Effect of Long-Term Feeding of DDT to Turkeys

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Adverse effects from the feeding of DDT to mammals (1) and birds (2,3) have been reported over the past several years. Most of the experiments have been conducted with mice (1,4) or rats (5), and many of these recent works are involved with the influence of o,p'-DDT on reproduction.

Evidence of an uterotropic (estrogenic) activity of o,p'-DDT was demonstrated in intact, immature rats by increased uterine weights following injection of the drug (5,6). The increased uterine weights, according to histologic observations, resulted from endometrial edema. In trials with immature rats, it was found that short-term feeding of high levels of o,p'-DDT caused early vaginal opening but low levels of o,p'-DDT did not have an estrogenic effect in long-term feeding trials (7). Recognition of the uterotropic properties of o,p'-DDT also was reported in earlier observations (8,9). It has been suggested that uterotropic activity might be related to the structural similarity of o,p'-DDT to diethylstilbestrol (DES) (10), and in support of this theory it was observed that feeding of "purified DDT" inhibited the testicular growth and development of secondary sex characteristics of cockerels (11).

Additional effects, other than estrogenic, result from the consumption of DDT. Bengalese finches develop apparent hyperthyroidism following consumption of p,p'-DDT for 6 weeks (12), and sublethal quantities of the same drug increase thyroid weight and reduce colloid content of follicles in homing pigeons (13). The latter observation was interpreted as hyper- or hypothyroidism. Thyroid weights were increased in rats fed o,p'- and p,p'-DDD, and this effect was interpreted as evidence of hypothyroidism (14).

The estrogenic activity of orally administered DES is evidenced dramatically in immature male and female Broad-Breasted turkeys (15). Readily recognizable clinical evidence consists of: Hyperlipemia, hypercholesterolemia, hypercalcemia, hypotension,

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hyperproteinemia, alteration of the albumin-globulin ratio and abrupt mortality from aortic ruptures. According to histologic studies, vascular rhexis is a consequence of DES-induced aortic atherosclerosis (16). In addition to the clinical manifestations which result from the feeding of DES to turkeys, there is histologic evidence of hypothyroidism, and also alteration of secondary sex characteristics manifested by strutting, gobbling and prolapsed rectums (17).

Long-term feeding trials with DDT in the class Aves have not been conducted (18). Because of this fact and the readily recognizable manifestations in turkeys of feeding a compound with estrogenic activity (DES), the effect of long-term feeding of o,p'- and p,p'-DDT to turkeys is reported in this paper.

METHODS AND MATERIALS

Twelve pens of 6-week-old Broad-Breasted White turkeys, each pen consisting of 2 females and 2 males, were fed a 23% protein diet which contained 6% animal fat and 264.6 ppm o,p'-DDT.* The same amount of p,p'-DDT was fed to an equal number of 6-week-old male and female poults allotted to 12 pens. An identical number of male and female poults were fed the 23% protein diet with 6% animal fat and no additional supplementation. One-half of the male and female poults from each of the 3 dietary regimens were killed at 13 weeks of age (7 weeks of feeding the experimental diets), and the remainder of the turkeys were killed at 21 weeks of age (15 weeks of feeding of experimental diets).

Several biologic parameters were determined from at least 6 turkeys per treatment regimen at both 13 and 21 week-old killing dates. These parameters included: Indirect blood pressure by use of an inflatable cuff on the leg, and plasma calcium and cholesterol levels by use of a Technicon autoanalyzer. In addition, a necropsy was performed on all turkeys at each date of sacrifice, and pieces of abdominal aorta, left ventricle, liver, testes, oviduct, ovary, thyroid and kidney from at least 6 poults per treatment regimen were fixed in 10% neutral formalin and stained with hematoxylin and eosin for histologic study. Moreover, sections of abdominal aorta and myocardium were cut at 8μ on a freezing microtome, and stained with oil red O stain for the demonstration of lipid. On the basis of this histologic technique, aortic and coronary atherosclerotic lesions were graded from 0-4, grade 4 being the most severe.

*Aldrich Chemical Company, Milwaukee, Wisconsin.

Additional evaluations were made with at least 6 turkeys per dietary regimen killed at 13 and 21 weeks of age. Disc electrophoresis on polyacrylamide gels of plasma lipoproteins (19) and total plasma proteins (20) was performed. Residue levels of DDT in the abdominal fat of turkeys was determined by gas chromatography (21). In this procedure, tissues were ground with sodium sulfate and extracted with petroleum ether in a Soxhlet apparatus. A Varian 2100 Gas Chromatograph equipped with an electron capture detector and a 6 foot x 1/4 inch glass column packed with 3% OV-17 on Chromosorb W with a flow of 100 ml/min Nitrogen was used for analysis. The injection port temperature was 210°, column 200° and detector, 215°C.

RESULTS

No birds died, regardless of the diet fed. Moreover, there were not alterations of the several tissues (liver, aorta, heart, kidney, thyroid, testes, oviduct) examined grossly and microscopically at either the 13 or 21 weeks of age autopsy dates. In particular, the testes and oviducts were of similar size in turkeys from all treatment groups.

