Chronic Toxicity to Quail and Pheasants of Some Chlorinated Insecticides

JAMES B. DEWITT
Fish and Wildlife Service,
U. S. Department of the Interior,
Laurel, Md.

Inclusion of 1 p.p.m. of aldrin, dieldrin, or endrin in diets fed growing quail resulted in high mortality rates, but the birds survived on diets containing 100 p.p.m. of DDT or 50 p.p.m. of strobane. Young pheasants survived on diets containing 50 p.p.m. of DDT or strobane, but failed to survive on diets containing 5 p.p.m. of aldrin, dieldrin, or endrin. No ill effects were noted when quail were fed winter diets containing 50 p.p.m. of strobane, or 1 p.p.m. of dieldrin or endrin, but nearly all birds died when fed diets containing 0.5 p.p.m. of aldrin. Mortality rates among pheasants fed 50 p.p.m. and of quail fed 100 p.p.m. of DDT were higher than for birds receiving normal diets, but none of the birds displayed symptoms characteristic of DDT poisoning. Egg production, fertility, and hatchability were relatively unaffected by inclusion of insecticides in diets fed breeding quail, but chicks from these matings showed high mortality rates even when reared on insecticide-free diets. Lowered viability of quail chicks was most pronounced in groups receiving DDT and strobane in the reproduction diets. Hatchability of pheasant eggs and viability of chicks were adversely affected by inclusion of aldrin, dieldrin, or endrin in the reproduction diets.

Use of chlorinated hydrocarbons for control of agricultural and forest insect pests necessarily involves the possibility that birds in the treated areas will ingest or absorb the toxicants with their food or in the course of dusting and preening. Immediate effects of such exposure may be slight when the insecticidal concentration does not exceed 2 pounds of DDT per acre (*3*), but heavy avian mortality associated with typical symptoms of DDT poisoning has resulted when this compound was applied at the rate of 5 pounds per acre (*4*). With pen-reared quail, these symptoms of acute poisoning occur when the birds receive single doses of 50 to 200 mg. of DDT per kilogram of body weight, or when fed diets containing approximately 500 p.p.m. of this insecticide (*1*).

Cumulative effects produced by repeated applications of low concentrations of DDT, or by continued ingestion of feeds containing sublethal quantities of this toxicant, also may act to decrease avian populations. Robbins, Springer, and Webster (*6*) found that five annual applications of 2 pounds DDT per acre resulted in a 26% decrease in numbers of nesting birds, although Hotchkiss and Pough (*4*) had shown that a single application at this rate had no apparent effect upon bird life. Mitchell, Blagbrough, and Van Etten (*5*) found considerable mortality among nestlings under conditions in which adult birds were unaffected by application of 3 pounds of DDT per acre, and DeWitt (*2*) reported abnormally low survival among quail chicks whose parents had received 200 p.p.m. of DDT in the reproduction diets.

Other chlorinated insecticides which are being or may be used under conditions offering possible hazards to wild birds are aldrin (1,2,3,4,10,10-hexachloro - 1,4,4a,5,8,8a - hexahydro - 1,4 - *endo, exo* - 5,8 - dimethanonaphthalene); dieldrin (1,2,3,4,10,10 - hexachloro - *exo* - 6,7 - epoxy - 1,4,4a,5,6,7,8,8a - octahydro - 1,4 - *endo, exo* - 5,8-dimethanonaphthalene); endrin (1,2,3,4,10,10-hexachloro-*exo*-6,7-epoxy-1,4,4a,5,6,7,8,8a - octahydro - 1,4 - *endo, endo* - 5,8 - dimethanonaphthalene); and strobane (mixed polychlorinated terpenes). Strobane appears less toxic than DDT to quail and pheasants, but aldrin, dieldrin, and endrin are highly toxic to these species (*2*). The present series of experiments was designed to furnish information on maximum dietary concentrations of these compounds which would permit growth and survival of quail and pheasant chicks, and on the effects of these low levels upon reproduction.

