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DDT THINS SHELLS OF EGGS FROM MALLARD DUCKS MAINTAINED ON *AD LIBITUM* OR CONTROLLED-FEEDING REGIMENS

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Mallard ducks were fed diets containing various levels of technical DDT or chemically pure *p,p'*-DDT, or dieldrin. Either technical DDT or *p,p'*-DDT at 20 ppm or greater, or dieldrin at 10 ppm caused a statistically significant reduction in eggshell thickness, weight, and calcium. Shells of eggs from ducks fed 40 ppm of *p,p'*-DDT were about 20% thinner than those from control ducks, and shells of eggs from ducks fed 10 ppm of dieldrin were about 6% thinner than those from controls. The reduction in eggshell thickness was linear with increasing dose of DDT to 40 ppm, and with increasing dose of dieldrin through all levels studies. Eggshell thinning occurred regardless of whether the diets containing DDT were fed under *ad libitum* or controlled conditions. DDT fed at 200 ppm was lethal to the ducks. Neither DDT nor dieldrin affected weight of the eggs or rate of egg production.

Heath *et al.* (1969b) first provided experimental evidence that DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] and DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene] caused eggshell thinning in mallard ducks (*Anas platyrhynchos*). In their experiments, shells of eggs from ducks fed diets containing 25 ppm of DDT or 40 ppm of DDE were about 14 percent thinner than shells of control eggs. Subsequently, Tucker and Haegele (1970) observed that shells of eggs from mallards fed 30 ppm of DDT were 4.9 percent thinner than those of control eggs, and Longcore *et al.* (1971) observed that shells of eggs from black ducks (*Anas rubripes*) fed 30 ppm of DDE were 23.5 percent thinner at the equator than those of control eggs. 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) fed at four ppm (Muller and Lockman 1972) and ten ppm (Lehner and Egbert 1969) of the diet has also been shown to thin shells of eggs from mallards by about four percent.

Smith *et al.* (1970) observed an 11 percent decrease in shell thickness of eggs from chickens fed ten ppm of DDT. Subsequent research by Cecil *et al.* (1972) and Davison and

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Sell (1972), in which the DDT levels included and greatly exceeded (range two to 300 ppm) those fed by Smith, revealed no measurable effects of DDT, DDE, or dieldrin on shell thickness of chicken eggs. About the same time as these reports were published, Sauter and Steele (1972) reported that ten ppm of dietary DDT reduced shell thickness in chicken eggs by nine percent, compared with the experimental controls.

The reasons for the variability among the findings of researchers concerning the effect of DDT on shells of eggs from chickens are not apparent. Even though the chickens used in all research cited were White Leghorns, it is possible that certain strains of this breed may be more susceptible to the effects of DDT than others. Also, it is possible that other factors, unknown and therefore uncontrollable, may interact to produce eggshell thinning in chickens under certain circumstances.

The present study was conducted to determine if shell thickness of eggs produced by ducks would be affected by dietary DDT when the ducks were kept under environmental conditions similar to those of the chickens. Two experiments were conducted—one in which ducks in pens were fed *ad libitum* diets containing various levels of technical DDT, chemically pure *p,p'*-DDT, or dieldrin, and one in which ducks in individual wire cages were fed various levels of *p,p'*-DDT in a fixed amount of feed.

Methods

Experiment 1. One hundred and eighty, two-year-old game farm mallard females (Frost Game Farm, Coloma, WI), were divided equally among 30 indoor pens. Each pen measured 5.5 m × 11 m and had a solid concrete floor that was covered with wood shavings. Each pen contained a suspended automatic waterer, suspended self-feeder, and three-compartment nestbox. Initially, two drakes were added to each pen, but, because of fighting, one drake was removed.

Ten treatments were randomly assigned, with three pens on each treatment, to the 30 pens of ducks. The treatments consisted of a control diet and the same diet supplemented with technical DDT[1] at 2, 20, and 200 mcg/g of diet (ppm); *p,p'*-DDT[2] at 2, 20, and 200 ppm; or dieldrin[3] at 1, 5, and 10 ppm. The insecticides were dissolved in acetone and premixed into three kg of feed. This premix was then mixed with 37 kg of feed to yield a 40-kg batch each time that feed was mixed. An equal amount of acetone was mixed in the control diet in the same manner. Mixing of both the premix and the final diet continued for approximately 15 min each while the acetone evaporated. The diets were assayed to verify addition of the insecticides. DDT and dieldrin were not detected in the control diet; limits of detection for this assay were about 0.02 ppm for DDT and 0.01 ppm for dieldrin.

