BIOACCUMULATION OF ENDRIN FROM NATURAL FOOD SOURCES IN THE EASTERN BOBWHITE QUAIL, *Colinus virginianus virginianus L.**

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SYNOPSIS

The study was undertaken to determine the fate of endrin in a food chain situation involving the soybean plant, *Glycine Max* L. (Leguminosae), the Mexican bean beetle, *Epilachna varivestis* Muls. (Coccinellidae), and the eastern bobwhite quail, *Colinus virginianus virginianus* L. (Perdicidae). Beetles contaminated with endrin were force-fed to birds at 1 mg/kg/bird, in both acute (4 hr) and chronic (5 day) exposures. Contaminated beans were force-fed to birds at 0.015 mg/kg/bird, in similar acute and chronic rates. Endrin concentrated primarily in the fat, liver, and gonadal tissues of the birds. Analyses of whole birds revealed retention of approximately 16% of the total acute dose administered, and 21% retention of the total chronic dose. At sensitivities used for analyses, no metabolites were detected in any component of the food chain.

The eastern bobwhite quail, *Colinus virginianus virginianus* L., long has been recognized as the most important game bird in South Carolina. During recent years concern has developed among conservationists regarding exposure of this and other birds to pesticide residues in the environment. This concern has been shared by many scientists (Bernard, 1963; DeWitt, 1956; DeWitt, Stickel and Springer, 1963; Eden, 1951; George, 1963; James and Davis, 1965; Moore, 1964; Rudd and Genelly, 1956; Rudd, 1964; Sherman and Rosenberg, 1954; and Stickel and Stickel, 1964).

Although endrin usage on soybeans, *Glycine Max* L., has never been recommended in South Carolina, it has been used for pest management in the past. Recently, insecticides of the chlorinated hydrocarbon type have been shown to be absorbed from soils into various plants (Marth, 1965). This is noteworthy since endrin has been used on cotton in South Carolina and could enter the soybean plant via soil accumulations.

During the last decade much data has been accumulated, with the aid of refinements in analytical techniques, to show that most chlorinated hydrocarbon insecticides pentrate plant tissues and are translocated at least to a limited extent (Beck *et al.*, 1962; Bruce, Lind and Decker, 1965; Lichtenstein and Schulz, 1960; Maier-Bode, 1965; and Popham and Hale, 1958). Endrin residues were found in about three fourths of the soybean samples from Arkansas and Mississippi (Pesticides Monitoring Journal, 1968).

Mexican bean beetles, *Epilachna varivestis* Muls., both in the larval and adult stages, feed readily on most varieties of the soybean plant (Pallister, 1949). Davison (1958) reported that plants supplied about 85% of the bobwhite's food, the other 15% being animal foods. Martin (1935) found that soybeans ranked ninth out of 46 food items in preference by bobwhite quail.

* Supported by funds provided by NIH Training Grant No. 1TL ES60 from the Division of Environmental Health Services.

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Thus, the study was conceived to investigate bioaccumulation of endrin in a natural food chain, involving the soybean plant, the Mexican bean beetle, and the eastern bobwhite quail.

Specific attention was given to possible tissue specificity as reflected by the analysis of 7 major tissues from the quail, and to the per cent retention of the endrin as reflected by total body burden in the birds at the end of the test periods.

MATERIALS AND METHODS

All biological materials and feed were screened for endrin content prior to inclusion in the subsequent tests. No endrin was detected at a level of sensitivity which would allow detection of endrin in the range of 1 ppb. The lack of even trace amounts of the test compound in samples of feed and in the birds prior to treatment removed one source of error from the final results. The fact that no endrin was found in any tissue from the untreated control pens indicated no source of contamination at any time.

This study was designed to investigate the accumulation of endrin from a natural food source by tissues of the eastern bobwhite quail, maintained under laboratory conditions. The food chains studied involved endrin transferal to quail either by exposure to contaminated beans directly, or by feeding upon contaminated beetles (Figure 1).

