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RESPONSES OF STARLINGS
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At weekly intervals, one of 26 groups of 16 starlings (Sturnus vulgaris) each was fed ad libitum a diet containing 0.00, 2.50, 5.00, 7.50, 10.00, 12.50, 18.75, 25.00, or 50.00 ppm p, p-DDT (2, 2-bis-(p-chlorophenyl)-1, 1, 1-trichloroethane) for 9 days. The birds were then subjected to a conditioning process whereby they had to respond to the flash of a red light by depressing two perches in sequence to obtain the treated food. On the 5th day of conditioning, 54 ten-second intervals were presented in which a correct response was rewarded with an opportunity to feed. The success of a bird in acquiring the response was measured by the number of intervals in which the feeding occurred on that day. On days 6 through 12 the birds were in holding cages and had free access to food without added DDT. On the 13th day, birds were retested to assess residual effects of DDT.

Dietary levels of 12.50, 25.00, and 50.00 ppm DDT significantly ($\underline{P} < 0.01$) inhibited the ability of starlings to acquire the conditioning response during May and June 1972. An analysis of data collected from July 1972 through April 1973 for birds fed 0.00, 2.50, 5.00, 7.50, 10.00, 12.50, 18.75, and 25.00 ppm DDT showed that DDT had no significant ($\underline{P} > 0.05$) effect on the ability of starlings to acquire the conditioning response. A comparison of data recorded during May and June 1972 with those recorded from December 1972 through April 1973 for birds fed 12.50 and 25.00 ppm DDT indicated that starlings were more sensitive to the effects of DDT during the normal period of intensive reproductive activity (May and June) than from December through April.

An analysis of data for retention of the conditioning response indicated that the ability of starlings to retain a conditioning response was not dependent on the concentration of DDT in the diet.

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Responses of Starlings

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TABLE OF CONTENTS

INTRODUCTION	1
METHODS AND MATERIALS	5
Capture and Care of Starlings	5
Treatments	5
Conditioning Equipment	6
Conditioning Technique	7
Test for Acquisition of Response	8
Test for Retention of Response	8
RESULTS	10
DISCUSSION	20
LITERATURE CITED	25

LIST OF TABLES

<u>Table</u>		<u>Page</u>
1.	Duration, frequency, and number of feeding intervals during conditioning.	9
2.	Analysis of variance of test scores for acquisition of the conditioning response by starlings fed a diet containing either 0.00, 12.50, 25.00, or 50.00 ppm DDT. Data were collected during May and June 1972.	12
3.	Treatment levels, observations per treatment, and mean scores for acquisition of the conditioning response by starlings. Data were collected during May and June 1972.	13
4.	Regression analysis of test scores for acquisition of the conditioning response by starlings fed a diet containing either 0.00, 2.50, 5.00, 7.50, 10.00, 12.50, 18.75, or 25.00 ppm DDT. Data were collected between June 1972 and May 1973.	14
5.	Treatment levels, observations per treatment, and mean scores for acquisition of the conditioning response by starlings. Data were collected between June 1972 and May 1973.	15
6.	Analysis of variance of test scores for acquisition of the conditioning response by birds fed a diet containing either 12.50 or 25.00 ppm DDT. Data recorded May and June 1972 were analyzed as separate treatments from data recorded from December 1972 through April 1973.	16
7.	Differences between means of test scores for acquisition of the conditioning response recorded during May and June 1972 and from December 1972 through April 1973.	17

Table

Page

8. Regression analysis of test scores for retention of conditioning response by starlings. These birds scored 36 or more on the test for acquisition of the response. Data were collected between May 1972 and May 1973. 18
9. Treatment levels, observations per treatment, and mean scores for retention of the conditioning response by starlings. These birds scored 36 or more on the test for acquisition of the response. Data were collected between May 1972 and May 1973. 19

EFFECTS OF DDT ON CONDITIONING RESPONSES OF STARLINGS

INTRODUCTION

This is a report on an investigation of the effects of *p, p'*-DDT (2, 2-bis-(*p*-chlorophenyl)-1, 1, 1-trichloroethane) on conditioning responses of starlings (*Sturnus vulgaris*).

