SUB - LETHAL EFFECTS OF SEED TREATMENT PESTICIDES ON BREEDING HEN PHEASANTS

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ABSTRACT

SUB-LETHAL EFFECTS OF SEED TREATMENT PESTICIDES ON BREEDING HEN PHEASANTS

By

Kenneth Lee Stromborg

Three seed treatment pesticides, the fungicide captan and the insecticides dieldrin and Diazinon,® were tested for sub-lethal effects on pheasants (*Phasianus colchicus*). In one experiment, laying female pheasants were fed sub-lethal daily doses of captan, dieldrin, Diazinon, captan plus dieldrin, and captan plus Diazinon. In a second experiment, the dose-response relationships of captan and Diazinon singly and in combination were investigated by giving a range of sub-lethal daily doses. The spring food habits of pheasants were investigated to determine whether they consume pesticide treated corn seed during the reproductive season.

In the single dose level experiment, food consumption was initially depressed by all single treatments and depressed additively by the combination treatments. Over the course of the experiment, food consumption declined in the control, dieldrin, and captan groups. The Diazinon group and the combination groups had the lowest initial

level and did not change over time. Decreased egg production was the only significant reproductive effect. Dieldrin and captan caused small decreases individually and the combination of the two caused an additive decrease. Diazinon alone had no reproductive effect, but when combined with captan, egg laying was almost totally inhibited.

In the dose-response experiment, neither a captan effect nor an interaction effect was observed on any parameter measured. Diazinon depressed food consumption as a function of dose above 1.05 mg/day during the 19 days that 14 pesticide treatments were administered. After termination of pesticide treatment, no food consumption differences were detected among the groups of birds previously given pesticides. During pesticide administration, the rate of egg laying decreased with increasing doses of Diazinon above 1.05 mg/day. The number of days to recovery from this effect increased with increasing doses above 2.10 mg/day. No Diazinon effects on hatchability or chick survival were detected. No reproductive effects were observed during a 12 day period commencing 13 days after the last pesticide dose. Birds which were given 7 pesticide treatments did not differ in egg laying rate or recovery interval from those given 14 doses implying no cumulative effect of Diazinon. During the treatment period, hens lost body weight in relation to dose above 1.05 mg/day. The weight loss was recovered after the end

of pesticide treatment. No differences were found between recovery intervals or body weight changes of pairs of birds receiving equal amounts of pesticide treated and untreated food. A difference approaching significance was found in egg laying rates which suggested a Diazinon effect beyond that attributable to reductions in food consumption alone. A threshold dose between 1.05 and 2.10 mg/day was observed in all cases where Diazinon affected the birds.

Analysis of the reproductive status and crop contents of pheasants collected in the field showed that agricultural foods made up the largest portion of the diet of breeding birds. Residue analysis showed that 45 percent of the birds sampled had consumed food contaminated with seed treatment pesticides.

On the basis of laboratory and field results, it was concluded that the use as a seed treatment of diazinon alone or in combination with captan represents a potential hazard to the reproductive performance of pheasants. The degree of hazard depends on the availability and utilization of treated seed by pheasants. The amount of treated seed necessary to reach an effective dose rate was estimated to be 6 percent to 12 percent of the daily food intake of a breeding hen pheasant.

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INTRODUCTION

Theoretical assessment of pesticide effects on avian populations have shown that adult mortality, reduced fecundity, and partial sterility induced by pesticides could differentially reduce reproductive potential according to the rate of population turnover (Young 1968). Because game bird populations generally have relatively rapid rates of turnover, the latter two effects could cause significant changes in such populations. Although Grolleau and Giban (1966) concluded that there is a low theoretical risk of mortality to adult game birds in relation to various seed treatment pesticides, reproductive impairment at sub-lethal doses has been demonstrated in studies on adult pheasants with alkyl mercurials (Adams and Prince 1972, Spann et al. 1972, Fimreite 1971, Borg et al. 1969) and dieldrin (DeWitt 1955, 1956; Genelly and Rudd 1956a; Atkins and Linder 1967; Baxter et al. 1969; Dahlgren and Linder 1974). Pheasants consume corn in varying quantities when it is available (Kopischke and Harris 1969); part of this consumption is seed corn (Fried 1940, Dambach and Leedy 1948, Trautman 1952) and thus provides a potential source of contamination by seed treatment pesticides. Tejning (1967) has shown that wild pheasants in Sweden accumulated mercury from treated seed grain. Fimreite et al. (1970) demonstrated similar accumulations of mercury in seed eating birds in the cereal growing regions of Alberta and Saskatchewan, Canada. In this case, the degree of mercury contamination was related to the extent with which mercurial seed treatments were used. Although Grolleau and Giban (1966) suggested that seed treatments applied in combinations may have effects greater than single compounds, Leedy and Cole (1950) observed no such effects after evaluating several such combinations. Recent studies on the interaction of pesticides have shown these effects occur between compounds of similar chemical structure (Deichmann et al. 1971, Street et al. 1969, Lichtenstein 1969, Tsao et al. 1953), between different classes of pesticides (Lichtenstein et al. 1973, Liang and Lichtenstein 1974, Plapp 1972, Hewlett 1968), and between pesticides and other causes of mortality (Friend and Trainer 1970).

This study was initiated to evaluate the reproductive effects of three seed treatment pesticides recommended for use on seed corn in Michigan. Pheasants were collected in the spring to test the hypothesis that pheasants consume treated seed corn. One experiment was conducted to test the hypothesis that the fungicide captan (cis-N-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide) does not have an interactive reproductive effect with either of the alternative insecticides 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 = HEOD and related compounds) or Diazinon (O,O-diethyl O-[2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate]. On the basis of an observed interaction between Diazinon and captan and the withdrawal of registration of dieldrin as a seed treatment, a second experiment was performed to test the hypothesis that Diazinon and captan interact and have the potential for altering pheasant reproduction at levels which might be consumed by birds in the field.

MATERIALS AND METHODS

Experiment 1

This experiment was conducted to test the hypothesis that neither dieldrin nor Diazinon have statistically interactive effects on pheasant reproduction when used in combination with captan. A cross-classified analysis of variance design was employed to test for the presence of such an interaction. The experimental birds were thus assigned to one of six treatment groups: control (no treatment), captan, dieldrin, or Diazinon alone, captan plus dieldrin, or captan plus Diazinon.

Forty-eight one-year old female pheasants were randomly assigned to forty-eight separate outdoor pens in mid-May. Groups of 4 individual hens in 2 replicates were randomly assigned the 6 treatments. Each of twelve one-year old males was randomly assigned to inseminate one group of 4 females on a 4 day rotation between individual hens. The experiment lasted a total of 42 days. During the first two weeks of treatment, the pesticides were sprayed onto pelletized food using acetone as a solvent. Thereafter, the pesticides were thoroughly mixed directly into ground food. Acetone was used in minimal quantities only for dieldrin treatments. No solvent controls were conducted because acetone residues have no reproductive effect (R. Ringer, pers. comm.). This was also verified in a later experiment. Pesticide formulations used were: Ortho Soybean Seed Protectant,® 25% captan, 75% inert ingredients; Diazinon MG50, 33.3% Diazinon, 66.7% inert ingredients; technical dieldrin, 85% HEOD, 15% related compounds. A single daily dose level was given for each pesticide calculated as active ingredient (dieldrin, 1 mg; Diazinon, 2 mg; captan, 40 mg) with the combination treatments consisting of the dose level for each of the two pesticides involved. The females in pens without males were given 20 g of pesticide treated food each evening beginning in mid-June. To avoid male effects, the food ration was doubled and treated food was not given when a male was present. Each morning, untreated food was given *ad libitum* to birds that had completely consumed the evening ration. Eggs were collected twice daily, stored no longer than 7 days at 11 C and 60% relative humidity before incubation in a Jamesway® incubator. Chicks were pedigree hatched, weighed, wing banded, and placed into brooder units. Food and water were supplied *ad libitum*. Chicks were reared for 4 weeks to monitor survival.