Experimental

Growth Period. One-day-old quail and pheasant chicks, whose parents had not been exposed to insecticides, were distributed by random selection into groups of 20 to 30 birds each, and placed in electrically heated brooders. Birds in the control groups received regular growth diets (25 to 28% protein), and the experimental groups were fed the same diets modified by the addition of small percentages of the test compounds. Feeding was on an *ad libitum* basis, and continued throughout the growth period of 16 to 20 weeks, or until all birds in the experimental groups died. All experiments were conducted in replicate, with two or more groups of 20 or more birds on each experimental diet. Data from these replicate studies have been combined for presentation in this report.

Winter Maintenance Period. Early in November when the birds used in the

Table I. Effects of Insecticides upon Quail and Pheasant Chicks

(Birds 1 day old at start of experiment)

Species	Compound	No. of Birds	Level in Diet, P.P.M.	Duration of Test, Days	% Mortality	Toxicant Consumed, Mg./Kg. Daily	Total
Quail	(Controls)	200	...	120	24.0	...	...
	Aldrin	40	50	6	100	1.4	8.4
		40	20	8	100	1.2	9.6
		60	10	13	100	0.9	11.1
		40	5	28	100	0.6	15.4
		80	1	47	100	0.18	9.0
	Dieldrin	40	20	40	100	1.6	62.0
		60	10	61	100	1.2	70.2
		80	5	87	100	0.7	50.2
		60	1	76	100	0.8	46.2
	Endrin	40	50	3	100	2.5	7.5
		40	20	8	100	2.0	16.0
		60	10	14	100	1.2	1.68
		40	5	21	72.6	0.4	8.4
		40	1	105	70.0	0.1	12.6
		40	0.5	41	40.0	0.2	9.0
	DDT	40	150	15	53.3	7.2	108
		80	100	120	30.0	10.5	1260
	Strobane	30	1000	6	100	77.2	465
		40	500	9	100	27.8	250
		53	50	120	20.8	5.3	620
Pheasants	(Controls)	200	...	103	28.0	...	...
	Aldrin	30	20	5	100	1.8	9.0
		40	5	46	100	0.7	31.3
	Dieldrin	40	5	68	100	0.6	42.2
	Endrin	40	20	4	100	1.7	8.5
		40	5	8	100	0.6	5.0
	DDT	40	100	51	100	21.2	1130
		101	50	103	37.0	4.6	475
	Strobane	100	50	103	23.0	4.3	445

growth studies were approximately 16 to 20 weeks old, the growth diets were replaced by maintenance rations containing 12 to 15% protein. Birds which had received 100 p.p.m. of DDT or 50 p.p.m. of strobane in the growth diets were fed the same levels of insecticides during the winter. Birds from the summer (growth period) control groups were redistributed into additional experimental groups receiving insecticides in the diets, and into controls receiving insecticide-free food. Feeding continued until March 15, and all groups contained 20 or more birds.

Reproduction Period. At the close of the winter studies, maintenance-type diets were replaced by high-protein (25 to 28%) reproduction diets. Part of the birds which had received insecticides during the winter received the same levels of toxicant in the reproduction diets; others were fed insecticide-free rations. Control groups, and the remaining experimental groups, were made up of birds which had not previously been exposed to the test compounds. In all cases, care was taken to ensure that birds in the control and experimental groups were from the same parent stock. Size of the experimental groups varied according to numbers of available birds, but not less than 10 birds were started in each group. All chicks resulting from these studies were fed insecticide-free diets.

Table II. Effects upon Quail and Pheasants of Insecticides Fed during Winter Maintenance Period

(Birds 16 to 20 weeks old at start of experiments)

Species	Compound	No. of Birds	Level, in Diet, P.P.M.	Duration of Test, Days	% Mortality	Toxicant Consumed, Mg./Kg. Daily	Total
Quail	(Controls)	200	...	162	8.7	...	...
	Aldrin	48	1	101	100	0.09	9.1
		40	0.5	127	97.5	0.04	5.1
	Dieldrin	40	1	162	17.5	0.12	19.4
		60	0.5	162	0	0.08	13.0
	Endrin	47	1	162	8.6	0.07	11.0
		60	0.5	162	10.0	0.04	6.5
	DDT[a]	54	100	162	20.2	7.3	1180
	Strobane[a]	80	50	162	7.5	3.7	600
		40	25	162	0	2.0	325
Pheasant	(Controls)	192	...	120	12.5	...	...
	DDT[a]	58	50	120	43.0	2.5	300
	Strobane[a]	68	50	120	20.6	3.0	360

[a] Birds also received insecticides at these levels during growth period.

