Residues in Eggs, Preening Glands, Liver, and Muscle from Feeding Dieldrin-Contaminated Rice Bran to Hens and its Effect on Egg Production, Egg Hatch, and Chick Survival

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During the rice milling season in Louisiana, rice bran is utilized in large quantities by poultrymen as a constituent of rations for laying and breeding flocks (approximately 20% of total diet). Dieldrin residues of 0.03 ppm are commonly found in the rice bran as a result of treatment of rice seed prior to planting with aldrin for rice water weevil (Lissorhoptrus oryzophilus Kuschel) control (1).

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One objective of the research reported herein was to ascertain if detectable residues would accumulate in eggs of hens fed a ration containing rice bran contaminated with 0.03 ppm dieldrin. The second objective of the research was to determine the effects of high levels of dieldrin residues in eggs of hens on egg production, hatch and survival of chicks. It had been found previously that eggs of the gallinules Porphyrula martinica L. and Gallinula chloropus L. nesting in rice fields contained dieldrin residues averaging 6.51 and 9.37 ppm, respectively (2). In field studies, the hatch of eggs containing these high levels of dieldrin was not significantly different from that of eggs with no detectable dieldrin residue. However, no observations on gallinule chick survival were made. Since some insecticide residues in birds' eggs seem to affect chick survival more than egg production or hatch, it was desirable to study the effects of dieldrin residues on chick survival (3).

Methods and Materials

Sixty 8-month-old White Leghorn hens were divided into 5 groups of 12 birds each. All groups were maintained on a basal diet (4) for 2 weeks prior to beginning treatment. After 2 weeks, groups II, III, IV, and V were placed on diets containing respectively, 0.006, 0.2, 2 and 5 ppm dieldrin for 16 weeks. In the dieldrin-contaminated diets, rice bran fortified with dieldrin at the rates of 0.03, 1, 10, and 25 ppm was substituted for pulverized oats to give the desired residue levels. The forti-

fication procedure was essentially the same as that reported by Cummings et al. (5). Group I was fed the basal diet for the entire period of 16 weeks and served as a control. Egg samples were taken for residue determination every 2 weeks from each group. A sample consisted of 6 eggs which are frozen and stored for analysis.

Each group of hens was kept in a 10 ft. x 10 ft. floor-type pen with feed and water ad libitum. A Rhode Island Red rooster, 10 months old, was added to each pen at the start of the experiment and the roosters were rotated weekly. Egg production of each group was recorded daily. From 30-60 eggs were collected from each group at the end of the 1st, 2nd, and 3rd months of the experiment and were incubated in a Jamesway automatic incubator. As they hatched, the chicks were transferred to a battery-type brooder and supplied with feed (4) and water ad libitum.

Chicks were held for 14 days to check survival. Six 14-day-old chicks from each treatment were analyzed for dieldrin on a whole body basis at the end of the 3rd month observations.

At the end of the experiment, the preening (oil) glands, livers, and a sample of breast muscle were removed from 6 hens in each group and frozen for analysis.

Extraction and Cleanup

Eggs samples were analyzed using the methods of Cummings et al. (5) and direct saponification as described by Shell

Chemical Company (6). Two gram samples were used for the Cummings technique and the second sample fraction was further cleaned by saponifying according to Mills (7). Sample extracts from the Shell method were chromatographed through florisil in the conventional manner. The second eluting solvent in both instances contained 20% ethyl ether in order to improve recoveries of dieldrin from the particular lot of florisil used in this study.

Preening glands and tissue samples were extracted and cleaned up following the methods of Mills (7) and Johnson (8).

All p,p' -DDT and p,p' -DDE are reported as DDE since the parent compound is converted to DDE by saponification. Reported values are averages of 2 to 5 separate analyses.

A florisil recovery and reagent blank were run with each series of samples. Recovery standards averaged 83.4 with a range of 74-95%. All residues analyses were completed by electron-capture gas chromatography using Varian-Aerograph Model 680 and 682 instruments. The columns were packed with 5% Dow-11 on Gas Chrom Q, operated at 185° C. with nitrogen as the carrier gas. Standard solutions of dieldrin, p,p' -DDT, and p,p' -DDE were injected prior to the samples to determine the elution time characteristic of each pesticide and to check for day-to-day changes in the behavior of the gas chromatographs. It was desirable to know how much DDT and its metabolites were present because of the effect they have on the storage and metabolism of dieldrin (9). The peak heights resulting from

residues detected in each sample were adjusted by dilution until they closely approximated the peak height produced by injection of a known amount of standard. Calculations were made by direct linear comparison of similar sized peaks. The sensitivity level for the three insecticides was 0.01 ppm. Random samples were selected for qualitative verifications of residues by thin layer chromatography using the technique described by Kovacs (10).

Results and Discussions

The accumulation of dieldrin in eggs of hens fed dieldrin at 4 dietary levels over a 16 week period is presented in Table 1.

TABLE 1

Accumulation of dieldrin in eggs (in ppm) of hens fed dieldrin-contaminated rations[a/].

Contamination Level (ppm dieldrin)	2	4	6	8	10	12	14	16
Control	ND[b/]	ND[b/]	0.01	0.01	0.01	0.01	0.01	0.01
0.006	ND[b/]	0.01	0.02	0.01	0.02	0.02	0.02	0.02
0.2	0.03	0.08	0.10	0.12	0.14	0.59	0.16	0.36
2.0	0.34	0.74	1.45	1.22	1.41	1.36	1.32	1.41
5.0	1.21	2.73	3.54	3.48	3.96	4.80	4.60	4.20

[a/] Dieldrin not detected in pretreatment sample.

