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Effects of Aldrin on Young Pen-Reared Pheasants

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EFFECTS OF ALDRIN ON YOUNG PEN-REARED PHEASANTS

BY

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Abstract

Three levels of encapsulated aldrin (0.5, 1.0, and 1.5 mg) were administered weekly to young pen-reared pheasants from 5 to 11 weeks of age to determine effects on growth and residue levels in brain, feathers, and whole body during and after treatment. During treatment, birds were weighed weekly. After treatment ceased, birds were weighed every other week until 21 weeks of age. Analysis of residues was by electron capture gas chromatography.

Growth of pheasants between 5 to 21 weeks of age was depressed (P 0.01) by administration of aldrin dosages of 1.0 and 1.5 mg. Aldrin values in the brain and whole body of birds sampled throughout the experiment varied little (0.02 ppm to 0.08 ppm) regardless of treatment level while dieldrin values increased proportionately with treatment levels during the period of administration. Ten weeks after treatment was terminated brain and whole body levels of dieldrin from birds surviving the experiment were comparable to controls. Administration of 1.5 mg of aldrin in a single dosage caused death of 11 of 22 five-week old pheasants within 48 hours. Aldrin plus dieldrin levels in these 11 birds were: brain, 2.29 ppm to 4.25 ppm and 2.72 ppm average; whole body, 1.45 ppm to 4.35 ppm and 2.38 ppm average; feathers, 0.56 ppm to 1.43 ppm and 0.81 ppm average.

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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.

Thesis Adviser	Date
Head, Wildlife and Fisheries Sciences Department	Date

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J. E. H.

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INTRODUCTION

The ring-necked pheasant (*Phasianus colchicus*) is a popular upland game species in South Dakota and is of major recreational and economic importance. Recent decline in wild pheasant populations has prompted workers to investigate possible causes for the decrease. Insecticides have been shown to be detrimental to wildlife. Scott et al. (1959) and Stickel et al. (1968) indicated that bird populations on an area treated with three pounds of dieldrin per acre suffered heavy losses. Approximately 58 percent of the corn acreage in the corn belt states during 1966 was treated with aldrin (Fitzsimmons 1968).

Gannon and Decker (1958) stated that conversion of aldrin to the more residual and equally toxic material, dieldrin, occurred on alfalfa, soybeans, and corn and may take place on other plants. Gannon and Bigger (1958) indicated aldrin converts to its epoxide, dieldrin, in soils treated for control of soil-inhabiting insects.

Aldrin and dieldrin, prior to 1964, were applied both to foliage and soil. Foliar applications were prohibited by federal law in 1964 and now these chemicals are approved only for crops without tuberous root systems (U. S. Dept. Agr. 1965). South Dakota permits aldrin for soil treatment of corn (Kantack and Berndt 1968).

This experiment was initiated to: (1) determine effects of relatively low levels of aldrin upon growth of young pen-reared pheasants, (2) determine accumulations of aldrin or dieldrin in tissues of treated birds, and (3) compare insecticide residues in brain, feathers and whole body during and after treatment.

METHODS AND MATERIALS

Aldrin was purchased from Shell Oil Company and after laboratory purification revealed no extraneous peaks when analyzed by electron capture gas chromatography (ECGC). Administration of dosage to birds was by No. 5 gelatin capsules containing 0.5 mg aldrin and 110 mg lactose. Control birds received capsules containing only lactose.

Pheasant chicks purchased from Bird Island Resort, Afton, Oklahoma, were tagged with No. 3 aluminum patagial wing bands (National Band and Tag Co., Newport, Ky.) and placed in brooders until 4 weeks old. They were then randomly divided into groups of 22 and placed in 20' x 20' outdoor pens. When chicks were 5 weeks of age, the groups were assigned weekly treatments of 0.0 (control), 0.5, 1.0, and 1.5 mg of aldrin. All dosages were given on the same day.

