Estrogenicity of *o,p'*-DDT in Rats

Helene C. Cecil,* Joel Bitman, and Susan J. Harris

The estrogenicity of *o,p'*-DDT was compared to estradiol by studying changes in uterine H_2O, RNA, and glycogen after a single injection, multiple injections, or feeding *ad libitum* to immature female rats or ovariectomized rats. The minimal effective single dose of *o,p'*-DDT was approximately 0.4 mg, while that of estradiol was 0.04 μg, with maximum uterine responses occurring with 10× these levels. The relative estrogenicity of *o,p'*-DDT is 1/10,000 that of estradiol and the uterine effect of *o,p'*-DDT could be blocked by either MER-25 or Actinomycin D. Feeding 0.5 ppm estradiol elicited a maximum increase in uterine weight, while 1000 ppm *o,p'*-DDT did not. 1000 ppm *o,p'*-DDT in the diet (equivalent to 5000 μg per day) elicited uterine responses comparable to feeding 0.1 ppm estradiol.

Recently, Levin *et al.* (1968), Welch *et al.* (1969), and Bitman *et al.* (1968) reported that *o,p'*-DDT was estrogenic, stimulating uterine growth and glycogen deposition in the uteri of rats and in the oviducts of chickens and quail, while *p,p'*-DDT was only weakly estrogenic. The present study follows the time course of changes in several biochemical constituents effected by *o,p'*-DDT in rats, determines dose-response relationships of estrogens and *o,p'*-DDT, and determines the inhibition of estrogenic effects of *o,p'*-DDT by MER-25 and actinomycin D.

MATERIALS AND METHODS

o,p'-DDT, 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane and *p,p'*-DDT, 1,1,1-trichloro-2,2,bis(*p*-chlorophenyl)ethane, both 99+% pure were obtained from Aldrich Chemical Co. MER-25, 1-(*p*-2-diethylaminoethoxyphenyl)-1-phenyl- 2*p*-methoxyphenyl ethanol was supplied by William S. Merrell Co. All chemicals were used as supplied without further purification.

Intact immature, albino, female rats, 22 days of age (35 to 50 g) or ovariectomized mature female rats (220 to 270 g) received the following treatments.

Time Course of Uterine Changes. Immature rats were injected subcutaneously with either 4 mg of *o,p'*-DDT or *p,p'*-DDT or 0.4 μg of 17β-estradiol in 0.4 ml of olive oil and killed at intervals between 6 and 72 hr. Mature ovariectomized rats injected subcutaneously with either 10 or 20 mg of *o,p'*-DDT or *p,p'*-DDT or 1 μg of 17β-estradiol were killed 6 or 18 hr later. Water, ribonucleic acid (RNA), glycogen, or glucose concentrations in the uterus were determined as previously described (Bitman *et al.*, 1963).

Dose-Response Curve. Immature rats were injected subcutaneously with 0.1 to 10 mg of *o,p'*-DDT or 0.0075 to 1.0 μg of 17β-estradiol or diethylstilbestrol. Eighteen hours after injection, uterine weight and glycogen were determined.

MER-25 Inhibition. Immature rats were injected subcutaneously 2 × daily (8 a.m. and 4 p.m.) for 2 days with 1.0 mg MER-25. At the time of the last injection of MER-25, either 4 mg of *o,p'*-DDT or 0.4 μg of estradiol was injected subcutaneously. Eighteen hours later, uterine weight and glycogen were determined.

Actinomycin D Inhibition. Immature rats were injected intraperitoneally with 50 μg of Actinomycin D per 0.2 ml of 0.9% NaCl and subcutaneously with either 4 mg of *o,p'*-DDT or 0.4 μg of estradiol. Twenty-four hours later, uterine weight and RNA content were determined.

***Ad libitum* Feeding of *o,p'*-DDT or Estradiol.** Immature rats were fed mash *ad libitum* containing 50, 100, 250, 500, or 1000 ppm of *o,p'*-DDT or 0.001, 0.01, 0.1, 0.5, 1.0, 10, and 50 ppm of 17β-estradiol. After 3 or 7 days of feeding, uterine weight and glycogen were determined.

