldrin before egg laying begins, residues will probably be present in all eggs of an average clutch. After completion of the clutch, the hen retains residue in her tissues. Further studies are necessary to determine if dieldrin residues have adverse effects on the pheasant population.

SUMMARY

1. Fat and egg yolks from pheasants administered different levels of dieldrin by capsule once a week for 13 weeks were analyzed for residues of the insecticide.

2. Residues in egg yolks increased each week following treatment to a maximum of 40.1 ppm and decreased during the fourteen days after the final treatment to a minimum of 6.9 ppm.

3. From 19 to 37% of the dieldrin administered was excreted via the egg yolk.

4. Following the egg laying period the dieldrin level in the fat was higher than in the egg yolks in all but one bird.

5. The difference in the level of residue in fat was found to be highly significant (0.01) between treatment groups while the levels in the egg yolks were not significantly different (0.05) even though the average dieldrin content was greater for the higher treatment.

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APPENDICES

Appendix A. Egg laying and treatment dates for hens in Treatment I (2 milligrams per week).

Date	Hen 1 Treatment	Hen 1 Egg	Hen 2 Treatment	Hen 2 Egg	Hen 3 Treatment	Hen 3 Egg
March 22						
23	1					
24						
25		X	1			
26		X			1	
27		X#		X		
28		X				
29						X
30	2	X				X
31		X				
April 1		X	2			X
2		X			2	
3						X
4		X				X
5		X				X
6	3	X				
7		X				
8			3			X
9		X			3	X
10		X				X
11		X				X

X - Eggs analyzed for dieldrin content.

Appendix A. (Continued) Egg laying and treatment dates for hens in Treatment I (2 milligrams per week).

Date	Hen 1 Treatment	Hen 1 Egg	Hen 2 Treatment	Hen 2 Egg	Hen 3 Treatment	Hen 3 Egg
April 12		X				
13	4	X				X
14						X
15		X	4			X
16		X			4	
17		X*				X
18						X
19		X				X
20	5	X				X
21						
22		X	5	X		X
23		X			5	X
24		X		X		X
25		X				
26		X		X		X
27	6	X		X		X
28		X				X
29		X	6	X		
30					6	X
May 1		X		X		X
2		X		X		X

* X - Eggs analyzed for dieldrin content.

Appendix A. (Continued) Egg laying and treatment dates for hens in Treatment I (2 milligrams per week).

Date	Hen 1 Treatment	Hen 1 Egg	Hen 2 Treatment	Hen 2 Egg	Hen 3 Treatment	Hen 3 Egg
May 3		X*				
4	7	X		X		
5		X		X		X
6		X	7			X
7		X		X	7	
8		X		X		X
9		X		X		X
10		X				X
11	8	X		X		X
12				X		X
13		X	8			
14		X			8	X
15		X		X		
16		X				
17		X		X		X
18	9	X		X		X
19		X		X		
20		X	9	X		
21		X		X	9	
22		X		X		
23		X		X		

* X - Eggs analyzed for dieldrin content.

Appendix A. (Continued) Egg laying and treatment dates for hens in Treatment I (2 milligrams per week).

Date		Hen 1 Treatment	Hen 1 Egg	Hen 2 Treatment	Hen 2 Egg	Hen 3 Treatment	Hen 3 Egg
May	24		X				
	25	10	X		X		X
	26		X		X*		X
	27		X	10	X		X
	28					10	X
	29		X				X
	30		X		X		
	31				X		X
June	1	11			X		X
	2		X		X		X
	3		X	11			
	4		X		X	11	X
	5		X		X		X
	6		X		X		X
	7		X		X		X
	8	12	X				
	9		X				X
	10		X	12	X		X
	11				X	12	X
	12		X				
	13				X		X

* X - Eggs analyzed for dieldrin content.

Appendix A. (Continued) Egg laying and treatment dates for hens in Treatment I (2 milligrams per week).