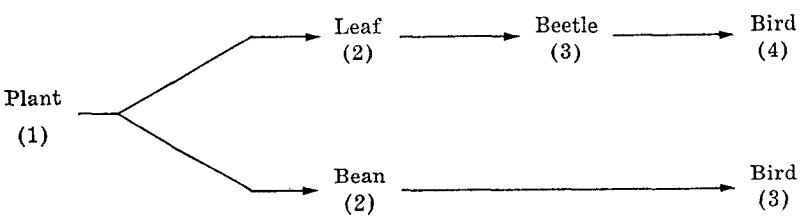

FIGURE 1. Routes of endrin transferal studies

Each of ten soybean plants, of approximately the same size, were sprayed uniformly with 1 ml of a 0.3% endrin solution. After 24 hours, the plants were made available to adult Mexican bean beetles. After feeding for 6 hours beetles were collected and analyzed for endrin content. The results indicated considerable variance so a mean value was used. In order that quantification of the data could be facilitated the beetles were dosed topically with 5 μg of endrin per beetle, using a microapplicator. An interim of 2-4 hours followed before beetles were force-fed to the quail.

Accumulation of endrin by beans in the field involved utilization of a 0.25 A control plot and a test plot of the same area. Four endrin spray applications (Aug. 5 & 9; Sept. 3 & 19) were programmed for the test plot at a level of 0.5 lb ai/A (1 kg/1.78 hectares). Beans from the control and test plots were harvested on Dec. 11, 1968 and residue determinations made. Based on the average weight of a soybean (0.140 $\pm$ 0.002 g) a total intake of 0.015 mg/kg of endrin for each bird resulted when a total of 200 contaminated beans were administered. This represented a much lower intake of endrin, as compared to that available when fed contaminated beetles.

Both beans and beetles were force-fed to the test animals, rather than being offered *ad libitum*. This was done so that uniformity of intake by all the birds was assured. The procedure by which the quail were force-fed could be performed by one person.

Forty bobwhite quail were separated into two groups on the basis of weight, and were used in the acute and "chronic" endrin exposures where beetles were utilized as the contaminated food source. Twenty birds, in the weight range of 180-197 g (X = 190 g), were randomly

separated into four cages (five birds per cage) and utilized in the acute dosage phase. The remaining twenty quail, with a weight range of 199-222 g (X = 210 g), were randomly placed into four cages and used in the chronic dosage test. Each test involved three treatment cages and one control cage.

The acute dosage test involved the administering of a single endrin dose to 15 quail in a 4 hour period. This was accomplished by force-feeding the quail with the number of beetles necessary to provide each bobwhite quail with 190 μg of endrin. This amount closely approximated the LD_{50} value (1 mg/kg) reported in the literature (DeWitt, stickel, and Springer, 1963). These birds were sacrificed 48 hours after the endrin was initially administered. Seven tissue samples were analyzed from four quail from each of the four cages. A fifth bird from each cage was analyzed *in toto* to determine total body burden of endrin.

In the chronic dosage test, endrin was administered in equal doses over a 5-day period, with the total consumed by each bird being 210 μg. The birds were force-fed beetles and received 42 μg of endrin per day over this 5-day period. After a waiting period of 48 hours following the final dose, the birds were sacrificed. Analyses were performed as described in the acute exposure test. The acute and chronic dosage tests, in which beetles were utilized, involved the analysis of 232 samples.

The acute and chronic dosage tests, in which soybeans were used, were carried out in a manner similar to the tests in which beetles were employed. The only change in procedure was that the quail were not separated into groups on the basis of weight. This was not deemed necessary, since the endrin dose administered via soybean consumption, had already been determined to be very low (0.015 mg/kg).

The instrument utilized for residue analyses was a Micro-Tek Model 2000MF gas chromatograph equipped with a ^{63}Ni electron capture detector. A 5 ft x ¼ inch o.d. (1.52 m x 0.64 cm) glass column packed with 3% OV-1 on Chromosorb W (60-80 mesh), AW-DMCS, was used for separations. Identifications were cross-checked with a 1.5% OV-17/1.95% QF-1 on Chromosorb W (60-80 mesh), AW-DMCS, column.

Operating parameters of the instrument were:

Injection Port Temperature: 195 C
Column Temperature: 185 C
Detector Temperature: 275 C
Carrier Gas: high purity Nitrogen Carrier Flow: 95 cc/min (STP)
Detector Operating Conditions:
DC mode
38 volts output
60 sec pulse rate of 6μ sec width
Electrometer:
Input Attenuator: 10
Output Attenuator: 32

Injection of microliter quantities was made with a Hamilton 10 μl syringe. Tests with endrin standards were run under these conditions, employing these materials, and reproducible results were obtained. Levels of endrin less than twice its standard error were not reported as a residue, but were indicated as a trace. Recovery tests were also run for endrin in bean and bird tissues, in order to ascertain the relation between the actual endrin content and the percent recovery by the techniques which were employed. The recovery efficiency of the analyses was 72% for bean tissue and 83% for bird tissue.