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[1]Obtained from Olin Matheson Chemical Co., and contained about 70% *p,p'*-DDT and 20% *o,p'*-DDT on analysis. No corrections for purity were made in the diets.

[2]Obtained from Aldrich Chemical Co., Inc., lot No. 100571, 99% pure as stated by the manufacturer.

[3]Obtained from Shell Chemical Co.; about 87% HEOD on analysis, but enough was used to supply 1, 5, or 10 mg HEOD/g of diet.

The basic diet fed during the laying season was formulated to contain about 19% protein, 2.7% calcium, 0.5% inorganic phosphorus, and 2.74 kcal metabolizable energy/g and consisted of ground corn, soybean meal, tallow, fish solubles, dehydrated alfalfa meal, limestone, dicalcium phosphate, DL-methionine, a salt and trace mineral premix, and a vitamin premix. The diet fed during the nonlaying season was formulated to contain about 14% protein, 2.6% calcium, 0.5% inorganic phosphorus, and 2.55 kcal metabolizable energy/g. This diet contained ground oats in addition to the ingredients mentioned previously. The diets were fed *ad libitum* at all times, and artificial lighting supplied 13 to 15 hr of light per day during the laying season.

Pesticide feeding was begun May 12, 1971, and terminated April 18, 1972. Eggs for experimental purposes were collected May 13 through June 23, 1971, and March 8 through April 18, 1972. Eggs were collected daily, dated, and stored in a cooler with a relative humidity of 70 to 75%. At biweekly intervals, the eggs were weighed individually, candled to reveal cracks, and arranged in numbered flats according to date laid. With the aid of a random-digits table, about one-third of the eggs were selected for shell thickness measurements. Thickness was measured at two locations along the equator of the egg, with the membrane removed (Davison and Sell 1972). Eggshells, including membranes, were then dried overnight in a forced-draft oven at 70°C and weighed. The dried eggshells were crushed, and their calcium content was determined after ashing by direct EDTA titration using hydroxynaphthol blue as an indicator (Hurwitz and Griminger 1960, Hurwitz 1969).

Experiment 2. Seventy-two, one-year-old game farm mallard females were placed in individual wire cages, 30 cm × 40 cm × 45 cm high, of a type used for laying hens. The ducks were randomly and equally allotted to one of six treatment groups. Treatments consisted of control A, control B, and technical DDT fed at 2, 20, 40, and 200 ppm. The higher protein diet used in Experiment 1 was fed, and DDT was added in acetone as previously described. Eighty grams of feed was fed daily to each duck beginning March 6, 1972, the start of the experiment, through May 23, 1972. Seventy grams of feed was fed daily to each duck from May 24, 1972, when laying had virtually ceased, through May 28, 1972, when the experiment was terminated.

Control group B was carried as a precaution. It was anticipated that ducks fed 200 ppm of DDT might not consume all feed offered because of perception of the DDT or intoxication. Should reduced feed consumption have occurred, feed offered ducks in control B would also have been reduced in an attempt to equalize nutrient intake.

Eggs were collected daily and weighed. At the end of the first biweekly period, one egg was taken at random from those laid by each duck and its shell thickness, weight, and calcium measured as described. Because of a low rate of egg production, shells of all eggs laid subsequent to the first biweekly period were measured. For statistical and graphical purposes, the data were examined at biweekly intervals. Thus, the biweekly value for each duck would represent an average obtained from one or more eggs.

Statistics. The data from both experiments were analyzed by least squares analysis of variance, using a model similar to that described by Davison and Sell (1972). Significance was assessed at probabilities of five percent or less, and significance among treatment means was determined by the method of Duncan (1955). In Experiment 1, data collected in 1971 and 1972 were analyzed separately because of the imbalance in the data created by the loss of five pens of ducks between 1971 and 1972.