Birds receiving a single dose of 1.5 mg aldrin suffered high mortality within 15 hours after first treatment. Therefore another group was introduced into the study which received three 0.5 mg capsules given on alternate days for a total of 1.5 mg per week. All treatments were terminated after 7 weeks. The experiment was terminated when the birds reached 21 weeks of age.

Only males were analyzed for insecticide residues to eliminate sex differences. Two male birds from each treatment group were sacrificed at 7, 11, 16, and 21 weeks of age. In the group suffering high mortality from a single dose of 1.5 mg, analyses included all birds that died of suspected insecticide toxicosis plus three of the birds that survived the experiment. All birds sacrificed or that died were kept frozen until analyzed.

An Aerograph HY-FI model 600-D (Varian Aerograph) equipped with a model S-R 1 mv Sargent recorder and an electron capture detector cell with an 8 millicuric nickel source was used for ECGC. Injector and detector temperatures were 210^0 C and 200^0 C respectively. For better identification of aldrin and dieldrin, all samples were run on two separate columns. A 1/8-inch o. d. x 5-foot borosilicate glass column packed with 5 percent Dow 11 Silicone on 60/80 mesh (HMDS) treated chromosorb W was operated isothermally at 185^0 C with a 45 ml per minute nitrogen carrier gas flow rate. A 1/8-inch o. d. x 10-foot borosilicate glass column packed with 2 percent QF-1 Silicone (Fluoro) on 60/80 mesh (HMDS) treated chromosorb W was operated isothermally at 150^0 C with a 40 ml per minute nitrogen carrier gas flow rate.

Identification of aldrin and dieldrin was accomplished by comparing retention time of the sample with the retention time of a known standard. Thin-layer chromatography as described by Breidenbach et al. (1964) was used for further identification of aldrin and dieldrin. Amounts of aldrin and dieldrin were calculated by triangulation of the peaks on the chromatograms and comparison to known standards. Parts per million were calculated using the following formula:

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

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
e = procedure efficiency correction

Procedure efficiency was determined by adding known amounts of aldrin and dieldrin to control tissue. By calculating amounts recovered it was possible to determine percent recovery. Procedure efficiency for brain, whole body, and feathers was 90 ± 6 percent.

Brains from experimental birds were removed and 1 g samples were extracted and purified for aldrin and dieldrin by the Florisil column cleanup method employed by Stern et al. (1964) and revised by Greichus et al. (1968).

Whole body samples consisted of the entire body minus the brain. The body was ground to a homogeneous mixture with a Toledo meat chopper. Subsamples from this mixture were pooled to form a 25 g sample. Subsamples were then taken from the 25 g sample to form a 1 g sample which was then extracted and purified. Duplicates varied ± 8 percent between extracted samples.

Feathers were analyzed for insecticide residues from birds receiving 1.5 mg aldrin in a single dosage. Feathers were plucked and basal sections of primaries, secondaries, and rectrices were discarded. They were air dried and ground in a Wiley mill to form a homogeneous mixture. A 1 g sample of this mixture was extracted and purified. The remaining portion was added to the whole body sample for insecticide analysis.

Birds in all groups were weighed to the nearest gram each week during treatment. After treatments ceased, birds were weighed every other week until the end of the experiment.

RESULTS AND DISCUSSION

Growth

Polynomial regression analysis revealed significant differences ($P < 0.01$) between slopes of lines for different treatments within sex. Experimental data indicated growth was depressed for high level treatments as compared to controls (Fig. 1). It was observed by Genelly and Rudd (1956) that growth was depressed in pheasants receiving high levels of dieldrin while those receiving low dosages varied little from controls and sometimes surpassed controls in weight.

Residues in sacrificed birds

Insecticide residues for birds sacrificed at different time intervals are presented in Table 1. None of these birds displayed signs of insecticide toxicosis.

Aldrin values in brain and whole body were low and varied little in all treatment groups. Dieldrin values in the controls were similar during the study for both brain and whole body and probably reflected dieldrin contamination in the environment. Dieldrin residues in brain and whole body of aldrin treated birds increased during the treatment period as dosage increased.