Experiment 2

On the basis of an observed interaction between captan and Diazinon in the first experiment, this experiment was designed to further elucidate the nature of the interaction effect. Hens were given 14 daily doses at five levels of captan and four levels of Diazinon singly and in combinations centered on the ratio of the compounds as they are recommended for use in the field (Fig. 1). The highest dose of each compound was chosen to be the maximum which a pheasant would ingest if it were eating only treated seed. The maximum dose of Diazinon was not used because it was felt that this dose would be lethal (Tucker and Crabtree 1969). In addition, the hypothesis that observed effects could be caused by the solvent was tested in one group of 4 birds given food treated only with acetone (negative controls). The possibility that the number of treatments would influence the response was tested by administering 7 doses rather than 14 to one group of 5 hens at an intermediate dose rate (4.2 mg/day of Diazinon and 10 mg/day of captan). Finally, a pair-fed design was used to test the influence of the restriction of food consumption by treated birds. Twenty-six hens were paired on the basis of body weight with birds receiving pesticides. Birds in the former group were then given the amount of food eaten by their pair member, but without pesticides added. A four day lag period was necessary to calculate intake by the pesticide treated birds.

Birds were assigned to pens completely at random except the untreated pair members which were assigned at

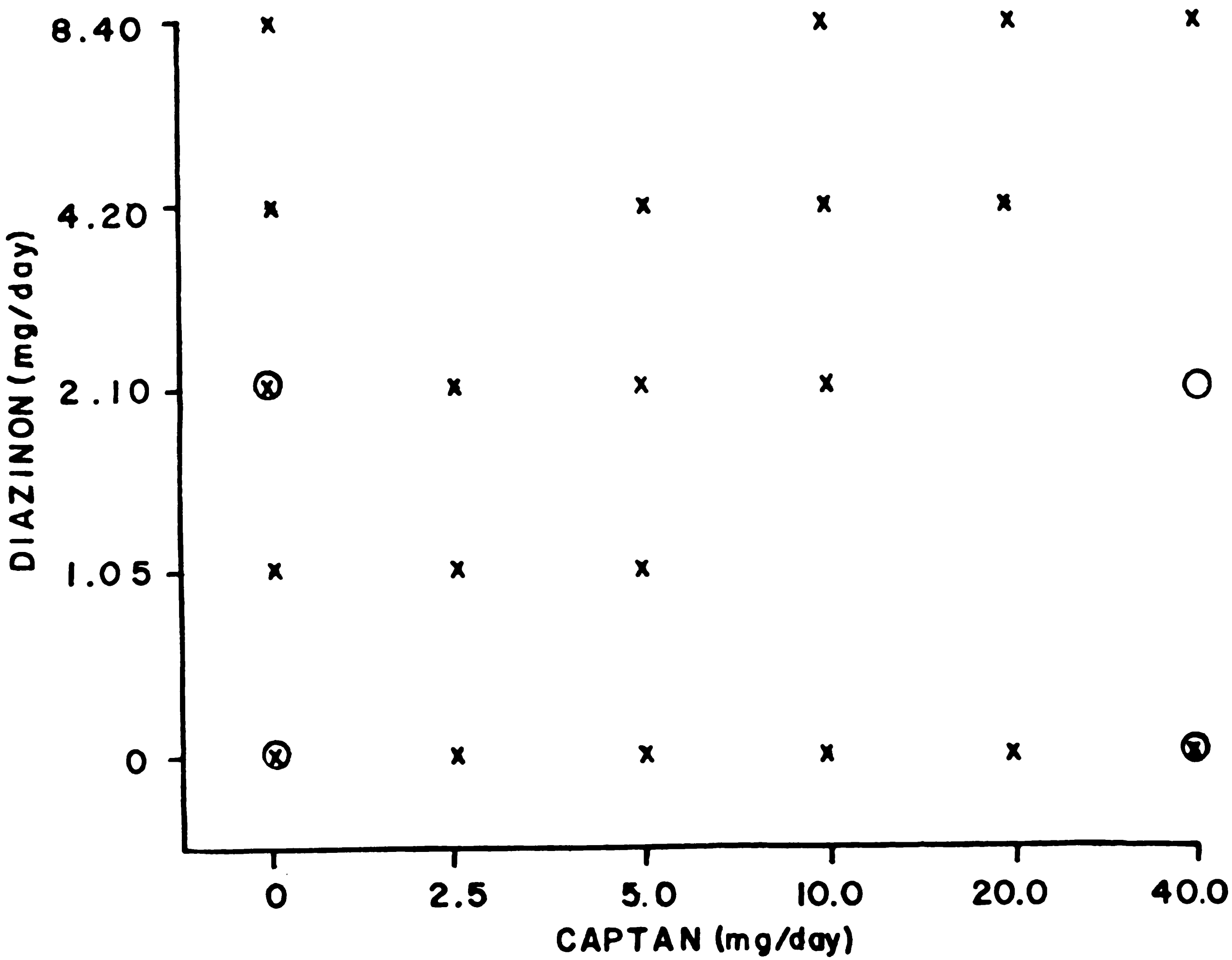

Fig. 1. Daily captan and Diazinon doses given to birds in the two experiments. (O = experiment 1; X = experiment 2).

random to pens within a separate unit. Pesticide treatments were then assigned randomly to birds. Pesticides were applied to pelletized food by dissolving them in equal measured quantities of acetone and spraying it onto the pellets. The mixture was then air dried to allow evaporation of the acetone. The pesticide formulations used were: technical Diazinon, 96.9% purity, and technical captan, 90% purity. Doses were calculated on the basis of active ingredient administered. The feeding regime of treated food in the evening and untreated food ad libitum in the morning was repeated in this experiment. Males were assigned to groups of 4 hens, rotated on a 4 day schedule and fed as before. After administering all 14 doses of treated food, birds were fed ad libitum on untreated food in the morning only. Eggs and chicks were handled as in the first experiment except that chicks were held for only 2 weeks because mortality after that time in the first experiment was all accidental. Hens were weighed prior to the beginning of the experiment, at the end of the 19 day treatment period, and at the end of the experiment on day 44.

RESULTS

Experiment 1

Two females receiving dieldrin treatment died during the experiment: one on day 27 and one on day 38. The food consumption reported is the daily individual mean of each group over 4 day intervals commencing on the first day of treatment (Fig. 2). Since the birds consumed almost all of the treated food presented, time on treatment reflects total pesticide consumed. Food consumption decreased over time ($P<0.05$) in the control, dieldrin, and captan groups. No decrease over time occurred in the Diazinon or combination groups. Initially, single pesticides decreased food consumption from 7 to 18 g/day below control levels. The combined pesticides depressed consumption from 24 to 30 g/day below control. This depression was approximately additive with Diazinon apparently responsible for the largest decrease. Although food consumption over time decreased at the greatest rate in the dieldrin group, this decrease was not significantly different from the control group ($0.20<P<0.30$).

During the 42 days of this experiment, the 48 hens produced 755 eggs, of which 92% were fertile, 472 hatched, and 236 chicks survived. Extreme variability in the number