The amounts of DDT administered were much less than reported toxic doses. Domenjoz (1946) reported the LD_{50} for mice fed DDT in olive oil to be 0.59 gm *p,p'*-DDT per kg body weight and 3.35 gm *o,p'*-DDT per kg body weight.

RESULTS

A single injection of 4 mg *o,p'*-DDT to the immature rat stimulated the same time course of uterine changes in weight, water, glycogen, and RNA as 0.4 μg of estradiol (Figures 1 and 2). The estrogenic effects of DDT occurred mostly with the *o,p'*-DDT isomer, as there was little effect of *p,p'*-DDT. *p,p'*-DDT elicited an increase in uterine water at 12 hr ($p < 0.001$), but only slight (nonsignificant) increases in uterine weight, RNA, and glycogen occurred. Treatment of mature ovariectomized rats with *o,p'*-DDT elicited uterine increases similar to those elicited by estradiol (Table I).

To be able to determine the estrogenic potency of *o,p'*-DDT, dose-response relationships for *o,p'*-DDT, estradiol, and diethylstilbestrol (a nonsteroidal estrogen) were compared. Since the glycogen response is nearly maximal at 18

Agricultural Research Service, Animal Science Research Division, U.S. Department of Agriculture, Beltsville, Md. 20705

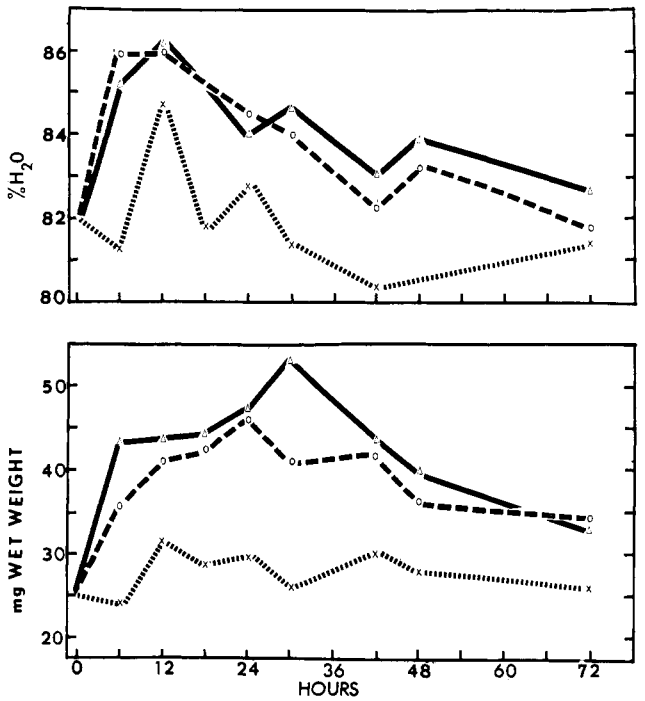

Figure 1. Changes in uterine wet weight and % H_2O after a single subcutaneous injection of estradiol or DDT in the immature rat. △—△, 0.4 μg 17β-estradiol; 0- - -0, 4 mg *o,p'*-DDT; ×- - -×, 4 mg *p,p'*-DDT. N = 16 for uterine wet weight and 8 for % H_2O for each point

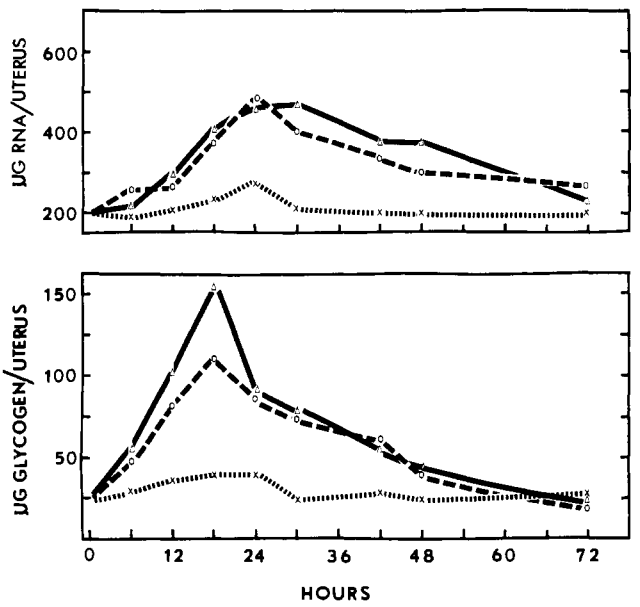

Figure 2. Changes in uterine RNA and glycogen after a single subcutaneous injection of estradiol or DDT in the immature rat. △—△, 0.4 μg 17β-estradiol; 0- - -0, 4 mg *o,p'*-DDT; ×- - -×, 4 mg *p,p'*-DDT. N = 8 for each point