Dieldrin values of aldrin treated birds from age 7 to 11 weeks were similar but tended to decrease. Pheasants receiving 0.5 and 1.0 mg aldrin had 6 days between treatments to rid themselves of insecticides whereas birds getting three 0.5 mg capsules on alternate days had only 2 days prior to sacrifice to eliminate residues. Data indicated that

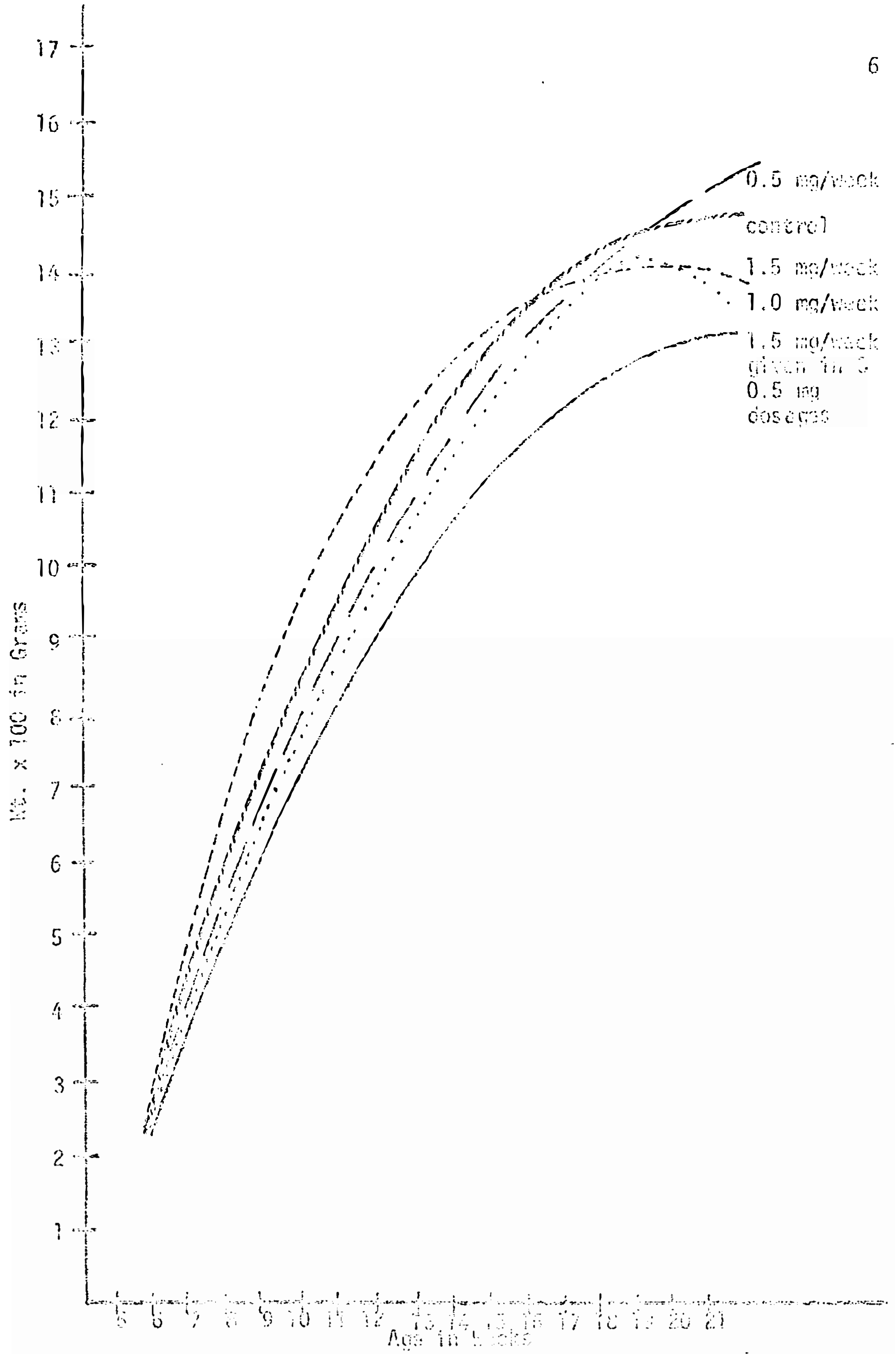

Figure 1. Polynomial regression lines of weight of male pheasants at five treatment levels of aldrin.

