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SOME SUBLETHAL EFFECTS OF pp'-DDT AND ITS METABOLITE pp'-DDE ON BREEDING PASSERINE BIRDS

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by

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ABSTRACT

A passerine bird, the Bengalese Finch, *Lonchura striata*, was used to study the effect of pp'-DDT on the various parameters of the reproductive cycle of a nidicolous species. One hundred and two birds were fed measured quantities of pp'-DDT in the diet for six weeks before pairing. The test diets were then continued until fledging of the young had occurred. A further fifty-eight birds were used as controls. Clutch sizes in the DDT-fed pairs were similar to those of the controls, but fertility, hatchability, and fledging success were reduced. Several other effects of the DDT were noted. These were longer periods prior to ovulation, longer incubation and rearing periods, lighter eggs and smaller newly-hatched young. Tests with a further fifty-two birds showed that feeding pp'-DDE produces similar sublethal effects to those noted with the parent product.

Introduction

The detection of residues of organochlorine insecticides in nearly all British avian wildlife specimens (Moore & Walker, 1964; Prestt, 1967) led to the British Nature Conservancy undertaking experimental work to evaluate their sublethal effects. pp'-DDE, the main metabolite of pp'-DDT, was found in 99% and pp'-DDT itself in 27 % of the specimens analysed. Thus research was concentrated on the effects of these two materials. The subject chosen for study was the reproductive success of the Bengalese Finch, a domesticated form of the Sharp-tailed Finch *Lonchura striata* (Passeriformes : Estrildidae).

Experimental method

Two hundred and twelve Bengalese Finches, 106 of each sex, were placed seperately in cages (61 × 41 × 38 cm) provided with grit, cuttlefish, water and canary and millet seed. The age of the birds was 200 - 300 days (i.e. sexually mature) and mean body weight was 14.13 g. The colony was maintained at 16° C. The rearing food vehicle for the insecticides, the method of dosing for 2 hours daily and the calculation of the daily dose rate for each bird were

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as used by Jefferies, 1967. Eleven concentrations of the pp'-DDT/rearing food mixture were prepared and fed to 102 birds (20, 18, 4, 14, 4, 4, 18, 10, 6, 2, and 2 on diets containing 75, 100, 150, 200, 300, 350, 450, 525, 600, 1200 and 10,000 parts per million of pp'-DDT by weight respectively). Ten concentrations of the pp'-DDE/rearing food mixture were prepared and fed to 52 birds (4, 6, 6, 10, 4, 4, 6, 2, 6 and 4 on diets containing 12, 25, 50, 100, 150, 300, 500, 1000, 2000 and 5000 ppm of pp'-DDE respectively). A further 58 birds were placed on control diet.

All birds were maintained on these test diets for six weeks before being paired with another fed at the same dietary level. The insecticide diet was then continued until fledging or death of the young had occurred. Certain pairs were allowed to lay a second clutch to increase data on egg weight but most were sacrificed at this point.

Two pairs of birds were sacrificed for analysis at 6 weeks and one pair with eggs were sacrificed at laying in both DDT and DDE-fed groups. Others were analysed at the end of the experiment. Analysis was by Gas-liquid chromatography (for method see Jefferies & French, 1969).

Dose rate groups and mortality

The 47 pairs fed pp'-DDT were divided into three dose rate groups ((A) up to 50, (B) 51-250 and (C) above 250 μg/day/bird) for purposes of analysis (see Table 1). Two pairs in group B laid no eggs and the females died one and two months after pairing. In the lethal dose rate group C (mean 1,205 μg/day) death occurred in one or both birds of each of the 5 pairs within 6 weeks. As Prestt *et al* (in press) have shown that Bengalese Finches at 16° C would consume 4.401 g of food daily, the mean dose rates, 34, 139 and 1205 μg/day amount to 8,32 and 274 ppm in the total diet.

The 24 pairs fed pp'-DDE were also divided into three dose rate groups ((D) up to 50, (E) 51-320 and (F) above 320 μg/day/bird). In the lethal dose rate group F (mean 840 μg/day) one or both birds of each of the 3 pairs died before eggs were laid. The mean dose rates, 18, 168 and 840 μg/day amount to 4,38 and 191 ppm in the total diet.