Examination of frozen sections of myocardium and aorta did not reveal evidence of coronary atherosclerosis, and grade 2 aortic atherosclerosis was seen in turkeys fed either the control or the DDT-supplemented diet. There was no difference in plasma cholesterol and calcium values or blood pressure measurements among the turkeys fed the 3 diets. Weight gains also were similar among all treatment groups. Diet did not influence the plasma lipoprotein patterns of turkeys killed at 13 and 21 weeks of age. All patterns were similar; there were no chylomicrons, a barely discernible pre-beta lipoprotein band and a distinct beta- and alpha lipoprotein band. The plasma electrophoretograms of turkeys fed DDT were similar to those of control turkeys; there was no hyperproteinemia and the albumin-globulin ratio was normal.

The residue of DDT in body fat of turkeys fed the 3 diets was related to the form of DDT fed (Table 1). Trace amounts of DDT were found in feed of birds fed the control diet. Minute quantities of o,p'-DDT and large amounts of p,p'-DDT were found in fat of turkeys fed p,p'-DDT, and the converse was true of fat of turkeys fed o,p'-DDT (5 times more o,p- than p,p'-DDT).

DISCUSSION

The immature male and female turkey, as shown in numerous works, is a highly sensitive indicator of

TABLE 1

DDT Residues in Abdominal Fat of Turkeys Fed 264.6 ppm o,p'- p,p'-DDT for 15 Weeks

	p,p'-DDT*				o,p'-DDT*			
Diet	DDE	DDD	DDT	Total	DDE	DDD	DDT	Total
Control	0.07	-	0.17	0.24	0.07	-	Trace	0.07
p,p'-DDT, Male	638.78	78.95	1960.34	2678.07	Trace	Trace	Trace	Trace
p,p'-DDT, Female	624.20	70.65	2055.87	2750.72	Trace	Trace	Trace	Trace
o,p'-DDT, Male	5.18	0.23	27.60	33.01	0.09	3.45	204.61	208.15
o,p'-DDT, Female	5.72	0.62	33.54	39.88	0.34	2.64	368.21	371.19

*ppm: average of 4 analysis

the estrogenic activity of 2 orally administered compounds, DES and dinestrol diacetate (22). Abnormalities have been induced in turkeys by treatments with estrogenic compounds. As an example, mortality from aortic ruptures (23) has been induced by feeding 176.2 ppm (8 gm/100 lbs feed) purified DES or with weekly injections of DES (22,24). Feeding of 83.1 ppm DES causes hyperlipemia, but not aortic ruptures.

In the present study with immature male and female turkeys, about 1.5 times more o,p'- or p,p'-DDT was fed than the amount of DES found necessary to produce adverse effects, including aortic ruptures (15). About 3 times more DDT was fed than the quantity of DES found to produce hyperlipemia but not vascular rhexis.

The lack of estrogenic response from feeding DDT to turkeys indicates that: 1) either insufficient quantities of DDT were fed, or; 2) the chemical does not have estrogenic activity in immature turkeys, or DDT is not estrogenic unless administered parenterally. In support of the first hypothesis is the observation in rats (6) that oral o,p'-DDT has 1/10,000 the estrogenicity of oral estradiol when fed for 7 days. Also in rats (7), it was found that long-term feeding of low levels of o,p'-DDT did not cause detrimental effects. It has been reported in chickens and quail (8) that o,p'-DDT is estrogenic when administered subcutaneously. In support of the second theory is the observation of Stephen et al. (18) that alteration of calcium deposition by chlorinated hydrocarbons does not appear to be a general phenomenon in the class Aves.

Higher residues of p,p'-DDT than o,p'-DDT were found in the body fat of male and female turkeys fed DDT for 15 weeks, even though each form was fed at the identical level (Table 1). It has been reported elsewhere that o,p'-DDT accumulates at about the same extent as p,p'-DDT in chickens (8). In the present work, the p,p'-DDT residue in the body fat of turkeys fed o,p'-DDT probably resulted from a mild impurity (0.4%) in Aldrich's o,p'-DDT, and did not result from the biological conversion of o,p'- to p,p'-DDT.

The work described herein indicates that immature male and female turkey poults can accumulate high levels of DDT in the body fat and not demonstrate estrogenic effects. Another relevant observation is the lack of good correlation between detection of high o,p'- and p,p'-DDT residues in body fat and the presence of gross, clinical or histologic evidence of adverse drug effects, including estrogenic influences.

SUMMARY

Diets containing 264.6 ppm o,p'- or p,p'-DDT were fed to 6-week-old male and female turkeys for 7 and 15 weeks. Such chronic feeding of DDT did not cause alterations of: Blood pressure; gross structure of body tissues; histology of heart, aorta, liver, testes, oviduct, ovary, thyroid, or kidney; plasma cholesterol or calcium levels; albumin-globulin ratio; or plasma lipoprotein patterns. The residue of DDT in body fat was determined. Trace amounts were found in birds fed the control diet. Minute quantities of o,p'-DDT and large amounts of p,p'-DDT were found in fat of turkeys fed p,p'-DDT, and the converse was true of turkeys fed o,p'-DDT.

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