Evaluation of the Possible Estrogenic Activity of Methoxychlor[1] in the Chicken by Means of Feeding Trials[2]

by T. S. FOSTER
Animal Research Institute
Research Branch, Canada Agriculture
Ottawa, Ontario K1A 0C6, Canada

Several investigators have suggested an estrogenic activity for the chlorinated insecticide, DDT and its analogs, in chickens, quail, mice, rats and mink, when administered by subcutaneous or intraperitoneal injection (BURLINGTON and LINDEMAN 1950, FISHER et al. 1952, LEVIN et al. 1968, BITMAN et al. 1968, WELCH et al. 1969, SINGHAL et al. 1970, COOKE 1970, BITMAN and CECIL 1970, CECIL et al. 1971, DUBY et al. 1971, WELCH et al. 1971). Estrogenic activity was based on decreased testes weights, increased uterine and oviduct weights, enhanced uterine glycogen content and several uterine enzyme activities, and increased uterine water and RNA. It was established that the estrogenicity was associated with o,p'-isomer[3] rather than the p,p'-isomer[4] of DDT. Similar findings have been observed when feeding studies were employed (TREON and CLEVELAND 1955, BERNARD and GAERTNER 1964, DEICHMANN and KEPLINGER 1966, WARE and GOOD 1967, WRENN et al. 1970, COOKE 1970, CECIL et al. 1971, DUBY et al. 1971).

FISHER et al. (1952) were unable to demonstrate estrogenic activity for methoxychlor in ovariectomized rats, whereas LEVIN et al. (1968), WELCH et al. (1969) and BITMAN and CECIL (1970) reported moderate estrogenicity, and by correlation of structure with activity, suggested that the active estrogens derived from o,p'-analogs of DDT were p-phenolic metabolites.

The banning of the organochlorine insecticide, DDT, has resulted in a greatly increased use of methoxychlor, which, in Canada, has been registered for practically all those uses formerly permitted for DDT. Methoxychlor residues are presently being detected in feeds and animal tissues. From an agricultural viewpoint, therefore, it seemed relevant to study whether methoxychlor produced estrogenic effects in the chicken.

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[1]Methoxychlor : 1,1,1-trichloro-2,2'-bis(p-methoxyphenyl)ethane.

[2]Contribution No. 456 from the Animal Research Institute.

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

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

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A test for oral activity of synthetic estrogens, based on increases in oviduct weight in domestic fowl and turkey, was reported by JAAP (1945). It is more customary to employ subcutaneous (DORFMAN and DORFMAN 1948, CECIL et al. 1969) or intramuscular (LORENZ et al. 1962) injections in statistical methods which employ the response of the chick oviduct for the assay of various estrogens. However, it is more probable that chickens would come in contact with methoxychlor via the feed, thus this method of administration was chosen for the studies which follow.

Methods

Experiment 1. Thirty-three 25 week-old White Leghorn pullets[5], at approximately 50% production, were divided into three equal groups, placed in individual cages and fed the following diets ad libitum for 4 weeks : (a) Basal ration (a practical type laying ration), (b) Basal ration containing 1 ppm methoxychlor[6], or (c) Basal ration containing 10 ppm methoxychlor[6]. They were maintained at 55°C and were given 16 hrs lighting.

Three pullets (2 laying and 1 not laying) from each group were killed by cervical dislocation at weekly intervals. The oviducts were removed, freed of all adhering fat and membranes and anatomically divided into four parts - the shell gland (uterovagina), isthmus, magnum and infundibulum. The latter was discarded as it was exceedingly difficult to obtain in its entirety. The other three parts were blotted dry and weighed. The data are presented in Table 1.

Experiment 2. One hundred, day-old, female chicks[7] were placed in a brooder and fed water and a practical type chick starter ration ad libitum. At the end of 6 weeks, 48 chicks were removed, randomly divided into three equal groups, placed in large holding pens and fed the following diets ad libitum for 5 weeks : (a) Basal ration (a practical type growing ration), (b) Basal ration containing 1 ppm methoxychlor[6], or (c) Basal ration containing 10 ppm methoxychlor[6].

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[5]Ottawa Control Strain.

[6]Methoxychlor (Tech.), Entomological Society of America insecticide reference standard, Nutritional Biochemicals Corporation.

[7]Ottawa Meat Control Strain.

The remaining chicks were fed Basal Grower ration ad libitum. At the age of 12 weeks, 48 chicks were removed and treated as above for the 6 week-old chicks.

At weekly intervals from both the 6th and 12th weeks, three chicks were removed from each group, weighed, killed by cervical dislocation and the oviducts removed, freed of adhering fat and tissues and weighed. The data are shown in Tables 2 and 3.

Experiment 3. One hundred and fifty day-old, unsexed chicks[7] were placed in a brooder and fed water and a practical type chick starter ration, ad libitum. At the end of 6 weeks, the surviving chicks were randomly distributed into four equal groups. They were fed the following diets ad libitum : (a) Basal Grower ration, (b) Basal Grower ration containing 1 ppm methoxychlor[6], (c) Basal Grower ration containing 10 ppm methoxychlor[6], or (d) Basal Grower ration containing 1 ppm diethylstilbestrol[8].