Widespread contamination of the biosphere resulted from extensive use of pesticides after WW II (Dustman and Stickel 1966, Westlake and Gunther 1966). It appears certain that the rate of introduction of pesticides into the environment will continue to increase in the foreseeable future (Westlake and Gunther 1966). Although nearly all living organisms harbor residues of persistent pesticides, the full impact of these chemicals in the environment is not known (Dustman and Stickel 1966). Environmentalists are concerned especially about the use of chlorinated hydrocarbon pesticides and the effects of these chemicals on wildlife. The residues of the chlorinated hydrocarbons are persistent (Macek 1970, Woodwell et al. 1971) and mobile (Cohen and Pinkerton 1966) in the environment. The lipophilic characteristic of these chemicals facilitates movement of residues from the environment into biological systems (Wurster 1969, Macek 1970). Residues of chlorinated hydrocarbon pesticides persist in and are concentrated by the biota (Macek 1970).

An obvious effect of pesticides on wildlife is death of contaminated animals (Rosene 1965, Wurster et al. 1965). Subtle and more deleterious effects are expressed in the form of reproductive inhibition and ensuing reduction of avian populations (Hickey and Anderson 1968, Wurster 1969, Cade et al. 1971). Hunt (1966) noted that animals containing residues of pesticides often exhibit no overt signs of intoxication, and he emphasized the need to determine real hazards to contaminated individuals, their progeny, and animals higher in the food chain.

Warner et al. (1966) suggested that measurement of pesticide-induced changes in behavior of organisms might provide data that would be useful in predicting the impact of pesticides on the environment and for diagnosing the class of pesticides in an animal population. Results of recent investigations indicate that measurement of changes in behavior caused by pesticides supplies useful data for assessing the effects of pesticides on animals. James and Davis (1965) reported that bobwhite quail (Colinus virginianus) maintained for 8 and 16 weeks on a diet containing 20 ppm DDT committed significantly more errors than controls in a visual discrimination program. Burt (in press) conditioned coturnix quail (Coturnix coturnix) to receive a reward for the first response after a specified period following the last reward (premature responses were not penalized).

The quail were then dosed with 8.4 mg 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)/kg body weight. A reduction in response rate and number of rewards received began 40 min later. A significant decrease in these parameters was noted 2 days after treatment. Burt (in press) reported that rats (Rattus norvegicus) dosed with 16.7 mg dieldrin/kg body weight also performed fewer responses and received fewer rewards in a similar situation. Burt (in press) conditioned rats to withhold responses for 32 sec after the last response (premature responses were penalized). The rats then were dosed with 2.5 and 5.0 mg dieldrin/kg body weight. These rats received significantly fewer rewards for up to 2 days after administration of the pesticide. Burt (in press) found that rats exposed to either an acute dose (16.7 mg/kg) or chronic doses (5 ppm for 120 days or 20 ppm for 60 days) of dieldrin experienced significant impairment of performance during maze training when compared with controls. Sandler et al. (1969) trained sheep (Ovis aries) to delay a conditioned response for 30 sec after the last response to obtain food. The sheep were then given daily doses of dieldrin (20 mg/kg) for 3 and 4 days. A large decrease in performance of the task followed. Three of four sheep tested recovered their pre-exposure response levels within 10 days. These sheep were then given daily doses of dieldrin (5 mg/kg) for 3, 5, or 7 days. Again,

a large decrease in performance of the task was observed.

Van Gelder et al. (1969) reported that sheep receiving daily doses of dieldrin (10 mg/kg for 66, 86, or 89 days) required significantly more trials than controls to relearn a visual discrimination task.

Anderson and Peterson (1969) exposed brook trout (Salvelinus fontinalis) to 20 ppb DDT in water for 24 hrs. When subjected to a conditioning process, untreated fish learned to avoid an electrical shock. Fish treated with DDT failed to acquire the response.

Warner et al. (1966) treated goldfish (Carassius auratus) for 4 days in water contaminated with 1.8 and 0.44 ppb toxaphene [mixture of chlorinated (67 to 69 percent) camphenes]. The fish treated with 1.8 ppb toxaphene responded more strongly to stimuli (a light and an electrical shock) than controls. After an additional 7 days in the toxicant, the fish treated with 1.8 ppb again responded more strongly than controls.