Effect of pp'-DDT on reproduction

Clutch size and characteristics of the eggs laid

The mean clutch size for all DDT-fed pairs was not significantly different to that of the control pairs (Table 1). However, there was a tendency for large clutches to be more common among pairs fed DDT (control 21 %, low dose rate 32 %, high dose rate 44 % of clutches were of 6/7 eggs).

TABLE 1

Effect of pp'-DDT and pp'-DDE on reproductive success

	Dose rate µg/day/bird (mean)	Days between pairing and laying (range)	Mean clutch size (total eggs)	Mean number fertile	Mean number hatching	Mean number fledging	Probabi of differe from con
Control (29 pairs)	None	13.9 (8–28)	4.86 (141)	3.83	3.72	3.59	
Low DDT dose (22 pairs)	1–50 (34)	17.5 (4–47)	4.82 (106)	3.41	3.00	2.27	P < 0.1
High DDT dose (20 pairs)	51–250 (139)	24.8 (4–73)	4.75 (95)	3.40	3.00	1.45	
All DDT dosed birds (42 pairs)	1–250 (84)	21.0 (4–73)	4.79 (201)	3.40	3.00	1.88	P < 0.1
Low DDE dose (15 pairs)	1–50 (18)	21.0 (7–99)	4.53 (68)	2.60	2.40	2.07	P < 0.1
High DDE dose (6 pairs)	51–320 (168)	26.5 (9–54)	6.17 (37)	2.17	1.83	0.83	
All DDE dosed birds (21 pairs)	1–320 (61)	22.6 (7–99)	5.00 (105)	2.48	2.24	1.71	P < 0.1

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The eggs were weighed on the day of laying (see Table 2). As the dose rate increased the eggs laid became lighter in weight. A Student's test showed this reduction in weight to be significant ($t = 2.509$; DF 490; $P < 2$ %). In the Bengalese Finch the weight of an egg is correlated with the order of laying as successive eggs laid tend to be progressively heavier (control : 1.119, 1.167, 1.175, 1.205, 1.222 g for eggs 1 to 5). However, the difference in weight between eggs laid by controls and dosed birds is not due to difference in clutch size as the eggs laid by dosed birds also follow an ascending weight series but are lighter in weight (1.096, 1.139, 1.161, 1.164, 1.194 g).

TABLE 2

Mean egg weight of control, DDT and DDE-dosed birds

Doserate of female	DDT		DDE	
	Number	Mean Wt. ± St E	Number	Mean Wt. ± St E
Control	160	1.185 ± 0.009	160	1.185 ± 0.009
1- 50 µg/day	212	1.162 ± 0.007	162	1.172 ± 0.010
51-100 µg/day	65	1.154 ± 0.015	9	1.094 ± 0.036
101-300 µg/day	55	1.146 ± 0.014	27	1.075 ± 0.026
All dosed birds	332	1.158 ± 0.006	198	1.156 ± 0.009

With regard to shell weight, previous work (Jefferies, 1969) has indicated that at a given egg weight (above 1.08 g) an egg laid by a DDT dosed bird has a higher percentage shell weight than one laid by a control.

Fertility

Only 70.8 % of the 106 eggs laid by the low dose rate group were fertile compared to 78.7 % of the 141 control eggs (Table 1). Surprisingly, this percentage was not increased in the high dose rate group and remained very similar at 71.6 %. The dose rates of DDT fed, appear to reduce fertility by about a tenth. The first and second eggs laid have the highest infertility rate (35 %, DDT; 26 % control).

Embryonic mortality

Only 3 of the 111 fertile eggs produced by the control pairs failed to hatch (97.3% hatching). In the DDT-fed birds this number was 17 out of 143 fertile eggs 88.1 % hatching). The percentage

hatching from all eggs laid was reduced from 76.6% to 62.7 %. Again there was a close similarity between the results obtained with both high and low dose rates of DDT. The dose rates of DDT fed appear to reduce hatching of fertile eggs by about a tenth. The dead embryos were mostly almost completely developed but showed no obvious teratisms.

Chick survival :

Four out of the 108 chicks reared by the control pairs died before fledging (96.3 % survival). In the DDT-fed groups, chick survival was reduced to 75.8 % and 48.3 % with low and high dose rates respectively (62.7 % overall). Thus survival was considerably lower than in the control group and is related to the dose rate (up to 30 μg/day, 3.0; over 150 μg/day, 0.9 fledglings per nest). The dose rates of DDT fed, reduce chick survival by about a third.

