the caking tendency of the fertilizer bagged after 4 weeks would not differ much from that bagged after 1 week. A comparison of Figure 5 with Figures 2 and 3, however, shows the marked beneficial effect of the additional 3-week curing period prior to bagging. This same effect can be seen by comparing Figure 6 with Figure 4 and by noting Figure 7.

Summary and Conclusions

Drop tests made on the bagged fertilizer indicated no reduction in the caking tendency of the fertilizer in the presence of either of the two surfactants on prolonged storage for several months.

When the fertilizer was bagged 1 week after manufacture and stored for 3 weeks, the anionic surfactant reduced noticeably the caking tendency of the bagged fertilizer stacked in the conventional manner. The beneficial effect of the anionic surfactant noted during this short storage period was not evident in the same bagged fertilizer several months later, nor in the fertilizer bagged 4 weeks after manufacture and stored for either a short or a long time. The effect of the additional 3-week curing was to obliterate the beneficial effect of the anionic surfactant.

This suggests that no reduction in caking tendency in bagged fertilizer can be expected from incorporating these surfactants into mixed fertilizers, if the fertilizer is to be bagged within 1 week of manufacture and stored for more than 3 weeks. Nor can any reduction in the caking tendency be expected from the presence of an anionic surfactant if the fertilizer has been cured for 4 weeks prior to bagging.

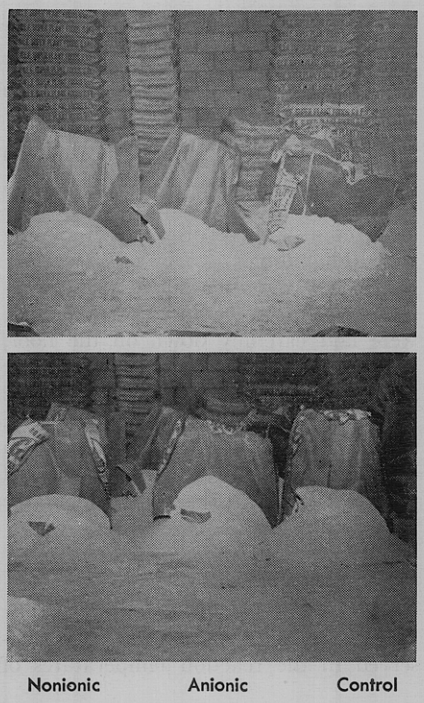

Figure 7. Thirteenth bag in stack for each formulation

Top. Fertilizer bagged 1 week after manufacture
Bottom. Fertilizer bagged 4 weeks after manufacture
Each bag was dropped five times flat and once on each end before slitting—considerably more handling than in standard drop test. Fertilizer in storage 7 months

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PESTICIDE TOXICITY

Effects of Chlorinated Hydrocarbon Insecticides upon Quail and Pheasants

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Effects upon wildlife of the insecticidal use of chlorinated hydrocarbons have not been fully determined. Coburn and Treichler (*2*) and Dahlen and Haugen (*3*) have published data on the acute oral toxicities to avian species of some of these compounds, and studies have been made on the immediate effects resulting from use of DDT for control of agricultural and forest insect pests. Hotchkiss and Pough (*6*) found that the use of 1 pound of DDT per acre had no apparent effect upon bird numbers, but that an experimental application at the rate of 5 pounds per acre resulted in marked reductions in avian populations. Similar findings were made by Robbins and Stewart (*10*), and Robbins, Springer, and Webster (*9*) found a 26% decrease in breeding birds following five annual applications at the rate of 2 pounds per acre.

These results suggest that prolonged exposure to DDT may affect breeding potential of birds even when no immediate effects are apparent. Under conditions of sublethal intake, DDT accumulates in various organs and body tissues (*7*), and DeWitt, Derby, and Mangan (*4*) showed that nonbreeding adult quail succumbed to DDT poisoning when concentration of the toxicant in muscle tissue exceeded 30 to 35 γ per gram. Coburn and Treichler (*2*) and Mangan (*8*) reared young quail on diets containing 0.01% DDT, and Mangan found that no ill effects were apparent under conditions of relatively low storage in tissues. However, the experiments were terminated at the end of 9 or 10 weeks, and no data are available on effects of more prolonged exposure.

