Distribution of Dieldrin-^{14}C in Pheasant Tissues after a Single Administration

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The distribution and excretion of dieldrin-^{14}C in 18 laying hen pheasants were studied by administration of a single gelatin capsule containing 1.4 mg. of dieldrin labeled with 5.5 microcuries. Dieldrin residues were detected using electron capture gas chromatography and liquid scintillation counting in tissues 2 hours after treatment. Highest average level of residues occurred in all tissues after 6 hours with fat having 6.45 p.p.m.; liver 1.56 p.p.m.; and brain, heart, and muscle less than 0.3 p.p.m. Three birds appeared to absorb 86, 89, and 97% of the 1.4 mg. of administered dieldrin. Birds killed 5 weeks after treatment excreted 26.5, 21.5, and 18.2% of the dieldrin in their feces; 11.5, 14.4, and 13.0% in their eggs; and 34.6, 21.2, and 32.7% remained in the body. Indirect evidence indicated water-soluble metabolites in the feces but not in egg yolks, while no evidence of ether-soluble metabolites was found in eggs or tissues.

Dieldrin and its residues are commonly found in the tissues of wild birds but only limited data are available on its distribution, excretion, and metabolism in birds. Robinson *et al.* (1967) reported on the pharmacodynamics of pigeons fed a diet containing 50 ppm of dieldrin for six months, then fed a normal diet and killed at intervals. Jefferies and Davis (1968) reported the passage of dieldrin from soil to earthworms to song thrushes and studied the pharmacodynamics of dieldrin in five song thrushes. The present experiment was conducted to study the metabolic fate of dieldrin-^{14}C fed to laying hen pheasants. The objectives were to determine: approximate time required for dieldrin-^{14}C to pass from the digestive tract to tissues, rate of deposition of dieldrin-^{14}C residues in brain, fat, heart, liver, muscle, and ovary, and rate of excretion of dieldrin-^{14}C residues by feces and eggs.

MATERIALS AND METHODS

Eighteen adult hen pheasants were placed in individual cages and induced to lay eggs by regulation of the photoperiod. The birds were randomly assigned to six groups of three birds per group, and a gelatin capsule containing 1.4 mg. of dieldrin labeled with 5.5 microcuries of carbon-14 was administered via stomach tube to each hen. In addition three control birds were given capsules containing no dieldrin and sacrificed one week later. The birds were fed a commercial pheasant breeder ration (Zip Feed Mills, Sioux Falls, S. D.) throughout the experiment. Electron capture gas chromatography (ECGC) analysis showed <0.01 ppm of dieldrin present in the ration. All birds appeared to have normal appetites and health throughout the experiment. Eggs and feces from each bird were collected daily until the birds were sacrificed as follows: Group 1, two hours; Group 2, six hours; Group 3, 12 hours; Group 4, three days; Group 5, six days, and Group 6, five weeks. Eggs, feces, and samples of the brain, fat, heart, liver, muscle, ovary, and whole body were stored in a deep freezer (−20° C.) for later analysis. Fat samples were removed from the lower abdominal region while muscle samples were dissected from the breast.

Samples were extracted and purified for dieldrin-^{14}C by the Florisil column cleanup method (Stemp *et al.*, 1964). Using samples from the same experimental birds, Greichus *et al.* (1968) reported a 95% procedure efficiency for extracting dieldrin-^{14}C with a Florisil column from pheasant tissues, eggs, and feces. Following extraction, samples were divided into two equal parts by volume. The first half was saved for electron capture gas chromatographic analysis to determine amounts of dieldrin present, and the second half was saved for liquid scintillation counting (^{14}C analysis).

The instrument used for ECGC analysis was a Wilkens Aerograph HY-FI model 600-D equipped with a model S-R 1-mv. Sargent recorder and an electron capture detector cell with a 250-millicurie tritium source. Injector and detector temperatures were 210° C. and 200° C., respectively. For better identification of dieldrin, all samples were run on two separate columns. A $^1/_8$-inch o.d. × 5-foot borosilicate glass column packed with 5% Dow 11 Silicone on 60/80 mesh (HMDS) treated chromosorb W was operated isothermally at 185° C. with a 45-ml.-per-minute nitrogen carrier gas flow rate. The relative retention time of dieldrin to aldrin was 1.95 and the peak emerged in 6.5 minutes. A $^1/_8$-inch o.d. × 10-foot borosilicate glass column packed with 2% QF-1 Silicone (Fluoro) on 60/80 mesh (HMDS) treated chromosorb W was operated isothermally at 150° C. with a 40-ml.-per-minute nitrogen carrier gas flow rate. The relative retention time of dieldrin to aldrin was 3.25 and the peak emerged in 7.5 minutes.