Results

Experiment 1. Results of Experiment 1 are shown in Table I. Indicated in the table is the number of ducks that survived through each of the egg-collection periods. Many deaths occurred, the majority after the end of the egg-collection period in 1971 and before the start of the egg-collection period in 1972. One duck fed 20 ppm of *p,p'*-DDT died the ninth day of the experiment for unknown reasons. Ducks fed 200 ppm of *p,p'*-DDT and 200 ppm of technical DDT began dying on the 35th and 48th days, respectively. The last duck fed 200 ppm of *p,p'*-DDT died on the 167th day, while one duck fed 200 ppm of technical DDT survived through the second breeding season. In nearly all of these cases tremors began a few days before death, and DDT poisoning was the probable cause of death. Seven deaths occurred at the start of the second breeding season; these deaths appeared to be related to fighting between males and females and among females. A duck fed two ppm of *p,p'*-DDT died in April 1972 for an undetermined reason.

Body weights of ducks surviving at the end of six weeks or at the end of the experiment were similar among all groups. However, carcasses of ducks that died with signs of DDT poisoning were emaciated. In 1971, feed consumption appeared greater for control ducks than for some of the ducks fed insecticides; however, due to variable feed wastage, feed consumption could not be measured accurately. In 1972, it was obvious that the duck and drake remaining on 200 ppm of technical DDT consumed less feed than any of the remaining groups of ducks.

Egg production did not appear to be affected by the insecticides in either 1971 or 1972. However, egg production was considerably higher in 1972 than in 1971. Average egg weight was about 62 g during both years, and egg weight was not affected by the insecticides. In 1972, more cracked or broken eggs were gathered from ducks fed 20 ppm of technical DDT or *p,p'*-DDT than from control ducks. The proportion of cracked or broken eggs from ducks fed 200 ppm of technical DDT was not so obviously increased, but only one female survived to lay eggs during the second year. The proportion of cracked or broken eggs collected from ducks fed two ppm of either kind of DDT or from ducks fed dieldrin was similar to that from control ducks. Statistics were not applied to the data on cracked eggs. In view of the effects of the insecticides on eggshell thickness, weight, and calcium, this was judged unnecessary.

Shells of eggs from ducks fed two ppm of technical DDT or *p,p'*-DDT, or one and five ppm of dieldrin were not significantly thinner or lighter than those from control ducks during either 1971 or 1972. In 1971, shells of eggs from ducks fed 20 ppm of

Table I. *Survival, Feed Consumption, Egg Production, and Some Characteristics of Eggs from Ducks Fed Ad Libitum Diets Containing Various Levels of DDT or Dieldrin for 343 Days (Experiment 1)*

Item	Control	Technical DDT		
		2 ppm	20 ppm	200 ppm
1971				
Surviving ducks[2]	18	18	18	18
Avg initial body weight of duck hens, kg	1.23	1.25	1.17	1.16
Avg body weight at end of 6th week, kg	1.10	1.15	1.12	1.10
Duck days[2]	756	756	756	756
Feed consumed/duck day, g	115	111	104	102
Eggs/duck day	0.27	0.25	0.29	0.20
Cracked or broken eggs, %	3.0	0	0	5.8
Avg egg weight, g	60.5	61.4	61.3	62.7
Avg eggshell weight, g	5.22	5.51	4.95	4.33A[3]
Avg eggshell thickness, mm $\times 10^{-2}$	31.9	31.8	29.9	25.1A
1972				
Surviving ducks[2]	16	18	17	1
Avg body weight at end of experiment, kg	1.23	1.26	1.23	1.48
Duck days[2]	676	756	720	42
Feed consumed/duck day, g	130	133	116	62A
Eggs/duck day	0.69	0.66	0.54	0.69
Cracked or broken eggs, %	2.6	2.6	5.4	3.5
Avg egg weight, g	61.2	63.0	62.1	58.7
Avg eggshell weight, g	5.45a	5.41a	4.89bC	3.86D
Avg eggshell thickness, mm $\times 10^{-2}$	33.0a	32.9a	30.1bC	26.1D
Avg eggshell calcium, %	36.6a	36.5a	36.5a	36.0bC

[1] Standard error of treatment means.

[2] Numbers for survivors and duck days apply only to the 6-wk periods while eggs were being collected.