At weekly intervals from the 6th week, one half of the chicks in each group were removed, weighed, killed by cervical dislocation and either the testes or the oviducts plus ovaries removed, freed of adhering fat and tissues and weighed. At the second killing, the ovaries and the oviducts were weighed separately. The data are indicated in Table 4.

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[6]Methoxychlor (Tech.), Entomological Society of America insecticide reference standard, Nutritional Biochemicals Corporation.

[7]Ottawa Meat Control Strain.

[8]Diethylstilbestrol, U.S.P., Mann Research Laboratories Incorporated.

TABLE 1

The effect of feeding methoxychlor on various parts of the oviduct in the laying and non-laying pullet[1].

Treatment	Time on Treatment (weeks)							
Methoxychlor (ppm)	1		2		3		4	
	L[2]	NL[2]	L[2]	NL[2]	L[2]	NL[2]	L[2]	NL[2]
	Shell gland (g)							
0	10.99	10.75	9.81	0.33	14.05	3.35	15.40	1.00
	9.90	-	11.95	-	11.96	-	-	-
1	11.21	8.53	11.95	-	13.50	-	13.03	-
	13.42	-	11.45	-	11.52	-	-	-
	-	-	-	-	15.85	-	-	-
10	13.55	1.44	14.60	4.54	10.71	1.15	-	-
	10.54	-	14.32	-	12.53	-	-	-
	Isthmus (g)							
0	3.70	2.49	5.39	0.35	5.10	0.68	4.55	0.25
	4.86	-	5.85	-	4.11	-	-	-
1	3.28	2.32	6.40	-	4.13	-	4.45	-
	4.91	-	2.82	-	4.15	-	-	-
	-	-	-	-	4.45	-	-	-
10	3.40	0.28	4.32	1.61	3.45	0.10	-	-
	4.26	-	3.86	-	4.03	-	-	-
	Magnum (g)							
0	23.32	9.94	24.36	1.12	25.93	1.35	21.05	0.95
	12.50	-	27.62	-	18.85	-	-	-
1	15.06	12.19	20.22	-	18.71	-	24.95	-
	28.51	-	22.79	-	20.43	-	-	-
	-	-	-	-	20.87	-	-	-
10	24.44	0.53	22.75	4.45	21.21	0.10	-	-
	20.89	-	22.47	-	22.73	-	-	-

[1]25 week-old White Leghorns, Ottawa Control Strain.
[2]L = laying pullets; NL = non laying pullets.

TABLE 2

The effect of feeding methoxychlor on body weight gains of meat-type pullets.[1]

Treatment	Time on treatment (weeks)[2]				
Methoxychlor (ppm)	1	2	3	4	5
	Weight gain (g/week)[3]				
	First Group				
0	148±10 (16)	142±5 (13)	181±6 (10)	160±7 (7)	150±14 (4)
1	151±6 (16)	142±5 (13)	177±10 (10)	158±11 (7)	132±19 (4)
10	155±6 (16)	149±6 (13)	172±5 (10)	131±15 (7)	146±9 (4)
	Second Group				
0	182±13 (15)	147±11[a] (12)	138±11[a] (9)	106±8[b] (6)	97±18[b] (3)
1	163±11 (16)	161±9 (13)	165±14 (10)	141±13[c] (7)	116±14 (4)
10	156±8 (16)	168±8 (13)	136±14 (10)	110±16[b] (7)	114±14[a] (4)

[1]Ottawa Meat Control Strain

[2]First Group placed on treatment when the pullets were 6 weeks old; Second Group placed on treatment when the pullets were 12 weeks old.

[3]Mean ± S.E.M. with number of pullets in parentheses.

[a]Significant at the 5% level when compared with data from the same treatment at the end of week 1.

[b]Significant at the 1% level when compared with data from the same treatment at the end of week 1.

[c]Significant at the 5% level when compared with control data of the same week.

TABLE 3

The effect of feeding methoxychlor on weight of oviduct and ovary in meat-type pullets.[1]

Treatment	Time on treatment (weeks)[2]				
Methoxychlor (ppm)	1	2	3	4	5
	Oviduct + Ovary Weight (mg./100 g body weight)[3]				
	First Group				
0	16±2 (3)	45±2[b] (3)	36±3[b] (3)	38±2[b] (3)	38±2[b] (4)
1	42±3[d] (3)	40±2 (3)	37±3 (3)	37±2 (3)	39±2 (4)
10	34±5[c] (3)	38±2 (3)	35±2 (3)	37±4 (3)	34±2 (4)
	Second Group				
0	40±3 (3)	40±4 (3)	46±2 (3)	37±1 (3)	34±5 (3)
1	45±4 (3)	38±5 (3)	45±9 (3)	33±2[a] (3)	34±2[a] (4)
10	39±1 (3)	37±1 (3)	40±2 (3)	43±3[e] (3)	43±5 (4)

[1]Ottawa Meat Control Strain.
[2]First Group placed on treatment when the pullets were 6 weeks old; Second Group placed on treatment when the pullets were 12 weeks old.
[3]Mean ± S.E.M. with number of pullets in parentheses.
[a]Significant at the 5% level when compared with data from the same treatment at the end of week 1.
[b]Significant at the 1% level when compared with data from the same treatment at the end of week 1.
[c]Significant at the 5% level when compared with control data of the same week.
[d]Significant at the 1% level when compared with control data of the same week.
[e]Significant at the 5% level when compared with data for 1 ppm methoxychlor at the end of the same week.