Table 1. Concentrations (ppm wet weight) of aldrin (A) and dieldrin (D) in male pheasant brains and whole body for four treatment levels and four age groups.

Age in Weeks	Tissue	Bird No.	Control A	Control D	Bird No.	0.5 mg/week A	0.5 mg/week D	Bird No.	1.0 mg/week A	1.0 mg/week D	Bird No.	1.5 mg/week A	1.5 mg/week D
*7	Brain	P-1	0.02	0.13	Y-1	0.02	0.24	R-1	0.03	0.59	B-1	0.03	0.72
	Whole Body	"	0.02	0.19	"	0.04	0.43	"	0.03	0.59	"	[illegible]	0.87
	Brain	P-2	0.05	0.20	Y-2	0.05	0.32	R-2	0.04	0.59	B-2	[illegible]	0.79
	Whole Body	"	0.06	0.24	"	0.03	0.48	"	0.03	0.71	"	0.06	0.27
†11	Brain	P-3	0.03	0.18	Y-3	0.04	0.25	R-3	0.05	0.35	B-3	0.04	0.39
	Whole Body	"	0.02	0.19	"	0.04	0.39	"	0.03	0.49	"	[illegible]	0.77
	Brain	P-4	0.06	0.13	Y-4	0.05	0.23	R-4	0.06	0.41	B-4	[illegible]	0.38
	Whole Body	"	0.06	0.21	"	0.03	0.31	"	0.05	0.52	"	[illegible]	[illegible]
					Cessation of Treatments								
‡16	Brain	P-5	0.02	0.20	Y-5	0.03	0.23	R-5	0.02	0.19	B-5	0.05	0.19
	Whole Body	"	0.03	0.24	"	0.03	0.30	"	0.03	0.30	"	0.05	0.39
	Brain	P-6	0.03	0.21	Y-6	0.05	0.24	R-6	0.04	0.25	B-6	0.03	0.16
	Whole Body	"	0.05	0.18	"	0.05	0.27	"	0.04	0.27	"	0.03	0.30
§21	Brain	P-7	0.04	0.24	Y-7	0.03	0.22	R-7	0.03	0.19	B-7	0.04	0.26
	Whole Body	"	0.04	0.23	"	0.05	0.27	"	0.07	0.37	"	0.04	0.35
	Brain	P-8	0.04	0.19	Y-8	0.03	0.13	R-8	0.04	0.22	B-8	0.05	0.19
	Whole Body	"	0.04	0.24	"	0.04	0.25	"	0.01	0.31	"	0.04	0.26

*Received 2 treatments and sacrificed 1 week after last treatment.
†Received 6 treatments and sacrificed 1 week after last treatment.
‡Received 7 treatments and sacrificed 5 weeks after last treatment.
§Received 7 treatments and sacrificed 10 weeks after last treatment.

between the 7th and 11th week in birds receiving the highest level of aldrin per week, elimination of the insecticide occurred rapidly enough that increased accumulation did not occur. Harvey (1967) administered 4.75 mg per day of ^{14}C-DDT to starlings for five days and found the birds absorbed less than 25 percent of the dosage. Ten days after treatment ceased less than 10 percent of the ingested DDT remained in the birds. He also indicated that the most rapid excretion occurred one week after treatment.

After treatment terminated, all birds at 16 weeks of age had been off dosage for 5 weeks and those at 21 weeks old were off for 10. At 21 weeks treated birds had levels of aldrin and dieldrin comparable to the levels in the controls. Administrations of 4.3 ug/day of aldrin-^{14}C by Ludwig et al. (1964) to male rats resulted in excretion of 99.5 percent of the total accumulative activity 12 weeks following the last dosage. They also found that after 8 weeks on treatment daily excretions equalled daily dosage.