[3] Means followed by different superscript letters are significantly different: for lower case letters, $P \leqslant 0.05$; for upper case letters, $P \leqslant 0.01$.

p,p'-DDT			Dieldrin			SE[1] of
2 ppm	20 ppm	200 ppm	1 ppm	5 ppm	10 ppm	means
18	17	16	18	18	18	
1.19	1.24	1.22	1.19	1.16	1.25	
1.06	1.11	1.12	1.12	1.09	1.16	
756	723	745	756	756	756	
115	115	105	106	106	111	± 6.6
0.32	0.30	0.24	0.25	0.30	0.39	± 0.04
3.7	4.4	4.1	3.8	1.8	1.4	
64.3	65.6	62.4	62.5	64.5	62.5	± 0.79
5.58	5.13	4.42[A]	5.45	5.07	5.23	± 0.19
31.2	30.3	26.8[A]	31.7	31.3	30.4	± 0.70
17	17	0	18	18	14	
1.32	1.28		1.23	1.26	1.33	
719	714		756	756	588	
131	131		119	129	122	± 6.0
0.66	0.68		0.71	0.79	0.77	± 0.04
2.1	12.1		3.2	3.0	3.5	
62.0	63.6		64.5	61.8	63.0	± 0.58
5.37[a]	4.75[bC]		5.58[a]	5.24[ab]	5.19[b]	± 0.11
32.3[a]	29.1[C]		32.8[a]	31.7[ab]	31.0[b]	± 0.49
36.5[ab]	36.3[b]		36.6[a]	36.5[ab]	36.3[b]	± 0.09

technical DDT or *p,p'*-DDT, or ten ppm of dieldrin were not significantly thinner or lighter than shells of eggs from control ducks. In 1972, however, shells of eggs from ducks fed 20 ppm of technical DDT or *p,p'*-DDT, or ten ppm of dieldrin were significantly thinner and lighter than those from control ducks. Shells of eggs from ducks fed 200 ppm of technical DDT or *p,p'*-DDT were significantly thinner and lighter than shells of eggs from all other groups of ducks in 1971, and shells of eggs from the duck fed 200 ppm of technical DDT were significantly thinner and lighter than shells of eggs from all other groups of ducks in 1972. Eggshell thinning amounted to about ten percent for 20 ppm and 20 percent for 200 ppm of either kind of DDT in the diet, and to about six percent for ten ppm of dieldrin.

Average shell thickness and weight for the respective groups did not differ appreciably between 1971 and 1972. Eggshell calcium, measured only in 1972, was significantly lower in shells of eggs from ducks fed 200 ppm of technical DDT, 20 ppm of *p,p'*-DDT, and ten ppm of dieldrin than in shells of eggs from controls.

Experiment 2. Results of Experiment 2 are shown in Table II. The ducks assigned to group control B were not needed for the purpose described. Treatment of these ducks was then identical to that of ducks in group control A; but since the data were collected from control B as a distinct group, data from control B are itemized in the table. One duck fed 200 ppm of DDT died during the tenth week of the experiment, and another died immediately after completing the experiment; both exhibited tremors prior to death.

The initial body weight of these ducks was lower than that of the ducks used in Experiment 1. Essentially, body weight was maintained on this restricted feeding regimen and was not affected by dietary DDT. Egg production was quite variable in this experiment and was lower than that observed in Experiment 1. As in Experiment 1, dietary DDT did not significantly affect egg production or weight. Beginning with 20 ppm in the diet, the proportion of cracked or broken eggs increased markedly with each increment of dietary DDT.

Shells of eggs from ducks fed two ppm of DDT were similar in thickness, weight, and calcium content to those from control ducks, while shells of eggs from ducks fed 40 ppm of DDT were significantly thinner, lighter, and lower in calcium than those from ducks fed 20 ppm of DDT. Shells of eggs from ducks fed 200 ppm of DDT were similar in thickness, weight, and calcium content to those from ducks fed 40 ppm of DDT.

The development of lighter and thinner eggshells with time on experiment is shown in Fig. 1. The effects of feeding 40 and 200 ppm of DDT were obvious by the second to fourth week, and the effects of feeding 20 ppm of DDT were clear by the sixth to eighth week.