Other chlorinated hydrocarbons which are being, or may be, used under conditions offering possible hazards to wildlife include aldrin (1,2,3,4,10,10-hexachloro - 1,4,4a,5,8,8a - hexahydro-1,4,5,8 - dimethanonaphthalene), dieldrin (1,2,3,4,10,10 - hexachloro-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 - 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 may be considered relatively nontoxic (*11*), but Eden (*5*), Arant (*1*), and Sherman and Rosenberg (*12, 13*)

Use of chlorinated hydrocarbon insecticides for control of agricultural insect pests subjects upland game birds to prolonged exposure to these toxicants. Studies were conducted to determine levels of acute and chronic toxicity, and effects of prolonged exposure upon reproduction. Feeding of diets containing 0.02% DDT to breeding quail had no apparent effects upon the adult birds, but resulted in decreased hatchability of eggs and viability of chicks. Similar results were obtained by feeding 0.001% dieldrin, but effects upon reproduction of short-term feeding of aldrin and endrin could not be determined. Adequate measures are needed to protect desirable birds during operations designed for insect control.

have shown that aldrin, dieldrin, and endrin are highly toxic to chicks.

Studies designed to furnish information on effects produced by prolonged exposure of quail and pheasants to low concentrations of chlorinated hydrocarbons were begun at the Patuxent Research Refuge in 1954. Experiments were conducted to determine tolerance limits for aldrin, dieldrin, endrin, and strobane, and to determine effects of these compounds and of DDT upon survival, growth, and reproduction. Chicks reared on diets containing these compounds will be utilized in breeding experiments during 1955.

Experimental

Adult Birds. Birds used in these studies were obtained by random selection from pen-reared stock. Approximately 30 days prior to the start of the breeding season, they were placed in wire-floored pens, and allowed free access to water and reproduction-type diets, modified by the addition of small percentages of aldrin, dieldrin, endrin, or DDT. Birds in the control groups received unmodified (insecticide-free) diets. Birds that died during the initial part of the reproduction period were replaced by birds that had received insecticide-free diets. Feeding was continued until all birds in the experimental groups died, or until the end of the breeding season (154 days). Eggs were collected daily, and stored at 65° F. until placed in the incubator. Maximum

Table I. Toxicity of Insecticides to Adult Quail and Pheasants

Compound	Species	No. of Birds	Level Fed		Toxicant Consumed, Mg./Kg.		Mortality, %	Survival Time, Days
			%	P.p.m.	Daily	Total		
Aldrin	Quail	10[a]	0.50	5000	12.0	46.4	100.0	4
		10	0.25	2500	11.8	46.3	100.0	4
		10	0.125	1250	5.5	20.8	100.0	4
		10	0.0625	625	2.2	9.1	100.0	4
		10	0.010	100	0.7	3.3	100.0	5
		10	0.005	50	1.9	9.7	100.0	5
		10	0.001	10	0.9	7.3	100.0	8
		10	0.0005	5	0.5	20.0	100.0	42
	Pheasants							
	Males	5	0.01	100	1.7	13.8	100.0	8
	Females	5	0.01	100	1.4	50.4	100.0	36
Dieldrin	Quail	10	0.50	5000	8.2	36.9	100.0	5
		10	0.25	2500	10.2	46.2	100.0	5
		10	0.125	1250	3.7	13.9	100.0	4
		10	0.0625	625	5.0	19.3	100.0	4
		10	0.010	100	2.8	15.3	100.0	6
		10	0.005	50	0.9	5.8	100.0	7
		10	0.001	10	0.8	31.1	100.0	39
		10	0.0005	5	0.5	29.2	100.0	59
	Pheasants							
	Males	5	0.010	100	2.4	24.0	100.0	10
	Females	5	0.010	100	2.0	62.0	100.0	31
Endrin	Quail	10	0.50	5000	62.9	92.5	100.0	2
		10	0.25	2500	7.2	13.9	100.0	2
		10	0.125	1250	2.6	9.2	100.0	4
		10	0.0625	625	1.8	4.5	100.0	3
		10	0.010	100	0.9	4.4	100.0	5
		10	0.005	50	0.6	2.8	100.0	5
		10	0.001	10	0.7	16.7	100.0	26
		10	0.0005	5	0.5	11.3	100.0	22
		10	0.0002	2	0.2	6.4	100.0	36
	Pheasants							
	Males	5	0.010	100	0.6	5.0	100.0	9
	Females	5	0.010	100	1.1	25.3	100.0	23
DDT	Quail	40	0.025	250	25.0	1100	100.0	45
		10	0.020	200	13.8	2125	10.0	154
	Pheasants	16	0.025	250	11.5	208	100.0	18
Strobane	Quail	8	0.05	500	38.0	3195	28.6	84
		8	0.025	250	10.6	890	25.0	84
		8	0.010	100	11.3	945	0	84
Controls	Quail	96	...	...	...	...	4.1	154
	Pheasants	108	...	...	...	...	3.6	100