The area of the dieldrin peak on the chromotograms was calculated by triangulation for each tissue sample. Then the average area of the dieldrin peak from each tissue of the control birds was subtracted before calculation of the amount of dieldrin. The instrument used for liquid scintillation analysis was a Packard Tri-Carb Series 3000, Liquid Scintillation Spectrometer.

To determine if ^{14}C activity remained with dieldrin or metabolites soluble in fat solvents, some samples were subjected to thin-layer chromatography (TLC) and counted for ^{14}C activity after the ECGC analysis. The thin-layer technique described by Breidenbach *et al.* (1964) was used.

Egg yolks were extracted for total lipids (Folch *et al.*, 1956) and counted for ^{14}C activity. To find if dieldrin was associated with any particular lipid class, lipids were separated by thin-layer chromatography (Mangold, 1961). Sections of the TLC plates containing the individual lipid classes were removed and counted.

Samples of feces were examined for water-soluble metabolites. One gram of feces was placed in 100 ml. of 1 to 1 (v. per v.) hexane and water and homogenized in a Sorvall mixer at

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low speed for 3 minutes. The hexane phase was washed with two additional 50-ml. portions of water which were added to the original water phase. The hexane phase was filtered, evaporated to 1 ml. and thin-layered (Breidenbach *et al.*, 1964). The water phase was filtered, evaporated to dryness, reconstituted in 1 ml. of methanol and thin-layered. Sections of each plate were removed and counted to determine if activity could be found in sections other than the dieldrin section.

Dieldrin-^{14}C with a specific activity of 2.5 millicuries per millimole was purchased from Nuclear Research Chemicals, Inc., Orlando, Fla. Nonradioactive dieldrin was obtained from Shell Chemical Company, Princeton, N. J. Thin-layer chromatography showed more than 95% of the activity of the dieldrin-^{14}C in the dieldrin band. Examination of the radioactive and nonradioactive dieldrin by ECGC revealed no extraneous peaks.

Florisil, 60/100 mesh, activated at 650° 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.

Hexane, petroleum ether (boiling range 30° to 60° C.), dichloromethane, and chloroform were Nanograde (Mallinckrodt Chemical Works). The maximum interfering gas chromatography peaks were no greater than that produced by 10 ng. per liter of heptachlor epoxide or 100 ng. per liter of parathion.

Carbon tetrachloride, ethyl ether, and methanol were distilled in glass (Burdick and Jackson Laboratories, Inc.), and had purity equal to Nanograde.

The apparatus for TLC was purchased from Desaga/Brinkmann including the 20- × 20-cm. glass plates precoated with 0.25 mm. silica gel without a fluorescent indicator.

The scintillation fluid consisted of 100 mg. of 1,4-bis-[2-(5-phenyloxazole)]-benzene, (POPOP) and 3 grams of 2,5-diphenyloxazole (PPO) in 1 liter of toluene. The POPOP and PPO were scintillation grade from Packard Instrument Company, Inc.

RESULTS AND DISCUSSION

ECGC analysis for dieldrin in the feces of three birds in Group 6 showed that 14, 11, and 3% of the administered dieldrin was excreted in the first 24 hours. Henry *et al.* (1933) using X-ray shadows reported the complete disappearance of feed from the digestive tract of hens within 16 to 25 hours. Therefore, assuming the administered dieldrin had sufficient time to travel completely through the digestive tract and that no losses other than those in the feces occurred, it appeared that approximately 86, 89, and 97% of 1.4 mg. of dieldrin was absorbed. Dieldrin which may have been absorbed, converted to metabolites, and eliminated in the feces would not be accounted for by ECGC analysis of dieldrin but would by ^{14}C analysis. Dieldrin residues found in the first 24 hours of feces after Florisil column extraction were 224, 187, and 49 μg. by ^{14}C analysis. Dieldrin found in the same samples using ECGC analysis was 202, 157, and 43 μg. These results suggested the possibility of residues in the form of metabolites.

Past research has demonstrated metabolites of dieldrin in the urine and feces of mammals. Korte and Arent (1965) isolated six metabolites from urine of rabbits administered dieldrin-^{14}C by means of a stomach tube. One metabolite accounted for 86% of the total metabolized dieldrin. It was identified as 6,7-transdihydroxy-dihydro-aldrin. Datta *et al.* (1965) reported the presence of two metabolites in the urine of rats fed dieldrin in their diets. These metabolites were usually distinguished from dieldrin by their increased hydrophilic properties.