Date		Hen 1 Treatment	Hen 1 Egg	Hen 2 Treatment	Hen 2 Egg	Hen 3 Treatment	Hen 3 Egg
June	14				X		X
	15	13	X		X		X
	16		X		X		
	17		X*	13	X		X
	18		X			13	X
	19				X		X
	20		X		X		
	21		X				X
	22		X		X		
	23		X		X		X
	24		X		X		X
	25		X				X
	26		X		X		X
	27		X		X		
	28		X		X		X
	29		X		X		X
	30						X
July	1				X		X
	2				X		X
	3						
	4						X

* X - Eggs analyzed for dieldrin content.

Appendix B. Egg laying and treatment dates for hens in Treatment II (4 milligrams per week).

Date	Hen 1 Treatment	Hen 1 Egg	Hen 2 Treatment	Hen 2 Egg	Hen 3 Treatment	Hen 3 Egg
March 22	1		1			
23					1	
24						
25						
26						
27				X		
28				X*		
29	2		2			
30				X	2	
31						
April 1				X		
2				X		
3						
4				X		
5	3		3	X		
6					3	
7				X		
8				X		
9				X		X
10				X		
11				X		X

* X - Eggs analyzed for dieldrin content.

Appendix B. (Continued) Egg laying and treatment dates for hens in Treatment II (4 milligrams per week).

Date	Hen 1 Treatment	Hen 1 Egg	Hen 2 Treatment	Hen 2 Egg	Hen 3 Treatment	Hen 3 Egg
April 12	4		4			
13				X	4	X*
14				X		
15				X		X
16						X
17				X		X
18				X		
19	5		5	X		X
20					5	
21				X		
22						X
23				X		X
24						X
25				X		X
26	6		6			
27					6	X
28				X		X
29				X		X
30						
May 1				X		
2				X		X

* X - Eggs analyzed for dieldrin content.

Appendix B. (Continued) Egg laying and treatment dates for hens in Treatment II (4 milligrams per week).

Date		Hen 1 Treatment	Hen 1 Egg	Hen 2 Treatment	Hen 2 Egg	Hen 3 Treatment	Hen 3 Egg
May	3	7		7	X		X*
	4		X			7	X
	5				X		X
	6				X		X
	7		X				X
	8				X		
	9		X		X		
	10	8		8	X		
	11		X			8	X
	12				X		X
	13		X				X
	14		X		X		
	15				X		
	16		X				X
	17	9	X	9	X		X
	18		X		X	9	X
	19				X		X
	20		X		X		X
	21		X				X
	22				X		X
	23		X		X		X

* X - Eggs analyzed for dieldrin content.

Appendix B. (Continued) Egg laying and treatment dates for hens in Treatment II (4 milligrams per week).

Date		Hen 1 Treatment	Hen 1 Egg	Hen 2 Treatment	Hen 2 Egg	Hen 3 Treatment	Hen 3 Egg
May	24	10	X	10	X		
	25		X			10	X*
	26				X		
	27		X		X		X
	28		X				
	29		X		X		X
	30				X		X
	31	11	X	11	X		
June	1		X			11	X
	2		X		X		
	3				X		
	4		X		X		X
	5		X				X
	6		X		X		
	7	12	X	12	X		X
	8		X		X	12	
	9		X		X		
	10						
	11		X		X		
	12		X		X		X
	13		X				X

* X - Eggs analyzed for dieldrin content.

Appendix B. (Continued) Egg laying and treatment dates for hens in Treatment II (4 milligrams per week).

Date		Hen 1 Treatment	Hen 1 Egg	Hen 2 Treatment	Hen 2 Egg	Hen 3 Treatment	Hen 3 Egg
June	14	13	X	13	X		X
	15				X*	13	X
	16		X				
	17		X		X		X
	18		X		X		X
	19				X		X
	20		X				X
	21		X		X		X
	22				X		X
	23		X				
	24				X		X
	25		X				X
	26		X		X		
	27		X				X
	28		X		X		X
	29						X
	30				X		

* X - Eggs analyzed for dieldrin content.