hr (Figure 2), this time period was used to establish dose-response relationships. In the immature rat the maximal uterine weight increase was attained with 4 mg of *o,p'*-DDT, but 6 mg was necessary to give a maximal glycogen increase at 18 hr (Figure 3). The minimal effective single dose of *o,p'*-DDT was approximately 0.4 mg (Figure 3). Intraperitoneal or oral administration of *o,p'*-DDT was as effective as subcutaneous injection. The minimal effective dose of estradiol or diethylstilbestrol was 0.04 μg, while the maximal effective dose of estrogen was 0.40 μg. Thus, these two estrogens had approximately 10,000 × the activity of *o,p'*-DDT.

Some nonsteroidal compounds have been developed which antagonize the action of natural estrogens, and these have been designated as antiestrogens. One such antagonist, MER-25, inhibits the uterine glycogen increase elicited by estradiol (Wood *et al.*, 1968). MER-25 also acts as an effective inhibitor of the action of *o,p'*-DDT (Figure 4), inhibiting the uterine glycogen synthesis of both *o,p'*-DDT and estradiol. MER-25 is presumed to act as a competitive inhibitor

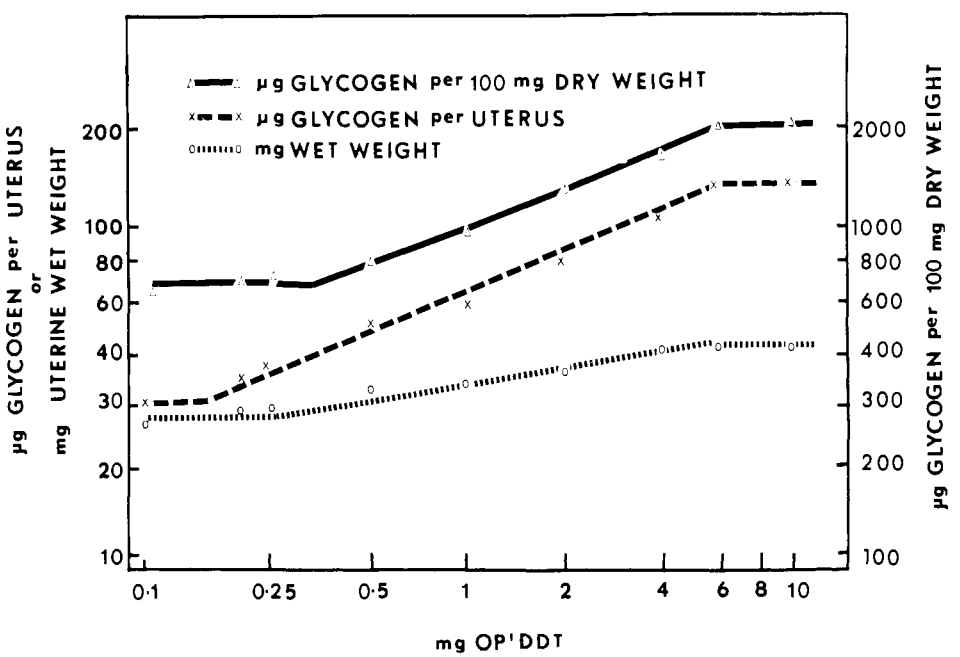

Figure 3. Uterine weight and glycogen content responses 18 hr after a single subcutaneous injection of *o,p'*-DDT. Control values were 631 ± 170 μg glycogen per 100 mg dry weight, 28.7 ± 1.1 μg glycogen per uterus and 24.7 ± 0.3 mg uterine wet weight. N = 5 to 10 for each point