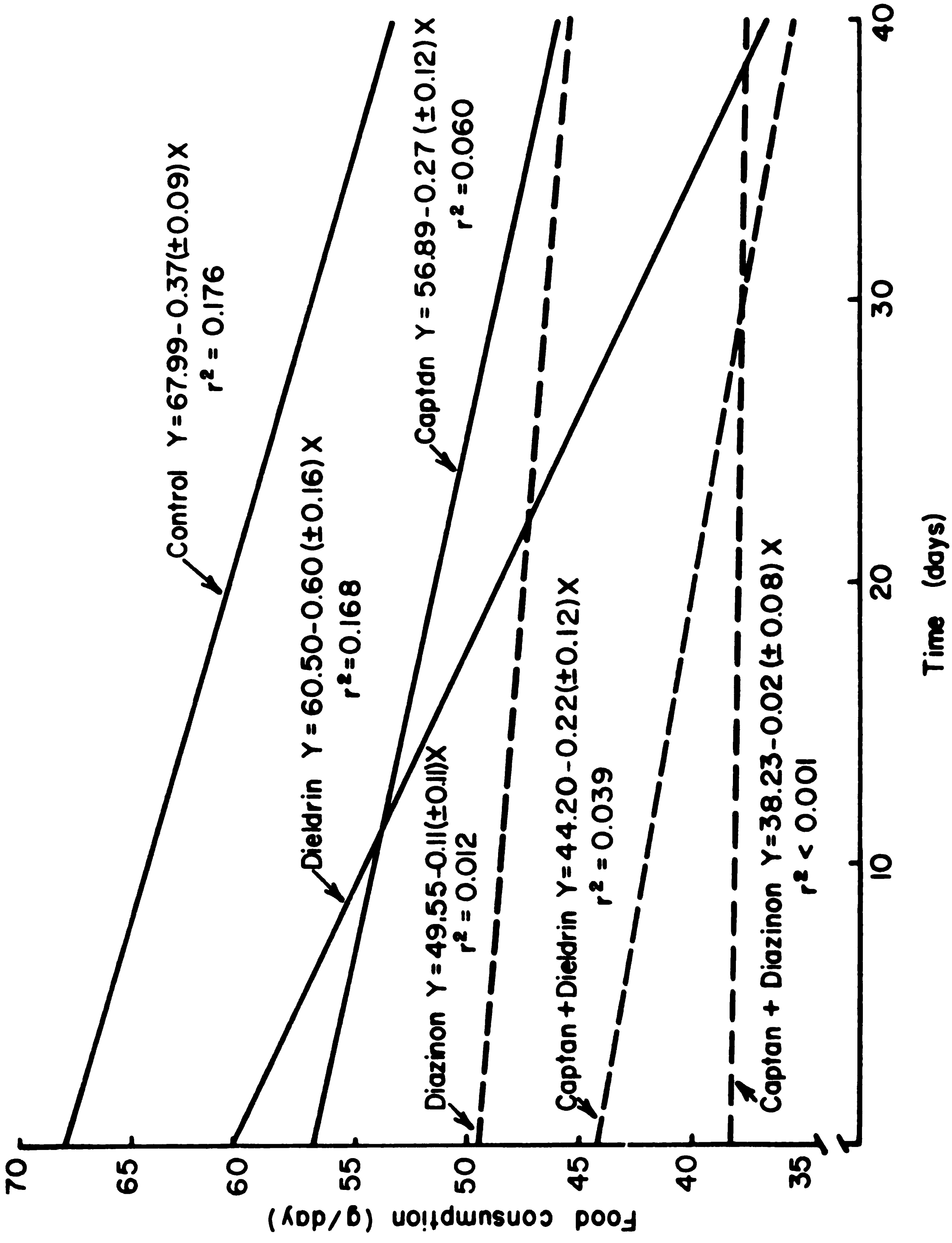

Fig. 2. Mean daily food consumption of pheasants given food treated with captan, dieldrin, and Diazinon singly and in combination.

of eggs and chicks produced by individual hens precluded analysis of mean egg and 18 hour chick weights. The differences between groups were small with the mean egg weights ranging from 28.4 g to 29.6 g and mean chick weights ranging from 18.6 g to 19.8 g. Bartlett's test for homogeneous variances was used to analyze the variances of egg and chick weights. Average treatment group variances of egg weights within hens weighted by the number of eggs produced were not different ($P>0.05$) but variances of egg weights among hens weighted similarly were significantly heterogeneous ($P<0.01$) (Table 1). This indicates that variation between eggs produced by an individual was influenced less by pesticide treatment than variation between individuals within a group. No significant differences ($P>0.05$) were observed in the variances of 18 hour chick weights.

Egg production data were transformed to square roots to obtain homogeneous variances for analysis. Using a cross-classified analysis of variance, significant interaction ($P<0.05$) was detected between the fungicide and insecticides. Further analysis using Scheffe's procedure (Snedecor and Cochran 1967:268-271) indicated that the interaction ($P<0.01$) occurred between captan and Diazinon. This amounted to a reduction in mean production per hen from 22.3 eggs for the control birds to 2.8 eggs for the group receiving a combination of captan and Diazinon (Table 2). Egg production in the group receiving a

TABLE 1.--Means and coefficients of variation of fresh egg weights and 18 hour chick weights from pheasant hens fed pesticides singly and in combinations. Daily treatments per hen were: dieldrin, 1 mg; Diazinon, 2 mg; captan, 40 mg.

Treatment	Fresh Egg Weights				18 Hour Chick Weights			
	n	mean (g)	C.V. eggs within hens	C.V.* eggs among hens	n	mean (g)	C.V. chicks within hens	C.V. chicks among hens
Control	166	29.6	3.58	36.42	107	19.8	4.85	26.01
Diazinon	162	28.4	9.72	92.25	113	18.6	6.24	46.88
Captan	131	29.5	3.29	34.71	83	19.1	5.71	29.69
Dieldrin	108	29.4	3.71	13.84	87	18.6	6.24	18.76
Captan and Dieldrin	82	29.1	5.33	34.92	55	18.6	3.49	27.85
Captan and Diazinon	28	29.3	7.37	9.18	18	19.1	7.02	34.45

*Variances significantly different ($P < 0.01$) by Bartlett's test for homogeneous variances.

TABLE 2.--Egg production, hatchability, chick survival, and calculated number of 4 week old chicks produced by pheasant hens given food treated with dieldrin, Diazinon, and captan, singly and in combination. Daily treatments per hen were: dieldrin, 1 mg; Diazinon, 2 mg; captan, 40 mg. Mean values and 95% confidence intervals per hen are presented.

Treatment	Reproductive Parameter				
	Eggs Laid		Percent Hatchability	Percent Survival	Calculated Number of 4 week old Chicks
	eggs/day	number			
Control	0.53 (0.43-0.65)	22.3 (18.1-27.1)	71.4 (59.0-82.4)	71.8 (56.7-84.6)	10.9 (7.7-14.5)
Diazinon	0.53 (0.42-0.65)	22.3 (17.8-27.3)	73.5 (60.6-84.5)	58.3 (38.2-77.1)	8.9 (5.2-13.7)
Captan	0.40 (0.19-0.69)	16.8 (7.8-29.1)	72.5 (62.8-81.2)	55.7 (18.5-89.5)	6.8 (1.8-14.6)
Dieldrin	0.32 (0.14-0.57)	13.6 (6.0-24.0)	87.9 (71.9-97.7)	50.1 (12.3-87.9)	3.2 (1.6- 5.4)
Captan and Dieldrin	0.22 (0.07-0.44)	9.1 (2.8-18.6)	78.3 (58.2-93.1)	57.2 (9.1-97.4)	2.6 (0.6- 5.6)
Captan and Diazinon	0.07 (0.01-0.16)	2.8 (0.4- 6.1)	73.3 (28.4-99.4)	19.3 (0* -75.4)	0.6 (0*- 2.1)

*Lower confidence limit was negative.

combination of captan and dieldrin declined additively from the control group.

The unequal number of eggs produced between treatment groups reduced the sample size for the analysis of hatchability and survival data (Table 2). These data were transformed by arcsin to obtain homogeneous variances. Although no significant effects ($P>0.05$) on hatchability or survival were detected, mean hatchability values for the pesticide groups were similar or slightly higher than controls while chick survival values of the pesticide groups were consistently lower than controls.

A calculated number of 4 week old chicks produced (Table 2) was generated for each bird by multiplying hatchability and survival percentages by the number of fertile eggs produced. Although this procedure removed the effects of cracked eggs and accidental mortality, it did not yield an independent measure of reproduction for statistical analysis. The smallest number of 4 week old chicks came from the group receiving a combination of captan and Diazinon. Reductions in the number of chicks produced were also observed in groups receiving a combination of captan and dieldrin and dieldrin alone.

Experiment 2

The planned administration of fixed dose levels of pesticides was not achieved at the upper levels of pesticides (Table 3). Although birds receiving 2.10 mg/day of Diazinon and less ate their entire daily ration of treated food, birds in the groups at 4.20 mg/day ate an average of 75% of their treated food. At 8.40 mg/day, the birds ate an average of about 50% of their treated ration. Consumption of treated food decreased as the planned dose rate at the upper three Diazinon dose levels increased. This relationship $(Y=(29.6 - 19.8(\log(X + 1.0)))\,g/day)$ was significant $(P<0.001)$ and accounted for 73% of the observed variation (r^2). No relationship between captan dose and consumption of treated food was observed. In light of the restricted dosage of birds in the upper two Diazinon groups (Table 3), the actual mean daily dose of Diazinon for each bird was used in all following analyses.

Food Consumption

Food consumption data (Table 4) were analyzed according to three time periods based on reproductive data. Because the males were rotated on a 4 day schedule, the 3 time periods were multiples of 4 days for the food consumption data. The periods represented the first 20 days during which 14 doses of pesticide were given to each hen, and the 24 days after treatment, the first 12 of which were designated as the period of recovery from pesticide effects, and the remaining 12 days which were designated the post-treatment period. Food consumption was screened by multiple

TABLE 3.--Means and standard errors of actual daily pesticide doses of birds grouped by planned dose rates of Diazinon irrespective of captan and planned captan dose rates irrespective of Diazinon.