Results and Discussion

Growth Period. Typical symptoms of DDT poisoning appeared in quail chicks fed diets containing 150 p.p.m. of this compound (Table I), but feeding of 100 p.p.m. had no apparent effect upon survival. A previous experiment (*2*) had shown that some depression of growth rate may have occurred during the first few weeks on test, but body weights were normal by the end of the tenth week. Pheasant chicks appear more susceptible than quail to the effects of this compound, but normal survival and growth were obtained with diets containing 50 p.p.m. Strobane appeared similar to DDT in its effects upon both quail and pheasants, but tolerance limits were not established.

Aldrin, dieldrin, and endrin are highly toxic to quail and pheasant chicks, and efforts to rear birds on diets containing these compounds have been unsuccessful. Average food consumption was reduced by the addition of 10 p.p.m. of aldrin or endrin, or of 20 p.p.m. of dieldrin, although some groups of birds readily accepted these treated feeds. Variations in rate of acceptance greatly exceeded those (15 to 20%) frequently found among chicks fed indentical untreated foods. Symptoms of acute poisoning appeared within 48 to 72 hours after initial feeding of diets containing 20 p.p.m. of aldrin or endrin, and food consumption was abnormally low during the rest of the test period. Initial symptoms included lack of muscular coordination, occasional tremors, bedraggled appearance, and a stiff-legged, hesitating movement in walking. In advanced stages, the birds made spasmodic leaps into the air, and went into violent cartwheels lasting for several minutes.

Onset of these symptoms was delayed when these compounds were fed at lower levels, and in all experiments involving feeding of dieldrin to chicks. Growth was depressed, but the birds appeared in relatively good condition until the last

Table III. Effects of Insecticides upon Reproduction of Quail

Compound	Level in Diets, P.P.M. Winter	Level in Diets, P.P.M. Reproduction	No. of Birds	Mortality, %	Eggs/Hen, Av.	Fertile, %	Hatch, %	Chicks Surviving at End of 2 weeks	Chicks Surviving at End of 6 weeks
(Controls)	...	...	32	6.25	52	89.0	83.9	88.9	83.3
Aldrin	0.5	0	16	25.0	61	79.4	67.0	100	77.8
	0.5	0.5	8	100[a]	..	...	...	...	...
	0	1.0	16	25.0	40	91.0	87.3	92.5	77.8
Dieldrin	1.0	0	16	0	53	70.6	84.2	100	62.5
	1.0	1.0	10	40[a]	..	...	...	...	...
	0	1.0	16	0	60	81.4	81.6	92.6	75.0
Endrin	1.0	0	16	25.0	45	84.9	70.1	80.8	50.0
	1.0	1.0	10	60[a]	..	...	...	...	...
	0	1.0	16	25.0	60	93.1	79.0	89.2	64.3
DDT	100	0	8	0	61	87.5	75.7	86.2	64.3
	100	100	12	25.0	65	66.9	75.3	67.7	7.1
	0	200	12	25.0	55	92.8	80.0	32.3	12.9
Strobane	50	0	10	0	69	86.6	91.9	100	85.0
	50	50	10	0	51	90.5	71.5	63.6	50.0
	0	100	10	0	36	76.8	73.0	100	62.5

[a] Mortality occurred prior to beginning of egg production. Survivors discarded.