Table I. Changes in Uterine Composition of the Mature Ovariectomized Rat 6 and 18 hr after Subcutaneous Administration of *o,p'*-DDT or 17β-Estradiol

	N	% H_2O ± SE	Uterine Weight mg ± SE	Glycogen μg/uterus ± SE	RNA μg/uterus ± SE
6 Hr					
Control	17	78.1 ± 0.3	129 ± 5	92 ± 8	748 ± 47
1 μg estradiol	17	83.3 ± 0.2[b]	201 ± 14[b]	260 ± 22[b]	798 ± 52
10 mg *o,p'*-DDT	25	83.2 ± 0.4[b]	229 ± 12[b]	345 ± 22[b]	879 ± 85
20 mg *o,p'*-DDT	10	83.6 ± 0.3[b]	201 ± 20[b]	193 ± 12[b]	680 ± 43
18 Hr					
Control	16	78.8 ± 0.2	136 ± 7	87 ± 6	611 ± 37
1 μg estradiol	18	82.9 ± 0.2[b]	205 ± 7[b]	648 ± 33[b]	979 ± 33[b]
10 mg *o,p'*-DDT	16	81.2 ± 0.3[b]	180 ± 14[b]	321 ± 25[b]	955 ± 51[b]
20 mg *o,p'*-DDT	8	81.6 ± 0.2[b]	235 ± 24[b]	523 ± 41[b]	801 ± 81[a]

[a] $p < 0.025$ when compared with control. [b] $p \leq 0.001$ when compared with control.

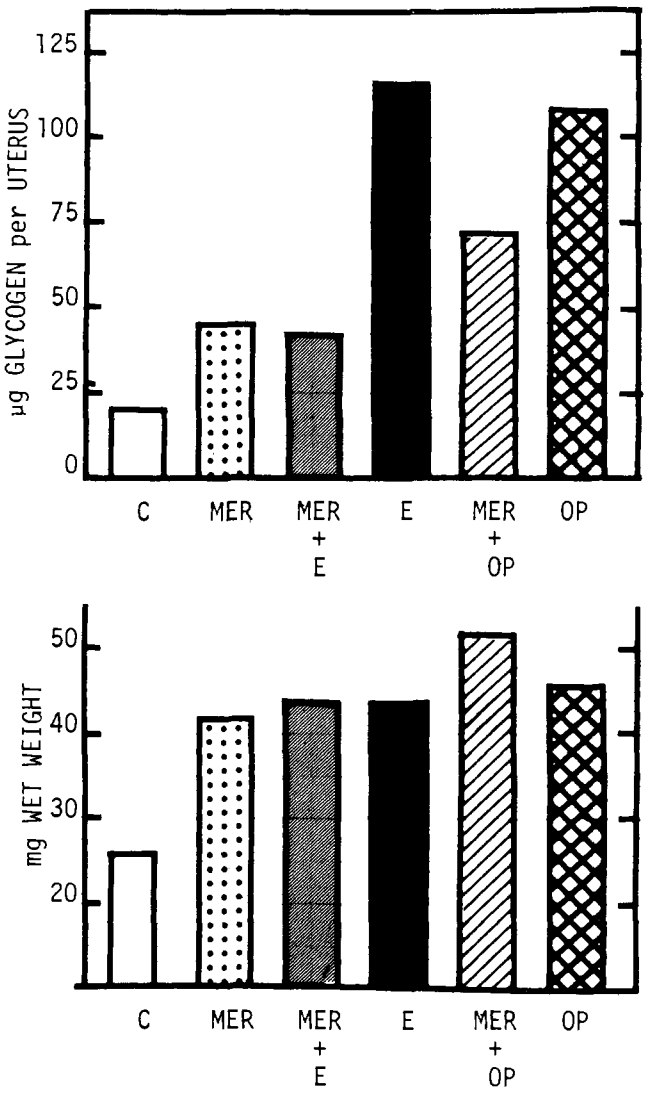

Figure 4. Changes in uterine weight and uterine glycogen after the administration of MER-25, MER-25 and estradiol (MER + E), estradiol (E), MER-25 and *o,p'*-DDT (MER + OP) or *o,p'*-DDT (OP). The range of the standard error of the mean for uterine weight was ±1.2 to ±2.7 and for μg glycogen per uterus ±1.1 to ±5.9. N = 5 for each group