Even though pesticide contamination in passerines is known to be extensive (Martin 1969), no data were available on behavioral toxicology of pesticides in perching birds. Such data should prove useful in assessing the impact of pesticides on passerines and consumers of passerines.

METHODS AND MATERIALS

Capture and Care of Starlings

Starlings were captured with mist nets placed among holly trees (Ilex spp.) in a winter roost at Wilsonville, Oregon. The starlings were banded and randomly assigned to a position in the experiment. Prior to being placed on treatments, the birds were held in 1.5- × 3.7-m aviaries. Turkey-starter ration and water were provided ad libitum.

Treatments

At weekly intervals, starlings were placed on diets of turkey-starter ration into which different amounts of DDT were incorporated. Treated food was prepared by tumbling 20 g peanut oil (into which the desired quantity of DDT was dissolved) with 980 g turkey-starter ration for 1 hour. Concentrations of DDT used in this study were 0.00, 2.50, 5.00, 7.50, 10.00, 12.50, 18.75, 25.00, and 50.00 ppm of 99⁺ percent pure 2, 2-bis-(p-chlorophenyl)-1, 1, 1-trichloroethane.

Sixteen starlings were divided into four groups of four birds each and housed in four 30- × 91- × 36-cm hardware cloth cages spaced 30 cm apart. One group served as a control and was provided with turkey-starter ration ad libitum; the other three groups were fed diets containing DDT. After 9 days, the birds were

transferred to individual cages in which they were required to perform a specific response to obtain food.

Conditioning Equipment

Equipment was available for measuring behavioral changes in perching birds. Langowski et al. (1969) and Arhart (1972) used the equipment to determine the repellent properties of acoustic and visual stimuli to starlings. Birds were conditioned to perch-hop on cue for a reward. Aversive stimuli were presented, and the time required to recover the conditioned response was used as a measure of the repellent properties of the stimuli. The equipment and techniques used by these investigators were modified for this study to enable quantification of pesticide-induced changes in behavior of starlings.

The 30- × 76- × 30-cm conditioning cages were arranged in two tiers of four cages in each of two 2.4 × 3.0- × 2.4-m sound-proofed rooms. Plywood partitions between cages prevented visual contact between birds. The conditioning cages also were constructed of hardware cloth. Each cage was equipped with a front perch, a rear perch, a food cup, and a water cup. The water cup was attached to the exterior of the rear panel of the cage; slots in the rear panel allowed free access to water. The food cup was located to the exterior of the front panel. A plexiglass door, operated by a 6-volt DC solenoid, barred access to the food cup. Two 10-watt red lights,

situated 90 cm apart and 90 cm in front of the cages, were readily visible from within all cages in each room.

Semi-automatic control of the conditioning equipment was accomplished by use of three timers. The master timer, activated from 0530 to 1930 each day, controlled power to overhead lights in the conditioning rooms and to two subordinate timers. One subordinate timer controlled the duration of an interval in which power was supplied to the conditioning cages, the red lights, and to two 20-pen event recorders. During this interval (the feeding interval), depression of the rear perch closed circuits to the solenoid and a pen of a recorder. The solenoid opened the plexiglass door, and for the rest of the interval, a bird could feed through the open door while standing on the front perch. The depression of the front perch also closed a circuit to a pen of a recorder. After the feeding interval, all doors closed and remained so until the first correct response during a subsequent interval. The second subordinate timer (an automatic reset type) controlled the time between feeding intervals.

Conditioning Technique

A flash of the red lights signaled the beginning of each feeding interval. During the first day of conditioning, a 30 sec feeding interval occurred once each minute. As a result of their spontaneous activity in the conditioning cages, birds inadvertently depressed the

rear perch during many feeding intervals and were able to obtain food. Some birds developed an association between the flash of the red lights, the depression of the rear perch, and access to food. These birds responded to the flash of the red lights by depressing the rear perch and quickly moving to the front perch. The response was then strengthened by decreasing the frequency and duration of the feeding interval at the end of each day of conditioning (Table 1).

Test for Acquisition of Response

The success of a bird in acquiring a conditioning response was measured by the number of intervals in which feeding occurred during the 5th day in the conditioning cage.