Pheasants that died or survived dosage of 1.5 mg aldrin per week given in a single dosage

Within 15 hours after treatments began on August 26, 1968, eight birds were dead and by August 28, three more died. Insecticide toxicosis was suspected because these birds exhibited a ragged appearance, walked with a hesitant and stiff-legged gait. They also showed a loss of muscular co-ordination, had quiescent periods, tremors, and convulsions. Brain levels of aldrin plus dieldrin in these birds were higher than those in all other birds analyzed. Kawano et al. (1967) observed young chickens affected by dieldrin dying within 5-10 minutes after the onset of convulsions. Experimental birds in the other treatment groups displayed no signs of insecticide toxicosis.

Six of the 11 birds that died from the effects of the insecticide were females. These limited data suggested there were no sex differences in relation to mortality in young birds due to insecticide toxicosis. Genelly and Rudd (1956), working with adult pheasants, indicated that females were more resistant to insecticides than males. Dahlen and Haugen (1954), using bobwhite quail, noted that females were more resistant than males to insecticides and that older birds were more resistant than younger ones. Lamb et al. (1967) found that the hen's ability to lay eggs was a large factor in passing dieldrin from the body. This may account for the hen's increased resistance to harmful effects of insecticides. The inability of sexually immature females to lay eggs eliminates a pathway for elimination of insecticides and may make them as equally susceptible as males of the same age to insecticides.

Brain

Brain residues of aldrin and dieldrin in birds that died from suspected insecticide toxicosis ranged from 2.13 ppm to 4.25 ppm (Table 2). Mean and standard deviations were 2.72 ppm $\pm$ 0.61 ppm. Stickel et al. (1968) using Japanese quail (*Coturnix coturnix*) concluded that death may occur with brain levels of 4 or 5 ppm of dieldrin. Adult birds were used in the experiment and different values could be expected for young birds (pers. comm. L. F. Stickel).

Table 2. Concentrations (ppm wet weight) of aldrin and dieldrin in brains, whole body, and feathers in pheasants suffering insecticide toxicosis and surviving birds.

Bird	Sex	Age in Weeks	Aldrin and Dieldrin Concentrations					
			Brain		Whole Body		Feathers	
			Aldrin	Dieldrin	Aldrin	Dieldrin	Aldrin	Dieldrin
B-2**	F	5	0.03	2.36	0.03	1.44	***	0.70
B-8**	F	5	***	2.41	1.23	1.52	***	0.61
B-12**	F	5	0.02	2.64	1.98	2.35	***	0.79
B-85**	F	5	***	2.29	0.51	1.51	***	0.56
B-95**	F	5	***	2.73	0.04	1.42	***	0.63
B-81**	F	5	0.02	2.52	0.04	1.51	***	0.91
B-4**	M	5	***	4.25	0.15	2.36	***	0.87
B-13**	M	5	0.03	3.01	0.73	1.99	***	0.93
B-90**	M	5	0.02	2.46	0.69	1.44	***	0.67
B-1**	M	5	***	2.34	***	1.45	***	0.81
B-81**	M	5	0.07	3.14	0.03	2.25	***	1.43
B-3*	M	21	0.02	0.10	0.01	0.11	0.13	0.19
B-91*	M	21	***	0.14	***	0.18	0.05	0.20
B-96*	M	21	0.02	0.18	0.02	0.17	0.06	0.14

*** Indicate values below experimental limits (less than 0.01 ppm).
* End of experiment birds
** Died within 48 hours of first treatment

Stickel et al. (1966) indicated brain levels were more useful than other tissues in diagnosing death from insecticides and that a range rather than an exact value was a more practical tool for diagnosis. Dosage or length of time on treatment had little effect on brain residue levels in birds that died from DDT (Stickel et al. 1966). This study revealed lower toxic insecticide levels in brains than those reported by Stickel et al. (1966). Young birds may be more susceptible to the insecticide and the brain levels may therefore be lower.