TABLE 4

The effect of feeding methoxychlor on body weight gain and sexual organs in meat-type chicks.[1]

	sex	age (wks)[2]	Treatment: controls	methoxychlor 1 ppm	methoxychlor 10 ppm	diethylstilbestrol 1 ppm
Weight gain[3] (g/week)	male	7	119±5 (21)	115±10 (20)	110±3 (24)	118±6 (16)
		8	122±8 (10)	138±5[a] (12)	130±6[a] (11)	124±10 (9)
	female	7	117±5 (14)	108±9 (16)	100±5 (12)	84±9 (19)
		8	94±11 (8)	115±12 (6)	121±12 (7)	99±7 (9)
Testes weight[3] (mg/100 g body weight)	male	7	27±3 (11)	36±5 (8)	42±12 (13)	30±5 (7)
		8	27±3 (10)	38±7 (12)	42±7 (11)	31±12 (9)
Ovary+Oviduct weight[3] (mg/100 g body weight)	female	7	31±3 (6)	40±1 (10)	39±3 (5)	39±2 (10)
		8	37±2 (8)	38±3 (6)	35±2 (7)	44±2 (9)
Ovary weight[3] (mg/100 g body weight)	female	8	28±3 (8)	28±10 (6)	26±9 (7)	31±12 (9)
Oviduct weight[3] (mg/100 g body weight)	female	8	9±1 (8)	10±1 (6)	9±1 (7)	12±1 (9)

[1] Ottawa Meat Control Strain.
[2] Chicks placed on treatment when they were 6 weeks old.
[3] Mean ± S.E.M. with number of chicks in parentheses.
[a] Significant at the 5% level when compared with control data or data from the same treatment at age 7 weeks.

Results and Discussion

Experiment 1. A tremendous increase in oviduct weight occurs coincident with sexual maturity. This is evident even from the limited data presented in Table 1. Secretion of estrogen maintains the reproductive tract in a functional state and controls the reproductive processes of the female fowl (RIDDLE and LAHR 1944, LORENZ et al. 1962). The 25 week-old pullets, 50% of which had laid their first egg and were thus sexually mature, were not good choices as an experimental vehicle in an estrogenic assay. However, it can be seen that feeding 1 and 10 ppm methoxychlor for periods of up to 4 weeks, did not significantly affect oviduct weights or anatomical portions thereof either in laying or non-laying hens. Two deaths occurred during the experiment both of which were tentatively diagnosed as Marek's disease.

Experiment 2. Data in Table 2 indicated that no significant differences in body weight occur as a result of feeding mechoxychlor for periods up to 5 weeks at 1 and 10 ppm levels to 6 week-old chicks. In the case of 12 week-old chicks, significantly decreased body weights with increasing age or time on experiment were evidenced. Since these differences occurred in the control group as well, they were probably a result of age rather than treatment. In Table 3, the data seem to show significant increases in the oviduct + ovary weights as a result of feeding methoxychlor for periods up to 5 weeks at 1 and 10 ppm levels to 6 week-old chicks, but not in the case of 12 week-old chicks. Similar increases occurred in the control group. However, no cumulative effects were seen with respect to age, time on trial, or level of pesticide fed. It was decided to repeat the experiment with larger numbers at the lower age limits. During the trials one undiagnosed death occurred in the control group.

Experiment 3. Data are presented in Table 4 for an increased number of chicks and for both sexes. Feeding 1 or 10 ppm methoxychlor or 1 ppm diethylstilbestrol evoked no significant increases in weight of testes or oviduct and ovary although there was a trend towards increased ovary and oviduct weights after feeding 1 ppm diethylstilbestrol for 2 weeks. Comparisons of oral potency of estrogens are influenced by differences in absorption, destruction in the gut, variations in feed consumption and systemic destruction and excretion, especially by the liver and kidneys (LORENZ et al. 1962). JAAP (1945) reported that the limit of detection of estrogenic activity of diethylstilbestrol was approximately 1 ppm as a result of feeding to day-old chicks for 17 days.

Conclusions

From the data presented here, it can be concluded that consumption of methoxychlor by chickens at levels as high as 10 ppm, which under current conditions should only occur as a result of an accident, would produce no significant estrogenic effects. No toxic effects would occur over a 5 week feeding period at this level.

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