Discussion

The effects of DDT on eggshell thinning were quite obvious and consistent in these two experiments. There were no detectable differences between the effects of chemically

Table II. *Survival, Egg Production, and Some Characteristics of Eggs from Ducks Fed Equal Amounts of Feed Containing Various Levels of p,p-DDT (Experiment 2)*

Item	Control[1] A	Control[1] B	*p,p'*-DDT 2 ppm	*p,p'*-DDT 20 ppm	*p,p'*-DDT 40 ppm	*p,p'*-DDT 200 ppm	SE[2] of means
Surviving ducks	12	12	12	12	12	11	
Avg initial body weight, kg	0.97	1.01	0.97	1.01	0.99	1.03	
Avg final body weight, kg	0.91	1.00	0.96	1.05	0.94	0.93	
Duck days $\times 10^{-2}$	10.08	10.08	10.08	10.08	10.08	9.93	
Eggs/duck day	0.112	0.094	0.092	0.072	0.045	0.094	± 0.015
Cracked or broken eggs, %	0	2.1	0	4.1	11.1	16.1	
Avg egg weight, g	51.4	55.2	51.9	54.7	57.4	53.8	± 1.09
Avg eggshell weight, g	4.40^{ab}[3]	4.55^{a}	4.28^{ab}	4.19^{b}	3.72^{c}	3.45^{c}	± 0.14
Avg eggshell thickness, mm $\times 10^{-2}$	31.0^{a}	30.2^{ab}	29.8^{ab}	28.5^{b}	24.6^{c}	24.7^{c}	± 0.72
Avg eggshell calcium, %	36.4^{a}	36.3^{a}	36.2^{a}	36.0^{a}	35.2^{B}	35.2^{B}	± 0.19

[1] Both control groups were randomly drawn and were treated identically during the experiment.

[2] Standard error of treatment means.

[3] Means followed by different superscript letters are significantly different: for lower case letters, $P \leqslant 0.05$; for upper case letters, $P \leqslant 0.01$.

pure *p,p'*-DDT and technical DDT on the parameters measured, nor did it seem to make any difference whether the DDT was fed in diets under *ad libitum* or controlled conditions. By inspection of the data in Tables I and II, one can see a remarkably linear relationship between dose of DDT up to 40 ppm and eggshell thickness. The mean shell thickness of eggs from ducks fed up to 40 ppm of DDT was subtracted from the respective control means for both experiments, and these values were used to calculate a regression of eggshell thinning *vs.* DDT concentration in the diet. The regression for change in shell thickness was Y (mm $\times$ 10^{-2}) = 0.09 + 0.133 X (ppm of DDT in the diet). A regression for change in shell thickness *vs.* dieldrin in the diet was calculated in the same manner, giving the equation Y (mm $\times$ 10^{-2}) = 0.05 + 0.172 X (ppm of dieldrin in the diet). Two things about these regressions stood out. First, linear regression gave a good fit, implying that, at least over the region investigated, the effects of DDT and dieldrin were expressed at lower levels of feeding as well as at higher levels. Second, the intercept was very small, suggesting that the regression could be extrapolated from the lowest feeding level to the level of the control diet. This implies that the effects of dieldrin or DDT on eggshell thinning do not change abruptly below the lowest feeding level and there is no threshold below which there are no effects. The effect of dietary DDT on eggshell thinning in