Test for Retention of Response

After 5 days in the conditioning cages, the birds were placed in holding cages identical in size and arrangement to the treatment cages. The starlings had free access to turkey-starter ration without added DDT. After 7 days, the birds were returned to their respective conditioning cages, and their performance was monitored on the next day. The success of a bird in retaining a conditioning response was measured by the number of intervals in which feeding occurred during that day.

Table 1. Duration, frequency, and number of feeding intervals during conditioning.

Day ^a	Duration of feeding interval (seconds)	Time between feeding intervals (minutes)	Feeding intervals per day
1	30	0.5	840
2	25	2.5	288
3	20	5.0	157
4	15	10.0	82
5 ^b	10	15.0	54
13 ^c	10	15.0	54

^aBirds were in holding cages from day 6 through 12.

^bNumber of intervals in which a bird fed during this day was used as a measure of the success of the bird in acquiring the conditioning response.

^cNumber of intervals in which a bird fed during this day was used as a measure of the success of the bird in retaining the conditioning response.

RESULTS

Test scores for acquisition of the conditioning response were recorded during May and June 1972 for 50 birds fed a diet containing either 0.00, 12.50, 25.00, or 50.00 ppm DDT. An analysis of variance of the data indicated that DDT in the diet had a highly significant ($P < 0.01$) effect on scores of the birds (Table 2). Studentized Range tests (Snedecor and Cochran 1967) revealed that the mean score of controls (0.00 ppm) was significantly ($P < 0.05$) greater than the mean scores of birds fed a diet containing either 12.50 or 50.00 ppm DDT (Table 3). The difference between the mean scores of controls and birds fed a diet containing 25.00 ppm DDT was not significant ($P > 0.05$). However, data from birds consuming DDT were pooled, and the difference between the mean of the pooled data and the mean score of controls was highly significant ($P < 0.01$) (Table 3).

To determine the maximum sensitivity of the experimental technique, concentrations of 2.50, 5.00, 7.50, 10.00, and 18.75 ppm DDT were added as treatments to be examined in the study. The concentration of 50.00 ppm was excluded from further study. Test scores for an additional 250 birds were obtained between June 1972 and May 1973. Regression analysis of these data showed that the test scores were not dependent on concentrations of DDT in the diet ($P > 0.05$) (Table 4). There were no significant ($P > 0.05$) differences

between mean scores for acquisition of the conditioning response of birds treated during this period (Table 5).

Mean scores of birds consuming 12.50 and 25.00 ppm DDT in the diet during this period were higher than the means of scores recorded during May and June 1972 for birds fed the same concentrations of DDT. An analysis of variance (Table 6) and tests for difference between means (Table 7) indicated that the mean of scores collected from December through April was significantly ($\underline{P} < 0.05$) greater than the mean of scores collected for birds consuming 12.50 ppm DDT in the diet during May and June. The difference between the means of scores of birds fed a concentration of 25.00 ppm in the diet was not significant ($\underline{P} > 0.05$).

Birds that obtained food during 36 or more of the feeding intervals on the 5th day of conditioning were considered to have successfully learned the response. One-hundred and seventy-nine of these birds were retested between May 1972 and May 1973 to measure the retention of the response. A regression analysis of these data revealed that the ability of starlings to retain the conditioning response was not significantly ($\underline{P} > 0.05$) dependent on the concentration of DDT in the diet (Table 8). There were no significant ($\underline{P} > 0.05$) differences between mean scores for retention of the conditioning response (Table 9).

Table 2. Analysis of variance of test scores for acquisition of the conditioning response by starlings fed a diet containing either 0.00, 12.50, 25.00, or 50.00 ppm DDT. Data were collected during May and June 1972.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	49	11099.50		
Treatment	3	3692.85	1230.95	7.65 ^a
Error	46	7406.65	161.01	

^aSignificant effect ($P < 0.01$).

Table 3. Treatment levels, observations per treatment, and mean scores for acquisition of the conditioning response by starlings. Data were collected during May and June 1972.

Treatment (ppm DDT)	Observations per treatment	Mean ^a score
0.00	10	40.10±4.01
12.50	11	16.45±3.82 ^b
25.00	17	28.10±3.08
50.00	12	18.75±3.66 ^b
All levels combined	40	22.10±2.01 ^c

^a± one standard error.