Aldrin plus dieldrin levels in the brain departed 22 percent from the mean, while percent departure from means of these residues in whole body and feathers was 32 percent and 33 percent respectively. It appeared that brain tissue in this experiment was a useful diagnostic tool for determining cause of death. All birds that survived treatments had brain residues of dieldrin less than 1.00 ppm.

Brain levels of insecticide in birds sacrificed at the end of the experiment ranged from 0.12 ppm to 0.20 ppm (Table 2). These low values indicated birds were able to rid themselves of the insecticides during the 10 weeks following termination of treatment. Harvey (1967) indicated that DDT appeared to be mobilized rapidly from neural tissue of starlings and individual birds that died were unable to eliminate insecticides rapidly.

Brain levels of aldrin were low and quite uniform for birds sacrificed at the end of the experiment and for birds that died of insecticide toxicosis, indicating rapid metabolic conversion of aldrin to dieldrin.

Whole body

Aldrin plus dieldrin residues ranged from 1.45 ppm to 4.33 ppm in birds that died within 48 hours. Mean and standard deviation of 2.38 ppm $\pm$ 0.76 ppm. Aldrin residues were higher and more variable in whole body than in brain or feathers. When the bird dies the metabolic conversion of aldrin to dieldrin ceases. Higher and more variable residues of aldrin in the whole body compared to brain and feathers may be explained by metabolic conversion of aldrin to dieldrin before deposition in the brain or in the lipids which are deposited on feathers via the uropygial gland. Aldrin plus dieldrin levels in the whole body of birds sacrificed at the end of the experiment were low and ranged from 0.11 ppm to 0.19 ppm which indicated the elimination of insecticides after treatment ceased.

Feathers

Dieldrin residues on feathers of birds that died within 48 hours ranged from 0.56 ppm to 1.43 ppm with a mean and standard deviation of 0.81 ppm $\pm$ 0.24 ppm. Aldrin levels were less than 0.01 ppm in all 11 birds. These data suggested rapid conversion of aldrin to dieldrin and deposition of lipids containing dieldrin in the uropygial gland. Uropygial secretions on feathers would account for the high levels of dieldrin found on the feathers and may act as another pathway for elimination of lipid soluble insecticides. Levels of DDT and its metabolites in the uropygial gland of waterfowl have been shown to be higher than 12 other tissues analyzed (Dindal and Peterle, 1968). Birds at the end of the experiment had much lower levels (0.14 ppm to 0.19 ppm) of dieldrin on the feathers.

CONCLUSIONS

1. Brain levels of dieldrin were a valuable diagnostic tool for determining death by insecticide toxicosis.
2. Dosages of 1.5 mg of aldrin per week given in a single dosage resulted in death of 60 percent of 6 week old pheasants within 48 hours. Aldrin plus dieldrin levels in birds which died were: brain, 2.13 - 4.25 ppm with a mean of 2.72 and a standard deviation ± 0.61; whole body, 1.45 - 4.33, mean 2.38, standard deviation ± 0.76; feathers 0.56 - 1.43, mean 0.81, standard deviation ± 0.24. The average dieldrin level on feathers of birds 10 weeks after treatment ceased was 0.17 ppm, indicating conversion of aldrin to dieldrin and elimination of dieldrin apparently through the uropygial gland.
3. Aldrin concentrations in brain and whole body varied little, 0.02 ppm to 0.08 ppm, regardless of treatment level indicating efficient conversion of aldrin to dieldrin. Dieldrin values in both brain and whole body increased proportionately to treatment levels during the treatment period. Within 10 weeks after the last treatment all tissue levels of aldrin and dieldrin were comparable to controls.
4. Under conditions of this study, total body growth of young ring-necked pheasants was adversely affected ($P < 0.01$) by aldrin dosages of 1.0 mg and 1.5 mg per week administered by capsules.

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