Table IV. Effects of Insecticides upon Reproduction of Pheasants

Compound	Level in Diets, P.P.M. Winter	Level in Diets, P.P.M. Reproduction	No. of Birds	Mortality, %	Eggs/Hen, Av.	Fertile, %	Hatch, %	% Chicks Surviving at End of 2 weeks	% Chicks Surviving at End of 6 weeks
(Controls)	..	...	128	0	48	86.6	57.4	94.8	89.7
Aldrin	0	10	8	100	8	85.7	30.0	62.5	62.5
	0	2	10	40	35	66.7	39.1	53.3	46.7
	0	1	10	20	40	86.0	55.6	95.5	81.8
Dieldrin	0	10	10	0	41	82.8	43.3	70.6	52.9
	0	2	10	0	25	88.9	51.6	95.2	71.4
	0	1	10	0	56	88.2	60.4	96.6	89.7
Endrin	0	10	10	100	11	81.7	40.6	37.5	31.3
	0	2	10	0	42	89.8	47.7	97.1	91.2
	0	1	10	0	45	92.6	56.6	91.7	91.7
	0	0.5	10	0	40	84.9	71.3	80.0	71.4
DDT	0	50	10	0	31	81.4	58.6	100	85.0
	50	50	10	0	18	77.5	80.6	100	93.3
	0	100	10	0	19	86.2	52.0	100	82.4
Strobane	50	50	10	0	37	79	51.9	75	62.5

few days of the experimental period. In most cases, all birds in an experimental group died within 5 days after the first appearance of toxic symptoms.

Maximum concentrations of aldrin, dieldrin, and endrin which will permit growth and survival of quail and pheasant chicks have not been established. Additional studies are being conducted, in which these compounds are being fed at levels ranging down to 0.1 p.p.m. On the basis of available data, it appears that they are at least 100 times more toxic to quail chicks, and at least 20 times more toxic to pheasant chicks, than is DDT fed under similar conditions.

Maintenance Studies. Dietary requirements of quail and pheasants during the winter months are less critical than during growth or reproduction, and satisfactory survival was obtained in all groups fed DDT, strobane, dieldrin, or endrin (Table II). Mortality rates among pheasants fed 50 p.p.m. and of quail fed 100 p.p.m. of DDT were significantly higher than those for the control group, but the differences may have reflected effects of previous exposure to the compound. Body weights of surviving birds in all groups were equal to those of the controls, and no symptoms of acute poisoning appeared in any of the birds receiving these compounds. Aldrin appeared more toxic than any of the other test materials, and the high mortality rates among quail fed this compound at 0.5 p.p.m. may be contrasted to the survival of birds fed 200 times this amount of DDT.

Reproduction Studies. No demonstrable effects upon egg production, percentage fertility, or percentage hatchability were produced by the inclusion of 200 p.p.m. of DDT, 100 p.p.m. of strobane, or 1 p.p.m. of aldrin, dieldrin, or endrin in diets fed quail which had not previously been exposed to these compounds (Table III). Differences between the control and experimental groups do not exceed those found among control birds during the past 10 years. Viability of chicks from these birds was markedly reduced in the DDT groups, where 87% of the chicks died within the first 6 weeks. Strobane and endrin also appeared to reduce chick viability, but aldrin and dieldrin had no appreciable effects.

Heavy mortality occurred among quail which had received aldrin, dieldrin, or endrin in both the maintenance and reproduction diets, and no eggs were obtained from these birds. Birds which had received 100 p.p.m. of DDT throughout growth, maintenance, and reproduction periods produced normal numbers of eggs, but fertility was significantly (25%) lower than that of the controls. Hatchability was only 10% below that of the control group, but chicks from these matings failed to survive even when fed insecticide-free diets. Heavy mortality also occurred among chicks whose parents had received 50 p.p.m. of strobane in the growth, maintenance, and reproduction diets. Similarly, some reduction in chick viability occurred in groups where the parents had been fed DDT, dieldrin, or endrin during the winter months, but received insecticide-free diets during the breeding season.