RESULTS AND DISCUSSION

Administration of Contaminated Beetles to Quail

The results obtained by administration of contaminated beetles to the quail (1 mg/kg of endrin per bird) in an acute dosage test are presented in Table I. Three of the tissues showed rather high concentrations of endrin residue. These tissues were fat (0.682 $\pm$ 0.059 ppm),

TABLE 1. Endrin distribution in the bobwhite quail following acute exposure[1] from feeding contaminated Mexican bean beetles at the rate of 1 mg/kg total endrin for each bird.

Tissue or organ analyzed [2]	Control [3] Avg. wet wt.	SE [5]	Treated Avg. wet wt.	SE	Endrin Content (ppm) Mean	SE
	g		g			
Brain	1.031	± 0.008	1.017	± 0.013	0.021	± 0.004
Fat	1.165	± 0.041	1.115	± 0.086	0.682	± 0.059
Kidney	1.044	± 0.012	1.026	± 0.022	0.017	± 0.001
Liver	6.985	± 0.559	6.539	± 0.275	0.145	± 0.008
Gonads	0.082	± 0.012	0.165	± 0.077	0.113	± 0.009
Breast muscle	4.588	± 0.125	4.690	± 0.241	0.005	± 0.000
Heart	1.058	± 0.020	1.103	± 0.061	trace	
Whole bird [4]	191		191	± 1.374	0.166	± 0.002

1 Total dose given in a single feeding, then birds sacrificed two days later.
2 Based on tissues from 12 birds.
3 Control birds contained no detectable endrin.
4 Based on three birds only.
5 SE = Standard error.

liver (0.145 ± 0.008 ppm), and gonadal tissue (0.113 ± 0.009 ppm). Brain, kidney, and breast muscle tissues contained smaller amounts of endrin, while heart tissue ranked the lowest. Analysis of whole birds revealed that 16.6% of the administered dose, or 0.168 ± 0.002 ppm, had been retained. The level of endrin in the liver closely reflected the whole body burden level for the test animals.

Feeding of contaminated beetles to quail (1 mg/kg of endrin per bird) in the chronic dosage phase yielded the results presented in Table II. Again, the same three tissues accumulated fairly high concentrations of endrin. These were fat tissue (0.421 ± 0.008 ppm), gonadal tissue (0.245 ± 0.024 ppm), and liver tissue (0.201 ± 0.004 ppm). Kidney, brain, breast muscle, and heart tissue contained lesser amounts of endrin. Whole bird analysis indicated that 22% of the administered dose, or 0.222 ± 0.006 ppm, had been retained. Again, the liver tissue most closely reflected the whole body burden for the birds.

A tissue by tissue comparison of the concentration of endrin in birds in both the acute and chronic tests, utilizing contaminated beetles, is

TABLE 2. Endrin distribution in the bobwhite quail following chronic exposure[1] from feeding contaminated Mexican bean beetles at the rate of 1 mg/kg total endrin for each bird.

Tissue or organ analyzed [2]	Control [3] Avg. wet wt.	SE [5]	Treated Avg. wet wt.	SE	Endrin Content (ppm) Mean	SE
	g		g			
Brain	1.107	± 0.016	1.117	± 0.022	0.022	± 0.003
Fat	1.912	± 0.127	2.032	± 0.035	0.421	± 0.008
Kidney	1.091	± 0.012	1.083	± 0.017	0.023	± 0.003
Liver	4.738	± 0.081	4.937	± 0.218	0.201	± 0.004
Gonads	0.078	± 0.000	0.171	± 0.068	0.245	± 0.024
Breast muscle	4.680	± 0.106	4.178	± 0.111	0.005	± 0.000
Heart	1.061	± 0.008	1.197	± 0.048	0.001	± 0.000
Whole bird [4]	214		210	± 3.014	0.222	± 0.006

1 Total dose subdivided into five daily doses, then birds sacrificed two days after final dose.
2 Based on tissues from 12 birds.
3 Control birds contained no detectable endrin.
4 Based on three birds only.
5 SE = Standard error.