^bSignificantly different from control mean ($\underline{P} < 0.05$).

^cSignificantly different from control mean ($\underline{P} < 0.01$).

Table 4. Regression analysis of test scores for acquisition of the conditioning response by starlings fed a diet containing either 0.00, 2.50, 5.00, 7.50, 10.00, 12.50, 18.75, or 25.00 ppm DDT. Data were collected between June 1972 and May 1973.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	249	106624.68		
Regression	1	550.23	550.23	1.29 ^a
Error	248	106074.45	427.72	

^aNot significant ($P > 0.05$).

Table 5. Treatment levels, observations per treatment, and mean scores for acquisition of the conditioning response by starlings. Data were collected between June 1972 and May 1973.

Treatment (ppm DDT)	Observations per treatment	Mean ^a score
0.00	63	38.59±2.61 ^b
2.50	34	35.88±3.55
5.00	31	34.90±3.72
7.50	33	38.39±3.80
10.00	30	35.60±3.78
12.50	15	35.33±5.34
18.75	27	27.26±3.98
25.00	17	39.71±5.02

^a± one standard error.

^bNo treatment means significantly different from the control mean ($\underline{P} > 0.05$).

Table 6. Analysis of variance of test scores for acquisition of the conditioning response by birds fed a diet containing either 12.50 or 25.00 ppm DDT. Data recorded during May and June 1972 were analyzed as separate treatments from data recorded from December 1972 through April 1973.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	59	29411.73		
Treatment	3	4038.38	1346.13	2.97 ^a
Error	56	25373.35	453.10	

^aSignificant effect ($P < 0.05$).

Table 7. Differences between means of test scores for acquisition of the conditioning response recorded during May and June 1972 and from December 1972 through April 1973.

Treatment (ppm DDT)	Treatment period	Observations per treatment	Mean score	Difference between means ^a
12.50	May-June 1972	11	16.45	18.88±8.45 ^b
12.50	Dec 1972-Apr 1973	15	35.33	
25.00	May-June 1972	17	28.12	11.59±7.30
25.00	Dec 1972-Apr 1973	17	39.71	

^a ± one standard error.

^b Significant difference ($P < 0.05$).

Table 8. Regression analysis of test scores for retention of conditioning response by starlings. These birds scored 36 or more on the test for acquisition of the response. Data were collected between May 1972 and May 1973.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Total	178	38450.03		
Regression	1	27.84	27.84	0.13 ^a
Error	177	38422.19	217.07	

^aNot significant ($P > 0.05$).

Table 9. Treatment levels, observations per treatment, and mean scores for retention of the conditioning response by starlings. These birds scored 36 or more on the test for acquisition of the response. Data were collected between May 1972 and May 1973.

Treatment (ppm DDT)	Observations per treatment	Mean ^a score
0.00	48	39.69±2.13 ^b
2.50	21	42.76±3.22
5.00	19	33.58±3.38
7.50	24	42.92±3.01
10.00	19	46.21±3.38
12.50	12	38.92±4.25
18.75	13	42.31±4.09
25.00	20	36.30±3.29
50.00	3	39.33±8.51

^a± one standard error.

^bNo treatment means significantly different from the control mean ($P > 0.05$).

DISCUSSION

During conditioning, mortality was 14, 15, 10, 18, 13, 31, 33, 24, and 75 percent of the starlings fed a diet containing 0.00, 2.50, 5.00, 7.50, 10.00, 12.50, 18.75, 25.00, and 50.00 ppm DDT, respectively. All birds appeared to be in good health when placed in the conditioning cages. Birds that acquired the response (scored 36 or more) maintained their health throughout conditioning. However, birds that failed to learn the response were unable to obtain an adequate supply of food. Most of these birds became severely emaciated and 59 percent of them died. It was assumed that all deaths resulted from failure of the birds to learn the response rather than as a direct result of DDT intoxication. Birds that died before the 5th day of conditioning were assigned a test score of 0.

An analysis of variance (Table 2) and comparison of treatment means (Table 3) provided strong evidence that dietary levels of 12.50, 25.00, and 50.00 ppm DDT inhibited the ability of starlings to acquire a conditioning response during May and June 1972. Comparable results were reported by James and Davis (1965). They found that 20 ppm DDT in the diet of bobwhites for 8 and 16 weeks adversely affected the performance of the birds in a visual discrimination program.