[a] Equal numbers of males and females were used in all groups.

Table II. Effect of Insecticides upon Reproduction of Quail

Compound	Level Fed, %	Egg/Hen/ Day	Fertility, %	Hatchability, %	Chicks Surviving[a], %		
					1 wk.	3 wk.	12 wk.
..... (Control)	0	0.53	88.6	82.3	90.0	87.5	78.3
DDT	0.02	0.35	93.6	66.8	43.8	36.2	19.8
Aldrin	0.001	0.08	85.7	83.3	100.0	80.0	76.0
Dieldrin	0.001	0.56	90.0	41.7	53.7	43.9	32.7
Endrin	0.001	0.37	92.9	84.6	100.0	89.3	63.0

[a] All chicks in these groups received normal (insecticide-free) diets.

storage time was 13 or 14 days. Chicks obtained from these groups were transferred from the incubator to electrically heated battery brooders, and fed regular growth diets, unmodified by the addition of insecticide.

Young Birds. Chicks used in these studies were obtained from the control groups or from other lots of birds which had not been exposed to the insecticides. All birds were housed in battery brooders, or runs providing access to the outside. Feeding of insecticide diets was begun during the first few days after hatching, and was continued in some groups throughout the winter months, or until all birds died. In other groups, the experimental diets were fed for short periods (7 to 14 days), and then replaced by normal diets. Feeding of insecticides was resumed at the end of 28 days, and continued for a second short period.

Results and Discussion

Data obtained in these studies are summarized in Tables I to V. All of the test materials produced toxic symptoms resembling those of acute DDT poisoning, but time required for development of these symptoms varied with changes in levels fed. Adult quail developed severe tremors, lack of muscular coordination, and extreme nervousness within 2 hours after being fed diets containing 0.50% endrin, and all birds in this group died within 48 hours. Feeding of this compound, and related materials at lower levels, resulted in a lethargic condition and bedraggled plumage prior to the onset of symptoms of acute toxicity.

Adult pheasants and quail succumbed to the effects of DDT poisoning when fed diets containing 0.025% of the compound (Table I), and no reproduction occurred in these groups. No ill effects were observed in quail which were fed diets containing 0.020% DDT throughout the breeding season of 154 days. Body weights remained normal, and the number of eggs per hen equaled that of some of the control birds (Table II). The percentage of fertile eggs was slightly higher than in the control groups, but the difference was not significant. Hatchability of fertile eggs was appreciably below that of eggs from the control group, and the difference approached significance ($P = 0.08$). Many embryos appeared to develop normally during the early stages of incubation, but died during the hatching period. Mortality among chicks from this group was extremely high, and more than 50% died within the first 5 days after hatching.

Aldrin, dieldrin, and endrin were highly toxic to adult quail and pheasants (Table I), and diets containing these compounds were poorly accepted. Pheasants refused to eat diets containing 0.025% or more of these compounds, but satisfactory acceptance occurred when the levels were reduced to 0.01%. All male pheasants in these groups died within 10 days after feeding of insecticides began, but females survived for longer periods. No eggs were produced in any of these groups.