TABLE 3. A comparison of endrin distribution in the bobwhite quail following acute and chronic exposure from feeding contaminated Mexican bean beetles at the rate of 1 mg/kg total endrin for each bird. Endrin content in tissues (ppm)

Tissue or organ analyzed [1]	Acute exposure [2] Mean	SE [5]	Chronic exposure [3] Mean	SE	Value of t	Level of significance
Brain	0.021	± 0.004	0.022	± 0.003	0.29	NS
Fat	0.682	± 0.059	0.421	± 0.008	6.21	0.01
Kidney	0.017	± 0.001	0.023	± 0.003	2.68	0.05
Liver	0.145	± 0.008	0.201	± 0.004	8.86	0.01
Gonads	0.113	± 0.009	0.245	± 0.024	7.25	0.01
Breast muscle	0.005	± 0.000	0.005	± 0.000	. . .	. . .
Heart	trace	. . . .	0.001	± 0.000	. . .	. . .
Whole bird [4]	0.166	± 0.002	0.222	± 0.006	12.53	0.01

1 Average of 12 samples.
2 Total dose given in a single feeding, then birds sacrificed two days later.
3 Total dose sub-divided into five daily doses, then birds sacrificed two days after final dose.
4 Based on three birds only.
5 SE = Standard error.

shown in Table III. The most marked difference was the amount of endrin residue in the fat tissue of the birds. The average content for the fat tissue in the acute dosage test was 0.682 ± 0.059 ppm, whereas, the endrin content of the fat tissue in quail subjected to chronic exposure was 0.421 ± 0.008 ppm. Another obvious difference was among the endrin levels in the liver and gonadal tissues from the two tests. Both the gonadal tissue (0.245 ± 0.024 ppm) and liver tissue (0.201 ± 0.004 ppm) from birds subjected to chronic exposure had considerably higher titers of endrin than did the corresponding tissues in the acute phase. The remainder of the tissues in both exposures varied little in comparative endrin content. The whole bird comparison indicated that birds subjected to chronic dosages accumulated a significantly greater percentage (22%) of the administered dose, than those of the acute dosage exposure which accumulated (17%) of the total dose.

ADMINISTRATION OF CONTAMINATED BEANS TO QUAIL

Results obtained when beans were fed to quail in an acute exposure test are shown in Table IV. These results were in general agreement

TABLE 4. Endrin distribution in the bobwhite quail following acute exposure [1] from feeding contaminated beans from the soybean plant at the rate of 0.015 mg/kg total endrin for each bird.

Tissue or organ analyzed [2]	Control [3] Avg. wet wt.	SE [5]	Treated Avg. wet wt.	SE	Endrin Content (ppm) Mean	SE
	g		g			
Brain	1.033	± 0.047	1.002	± 0.011	ND [6]	. . . .
Fat	1.151	± 0.101	1.096	± 0.094	0.014	± 0.002
Kidney	1.037	± 0.015	1.019	± 0.018	ND	. . . .
Liver	5.973	± 0.146	6.133	± 0.189	0.004	± 0.000
Gonads	0.127	± 0.034	0.159	± 0.020	trace	. . . .
Breast muscle	5.111	± 0.206	4.987	± 0.255	ND	. . . .
Heart	1.072	± 0.010	1.113	± 0.051	ND	. . . .
Whole bird [4]	194	. . . .	189	± 1.531	0.002	± 0.000

1 Total dose given in a single feeding, then birds sacrificed two days later.
2 Based on tissues from 12 birds.
3 Control birds contained no detectable endrin.
4 Based on three birds only.
5 SE = Standard error.
6 ND = Not detectable.

TABLE 5. Endrin distribution in the bobwhite quail following acute exposure[1] from feeding contaminated beans from the soybean plant at the rate of 0.015 mg/kg total endrin for each bird.