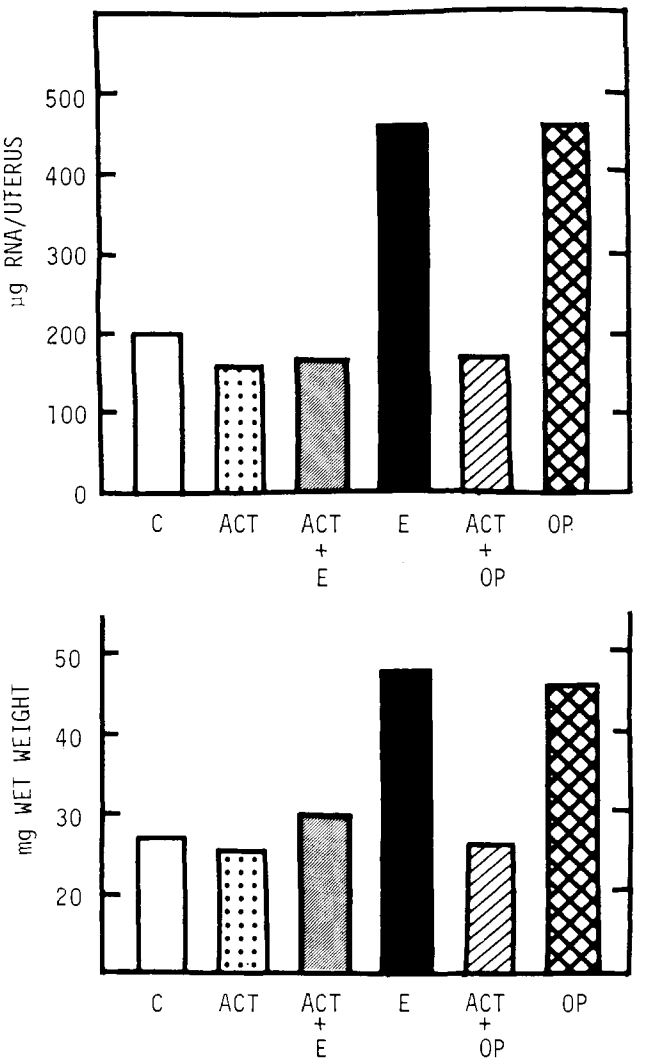

Figure 5. Changes in uterine weight and RNA after the administration of actinomycin D (ACT), actinomycin D and estradiol (ACT + E), estradiol (E), actinomycin D and *o,p'*-DDT (ACT + OP) or *o,p'*-DDT (OP). The range of the standard error of the mean for uterine weight was ±0.7 to ±4.0 and for μg RNA per uterus ±4 to ±23. N = 5 for each group

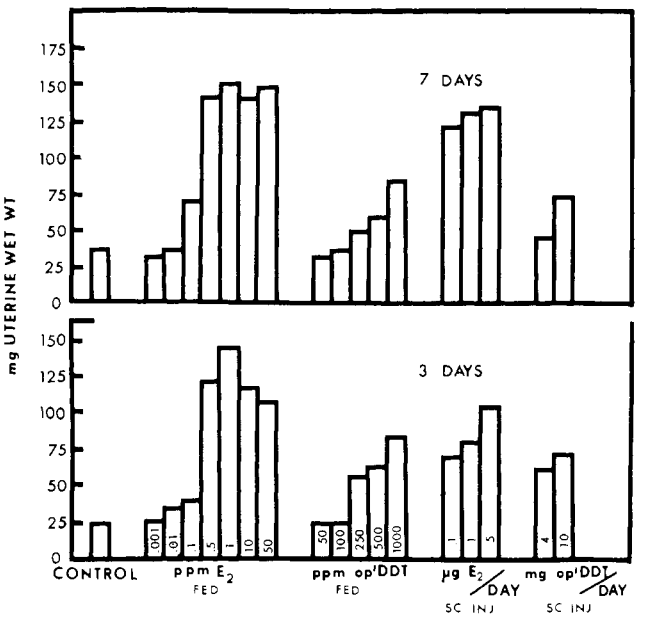

Figure 6. Effect of feeding of daily injections of estradiol or *o,p'*-DDT for 3 or 7 days on uterine weight of immature rats