Feeding of aldrin, dieldrin, and endrin to quail was commenced prior to the start of the breeding season, but all birds

Table III. Toxicity of Insecticides to Young Quail and Pheasants

(Continuous feeding)

Compound	Species	No. of Birds	Age at Start, Days	Duration of Test, Days	Level Fed, %	Toxicant Consumed, Mg./Kg.		Mortality, %
						Daily	Total	
Aldrin	Quail	10	1	6	0.002	0.62	3.3	100.0
		40	1	13	0.001	1.21	5.7	100.0
		17	16	37	0.0005	0.32	11.1	100.0
		10	1	47	0.0001	0.08	5.6	100.0
		32	15	70	0.0001	0.07	5.8	68.8
		20	1	14	0.0001	0.11	1.5	60.0
	Pheasants	20	1	46	0.0005	0.58	27.2	100.0
Dieldrin	Quail	10	1	40	0.002	1.14	25.0	100.0
		10	1	60	0.001	1.19	58.4	100.0
		16	16	87	0.0005	0.75	44.2	100.0
		10	1	76	0.0005	0.81	46.2	100.0
		20	1	7	0.001	1.87	13.1	75.0
		22	1	14	0.0001	0.17	2.4	27.3
		32	1	7	0.00005	0.11	0.8	0
	Pheasants	20	1	90	0.0005	0.53	47.7	100.0
Endrin	Quail	10	1	2	0.005	1.88	3.2	100.0
		10	1	5	0.002	1.96	10.0	100.0
		10	1	6	0.001	1.35	6.9	100.0
		20	1	19	0.0005	0.38	6.7	100.0
		20	16	7	0.0005	0.40	2.7	55.0
		32	1	14	0.0001	0.12	1.7	21.9
		22	1	120	0.00005	0.06	7.2	13.6
	Pheasants	20	1	5	0.0005	0.36	2.0	100.0
DDT	Quail	80	1	120	0.01	10.5	1260	30.0
	Pheasants	20	1	120	0.005	4.6	475	35.0
Strobane	Quail	20	1	6	0.10	77.2	420	100.0
		20	1	9	0.05	27.8	250	100.0
		20	1	120	0.005	5.3	620	20.0
	Pheasants	20	1	103	0.005	4.3	448	25.0
Control	Quail	200	1	120	...	...	...	28.5
	Pheasants	200	1	120	...	...	...	31.5

Table IV. Toxicity of Insecticides to Young Quail

(Intermittent feeding, 28 days between tests)

Compound	Level Fed, %	Initial Feeding: Duration, days	Initial Feeding: Toxicant Consumed, Mg./Kg. Daily	Initial Feeding: Toxicant Consumed, Mg./Kg. Total	Initial Feeding: Mortality, %	Second Feeding: Duration, days	Second Feeding: Toxicant Consumed, Mg./Kg. Daily	Second Feeding: Toxicant Consumed, Mg./Kg. Total	Second Feeding: Mortality, %
Aldrin	0.0001	14	0.105	1.47	58.8	7	0.016	0.86	100.0
Dieldrin	0.001	7	1.87	13.1	74.0	7	0.82	5.7	100.0
	0.0001	14	0.17	2.4	25.8	14	0.09	1.2	100.0
	0.00005	7	0.11	0.80	0	7	0.04	0.28	6.5
Endrin	0.001	7	1.35	9.4	81.7	7	1.20	6.1	100.0
	0.0005	7	0.38	2.7	55.2	7	0.19	1.3	69.7
	0.00001	14	0.12	1.7	20.7	14	0.09	1.3	26.0
Controls	...	7	...	...	4.0				
	...	14	...	...	4.0				
	...	42	...	...	22.0				