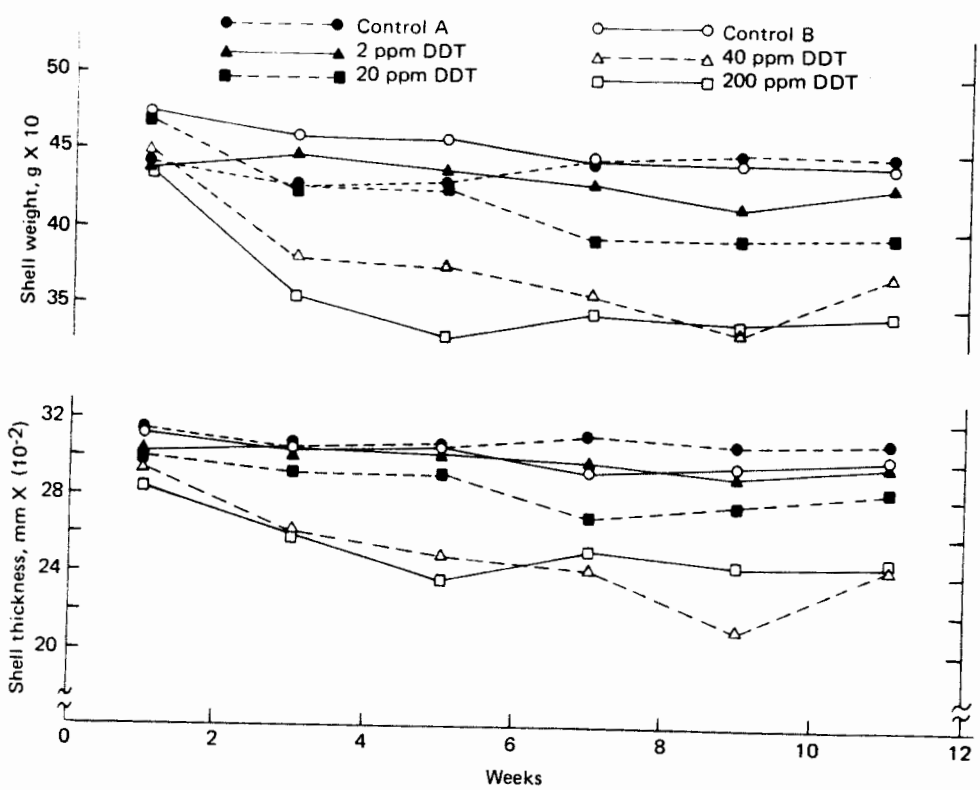

Fig. 1. Shell weight and thickness of eggs from ducks fed chemically pure *p,p'*-DDT. Interaction of DDT level X time significant, $P < 0.01$.

mallards was linear to a point somewhere between 40 and 200 ppm, and the effect of dietary dieldrin was linear to a point in excess of ten ppm. Dieldrin was not fed in amounts greater than ten ppm because we anticipated that it might then be lethal (Davison *et al.* 1970).

DDT fed at 200 ppm ultimately proved to be lethal to the ducks but did not cause measurable shell thinning of eggs produced prior to death above that caused by 40 ppm of DDT. Heath *et al.* (1969b) have reported that dietary DDT at 40 ppm was lethal to ducks. We did not observe deaths when 40 ppm of DDT was fed; but deaths might have occurred had we fed 40 ppm of DDT for a period of time longer than 12 weeks. Heath *et al.* (1969) did not report how long they fed 40 ppm of DDT before deaths occurred.

Eggshell weight and total calcium (g) per eggshell were diminished by dietary DDT or dieldrin by approximately the same percentage magnitude as shell thickness. On the other hand, while the percentage of calcium contained in the dried eggshells was significantly reduced by dietary DDT or dieldrin, the magnitude of change of this percentage figure was very small. Eggshell thinning obviously contributed to increased cracking and breaking of the eggs.

Effects of DDT and dieldrin on reproduction of the ducks will be reported later. Residues of total DDT, DDD, and DDE in the plucked and ground carcasses of the ducks of Experiment 1 averaged 0.26 ppm (fresh weight basis) in the controls; 4.4, 58.5, and 508.8 ppm in ducks fed 2, 20, and 200 ppm of technical DDT; and 5.2 and 52.2 ppm in ducks fed 2 and 20 ppm of *p,p'*-DDT. Dieldrin residues averaged 3.0, 11.5, and 17.9 ppm in the carcasses of ducks fed 1, 5, and 10 ppm of dieldrin; dieldrin residues were not detected in carcasses of control ducks. In a nationwide survey conducted in 1965-66, Heath (1969a) detected only trace amounts (below 0.05 ppm) of dieldrin and detected an average of 1.04, 0.31, 0.17, and 0.84 ppm for total DDT, DDD, and DDE in wings of wild mallards from the Atlantic, Mississippi, Central and Pacific flyways, respectively. Our duck carcasses averaged 16% lipid and 62% moisture, and Heath's duck wings averaged 11% lipid and 36% moisture. If we assume that the DDT and dieldrin residues were uniformly distributed in the lipid of duck carcasses and wings, the average residues found in our experimental ducks fed two ppm of DDT or one ppm of dieldrin would be higher than residues reported by Heath (1969a), and it would appear that wild mallards would normally be ingesting smaller amounts of DDT and dieldrin than were fed in our experiments.

These results substantiate and extend the observations and conclusions of previous investigators (Heath et al. 1969b, Tucker and Haegele 1970, Longcore *et al.* 1971, Muller and Lockman 1972, Lehner and Egbert 1969) that both DDT and dieldrin cause thinning of shells of eggs from mallard ducks. In experiments with ducks, dietary DDT has consistently caused eggshell thinning. The results of these experiments contrast with results of experiments with chickens in which dietary DDT has not consistently caused eggshell thinning.

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Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

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