Pesticide	Planned Daily Dose (mg)	Actual Dose ($\bar{x}$ ± S.E.)	n
Diazinon	1.05	1.05*	8
	2.10	2.10*	10
	4.20	3.21 ± 0.23	10
	8.40	4.38 ± 0.34	10
Captan	2.5	2.5*	8
	5.0	4.8 ± 0.2	10
	10.0	7.9 ± 0.9	10
	20.0	16.2 ± 1.1	8
	40.0	32.2 ± 4.8	5

*No deviation from planned dose.

TABLE 4.--Actual doses, means, standard deviations, and sample size ( ) of daily food consumption for untreated controls and planned Diazinon dose rates irrespective of captan and planned captan dose rates irrespective of Diazinon.

Pesticide and Dose (mg/day)		Mean Daily Individual Food Consumption (g)		
		Treatment Period	Recovery Period	Post-treatment Period
Diazinon				
Planned	Actual			
0	0	57.6 ± 8.1 (15)	71.0 ± 9.7 (15)	70.4 ± 6.1 (14)
1.05	1.05	60.0 ± 9.6 (7)	68.0 ± 15.4 (7)	70.7 ± 12.8 (5)
2.10	2.10	53.0 ± 5.3 (10)	71.9 ± 12.1 (9)	67.0 ± 13.1 (8)
4.20	3.21	33.0 ± 5.4 (10)	79.4 ± 18.5 (8)	73.3 ± 8.9 (7)
8.40	4.38	28.3 ± 3.3 (10)	74.7 ± 19.5 (10)	69.8 ± 11.9 (10)
Untreated Controls		59.1 ± 6.6 (8)	71.7 ± 10.5 (7)	68.1 ± 11.2 (7)
Captan				
Planned	Actual			
0	0	42.2 ± 14.5 (12)	73.7 ± 12.0 (10)	73.3 ± 8.7 (9)
2.5	2.5	61.7 ± 7.6 (7)	65.7 ± 12.9 (7)	71.0 ± 10.3 (5)
5.0	4.8	52.2 ± 10.9 (10)	69.7 ± 9.7 (10)	64.5 ± 11.7 (9)
10.0	7.9	41.2 ± 13.1 (10)	80.0 ± 18.5 (9)	70.6 ± 11.7 (8)
20.0	16.2	41.7 ± 16.8 (8)	72.4 ± 7.9 (8)	71.7 ± 9.0 (8)
40.0	32.2	44.4 ± 14.6 (5)	79.0 ± 8.7 (5)	70.8 ± 6.9 (5)

regression techniques (Cooley and Lohnes 1971:66-76) to isolate the effects of captan and Diazinon. Only during the treatment period were differences detected which were attributable to pesticides. Diazinon was the most important variable (Table 5). Food consumption decreased as the dose of Diazinon increased above 1.05 mg/day. The regression ($P<0.001$) of food consumption on Diazinon dose accounted for 58% (r^2) of the observed variation (Fig. 3).

Reproductive Effects

The experiment was divided into 3 time periods for analysis of reproductive data. The treatment period was the first 19 days. The next 13 days was a recovery period. Recovery was established by applying the criterion of an affected hen laying 2 eggs on 3 consecutive days. Recovery so defined was a clear-cut phenomenon and all non-laying birds recovered within the 13 days following treatment. The last 12 days (days 14-25 after treatment) were designated as the post-treatment period. Seven reproductive parameters were recorded: egg laying rate, percent hatchability, and percent survival during the treatment and post-treatment periods and the number of days to recovery from pesticide effects (Table 6).

The influence of the solvent on reproduction was evaluated by comparing the egg production of four birds receiving acetone treated food (negative controls) with

TABLE 5.--Multiple regression analysis of the variation in food consumption attributable to pesticide intake rates.

Time Period	n	Contribution to Multiple R^2		
		Diazinon	Captan	Total
Treatment	60	0.546	0.009	0.555
Recovery	56	0.045	0.014	0.059
Post-treatment	51	0.003	0.000	0.003

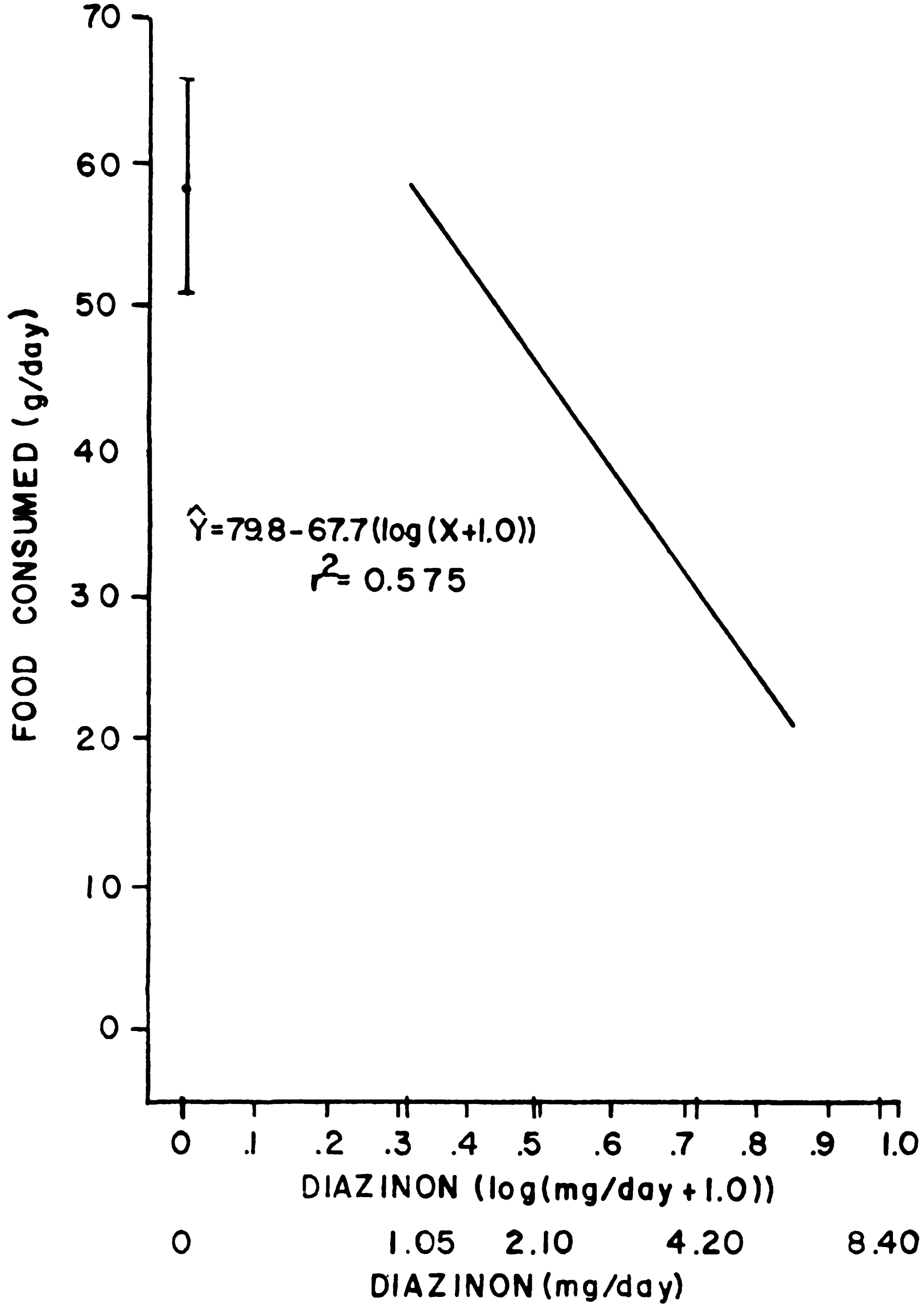

Fig. 3. Food consumption during pesticide treatment and Diazinon intake.

TABLE 6.--Actual doses, means, standard deviations, and sample size ( ) of reproductive parameters for untreated controls and planned Diazinon dose rates irrespective of captan and planned captan dose rates irrespective of Diazinon.