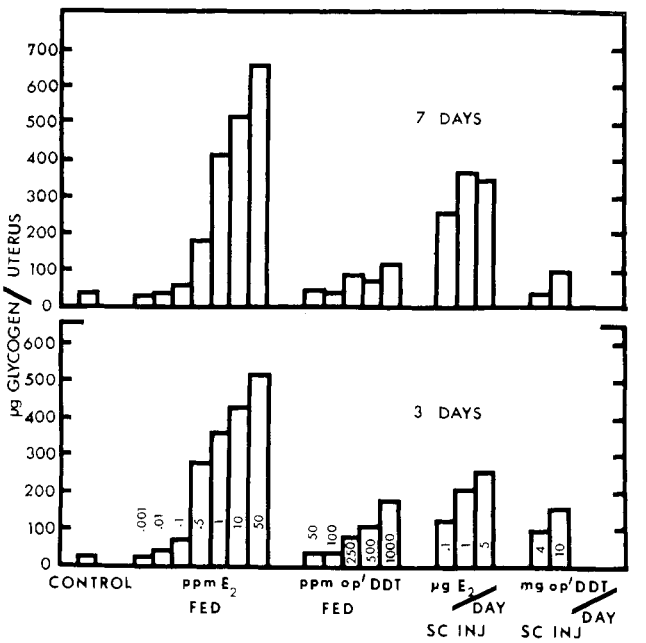

Figure 7. Effect of feeding or daily injections of estradiol or *o,p'*-DDT for 3 or 7 days on uterine glycogen content of immature rats

at the site of action of estradiol in the uterus. Consequently, since *o,p'*-DDT's action could also be blocked by MER-25, it appears that *o,p'*-DDT (or its metabolite) probably acts at the same site in the uterus as estradiol.

The early action of estradiol can also be blocked by antibiotics which inhibit protein synthesis. Actinomycin D which has been used to block estrogen-stimulated uterine responses (Valadares *et al.*, 1968) was also capable of inhibiting the action of *o,p'*-DDT (Figure 5). Actinomycin inhibited both the uterine weight response and RNA synthesis induced by *o,p'*-DDT or estradiol.

An attempt to simulate environmental exposure to pesticides was made by acute feeding of *o,p'*-DDT. The estrogenicity of *o,p'*-DDT was determined by comparing uterine responses of *o,p'*-DDT fed rats with those of estradiol fed rats (Figures 6 and 7). Intake for all groups was approximately 5.3 g per rat per day, while the gain was 2.7 g per rat per day with body weights of all groups comparable to controls. Feeding 0.5 ppm of estradiol (equivalent to 2.5 μg estradiol per day) elicited a maximum increase in uterine weight (Figure 6). Even 1000 ppm *o,p'*-DDT in the diet did not elicit a maximum increase in uterine weight. After 7 days, the uterine weight increase produced by feeding 1000 ppm *o,p'*-DDT (equivalent to 5300 μg per day) was comparable to that elicited by 0.1 ppm estradiol (comparable to 0.53 μg per day), a 10,000 fold difference. Injection of 10 mg *o,p'*-DDT subcutaneously daily resulted in a uterine weight response equivalent to feeding 1000 ppm *o,p'*-DDT. These results with multiple injections and feeding of *o,p'*-DDT were quite different from those after a single injection of *o,p'*-DDT. A single subcutaneous injection of 6 mg of *o,p'*-DDT gave a maximum uterine response at 18 hr (Figure 3), while multiple injections of 10 mg per day for 7 days or continuous feeding of 1000 ppm for 7 days gave a weight increase only 50% of maximum, and the effect on the uterine glycogen content was slight.

