# The Effect of o,p'-DDT[1] on Japanese Quail

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Technical DDT increases uterine wet weight in rats (1). The most active constituent is not p,p'-DDT[2] but the o,p'-isomer, which, it was suggested, could compete with labelled oestradiol for binding sites on the uterus. It has now been reported (2) that administration of o,p'-DDT can cause oestrogenic-type responses in the weight and glycogen content of chicken and quail oviducts and rat uteri.

If o,p'-DDT is a potent oestrogen, large scale treatment of the environment could produce a serious hazard since the commercial DDT mixture contains 11-29% of the o,p'-isomer (3). Oestrogens increase serum calcium and lipid levels in birds (4) and the effect of o,p'-DDT on Japanese quail (Coturnix coturnix japonica) has been studied, paying particular attention to any changes in these parameters.

## Experimental methods

Experiment 1. Two groups of 6 male, and two of 6 female, Japanese quail (age 33 days, weight 60-80 g) were randomly selected. Each bird in one group of each sex was treated daily for 4 days with o,p'-DDT[3] (5 mg in 0.2 ml olive oil, by intramuscular injection). Birds in the control group were injected

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[1] o,p'-DDT: 1,1,1-trichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane

[2] p,p'-DDT: 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane

[3] Aldrich Chemical Co., Milwaukee, Wis.



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with olive oil only. Blood samples were taken from the jugular vein of anaesthetised female birds 72 hours after the final injection, and from the males 24 hours later. Birds were sacrificed and either oviduct and ovary weight or testes weight recorded. Samples of breast muscle were analysed for organochlorine residues.

Experiment 2. Six adult male quail (age 73 days, weight 94-108 g) were each orally dosed by means of a catheter, with 10 mg o,p'-DDT in 0.2 ml olive oil, 3 doses per week being administered for 3 weeks. A further group of 6 quail was similarly treated with olive oil only. Blood samples were withdrawn from the brachial vein prior to dosing and again at the end of the 3 week period. After the second blood sample was taken, birds were sacrificed and liver and testes were weighed. Liver samples were analysed for organochlorine residues.

Analysis. All serum samples were analysed for total calcium (5) and those from the females were analysed for total lipid (6).

Tissue samples for organochlorine analysis were ground with sand and anhydrous sodium sulphate and extracted with hot, redistilled n-hexane, followed by redistilled acetone. Extracts were passed through an alumina column and residues determined by gas-liquid chromatography using an electron capture detector.

## Results

Results for Experiments 1 and 2 are given in Tables 1 and 2 respectively. In both experiments the mean testes weight of the treated birds was less than that of the corresponding controls, but because of variation within the groups, neither difference is statistically significant. No significant differences occurred between respective treated and control groups for any of the organs studied. In Experiment 2 serum calcium levels for birds in the o,p'-DDT group were virtually the same before and after treatment, while in Experiment 1, mean levels for the treated birds were slightly higher than for the control birds, but the differences were not significant. The percentage gain in weight of females treated with o,p'-DDT was lower ($P<0.05$) than that of the corresponding control group, but no difference was observed in the serum lipid levels of the two groups.

TABLE 1

Effect of o,p'-DDT on young Japanese quail. For experimental details see text. Standard errors are given whenever appropriate.

| Sex | male | male | female | female |
|---|---|---|---|---|
| o,p'-DDT to each bird | 0 | 4x5mg | 0 | 4x5mg |
| Mean initial weight (g) | 73±2 | 71±2 | 70±3 | 72±3 |
| Mean final weight (g) | 91±2 | 89±2 | 87±3 | 85±3 |
| Mean testes weight (g) | 0.29±0.05 | 0.16±0.05 | | |
| Mean ovary weight (g) | | | 0.058±0.012 | 0.046±0.0 |
| Mean oviduct weight (g) | | | 0.014±0.003 | 0.016±0.0 |
| Mean serum calcium (mg%) | 10.6±0.2 | 10.9±0.2 | 11.1±0.7 | 11.5±1.0 |
| Mean serum lipid (mg%) | | | 1260±30 | 1250±30 |
| Mean content of o,p'-DDT in breast muscle (ppm) | <0.02 | 2.3±0.5 | <0.02 | 2.1±0.6 |

TABLE 2

Effect of o,p'-DDT on adult male Japanese quail. For experimental details see text. Standard errors are given whenever appropriate.