Pesticide Dose (mg/day)		Treatment Period			Days to Recovery	Post-treatment Period		
		Eggs/Day	Percent Hatchability	Percent Survival		Eggs/Day	Percent Hatchability	Percent Survival
Diazinon								
Planned	Actual							
0	0	0.68 ± 0.20 (15)	68.7 ± 21.5 (14)	70.5 ± 25.0 (14)	0.9 ± 3.6 (15)	0.82 ± 0.07 (14)	57.2 ± 25.0 (14)	73.9 ± 32.6 (13)
1.05	1.05	0.63 ± 0.18 (7)	80.7 ± 24.8 (7)	94.7 ± 7.3 (7)	2.6 ± 6.8 (7)	0.58 ± 0.19 (5)	35.0 ± 23.8 (5)	75.0 ± 50.0 (4)
2.10	2.10	0.64 ± 0.13 (10)	76.6 ± 20.7 (10)	77.2 ± 29.6 (10)	0.7 ± 1.4 (9)	0.58 ± 0.24 (8)	77.7 ± 17.5 (7)	73.9 ± 37.2 (7)
4.20	3.21	0.30 ± 0.18 (10)	58.7 ± 42.3 (10)	77.3 ± 26.8 (8)	5.1 ± 3.9 (9)	0.78 ± 0.16 (8)	45.0 ± 20.9 (8)	75.3 ± 23.7 (8)
8.40	4.38	0.15 ± 0.11 (10)	38.5 ± 37.5 (8)	88.9 ± 19.2 (3)	9.9 ± 4.0 (10)	0.68 ± 0.27 (10)	46.7 ± 31.4 (10)	86.0 ± 15.5 (8)
Untreated Controls		0.70 ± 0.18 (8)	57.3 ± 31.8 (8)	86.5 ± 13.8 (7)	0 (8)	0.70 ± 0.22 (7)	57.5 ± 33.6 (7)	87.8 ± 19.1 (6)
Captan								
Planned	Actual							
0	0	0.38 ± 0.26 (12)	71.3 ± 33.7 (10)	86.6 ± 15.7 (9)	6.0 ± 7.1 (12)	0.63 ± 0.24 (10)	53.0 ± 26.0 (10)	80.8 ± 21.3 (9)
2.5	2.5	0.72 ± 0.12 (7)	67.6 ± 24.6 (7)	90.1 ± 7.5 (7)	0.3 ± 0.8 (6)	0.80 ± 0.13 (5)	61.9 ± 18.7 (5)	69.8 ± 39.8 (5)
5.0	4.8	0.61 ± 0.21 (10)	79.9 ± 31.2 (10)	72.3 ± 23.7 (9)	0.4 ± 1.3 (9)	0.68 ± 0.26 (9)	61.3 ± 28.5 (9)	72.1 ± 36.4 (9)
10.0	7.9	0.51 ± 0.30 (10)	50.9 ± 35.4 (10)	60.0 ± 40.6 (8)	6.2 ± 5.4 (9)	0.74 ± 0.16 (8)	50.1 ± 20.7 (7)	63.5 ± 36.1 (7)
20.0	16.2	0.31 ± 0.27 (8)	50.1 ± 33.9 (7)	96.0 ± 8.9 (5)	5.2 ± 5.5 (8)	0.74 ± 0.25 (8)	38.6 ± 31.6 (8)	88.3 ± 20.4 (6)
40.0	32.2	0.46 ± 0.24 (5)	68.6 ± 20.7 (5)	71.4 ± 9.5 (4)	3.6 ± 4.9 (5)	0.78 ± 0.05 (5)	59.0 ± 34.9 (5)	86.9 ± 18.9 (4)

the production of four birds receiving only untreated food (positive controls). No difference was detected ($F=1.07$, $P>0.25$) and the two control groups were pooled in all further analyses.

The 7 reproductive measures were initially analyzed by multiple regression techniques to separate the effects of the independent variables. These variables were the actual Diazinon and captan dose rates, and in the case of egg production rates and recovery intervals, the egg laying performance of the birds on the 4 days preceding the experiment. Performance was coded as good, intermediate, or poor. The results of these 7 analyses showed that only laying rate during the treatment period and the days to recovery from pesticide effects were significantly ($P<0.001$) affected by the pesticide doses (Table 7). In addition, the variable contributing the majority of the explained variation was Diazinon in both cases. The multiple coefficient of determination (R^2) for the post-treatment laying rate was also significantly different ($P<0.001$) from zero. In this case, the pre-treatment laying performance explained most of the variation. This result was as expected: a bird's post-treatment performance was similar to its pre-treatment performance.

A decrease in laying rate with increasing doses of Diazinon began at or just beyond the 1.05 mg/day dose (Fig. 4). Therefore, a regression of egg laying rate

TABLE 7.--Multiple regression analysis of the variation in reproductive parameters attributable to predictor variables of pesticide ingestion and reproductive history of individual birds.

Reproductive Parameter	n	Contribution to Multiple R^2			
		Diazinon	Captan	Pre-treatment Performance	Total
Treatment Period Laying Rate	60	0.446	0.002	0.022	0.470
Treatment Period Hatchability	57	0.004	0.008	--	0.012
Treatment Period Survival	50	0.040	0.016	--	0.056
Days to Recovery	59	0.286	0.000	.0782	0.364
Post-treatment Laying Rate	52	0.059	0.049	0.198	0.306
Post-treatment Hatchability	51	0.011	0.011	--	0.021
Post-treatment Survival	42	0.016	0.006	--	0.023

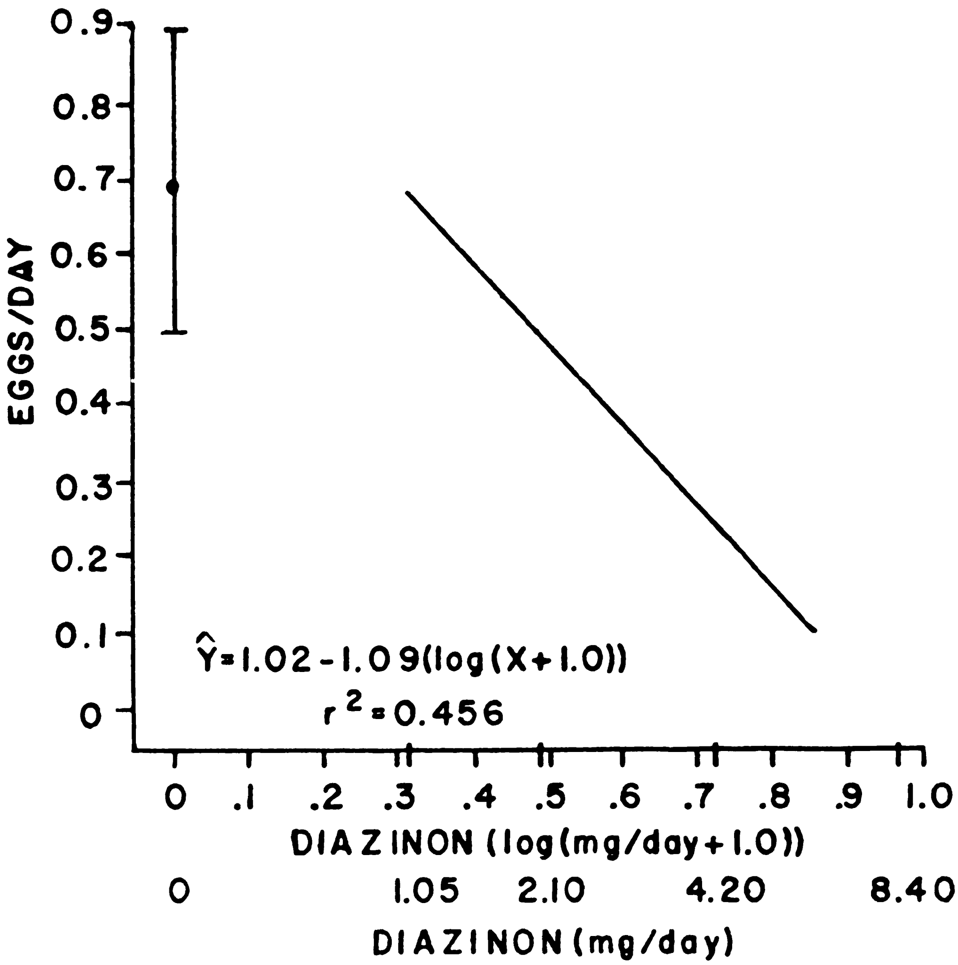

Fig. 4. The rate of egg production during pesticide administration and the daily consumption of Diazinon.

versus actual Diazinon dose was performed on those birds at the 1.05 mg/day and higher dose rates. This regression was significant ($P<0.001$) and explained 46% (r^2) of the variation. Doubling the dose rate decreased the laying rate by approximately 0.22 eggs/day.