Table V. Effects of Insecticides upon Growth and Survival of Young Quail

Weeks on Test	Controls Surv., %	Controls Wt., g.	Aldrin (0.0001%) Surv., %	Aldrin (0.0001%) Wt., g.	Dieldrin (0.0001%) Surv., %	Dieldrin (0.0001%) Wt., g.	Endrin (0.0001%) Surv., %	Endrin (0.0001%) Wt., g.	DDT (0.01%) Surv., %	DDT (0.01%) Wt., g.	Strobane (0.005%) Surv., %	Strobane (0.005%) Wt., g.
						Compound						
1	96	16	94	12	91	12	85	14	100	13	97	13
2	96	26	94	23	91	24	80	24	100	23	97	23
3	96	50	94	38	91	48	80	41	100	34	91	42
4	90	70	94	51	91	66	80	55	100	52	78	58
5	82	90	94	70	91	82	78	68	97	72	75	76
6	78	110	94	94	34	100	61	80	97	92	75	97
7	78	124	94	105	0	...	49	94	97	112	75	114
8	78	130	0	...	..	...	49	110	97	130	75	130
9	78	155	..	...	..	...	44	128	97	145	75	142
10	78	163	..	...	..	...	0	...	97	160	75	156

in the initial groups died before producing eggs. As each group died, it was replaced with birds from the control pens, and the diets were changed to lower concentrations of insecticide. No reproduction occurred until insecticide concentration had been dropped to 0.001%, and the birds had been producing eggs prior to being placed on experiment. Tests at 0.005% were not begun until the end of the reproduction period.

All birds which were fed diets containing 0.001% aldrin died within 8 days after the start of the experiment, and no eggs were produced after the third day. Fertility and hatchability of eggs from this group, and viability of chicks, were equal to those of the controls, but the experimental period was too short to permit determination of possible effects of aldrin upon reproduction. Dieldrin produced no visible ill effects during the first 4 weeks when fed at the level of 0.001%, but toxic symptoms appeared near the end of the fifth week. Egg production and percentage of fertile eggs during the first 4 weeks remained at the pre-experimental level, but hatchability dropped from 87% to less than 42%. Very few chicks were hatched from eggs laid during the latter part of the period, and chick mortality was high. Most of the chicks which survived more than 3 weeks were produced from eggs laid during the first 2 weeks after feeding of the insecticide was commenced.

Feeding of 0.001% endrin to breeding quail produced no visible symptoms of intoxication until the 23rd day, but all birds died within 26 days after start of the experiment. Egg production, fertility, hatchability, and viability of chicks remained at pre-experimental levels.

Feeding of strobane at 0.01, 0.025, and 0.05%, and of aldrin, dieldrin, and endrin at 0.0005% was begun after the close of the reproduction season. Strobane appears somewhat less toxic than DDT to quail, but additional studies will be required to establish long-range effects. All birds receiving the chlorinated dimethanonaphthalenes died within a relatively short period, under conditions where the daily intake was approximately 0.5 mg. of toxicant per kilogram of body weight. It appears that the limit of tolerance for these compounds is somewhere below this figure. No difference was noted in the resistance of male and female quail to the effects of the insecticides.

Feeding of diets containing 0.01% DDT or 0.005% strobane had little effect upon survival or growth of quail chicks (Tables III and V). Some depression of growth may have occurred during the first 5 weeks of the experimental period, but individual variations within the experimental and control groups indicate that the differences may not be significant. The low mortality among birds receiving 0.01% DDT during the first 10 weeks of life is similar to that found by Mangan (*8*), but the percentage of birds in this group at the end of 16 weeks was approximately equal to that in the controls. Young quail appear more susceptible than adults to the effects of continued ingestion of strobane.

Continuous feeding of diets containing 0.0005% aldrin, dieldrin, or endrin was lethal to young quail and pheasants, and short-time exposure to even lower levels produced a high percentage of kills. Tolerance limits were not established, although mortality was low among quail receiving 0.00005% endrin. Pheasants apparently are more resistant than quail to the effects of aldrin and dieldrin.

Cumulative effects produced by intermittent feeding of insecticides (Table IV) were more severe in some cases than those produced by continuous feeding at the same level. Thirty per cent of a group of young quail survived after ingesting a total of 5.8 mg. of aldrin per kilogram of body weight during 70 days of continuous feeding on diets containing 0.0001% of the toxicant. Intermittent feeding at this level produced 100% mortality within 21 days of total exposure time, when the total ingestion of aldrin was 2.3 mg. per kg. Similar results were obtained with dieldrin, where intermittent feeding at 0.001% during two periods of 7 days each produced 100% mortality (total toxicant ingested, 18.8 mg. per kg.). Continuous feeding of dieldrin at this level required 60 days to produce 100% mortality.