[b/] Level of detection was 0.01 ppm.

Dieldrin was not detected at 0.01 ppm in a pretreatment sample of eggs. After 2 weeks, detectable residues of dieldrin were found

in eggs of hens fed 0.2, 2, and 5 ppm dieldrin (0.03, 0.34, and 1.21 ppm, respectively). Dieldrin (0.01 ppm) was found in eggs of hens fed 0.006 ppm after 4 weeks and in eggs of hens fed the basal diet at the end of 6 weeks and throughout the remainder of the experiment.

Dieldrin accumulated rapidly in the eggs of hens fed 0.2, 2 and 5 ppm and gradually approached plateaus approximating the levels in the feed (Table 1). The plateaus were reached in 10-14 weeks and were more or less constant during the remainder of the experiment. Similar findings were recorded by Cummings et al. (5). At the 0.006 ppm level, dieldrin apparently was concentrated in eggs at a level approximately 3-fold that in the diet. Apparently some dieldrin was present in the basal diet since it was detected at the 0.01 ppm level in eggs and also at 0.09 ppm in preening glands of hens in the pretreatment sample. Another possible source of contamination was the sperm and accessory fluids from the roosters that were alternated between treatments. The amount of p,p' -DDE in the eggs ranged from 0.20 to 0.47 ppm during the course of the experiment. The contamination level or length of feeding of dieldrin did not appear to affect the amount of p,p' -DDE in the eggs.

Feeding hens dieldrin at levels up to 5 ppm had no effect on egg production or egg hatch (Table 2).

TABLE 2

Egg production and hatch from hens fed dieldrin-contaminated rations.

Contamination Level (ppm dieldrin)	Eggs/ Female/ Day	Egg Hatch					
		1st Month		2nd Month		3rd Month	
		Hatch/ Set	% Hatch	Hatch/ Set	% Hatch	Hatch/ Set	% Hatch
Control	0.80	45/52	87	28/30	93	28/30	93
0.006	0.74	43/51	84	28/30	93	21/30	70
0.2	0.74	43/52	83	26/30	87	24/30	80
2.0	0.79	54/60	90	28/30	93	27/30	90
5.0	0.80	51/60	85	27/30	90	29/30	97

The chicks were held for 14 days after hatch and no mortality was recorded at any time. Thus contamination of eggs with dieldrin up to 4.8 ppm (Table 1) had no effect on egg hatch or chick survival over a 14-day period. After 14 days, chicks that hatched from eggs of hens fed 0.2, 2, and 5 ppm dieldrin contained 0.06, 0.53, and 1.91 ppm dieldrin on a whole body basis. No residues were found in chicks from the control and 0.006 ppm dieldrin contamination levels. DeWitt (11) demonstrated that 10 ppm dietary dieldrin adversely affected reproduction of quail and pheasants. In the case of quail, hatchability was about one-half that of the controls whereas quail chick viability was less than one-half that of the controls.

Dieldrin was concentrated in the preening glands of hens fed 0.2, 2, and 5 ppm dieldrin at a level 8-12 times that in

the diet (Table 3). The concentration factor was much

TABLE 3

Residues of dieldrin in preening glands, liver and breast muscle of hens fed dieldrin-contaminated rations for 16 weeks.

Contamination level (ppm dieldrin)	ppm		
	Preening glands[a]	Liver	Breast muscle
Control	0.22	0.05	ND[b]
0.006	1.04	0.05	ND[b]
0.2	1.62	0.27	ND[b]
2.0	16.98	2.20	0.05
5.0	60.88	4.56	0.28

[a] Dieldrin present at 0.09 ppm in pretreatment sample

[b] Level of detection was 0.01 ppm

higher (173-fold) in hens fed at the 0.006 ppm level. Dieldrin was found also in a pretreatment sample (0.09 ppm) and in the control sample (0.22 ppm). The amount of p,p' -DDE (ppm) in the preening glands were as follows: control, 1.18; 0.006 level, 2.02; 0.2 level, 1.06; 2.0 level, 1.00; and 5.0 level, 1.07.

Feeding hens 2 and 5 ppm dieldrin for 16 weeks resulted in contamination of the breast muscle at 0.05 and 0.28 ppm, respectively (Table 3). The storage ratios (ppm in tissue/ppm in diet) for these 2 levels were similar and averaged approximately 0.04. Dieldrin was not detected in the breast muscle of hens fed 0.006 ppm, 0.2 ppm or in the control hens.

Dieldrin was found in the liver tissue of hens from all treatments including the control (Table 3). The amounts found corresponded to the dietary level at the 3 highest levels of fortification.

Based on the results of this study, rice bran contaminated with dieldrin at detectable levels should not be fed to laying hens since it will accumulate in the eggs at a level equal to or higher than the dietary level.

If the data obtained in this study on the hatchability of eggs contaminated with approximately 5 ppm dieldrin and the survival of chicks hatching from these eggs is pertinent to the contamination of eggs of wild birds such as purple and common gallinules, then there would appear to be little or no hazard to wild gallinaceous birds contaminated at similar levels.

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