In the low and high DDT dose rate groups 33 % and 52 % of the chick deaths occurred within a day of hatching whereas none of the control chicks died during this period. The crops of dead chicks were always found empty and no muscular trembling of the type noted with adults dying of DDT poisoning was seen in chicks of any age.

The weights of newly hatched chicks were taken to ascertain whether the lighter eggs of the DDT-fed birds produced smaller chicks than those of the controls. Each nest was examined at 09.00 hours each day and the first chicks hatched were weighed and compared to their egg weight at laying (each egg was numbered to facilitate identification). A comparison of chick to egg weights in the control pairs showed that as the egg weight decreased so did that of the chick hatched (Table 3). Although this same relationship was found to exist in DDT-fed groups, the chicks weight from a standard egg of 1.0 g decreased from 0.799 (control) to 0.753 (low dose) and 0.699 g (high dose). Thus, as well as the chicks of DDT-fed pairs being smaller because of their lighter eggs, it appears that they are smaller at corresponding egg weights.

Duration of the breeding cycle :

The time taken to complete the three periods of the breeding cycle, pairing to laying, incubation and rearing the young, was measured for each of the pairs used.

The relationship between dose rate of DDT and the time taken from pairing to ovulation has already been discussed by Jefferies (1967). As the dose rate of the female increases there is significant ($P < 1$ %) increase in the delay between pairing and ovulation. The present experiment confirms this conclusion (Table 1).

With regard to incubation times, these vary with the clutch size and the position of the egg in the clutch. Eggs are usually laid

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TABLE 3

The relationship between egg weight and weight of newly hatched young from pairs of birds fed control, DDT and DDE diets

	Equation	Significance of correlation coefficient	egg wt. in g. (x)			
			0.8	1.0	1.1	
Control	$y = 0.524x + 0.275$	$P < 1\%$	0.694	0.799	0.851	—
DDT 1- 50 µg/day	$y = 0.742x + 0.011$	$P < 1\%$	0.605	0.753	0.827	— Chick wt.
DDT 51-250 µg/day	$y = 0.754x - 0.055$	$P < 0.1\%$	0.548	0.699	0.774	— in g. (y)
DDE dosed	$y = 1.096x - 0.387$	$P < 0.1\%$	0.528	0.709	0.819	—

TABLE 4

The mean concentrations of DDT + DDE in the livers of test birds at the time of pairing, laying (♀ only) and fledging of the young, in one complete clutch taken at time of laying, in non-hatching eggs and in dead one-day-old chicks are given together with lethal concentrations for DDT and DDE and the contamination level of the control. The mean dose rate given is for the individuals analysed or for the parents of the eggs and chicks.

Dose rate	Specimen	Mean dose rate (µg/day)	Mean days on dose	Number tested	ppm DDT + DDE
Control	At fledging	—	108	10	0.08 + 0.69
	Non-hatching eggs	—	—	13	0.17 + 0.65
DDT low dose	At pairing	29	42	2	13.9 + 5.8
	At laying	48	64	1	8.6 + 9.7
	At fledging	32	103	10	10.3 + 10.0
	1 complete clutch	48	—	5	21.4 + 19.1
	Non-hatching eggs	33	—	17	17.9 + 24.5
DDT high dose	At pairing	161	42	2	16.9 + 8.1
	At laying	189	87	1	37.2 + 29.6
	At fledging	134	100	10	39.8 + 36.2
	1 complete clutch	189	—	6	124.8 + 61.7
	Non-hatching eggs	155	—	20	66.9 + 42.8
	Dead chicks	197	—	18	114.7 + 97.4
DDT lethal dose	Dying	734	43	10	176.1 + 84.5
DDE low dose	At pairing	34	42	2	— 21.8
	At fledging	18	102	5	— 15.8
	Non-hatching eggs	18	—	13	— 43.9
DDE high dose	At pairing	109	42	2	— 151.3
	At fledging	145	142	5	— 119.4
	Non-hatching eggs	53	—	1	— 98.3
DDE lethal dose	Dying	869	122	5	— 658.4

at 24 hour intervals until the full clutch has been produced. Incubation takes just over 16 days (control, 16.22 days; DDT, 16.21 days in 4, 5, 6 egg clutches) except for the first two eggs which take longer as incubation does not start until the third egg has been laid. With 4,5 and 6 egg clutches the mean incubation time for the firstegg was 16.8, 17.3 and 18.0 days respectively in the control and 18.0, 18.1 and 18.3 days in DDT-fed pairs. This difference is present but not so marked in the second egg and suggests that incubation times for the first two eggs are longer in DDT-fed pairs than in controls.