Table I. Dieldrin Residues Excreted in Feces for 5 Weeks Following a Single 1.4-mg. Treatment

^{14}C and ECGC Analysis

	Bird 1	Bird 2	Bird 3
Total dieldrin administered (g.)	1400	1400	1400
Wt. of dieldrin (g.) in feces	278	221	143
% of administered dieldrin	20	16	10
Wt. of dieldrin metabolites (g.) in feces	93	79	112
% of administered dieldrin	7	6	8
Wt. of total residues (g.) in feces	371	300	255
% of administered dieldrin	27	22	18

In the present study, a hexane and water partitioning technique was employed to separate dieldrin-^{14}C from its hydrophilic metabolites. Feces collected from three birds in Group 6 after the first and third days were used. The ^{14}C activity found in the water phase from feces collected one day after dieldrin-^{14}C administration had 6, 6, and 21% (average 11%) of the total activity while feces from the third day's collection had 46, 48, and 63% (average 53%). The percentage differences of activity found on the two days can be explained by unabsorbed dieldrin-^{14}C in the first day's collection. Of the total activity found in the hexane phase of the one-day collection, 97, 98, and 95% had the same TLC R_f value as dieldrin. Of the total activity in the water phase, 89, 94, and 99% had different R_f values than dieldrin when thin-layered. Metabolites appeared to be almost entirely in the water phase, while most of the unchanged dieldrin appeared to be in the hexane phase. Therefore, the metabolites of pheasant excreta appeared mainly as hydrophilic compounds. ECGC, TLC, and ^{14}C analyses of the hexane and water phases of partitioned feces made it possible to calculate approximate amounts of dieldrin and metabolites excreted in the feces. Metabolites in the first 24-hour collection accounted for about 11% of the activity in the feces while about 53% of the activity was due to water-soluble metabolites in the third day's collection. By knowing the total amount of feces excreted in five weeks, the amount of ^{14}C activity in the feces and the approximate amount of ^{14}C activity due to dieldrin metabolites, it was possible to calculate that 371, 300, and 255 μg. of dieldrin residues were excreted (Table I).

Samples of whole eggs (excluding shells) laid by hens in Group 6 were analyzed. Highest level of dieldrin residue found was 0.81 ppm. A rise in residue content occurred in eggs laid between the second day and the fifth day after treatment on May 5 (Figure 1). From May 20 to June 7, the level of residues generally declined with eggs from all hens ranging from 0.47 to 0.13 ppm.

Ware and Naber (1961) stated that 51 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 eggs and tissues of laying chickens contained residues of dieldrin 26 weeks after exposure to low levels. In this study after 35 days the residues present were 0.13, 0.20, and 0.15 ppm after birds 1, 2, and 3 of Group 6 had laid 30, 27, and 28 eggs.

Number of eggs laid was important in determining the amount of residues eliminated *via* the egg. The only sharp change in egg residue levels occurred with bird 3 between May 7 and 11 (Figure 1). Egg laying pattern appeared to

Table II. Average Dieldrin Residues (p.p.m.) in Tissues of Birds after 1.4-Mg. Treatment ^{14}C and ECGC Analysis

Time	Fat		Brain		Heart		Muscle		Liver	
	^{14}C	ECGC	^{14}C	ECGC	^{14}C	ECGC	^{14}C	ECGC	^{14}C	ECGC
2 hours	0.14	0.15	0.03	0.14	0.04	0.06	0.01	0.01	0.85	0.62
6 hours	6.45	6.05	0.09	0.11	0.28	0.27	0.06	0.07	1.56	1.58
12 hours	5.51	5.80	0.04	0.05	0.11	0.12	0.05	0.05	0.85	0.73
3 days	3.96	4.24	0.04	0.06	0.08	0.14	0.04	0.07	0.65	0.81
6 days	3.34	3.81	0.05	0.07	0.12	0.15	0.02	0.08	0.50	0.68
5 weeks	1.72	1.99	0.02	0.04	0.04	0.12	0.01	0.02	0.33	0.36

have no effect on changes in egg residue levels. Lamb *et al.* (1967) found that, if birds did not lay for a number of days, the next egg did not show an extreme increase or decrease in residue content.

Average weights of eggs, laying records, and analysis results were used to calculate the total micrograms of dieldrin residues deposited in eggs of each bird of Group 6 during five weeks. The average weight of eggs was 26.8, 25.8, and 22.3 grams for birds 1, 2, and 3. Similar results of ECGC and ^{14}C analyses indicated the presence of no appreciable amount of metabolites in the eggs. Thin-layer chromatography showed at least 95% of the ^{14}C activity in the same section as the dieldrin standard. The total amount of ^{14}C-active residues present in eggs laid during the five-week period was 162, 201, and 183 μg. for birds, 1, 2, and 3 in Group 6. These amounts accounted for 11.5, 14.4, and 13.0% of the administered dieldrin-^{14}C.

When total lipids were extracted from egg yolks and separated in lipid classes by thin-layer chromatography, the ^{14}C activity was found in the same section as standard dieldrin. This indicated that dieldrin probably was not associated with any particular lipid.