Reproduction of pheasants was adversely affected by the inclusion of aldrin, dieldrin, or endrin in the breeding diets (Table IV). Mortality occurred in all groups receiving aldrin, and surviving birds lost approximately 30% body weight during the breeding season. Egg production in the groups receiving 1 or 2 p.p.m. of aldrin remained at normal levels during the first 6 weeks of the test period, but had virtually ceased by the end of the tenth week. Hatchability appeared decreased by feeding of 10 p.p.m. of aldrin, dieldrin, or endrin, and chicks from these groups had unusually high mortality during the first 2 weeks. Egg production by birds fed 50 or 100 p.p.m. of DDT was below that of the controls, but fertility, hatchability, and chick viability appeared unaffected. Feeding of 50 p.p.m. of strobane appeared to reduce chick viability.

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AMINO ACIDS IN FERMENTATION

Pilot Plant Study of Utilization of Leucine by *Saccharomyces cerevisiae*

J. W. SPANYER, JR., and ALAN T. THOMAS

Brown-Forman Distillers' Corp., Louisville 1, Ky.

Much of the flavor of whisky results from esters of the higher alcohols and organic acids present in the distillate. The higher alcohols are normally analyzed as a group and called fusel oil. Isoamyl alcohol is a major constituent of this fraction. Much evidence has indicated that yeast deaminates and decarboxylates α-amino acids to form alcohols. This study with pilot fermentors has verified that hypothesis. The leucine–isoamyl alcohol system has been studied under standardized conditions. The results indicate that the amount of fusel oil formed is approximately a linear function of the amount of leucine added.

During the fermentation of sugars by yeast, many organic compounds, other than ethyl alcohol, are produced. Some are yeast-metabolic products or intermediary products in the fermentation cycle; others are formed by foreign organisms or materials in the medium. The higher alcohols that are present in new whisky are lumped together and called fusel oil.

Ehrlich (*1–3*) showed that the fusel oil content of spirits could be increased by the addition of amino acids and concluded that higher alcohols are formed from their corresponding amino acids by deamination and decarboxylation.

$$RCH\,(NH_2)\,COOH \longrightarrow RCH_2OH + NH_3 + CO_2$$

This hypothesis, as modified by Neubauer and Fromherz (*4*), is known as the Ehrlich mechanism and suggests the following sequence of steps.

$$RCH\,(NH_2)\,COOH \longrightarrow RCOCOOH + NH_3 \text{ (oxidation)}$$

$$RCOCOOH \longrightarrow RCHO + CO_2 \text{ (decarboxylation)}$$

$$RCHO \xrightarrow{2H} RCH_2OH \quad \text{(reduction}$$

$$RCHO \xrightarrow{O} RCHOH \quad \text{or oxidation)}$$

Strickland (*7–9*), working with bacteria, found that amino acids could be classified as either hydrogen donors or acceptors. The reaction occurring between a pair of opposite types is:

$$R_1CH(NH_2)COOH + R_2CH(NH_2)COOH \xrightarrow{H_2O} R_1COCOOH + R_2CH_2COOH + 2NH_3$$

Thorne (*10, 11*) confirmed Ehrlich's postulations for the formation of several alcohols and succinic acid, and reasoned that if deamination of α-amino acids is the normal mechanism for nitrogen assimilation by yeast, then ammonia should be superior to any amino acid. He found that ammonium phosphate was superior to all single amino acids except aspartic and glutamic acids, but that complex amino acid mixtures were much superior to the ammonium salt as a yeast nutrient. Thorne concluded that, when all of the necessary amino acids are present, they are integrated intact into the yeast protein, and that deamination takes place only to synthesize unavailable nitrogenous compounds. Thorne also presented evidence that yeast could, at least to a small extent, utilize the Strickland mechanism as a source of nitrogen.

To simulate normal distillery operations and check the utilization of leucine by yeast, a series of fermentations was conducted with varying amounts of leucine in pilot plant fermentors.

Fermentation and Distillation

Ten liters of medium were fermented in two 40-liter stainless steel fermentors, equipped with agitators and automatic temperature controls (Figure 1). The fermentors were agitated at 250 r.p.m. for approximately 4 hours after setting, then the agitators were turned off.