Although the mean score of birds consuming 25.00 ppm DDT

during May and June 1972 was not significantly ($\underline{P} > 0.05$) different from controls (Table 3), the average score of the last 11 observations of birds fed 25.00 ppm was 19.82. Therefore, it appeared likely that with a larger sample size, the mean score of birds fed 25.00 ppm would approach the mean score of birds fed 12.50 and 50.00 ppm DDT.

A regression analysis (Table 4) of the scores collected between June 1972 and May 1973 showed that DDT had no effect on the ability of starlings to acquire a conditioning response. The observations for birds consuming 12.50 and 25.00 ppm DDT were obtained from December 1972 through April 1973. A comparison of these data with those collected in May and June 1972 (Table 7) showed a significant increase in the score of birds fed 12.50 ppm DDT. Although the increase in the score of birds fed 25.00 ppm DDT was not significant ($\underline{P} > 0.05$), the mean score (39.71) of these birds was larger than the mean score (37.90) of controls for December through April.

These results suggested that starlings were more susceptible to the effects of DDT in May and June than in December through April. Davis et al. (1964) reported that rufous-sided towhees (Pipilo erythrophthalmus) were more susceptible to doses of DDT in March than in November.

Because this study spanned 11 months, differences existed in age and maturity of groups of birds. It is not known if these differences contributed to the seasonal effect indicated by the results.

Schwab et al. (1968) reported seasonal changes in the susceptibility of starlings to DRC-1339 (3-chloro-p-toluidine hydrochloride). They found that mortality among adult and juvenile starlings of known age dosed with the avicide was greatest in July and least in September; mortality among birds treated in December and March was intermediate to mortality observed in July and September. These investigators suggested that the seasonal rhythm in susceptibility of birds to the avicide was not a function of age.

May and June is a period of intensive reproductive activity for starlings (Kessel 1957). The starlings in this study did not breed, lay eggs, or nest while in captivity. However, the birds apparently were more sensitive to the effects of DDT during this normal period of reproductive activity (May and June). Wurster et al. (1965) reported that mortality among robins (Turdus migratorius) in an area treated with DDT was greatest during the breeding season and diminished during early summer. In contrast to these findings, Gish and Chura (1970) found that during the first 10 days on treatments of 700, 922, 1214, and 1600 ppm DDT, Japanese quail in breeding condition were affected less than birds not in breeding condition.

Starlings were captured in a winter roost in 1971 and again in 1972. Birds traveled up to 20.6 miles daily from the roost in search of food. (O. E. Bray, personal communication, 6 May 1974). Starlings collected in a nearby area in the winter of 1968 averaged 3.78

ppm DDT and metabolites plus 0.93 ppm dieldrin and 0.21 ppm heptachlor epoxide (1, 4, 5, 6, 7, 8, 8, -heptachloro-2, 3, -epoxy-2, 3, 3a, 4, 7, 7a-hexahydro-4, 7-methanoindane) (Martin 1969). When DDT was removed from the diet of starlings, they excreted 75 percent of the assimilated DDT within 2 weeks (Harvey 1967). All birds used for tests in May and June were held in captivity at least 3 months before treatments began. Time in captivity for birds used for tests in December through April ranged from 3 days to 2 months. Although no analysis of residues in tissues of birds was conducted in this study, it seems reasonable to assume that residues were excreted while the birds were maintained in captivity, and that immediately before treatments, residues were below those reported by Martin (1969). No apparent differences occurred in the scores of controls when newly captured birds were tested. Therefore, it appeared likely that residues in birds at the time of capture had little influence on the results of this study.

An analysis of the retest data provided no evidence to suggest that the ability of starlings to retain a conditioning response was effected by DDT in the diet. Goldfish exposed to 1.8 ppb toxaphene exhibited a stronger retention of a conditioned aversive response than controls (Warner et al 1966). In contrast to these findings, Van Gelder et al. (1969) reported that sheep exposed to dieldrin were less able to recall a visual discrimination task than controls.

Results of this study indicate that future investigations of the effects of pesticides on animals should account for the seasonal susceptibility of animals to these effects.

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