ECGC and ^{14}C analyses agreed closely on parts per million in all tissues, suggesting that no appreciable amount of metabolites was lost on the Florisil column (Table II). Agreement of extraction efficiencies obtained by dieldrin fortified tissues analyzed by ECGC and efficiencies obtained by comparison of liquid scintillation counting before and after Florisil column chromatography also led to the same conclusion. Thin-layered samples showed that the section containing at least 95% of the ^{14}C activity was in the same section as standard dieldrin.

Dieldrin residues were found in tissues from one bird in Group 1, two hours after capsule administration. The liver contained 1.72 ppm by ^{14}C analyses and 2.51 ppm by ECGC analysis, while other tissues had levels below 0.45 ppm. The analysis results of the other two birds in Group 1 showed no evidence that ^{14}C was incorporated in their tissues two hours after administration. Low levels were found in the liver by ECGC analysis.

Backstrom *et al.* (1965) injected dieldrin-^{14}C intramuscularly into mice and found the greatest concentration of dieldrin in the fat deposits within 24 hours. They also found high concentrations in liver, intestine, bone marrow, gall bladder, and mammary glands. Robinson *et al.* (1967) reported storage ratios of dieldrin in tissues of pigeons fed a diet containing 50 ppm of dieldrin for six months. Ratios of dieldrin in tissue to dieldrin in diet, were 9.0, 0.65, 0.33, and 0.15 for fat, liver, muscle, and brain.

In the present study, concentrations of dieldrin-^{14}C residues were highest in fatty tissue. Liver contained residues lower than those in the fat with heart levels much lower than liver. Brain and muscle levels generally remained below those of fat, liver, and heart (Table II). The pattern of deposition and excretion was similar for all tissues. Highest average levels were present six hours after capsule administration, then all tissues showed a slow decline throughout the five-week period. The pattern was more evident in fat, liver, and heart.

Samples of whole body with feathers from birds in Group 6

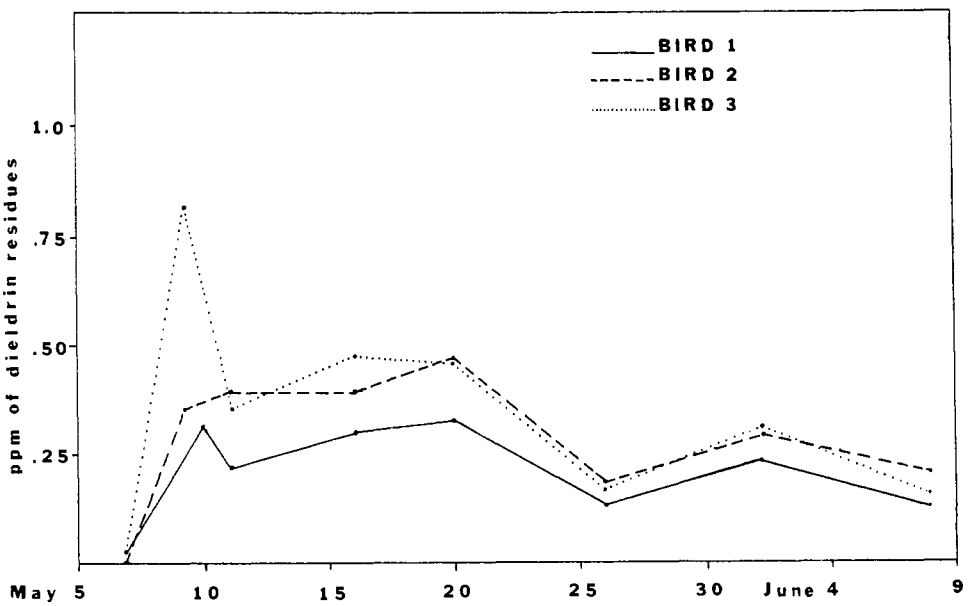

Figure 1. Dieldrin residues in eggs laid during 35 days following a single 1.4-mg. treatment

contained 0.37, 0.33, and 0.40 ppm of dieldrin residues. The birds weighed 1303, 897, and 1145 grams which accounted for 484, 296, and 458 μg. of dieldrin. Thus, these birds contained 34.6, 21.2, and 32.7% of the 1.4-mg. dieldrin-^{14}C treatment after five weeks of dieldrin residue elimination *via* eggs and feces. Therefore, the recovery of dieldrin-^{14}C was 72.6, 57.1, and 63.9%. Some losses may be accounted for in the unanalyzed egg shells, by dieldrin vaporization from the feathers and adherence to soil particles during dusting.

Robinson *et al.* (1967) suggested that a dynamic equilibrium existed between concentrations of dieldrin in tissues of pigeons. The equilibrium supposedly was due to circulation of dieldrin in the blood. In the present study there was a uniform decline of tissue residue concentration as residues were eliminated in eggs and feces.

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