Summary and Conclusions

Previous studies had shown that heavy or repeated applications of DDT resulted in decreases in bird populations, but long-range effects of this and other

chlorinated hydrocarbon insecticides had not been fully evaluated. Experiments were conducted to determine toxicity to quail and pheasants of aldrin, dieldrin, endrin, and strobane, and to determine effects of these compounds upon survival, growth, and reproduction.

Feeding of diets containing 0.02% DDT to breeding quail resulted in significant decreases in hatchability of eggs and in viability of chicks. Similar results were obtained by feeding 0.001% dieldrin, but effects upon reproduction of short-term feeding of aldrin and endrin could not be determined.

Aldrin, dieldrin, and endrin were lethal to both male and female quail when fed at levels of 0.0005% in the diets. Female pheasants appeared more resistant than males to the effects of these compounds.

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PESTICIDE RESIDUE ANALYSES

Preassay Purification of Tissue Extracts by Wax Column

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A reverse-phase partition column for removal of interfering extractives prior to bioassay or chemical determination of residual insecticides has been used with organic insecticides occurring as residues in plant and animal tissues. The column consists of finely ground alumina coated with a 2 to 1 mixture of petrolatum and low melting paraffin wax. The eluting liquid is 60% aqueous acetonitrile solution in most cases, but by successive use of 40, 60, and 75% solutions the toxicants may be separated into groups for easier identification. Recovery is satisfactory with most toxicants, and bioassay with houseflies is being used regularly with a wide range of produce.

THE FIRST STEPS in the determination of an organic insecticide or acaricide residue in a plant or animal product are subdivison of the material and extraction with an organic solvent. The resulting extract seldom contains the toxicant in a sufficiently pure state for analysis, but in addition to the residue in question, holds various fats, oils, waxes, and colored materials such as chlorophylls and carotenoids. These all interfere in varying degrees with such methods as colorimetric chemical determinations or bioassay involving response of a sensitive living organism. It is necessary therefore to purify the extract—i.e., in so far as practicable to separate the residual toxicant from the plant or animal extractives which have also passed into the organic solvent.

A number of purification methods have been advanced for cleaning up organic solvent extracts, but most of them are applicable to a limited number of toxicants. Sulfonation of the unsaturated fats, waxes, and colored materials, as in the Celite–sulfuric acid column (*7*) can be used only for acid-stable toxicants such as DDT. Saponification of the fats and removal as water-soluble soaps can be employed only with alkali-stable compounds such as aldrin or dieldrin. Adsorption columns with their highly specific characteristics must be developed individually for each toxicant and the ordinary partition columns of clays, cellulose, etc., work only with solutes that have considerable solubility in water. It seemed possible to develop a reverse-phase partition column that would work with extracts containing interfering extractives and any toxicants of low water solubility.

In reverse-phase partition columns, the stationary phase is of lower polarity than the moving one. Inclusion of water in the moving phase ensures this condition, but pairs of organic liquids of markedly different polarity and hence of low mutual solubility also give the same effect—e.g., hexane and acetonitrile. Such columns are suitable for holding back and separating compounds which are soluble in the typical organic solvents. At first sight, it might seem that such a column could be made without difficulty by treating some supporting solid with the chosen organic liquid and passing through any chosen water-solvent solution as developing liquid, but the situation is considerably more complicated.

The essential structure of a column having a relatively fixed polar phase—for example, a cellulose-water column—is that the water through hydrogen bonding forms a layer many molecules thick over all the surface of the substrate, which accordingly plays only a minor part in determining the distribution of solutes between this fixed aqueous phase and the external moving organic phase. The reverse of this situation would be a correspondingly thick layer of adsorbed organic liquid of low polarity held upon the supporting substrate. But such liquids usually are not capable of forming polymolecular layers, as they lack the necessary bonding atoms or groups. Ac-