Two results for the duration of the rearing period were obtained for each pair. The time taken from the hatching of the first egg to the fledging (i.e. voluntarily leaving the nest box) of the first young and the corresponding time for the last bird. In the control pairs, the mean rearing time was 25.1 $\pm$ 0.3 days (range 19 - 29) and in the DDT-fed pairs, 26.2 $\pm$ 0.4 days (range 22-34). The difference is significant ($t = 2.1598$; 80 DF; $P < 5\ \%$).

Overall effects on numbers fledged :

Chi-squared tests comparing the numbers of eggs laid to young fledged in control and DDT-fed pairs showed a significant difference even at the lowest dose rate (Table 1).

Second generation test :

A small test of the effects of DDT on the breeding ability of the second generation was made by using 32 of the fledglings. These birds had developed in a milieu contaminated with DDT from the first cell division until they were nearly sexually mature. Twenty-two fledglings from the low dose rate group (mean dose rate of parents 24 µg/bird/day) were given their DDT diets until they were 8 weeks old. They were then fed a control diet until at 5 months of age they were paired with another fledgling and their control diet continued. Ten 5-month old birds from control parents were also paired. Mean clutch size, number fertile, hatching and fledging was 4.80, 4.60, 4.60 and 3.80 respectively in the controls and 4.64, 4.18, 4.00 and 3.45 in the DDT fledglings. Although these results are below the success of the control pairs they are similar to the normal success of control pairs in the main experiment. One noticeable effect was the considerably longer delay before ovulation (Control 13.8 days, range 9-16; DDT 18.7 days, range 7-55). Analyses of fledglings at 2 months showed a mean liver residue of 17.78 ppm DDT + 8.96 ppm DDE. These figures were 2.51 + 9.08 at 5 months. Two infertile eggs contained a mean of 7.81 ppm DDT + 18.57 ppm DDE.

Effect of pp'-DDE on reproduction

Feeding pp'-DDE to Bengalese Finches produced the same

sublethal effects as those noted with pp'-DDT. There was a significant reduction in the number of young fledged (Table 1), reduced fertility and hatchability (Table 1), a delay in ovulation (Table 1), a reduction in egg weight (Table 2) and a reduction in chick weight (Table 3).

Two of the 11 dead chicks died with peck wounds on the abdomen (as did two of the DDT chicks — see also Jefferies, 1967) and one had both legs torn off. Extremely aggressive behaviour was also noted in one of the dosed males. This aggressive behaviour against chicks and mate has never been seen in any control or stock pairs. Soft-shelled and mis-shaped eggs were only laid by DDE-dosed birds.

Analyses

Analyses of livers, eggs and chicks were made for purposes of correlation with field samples. The results are shown in Table 4.

Discussion

Nidicolous species form the majority of birds and yet they are not usually used in laboratory trials of the effects of organochlorines on breeding (Galliform birds are generally used and with electric incubators). In such species, incubation and rearing of young stresses the test birds and approximates more closely to field conditions. Also the use of a known dose rate throughout the whole cycle has obvious advantages for comparison with field doses. In the present experiment the use of this technique showed significant reductions in fledging success at dietary levels lower than those used by other workers (8 ppm DDT and 4 ppmDDE). In California, where the Peregrine Falcon *Falco peregrinus* has disappeared, muscle and whole body analyses of raptor food species show concentrations higher than 4 ppm DDE (Risebrough *et al*, 1967).

The high mortality of one-day-old chicks could be due to (i) the large quantity of pesticide absorbed from the yolk (ppm increases as the chick is smaller than the egg) and the stress of hatching, (ii) parents not feeding or brooding them or (iii) reduced weight at hatching reducing chances of survival. The amount of DDT fed by the parents during the 2-hour test period of the first day must be extremely small compared to the content absorbed from the egg.

The reduction in chick weight at each egg size could be due to smaller yolks within each egg from DDT-fed pairs or again it could be related to reduced feeding by DDT parents during the first hours after hatching and before weighing at 09.00 hours.

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