The recovery interval increased with dose rate above 2.10 mg/day. The regression of recovery interval versus Diazinon level at 2.10 mg/day and higher doses was significant ($P<0.001$) and explained 57% (r^2) of the variation (Fig. 5). Doubling the dose rate increased recovery by approximately 7 days.

Whether the number of treatments influenced the pesticide effect was evaluated by comparing groups which received 4.20 mg/day of Diazinon and 10.0 mg/day of captan for 7 and 14 treatments. The two reproductive parameters affected by pesticide dose, laying rate during treatment and recovery interval, were used for this comparison. No significant differences were found for either measure between the two groups ($t=1.03$, 13 d.f. for laying rate and $t=-0.53$, 12 d.f. for recovery interval).

Body Weight Changes

Data on the body weights of the hens in the experiment (Table 8) were analyzed in two time periods: the treatment period and the remainder of the experiment. Screening by multiple regression showed that weight changes during both periods were influenced by Diazinon (Table 9).

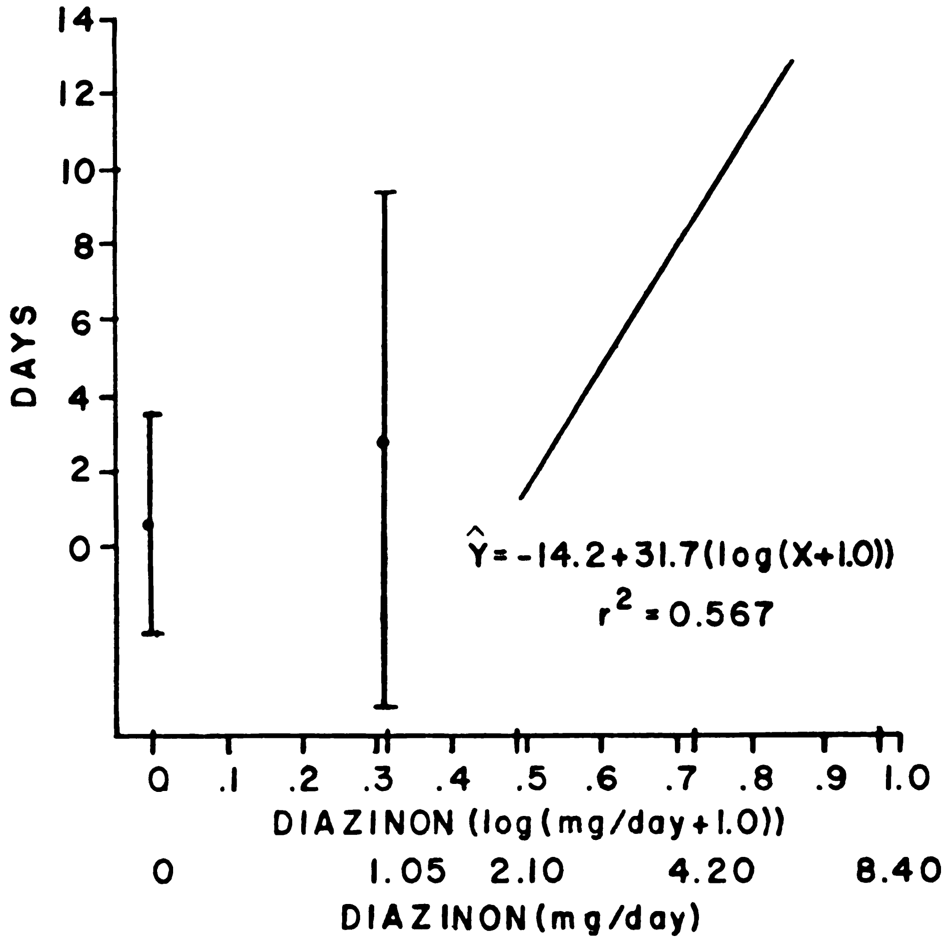

Fig. 5. The number of days to recovery from reproductive inhibition and the daily intake of Diazinon.

TABLE 8.--Actual doses, means, standard deviations, and sample size ( ) of percentage body weight changes for untreated controls and planned dose rates of Diazinon irrespective of captan and planned dose rates of captan irrespective of Diazinon.

Pesticide and Dose (mg/day)		(Weight Change/Pre-treatment Weight) X 100	
		Treatment Period	Post-treatment Period
Diazinon			
Planned	Actual		
0	0	-13.8 ± 6.2 (15)	0.7 ± 5.8 (15)
1.05	1.05	-14.0 ± 9.0 (7)	1.4 ± 19.2 (7)
2.10	2.10	-16.9 ± 6.9 (10)	-1.7 ± 5.4 (8)
4.20	3.21	-25.9 ± 6.1 (10)	12.8 ± 7.8 (9)
8.40	4.38	-32.9 ± 6.2 (10)	15.7 ± 6.8 (10)
Untreated Controls		-12.5 ± 4.4 (8)	2.3 ± 8.5 (8)
Captan			
Planned	Actual		
0	0	-23.9 ± 10.0 (12)	0.7 ± 19.5 (11)
2.5	2.5	-12.6 ± 10.3 (8)	-7.8 ± 13.2 (7)
5.0	4.8	-17.1 ± 6.1 (9)	-0.5 ± 8.7 (9)
10.0	7.9	-23.2 ± 8.2 (10)	7.3 ± 8.8 (9)
20.0	16.2	-23.9 ± 12.3 (8)	9.4 ± 7.2 (8)
40.0	32.2	-19.1 ± 8.8 (5)	0.7 ± 15.7 (5)

TABLE 9.--Multiple regression analysis of the variation in weight changes attributable to pesticide intake levels.

Time Period	n	Contribution to Multiple R^2		
		Diazinon	Captan	Total
Treatment	60	0.444	0.001	0.445
Post-treatment	56	0.198	0.029	0.227

A significant negative regression ($P<0.001$) of weight change versus actual Diazinon dose at 1.05 mg/day and above was found during the treatment period which accounted for 43% (r^2) of the variation (Fig. 6). During the remainder of the experiment, the relationship was also significant ($0.001<P<0.005$), but positive, and accounted for 26% (r^2) of the variation (Fig. 6). This latter relationship was reflective of recovery from the weight losses which occurred during the treatment period. The net result of these weight changes was a loss of approximately 13.5% for all birds, including controls.

Paired Data

Whether decreased food consumption adequately explained the decrease in laying rate, increase in recovery interval, and body weight changes was tested by analyzing the differences between pairs of birds receiving equal amounts of food with and without pesticides (Table 10). If food consumption adequately explained the observed effects, then there should have been no consistent differences in the performance of the pairs. On the other hand, a difference between the pair members which could be explained by pesticide dose would indicate an effect attributable to pesticides beyond that attributable to food consumption alone. Initial screening by multiple regression techniques showed that only egg laying rate