| o,p'-DDT to each bird | 0 | 9x10mg |
|---|---|---|
| Mean initial weight (g) | 101±2 | 106±2 |
| Mean final weight (g) | 109±3 | 114±3 |
| Mean testes weight (g) | 2.54±0.17 | 2.18±0.06 |
| Mean liver weight (g) | 2.10±0.21 | 2.10±0.13 |
| Mean initial serum calcium (mg%) | 9.4±0.8 | 9.8±0.4 |
| Mean final serum calcium (mg%) | 11.2±0.8 | 9.7±0.3 |
| Mean content of o,p'-DDT in liver (ppm) | <0.03 | 5.1±0.9 |

The series of oral doses of o,p'-DDT applied to the adult male quail did not cause them to vomit, as has been observed after dosing pigeons (7), and the general health of these birds and of the young quail treated in the first experiment seemed unimpaired. When mated with a female quail at the end of the 3 week treatment period, all the adult males treated with o,p'-DDT attempted copulation within 12 seconds, as did the corresponding control birds.

Serum calcium levels of 3 adult male quail, 24 hours after each received an oral dose of 1 mg diethylstilboestrol in 0.2 ml olive oil, were 11.5, 11.5 and 12.7 mg%. The mean, 11.9 mg%, is 18 per cent higher than the mean of 10.1 mg% for all samples from untreated birds in Experiment 2. Three more males, being used in a different experiment to study the effect of oestrogen on mating behaviour, were similarly dosed for 4 days and were then injected intramuscularly with the same amount of diethylstilboestrol for a further 6 days. Serum calcium levels of these 3 birds were then 101.8, 87.9 and 78.4 mg%.

## Discussion

In the first experiment, the work of Bitman et al on Japanese quail (2) has been extended. These authors reported increased oviduct weight after treatment with o,p'-DDT. While there is little difference in Experiment 1 between mean oviduct weights, there were reductions in mean ovary and testes weights (in both experiments) for the treated birds. Although these differences are not significant, they are consistent with changes that would occur if o,p'-DDT acted as an oestrogen. Since o,p'-DDT can act directly in an oestrogenic manner at uterine sites in ovariectomised rats (1), it might suppress the pituitary gonadotrophin secretion of quail used in the present experiment by means of a feedback mechanism (4) so reducing ovary weight and testes weight. Differences in the weights of the gonads do not, however, necessarily reflect true oestrogenic activity. For instance, testes growth in cockerels is suppressed by feeding p,p'-DDT (8), a fact which led the authors to suggest that the p,p'-isomer may be oestrogenic. Subsequent experiments, however, demonstrated that it does not produce other oestrogenic responses such as elevation of blood calcium levels (9). Although o,p'-DDT can produce a more varied range of oestrogenic responses than the p,p'-isomer, the elucidation and interpretation of the

possible oestrogenic properties of these two compounds have progressed along parallel lines. Blood calcium and lipid levels are sensitive indicators of oestrogenic activity(4) and since little or no change has been detected in either parameter during the present study, it would suggest that o,p'-DDT may exert an effect on the endocrine system, without displaying all the properties of a potent oestrogen. Observations just published by Bitman and his coworkers (10) also support this, since adult female quail dosed with o,p'-DDT lay eggs with thinner shells, but blood and skeletal calcium are unaffected. Furthermore, Fisher, Keasling and Schueler (11) reported that the p,p'-DDT molecule does not possess the full steric requirements for an oestrogen, an argument that may also be applied to o,p'-DDT.

In the wild state an organism obtains o,p'-DDT via applications of technical DDT. Since p,p'-DDT is present in concentrations of up to 80% in this mixture (3), its effect on the organism must also be considered. For the bobwhite quail (Colinus virginianus), the acute oral toxicity of p,p'-DDT in oil solution is 60-85 mg/kg (12). Adult Japanese quail in the present study received 9 doses each of 90-110 mg o,p'-DDT/kg. Had the p,p'-isomer been present at the same relative concentration found in technical DDT, evidence from the mortality study on a related species indicates that each of the 9 doses would probably have been sufficient to kill several individuals.

Bitman et al (10) have recently reported a decrease in egg shell thickness when quail are treated with p,p'- or o,p'-DDT, although, as these authors rightly pointed out, the pesticide intake of these quail was far in excess of that ingested by seed-eating birds in the field. o,p'-DDT is readily lost by birds(7) and very few wildlife samples contain detectable amounts. The maximum concentration found in over a thousand wildlife samples analysed for the Nature Conservancy during the last few years was 0.2 ppm in a liver sample (7), so that the intake of o,p'-DDT by raptorial species is probably negligible, the compound having been lost further down the food chain. Since the initial work of Ratcliffe (13), considerable interest has been shown in the decrease in egg shell thickness of several raptorial species. The evidence would suggest, however, that o,p'-DDT cannot be implicated in this decline, and it probably does not constitute a hazard to avian populations

in the field, especially when compared with several other organochlorine pesticides.

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