Tissue or organ analyzed[2]	Control[3] Avg. wet wt.	SE[5]	Treated Avg. wet wt.	SE	Endrin Content (ppm) Mean	SE
	g		g			
Brain	1.002	± 0.006	1.010	± 0.008	ND[6]	....
Fat	1.223	± 0.101	1.114	± 0.087	0.010	± 0.001
Kidney	0.987	± 0.033	1.075	± 0.025	ND	....
Liver	6.137	± 0.273	6.824	± 0.201	0.007	± 0.000
Gonads	0.091	± 0.008	0.097	± 0.009	trace	....
Breast muscle	4.676	± 0.203	5.021	± 0.326	ND	....
Heart	1.059	± 0.052	1.161	± 0.062	ND	....
Whole bird[4]	185	....	193	± 1.803	0.003	± 0.000

1 Total dose subdivided into five daily doses, then birds sacrificed two days after final dose.
2 Based on tissues from 12 birds.
3 Control birds contained no detectable endrin.
4 Based on three birds only.
5 SE = Standard error.
6 ND = Not detectable.

with those of the acute exposures of the beetle-fed birds. However, there was much less endrin available to the birds via bean intake. Three of the tissues showed the presence of endrin, while in the remainder of the tissues endrin was not detectable. The tissues which contained endrin in detectable amounts were fat (0.014 ± 0.002 ppm), liver 0.004 ± 0.000 ppm), and gonadal tissue which only contained trace quantities. Analyses of the whole birds revealed that 13.3% or 0.002 ± 0.000 ppm, had been retained. Again, liver tissue was most nearly indicative of total body burden.

Results of feeding contaminated beans to quail in a chronic fashion are shown in Table V. Similarly, the same three tissues contained detectable endrin residues while endrin was not detectable in the remainder of the tissues. Fat tissue contained 0.010 ± 0.001 ppm endrin, liver tissue 0.007 ± 0.000 ppm, and gonadal tissue contained trace quantities. Whole bird analyses revealed that 20% or 0.003 ± 0.000 ppm, had been retained.

A tissue by tissue comparison profile of the concentration of endrin in birds involved in the acute and chronic feeding tests, employing contaminated beans, is presented in Table VI. Again, there was difference in the amount of endrin residue in the fat tissue in quail of the two tests. The average content for the fat tissue in the acute dosage test was 0.014 ± 0.002 ppm, whereas, the endrin content of the fat tissue in the chronic dosage test was significantly less at 0.010 ± 0.001 ppm. On the other hand, the liver tissue from birds in the chronic dosage test averaged significantly higher residue content (0.007 ± 0.000 ppm), than the same tissue from acute exposure (0.004 ± 0.000 ppm). Gonadal tissue from both exposures contained only trace amounts of endrin. As before, analyses of entire birds indicated that quail subjected to chronic dosages retained a significantly greater percentage (20%) of the administered dose, than birds of the acute exposures which retained 13% of the total dose administered.

This study was undertaken to investigate the fate of endrin in a simplified food chain, involving the soybean plant, the Mexican bean beetle, and the eastern bobwhite quail. The results obtained from this study indicated that detectable amounts of endrin were incorporated and transferred along the components of the food chains investigated. The bobwhite quail, which represented the final link in both chains, contained detectable endrin in at least three tissues from all tests investigated.

It was noted that the highest concentration of endrin from all tests, was in the fat, liver, and gonadal tissues. The liver tissue, from all

TABLE 6. A comparison of endrin distribution in the bobwhite quail following acute and chronic exposure from feeding contaminated beans from the soybean plant at the rate of 0.015 mg/kg total endrin for each bird.

Tissue or organ analyzed [1]	Acute exposure [2] Mean	SE [5]	Chronic exposure [3] Mean	SE	Value of t	Level of significance
Brain	ND [6]		ND			
Fat	0.014	± 0.002	0.010	± 0.001	2.53	0.05
Kidney	ND		ND			
Liver	0.004	± 0.000	0.007	± 0.000	°°	0.01
Gonads	trace		trace			
Breast muscle	ND		ND			
Heart	ND		ND			
Whole bird [4]	0.002	± 0.000	0.003	± 0.000	°°	0.01

1 Average of 12 samples.
2 Total dose given in a single feeding, then birds sacrificed two days later.
3 Total dose sub-divided into five daily doses, then birds sacrificed two days later.
4 Average on three birds only.
5 SE = Standard error.
6 ND = Not detectable.

tests, was the best index for indicating the total body burden of the birds. Breast muscle, the edible portion of the bird, was consistently one of the lowest in endrin content. These findings are in agreement with those of Moore and Walker (1964) who found that residue concentrations of organic chlorine insecticides varied widely from tissue to tissue, but were richest in fat and poorest in breast muscle.