Using vaginal opening as a criterion of estrogen action, vaginae were closed after 3 days of feeding 500 ppm or 1000 ppm *o,p'*-DDT, although fluid accumulation had occurred in the uteri of rats fed 1000 ppm. After 3 days of feeding 50 ppm estradiol, three rats had open vaginae and three had closed vaginae, but the uteri were filled with fluid. However, after 7 days of feeding, 60% of the rats fed 500 ppm *o,p'*-DDT had open vaginae and all rats in the 1000 ppm *o,p'*-DDT group had open vaginae. The 500 ppm *o,p'*-DDT results are similar to those from rats fed 0.5 ppm estradiol, while the 1000 ppm *o,p'*-DDT feeding elicited results comparable to rats fed 1 ppm estradiol. The estrogenicity of *o,p'*-DDT is not as apparent in the vaginal opening test, as compared to the uterine weight test.

DISCUSSION

These studies agree with earlier reports that *o,p'*-DDT possesses estrogenic properties (Bitman *et al.*, 1968; Welch *et al.*, 1969). *o,p'*-DDT stimulated uterine weight, water, glycogen, and RNA changes similar to estradiol. In addition, substances which block estrogen action also inhibited *o,p'*-DDT action in the uterus. The blocking of the uterine glycogen increase by MER-25 indicates that *o,p'*-DDT's action is inhibited by a substance which acts as a competitor for estrogen binding sites in the uterus. Welch *et al.* (1969) have reported that *o,p'*-DDT competes with estradiol for binding sites in the immature rat uterus. Studies on the interactions of *o,p'*-DDT and estradiol are planned to determine the antiestrogenic properties of *o,p'*-DDT. Actinomycin D, an inhibitor of protein synthesis, also blocks the uterine RNA increase elicited by *o,p'*-DDT or estradiol, thus suggesting that both *o,p'*-DDT and estradiol stimulate early protein synthesis.

The relative estrogenic activity of *o,p'*-DDT is $^{1}/_{10,000}$ that of estradiol. A single injection of 6 mg of *o,p'*-DDT produced a maximum uterine response comparable to 0.4 μg of estradiol, while feeding 1000 ppm *o,p'*-DDT for 7 days induced a uterine response comparable to feeding 0.1 ppm estradiol. However, a different intensity of response occurred with injecting *o,p'*-DDT daily for 7 days than with a single injection. Injections of 10 mg *o,p'*-DDT daily did not give a maximum

response, while 0.1 μg estradiol daily does. This would be more than a 1 to 100,000 difference in eliciting a maximum response with multiple injections, while a 1 to 10,000 ratio gave a maximum response 18 hr after a single injection. This indicates that it is not digestion or absorption that is the limiting factor. The ineffectiveness of the *o,p'*-DDT after administration for several days may be due to increase in activity of liver enzyme systems that metabolize DDT, so that less DDT is available for action.

From these studies it appears that either *o,p'*-DDT or one of its metabolites is an active estrogen. *o,p'*-DDT has estrogenic activity that is $^{1}/_{10,000}$ that of estradiol. Current research in our laboratory demonstrates that several compounds with structures similar to *o,p'*-DDT have an estrogenic effect of the same magnitude as *o,p'*-DDT (Bitman and Cecil, 1970).

ACKNOWLEDGMENT

The authors thank William S. Merrell Co. for supplying the MER-25, and Merck, Sharp & Dohme for supplying the actinomycin D.

LITERATURE CITED

Bitman, J., Cecil, H. C., J. AGR. FOOD CHEM., **18,** 1108 (1970).
Bitman, J., Cecil, H. C., Gilliam, D. R., Wrenn, T. R., *J. Dairy Sci.* **XLVI,** 941–6 (1963).
Bitman, J., Cecil, H. C., Harris, S. J., Fries, G. F., *Science* **162,** 371–2 (1968).
Domenjoz, R., *Helv. Chim. Acta* **29,** 1317–22 (1946).
Levin, W., Welch, R. M., Conney, A. H., *Fed. Proc.* **27,** 649 (1968).
Valadares, J. R. E., Singhal, R. L., Parulekar, M. R., *Arch. Biochem. Biophys.* **123,** 417–9 (1968).
Welch, R. M., Levin, W., Conney, A. H., *Toxicol. Appl. Pharmacol.* **14,** 358–67 (1969).
Wood, J. R., Wrenn, T. R., Bitman, J., *Endocrinology* **82,** 69–74 (1968).

Received for review June 9, 1970. Accepted October 2, 1970.