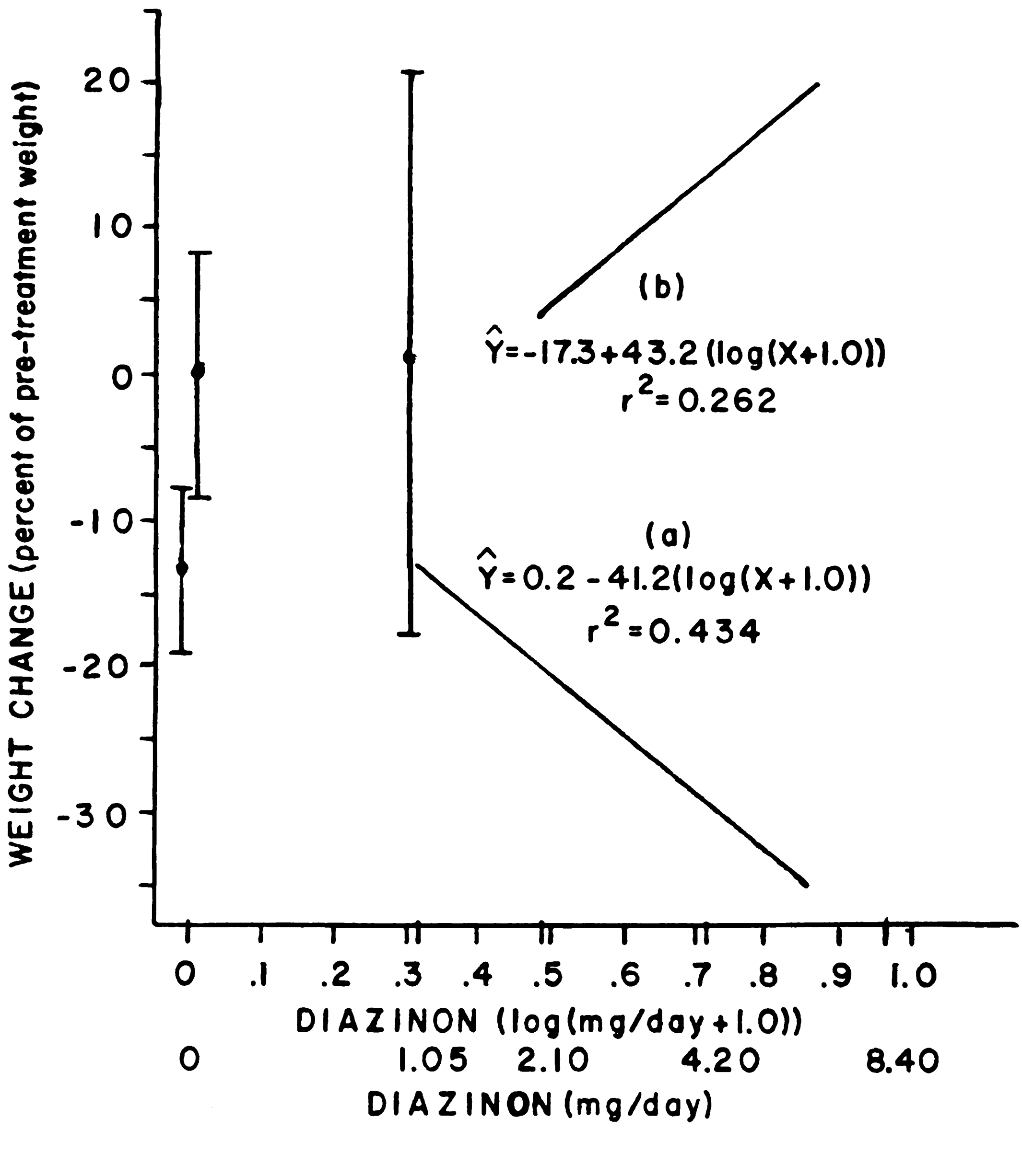

Fig. 6. Body weight changes of hens during (a) and after (b) pesticide administration and intake of Diazinon.

TABLE 10.--Means, maxima, and minima of measured differences in pesticide affected variables of birds paired by food consumption rates with one pair member receiving the indicated Diazinon dose rates, irrespective of captan dose rate, and the other receiving untreated food.

Diazinon Dose (mg/day)		Food Consumption (g/day)		Differences Between Treated and Untreated Pair Members			
						Percent Body Weight Change	
Planned	Actual	Treatment Period	Post-Treatment Period	Eggs/Day Treatment Period	Days to Recovery	Treatment Period	Post-treatment Period
0	0	59.7	70.2	0.15 (0.74, -0.16) (n=9)	0 (n=9)	5.4(19.2, -2.1) (n=9)	3.7(10.2,-3.8) (n=6)
1.05	1.05	58.4	65.8	-0.09 (0.32, -0.26) (n=4)	3.0(-6,18) (n=4)	2.1(17.2,-10.6) (n=4)	24.0(46.0, 2.0) (n=2)
2.10	2.10	53.9	64.8	-0.07 (0.11, -0.26) (n=4)	1.0(0, 4) (n=4)	1.3(6.8, -6.8) (n=4)	-0.3(2.1,-2.0) (n=3)
4.20	3.62	34.4	76.1	-0.12 (0.16, -0.37) (n=4)	2.3(-7,10) (n=3)	1.6(4.7, -1.1) (n=3)	3.7(7.8,-0.4) (n=2)
8.40	4.65	26.8	72.8	-0.21 (-0.11,-0.37) (n=3)	5.0(0, 10) (n=2)	-13.8(17.6,-17.2) (n=3)	8.1(18.3,-2.1) (n=2)

during the treatment period was influenced by pesticide treatment (Table 11). The multiple R^2 was low, but significantly different from zero ($P<0.05$). An analysis of covariance (Snedecor and Cochran 1967:420-425) was performed on the two groups and no difference between the slopes of the regressions of response on the Diazinon dose of the treated pair members was found. However, the difference in the heights of the lines corrected for the regression effect, while not significant, was suggestive of a possible effect ($0.05<P<0.10$). No difference was detected in recovery interval pair differences, but small sample sizes limited the statistical power to detect real differences. Body weight change pair differences were also analyzed, but no differences were detected.

TABLE 11.--Multiple regression analysis of the variation in differences between birds paired by food consumption levels attributable to the predictor variables of pesticide doses received by one member of each pair and the difference between the reproductive history of the pair members.

Pair Difference Measurement	n	Contribution to Multiple R^2			
		Diazinon	Captan	Pre-treatment Performance	Total
Treatment Period Laying Rate	24	0.207	0.078	0.048	0.333
Recovery Interval	22	0.020	0.127	0.038	0.185
Treatment Period Body Weight Change	23	0.035	0.048	--	0.083
Post-treatment Body Weight Change	16	-0.001	0.303	--	0.302

DISCUSSION

The decline in food consumption observed in the first experiment was probably caused by seasonal changes in energy expenditure for egg production. The faster rate of decline in the group receiving dieldrin agrees with the findings of Atkins and Linder (1967) and Genelly and Rudd (1956a,b), although no decline was evident with the dieldrin plus captan group. The absence of a decline in three of the groups was probably related to their initially low levels of consumption. The continued production of eggs by the Diazinon group despite its low food intake was surprising because of the additional energy needs of females producing eggs. Reduced food consumption during pesticide treatment was seen in the second experiment as well and it appears to be one of the primary effects of captan, dieldrin, and particularly Diazinon. In fact, the paired experiment results provide no evidence for an added effect attributable to pesticide treatment alone. This interpretation is made with caution, however, because the analysis of covariance on pair differences in laying rate during the treatment period was suggestive of such an added effect. In addition, the additive nature of food consumption effects as opposed to the greater than additive reproductive effects seen in the first experiment implies that more than energetics was involved in the lowered reproduction of the birds receiving the captan-Diazinon combination.

The relative magnitudes of reproductive effects in the first experiment (egg production>chick survival>hatchability) contrasts with the effects of alkyl mercurials (hatchability>egg production>chick survival) reported by Adams and Prince (1972) and Borg et al. (1969), and also the effects reported by Spann et al. (1972) (egg production>hatchability>chick survival). Previous studies on dieldrin have shown no consistency in the relative magnitudes of effects on these parameters. In the second experiment, egg production was also influenced, whereas hatchability and survival were not. The recovery of birds from pesticide induced reproductive inhibition in the second experiment shows that the effects of captan and Diazinon are not irreversible, rather they are short-lived and birds receiving these pesticides are not rendered permanently non-reproductive. The results of the 7 treatment experiment also support the non-cumulative nature of these pesticide effects in that no difference was detected between effects on birds receiving 7 or 14 treatments.

The absence of an observable interaction between Diazinon and captan in the second experiment as opposed to the strong interaction seen in the first poses an apparent contradiction in results. However, the two experiments were not strictly comparable in that the dose level used in the first experiment for the combination treatment group was not replicated in the second in favor of evaluating dose ratios close to those used in the field. In addition, the experimental birds came from two different sources and the pesticide formulations used were different. These considerations raise three possible explanations of the difference in results. First, the strains of birds might have had different susceptibilities to the pesticides. Second, differences in the inert fractions of the pesticide formulations might have influenced the results. Third, the interaction effect may be real, but in the second experiment, it might have been obscured by the strong Diazinon effect in the upper dose levels of Diazinon. Were the latter the case, then combinations of a relatively high dose of captan with a dose of Diazinon at or below the 2.10 mg/day level for Diazinon would be expected to show reproductive effects not predicted from the doses of the pesticides alone. In all cases of observed pesticide effects, it appears that a threshold at doses between 1.05 and 2.10 mg/day exists for Diazinon effects.