In a similar study El Sayed, Graves, and Bonner (1967) investigated chlorinated hydrocarbon levels in wild birds. They found that for fat tissue there was no apparent pattern in the amount of residues in the various tissues sampled. The fat tissue usually contained the highest levels of residues. They analyzed various insects in the test areas in order to establish the source of pesticides accumulated by the birds. Residues were found but could not be directly correlated with bird uptake, since the exact diet of the wild birds was unknown. It is interesting to note that they did not report finding endrin in birds, but it was at the highest level of six pesticides reported found in adult mayflies (Ephemeroptera).

Birds administered acute and chronic exposures of endrin by feeding upon beetles received much more of the pesticide (1 mg/kg/bird) than did birds in the dosage tests which were fed contaminated beans (0.015 mg/kg/bird). The results showed, however, that the trends of endrin concentration and retention by birds from the respective exposures were similar. Birds from the two acute exposure tests contained higher concentrations of endrin in their fat tissue than birds of the chronic exposure tests. Conversely, birds from the chronic dosage tests evidenced higher concentrations of endrin in the liver and gonadal tissues. The whole body burdens for the acute exposures were lower than for the chronic exposures. The results indicated, but did not porve, that endrin first concentrated in the fat tissue, then translocated to other tissues of the bird.

The results showed that birds which fed upon contaminated beetles incurred significantly higher endrin burdens than would be imposed upon them by equivalent soybean consumption. The bean plants on which the beetles fed were sprayed only once, as compared to four equivalent sprays to the bean plants from which soybeans were harvested. The beetles were directly exposed to the endrin residue on the leaf, whreas, the immature beans were protected by the pod and apparently not exposed to as high a level of endrin. Also, they were harvested weeks after the last exposure. From either route of entry, endrin and related chlorinated hydrocarbons which act similarly in the environ-

ment, could pose a hazard to these birds. Moore and Walker (1964) agree that in both terrestrial and aquatic birds those whose diet is mainly flesh contained much higher residues of the organic chlorine insecticides than did those whose diet was mainly plant material.

In conclusion, it has been shown that endrin and probably any similar fat-soluble, residual pesticide can accumulate in birds (the bobwhite quail) from the environment through their food even though the birds may not be directly exposed to the pesticide. While toxic levels of endrin were purposely not reached in this study, it was shown that contaminated food could contribute to the burden of pesticide acquired by the bird.

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ABSRACT

This study was concerned with the fate of endrin in a simplified food chain situation, involving the soybean plant, the Meximan bean beetle and the eastern bobwhite quail. Specific attention was given to the amount of accumulation, possible tissue specificity, and to the percent retention of endrin as indicated by the total body burden of the birds.

One group of quail was exposed to acute or chronic endrin concentrations by being force-fed contaminated beetles at the rate of 1 mg endrin/kg/bird. Another group of birds were exposed to acute and chronic dosages of endrin by being force-fed contaminated beans at the rate of 0.015 mg/kg/bird. Two days following treatment all birds were sacrificed and the whole bird or selected part was lyophilized and extracted in (1:1) hexane-acetone solution. The extract of each sample was analyzed by gas-liquid chromatography.

Fat, liver, and gonadal tissues, from both acute and chronic dosage tests, involving beans and beetles, consistently contained significantly higher endrin concentrations. Fat tissue from the acute dosage tests involving beetles contained 0.682 $\pm$ 0.059 ppm of endrin, while the same tissue from the chronic dosage test contained 0.421 $\pm$ 0.008 ppm of endrin. Liver and gonadal tissues from the acute dosage test utilizing beetles contained 0.145 $\pm$ 0.008 and 0.113 $\pm$ 0.009 ppm endrin, respectively. Fat tissue from the acute dosage test utilizing beans contained 0.114 $\pm$ 0.002 ppm of endrin, while the same tissue from the chronic dosage test contained 0.010 $\pm$ 0.001 ppm of endrin. Liver tissue from the acute test averaged 0.004 $\pm$ 0.000 ppm endrin, and the same tissue from the chronic phase contained 0.007 $\pm$ 0.000 ppm endrin. Gonadal tissue contained endrin only in trace amounts.

Analysis of whole birds, from all tests, revealed retentiton of 16% of the total acute dose administered, and 21% retention of the total chronic dose administered.

Apparently the compound was not metabolized by any component of the food chain, but was accumulated and transferred in the original form.