SPRING FOOD HABITS OF PHEASANTS

The pheasant is primarily associated with agricultural environments. Several investigations have shown that pheasants eat seed corn during the spring planting season (Fried 1940, Dambach and Leedy 1948, Trautman 1952). Agricultural seed has been implicated as a source of alkyl mercurial contamination of pheasants in both Sweden (Tejning 1967) and the Canadian prairie provinces (Fimreite et al. 1970). This study of the nesting season food habits of pheasants was undertaken to determine whether currently recommended non-mercurial seed treatments might be a potentially similar source of pesticide contamination.

METHODS

Pheasants were collected during May and June of 1972 and 1973 in the southwestern quarter of Ingham County and the eastern half of Hillsdale County, Michigan. Birds were collected from one hour after dawn until noon and from 1600 hours until dusk to maximize the probability of their having fed recently. During 1972, 14 days were spent collecting, while in 1973, 20 days were spent. Collection sites were chosen such that a freshly planted field with unsprouted seed, or sprouted corn less than 4 inches tall was within 1/8 mile. Body weights were recorded and crops and proventriculi were removed in the field for later quantification and pesticide residue analysis of the contents. Carcasses and digestive organs were immediately frozen on dry ice and later transferred to a freezer for storage.

The crop contents of each bird with more than 1.0 g of food were sorted into major categories and weighed. For each sex, weights of food items were then summed within categories and expressed as percentages of the total crop contents. The frequency of occurrence of food items was also recorded for each sex, including in this case, trace items (<0.1 g) which were identifiable, but not included in the weight analysis. The crop contents of those birds containing measurable quantities of corn and soybeans were analyzed for pesticide residues by the Pesticide Analytical Laboratory at Michigan State University. Analytical samples (entire crop contents or 8 g subsamples, whichever was smaller) were shaken for 15 minutes with 50 ml redistilled hexane in a 250 ml stoppered flask. The hexane was decanted into a beaker, an additional 15 ml hexane was added to the flask, swirled, and added to the original 50 ml of hexane. The hexane was then condensed to 10 ml and 1-5 μl was injected into a Beckman 72-5 gas chromatograph equipped with a 6' x 1/8" glass column packed with 3% SE-30 on 60/80 Gas Chrom Q. A Beckman electron capture detector was used under the following operating conditions:

Column temperature	210 C
Injector temperature	265 C
Detector temperature	290 C
Column flow (HE)	40 cc/min
Discharge flow (HE)	120 cc/min
CO_2 flow	0.9cc/min

The detection limit was approximately 0.05 ppm.

RESULTS

A total of 19 males and 16 females were collected approximately uniformly over time. Seven of these birds were collected in 1972 and 28 in 1973. Males averaged 1295 ± 20 g ($\bar{x}$ ± S.E.) whole body weight and females averaged 1052 ± 30 g. Body weights of males showed no trends over time. Testes weights decreased by 0.6 g/week ($0.01<P<0.025$) from May 11 to June 23. Whole body weights, ovary weights and the number of mature follicles (>6 mm) showed no evident decrease over time for the 12 hens which were in laying condition. The 4 hens which were not laying (no mature follicles) had ovary weights less than 2.0 g. Two of these birds collected during the first week of June had body weights close to the average for laying hens, while the two collected during the third and fourth weeks of June had weights 200 and 230 g below the lowest of the laying hens (930 g).

The contents of those crops and proventriculi containing food averaged 13.9 ± 2.8 g for 15 males while contents from 11 females averaged 16.2 ± 4.5 g. Agricultural foods (corn and soybeans) were most abundant in both sexes by weight and frequency (Table 12). The frequency of occurrence of food items was similar between

TABLE 12.--Crop contents of 15 male and 11 female pheasants collected during May and June of 1972 and 1973.

Food Item	Males		Females	
	Percent by Weight	Percent Frequency	Percent by Weight	Percent Frequency
Corn	62.8	93.3	37.3	81.8
Soybeans	18.6	60.0	31.4	54.5
Total Agricultural Food	81.4	93.3	68.7	81.8
Weed Seeds	15.4	20.0	tr	9.1
Unidentified Vegetation	3.2	80.0	14.2	72.7
Total Plant Material	100.0	100.0	82.9	100.0
Insects	tr	26.7	3.0	45.4
Snails			14.2	27.2
Earthworms			tr	18.2
Total Animal Material	tr	26.7	17.2	54.5

sexes except for animal materials which appear to be greater for females. Although the percentage by weight of food items between sexes appear to be different, the 2.3 g greater weight of crop contents in females was about equal to the weight of animal material which constituted 17.2 percent by weight of their diet. The mean weight of corn and soybeans was similar (11.3 g for males and 11.1 g for females) as was the mean weight of all vegetative material (13.9 g for males and 13.4 g for females).

The contents of crops from 13 male and 9 female pheasants containing corn and soybeans were analyzed for pesticide residues. Ten of these samples (6 males and 4 females) had detectable residues of one or more of the seed treatment pesticides, captan, Diazinon, and methoxychlor (Table 13). No dieldrin was detected. The ten birds with pesticide residues in their crop contents were collected relatively uniformly over the course of the collection period when the only probable source of these pesticides was treated corn seed.

TABLE 13.--Pesticide residue analysis of pheasant crop contents which had measurable quantities of pesticides.

Sex	Date Collected	Weight of Crop Contents (g)	Percentage of Agricultural Seed		Pesticide Residues (ppm)		
			Corn	Soybeans	Captan	Diazinon	Methoxychlor
Female	5/16/73	32.0	0.6	87.2		0.8	
Female	6/ 9/72	10.2	36.0		0.6		
Female	6/12/73	53.4	0.9	58.1	4.0		
Female	6/23/73	12.8	87.5		6.0		
Male	5/11/73	22.9	90.2	8.3	4.0		
Male	5/16/73	6.1	77.9	18.9	10.0	0.5	
Male	5/21/73	23.9	48.5	47.7	21.0		0.9
Male	6/ 6/73	4.6	87.9		0.2		
Male	6/23/73	8.5	82.6		1.0		
Male	6/23/73	26.5	89.2	10.8	38.0		0.8
Mean ($\bar{x} \pm$ S.E.)		20.1±4.8	60.1±11.5	38.5±12.8	9.4±4.2	0.7	0.9
n		10	10	6	9	2	2

DISCUSSION

The food habits observed in this study are in general agreement with previously published studies (Kopischke and Harris 1969, Fried 1940, Dambach and Leedy 1948, Trautman 1952), although the utilization of agricultural foods was somewhat higher. The specificity of collection sites probably influenced this result. The greater quantities of animal material by weight and frequency in females might represent an increased demand for protein by laying hens (Scott 1973).

Consideration of laboratory and field findings leads me to the conclusion that seed treatment pesticides represent a potential hazard to the reproductive performance of wild pheasants. Both sexes of pheasants were found to consume treated seed and thus, depending on the quantity of such seed available and actually consumed, the seed treatment pesticides currently in use represent a potential source of contamination to breeding pheasants similar to that documented for alkyl mercurials (Borg et al. 1969, Fimreite et al. 1970, Tejning 1967). Depending on the availability of treated seed, pheasants could consume Diazinon at levels exceeding the apparent threshold of effectiveness which lies somewhere between 1.05 and 2.10

mg/day. These levels correspond to a 70 g/day food intake, with 1/16 and 1/8 of it treated at the recommended rate, respectively. In addition, if captan decreases the level of Diazinon necessary for an effect, as suggested by the results of the first experiment, the widespread use of captan combined with limited use of Diazinon might represent an additional hazard.

Based on the population data summarized by Wagner et al. (1965), reduced laying rate or temporary reproductive inhibition induced by seed treatments might alter the recruitment rate of a population by causing reduced clutch size, nest abandonment due to an insufficient number of eggs in the clutch to elicit incubation behavior, or a delay in nesting phenology. Any one or all of these alterations would be manifested as a reduction in fall densities and age ratios.

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