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STIMULATION OF UTERINE DEOXYRIBONUCLEIC ACID SYNTHESIS BY 1,1,1-TRICHLORO-2-(*p*-CHLOROPHENYL)-2-(*o*-CHLOROPHENYL)ETHANE (*o,p'*-DDT)*

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Abstract—The administration of 1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl) ethane (*o,p'*-DDT) to immature female rats produced a maximum increase in uterine wet weight and DNA synthesis measured by tritiated thymidine incorporation into uterine DNA 24 hr after treatment. These responses were maximal at a dose of 250–1000 mg/kg of *o,p'*-DDT; half-maximal responses occurred in the range of 10–40 mg/kg. These responses were specific for *o,p'*-DDT, since *p,p'*-DDT was much less potent. 17β-Estradiol and *o,p'*-DDT also produced increases in total uterine DNA content and in total uterine protein content. The uterine responses to maximum doses of 17β-estradiol and *o,p'*-DDT were not additive, suggesting that both compounds interact with the same receptor. In a series of experiments, the administration of 250 mg/kg of *o,p'*-DDT produced increases in uterine weight that were the same as those seem after 17β-estradiol treatment, increases in DNA synthesis that were 60–80 per cent of those produced by 17β-estradiol and an increase in total uterine DNA that was 66 per cent of that observed after 17β-estradiol treatment. 17β-Estradiol and *o,p'*-DDT also produced significant increases in uterine wet weight and DNA synthesis in ovariectomized/adrenalectomized rats, indicating that the effects of the pesticide are not mediated via adrenal steroids.

DDT† and other pesticides have been reported to exhibit estrogenic properties following *in vivo* administration to a number of mammalian species (reviewed in Refs. 1–3). One such property that has been studied is the stimulation of the rat uterus. It has been demonstrated that *o,p'*-DDT competitively inhibits the binding of 17β-estradiol to the uterine estrogen receptor [4–6], and thus presumably binds to the receptor itself. It has also been shown that *o,p'*-DDT produces increases in uterine wet weight and other variables normally associated with estrogenic stimulation [4, 5, 7–10].

The response of the rat uterus to the naturally occurring hormone 17β-estradiol is a complex process involving a temporally ordered sequence of individual events which culminate in increased dry mass of the tissue, DNA synthesis and cell division [11–13]. However, some estrogens, e.g. estriol, can stimulate increases in uterine wet weight and in certain enzyme activities without causing increases in dry mass of the tissue, DNA synthesis or DNA content [11, 13]. To date, there have been no reported studies of the effects of DDT on uterine DNA synthesis. This is obviously a critical parameter to consider when evaluating the potential toxicities of DDT and related compounds.

The studies reported in this work, therefore, were undertaken to determine if *o,p'*-DDT stimulates uterine DNA synthesis and to investigate the time course, specificity and dose–response relations.

METHODS

Immature female rats (20–21 days old, 35–40 g), from the Texas Inbred Mouse Co., Houston, TX, were ovariectomized 3–4 days prior to use. For studies with adrenalectomized rats, animals were ovariectomized and adrenalectomized at the same time (i.e. 3–4 days prior to use) and given 0.9% saline in the drinking water. Hormones and pesticides were administered as intraperitoneal injections in 0.1 ml of DMSO as the solvent; control animals received the vehicle alone. At the times indicated in the text, animals were decapitated and the uteri were removed, stripped of adhering fat and mesentery, and weighed on a Cahn electrobalance.

Uteri were then quickly placed in Eagle's basal medium containing tritiated thymidine for the measurement of DNA synthesis by the method of Kaye *et al.* [14], as described previously [15]. DNA content was measured by the diphenylamine reaction [16], as described previously [15]; total uterine protein content was also measured as described previously [15].

17β-Estradiol and tritiated thymidine were obtained from Schwarz/Mann, Orangeburg, NY; *o,p'*-DDT and *p,p'*-DDT were obtained from the Aldrich Chemical Co., Milwaukee, WI; all other reagents used were the highest grade commercially available.

Statistical analyses were performed by Student's two-tailed *t*-test.

Competitive hormone binding studies were performed as described by Nelson [4] using a

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† Abbreviations: DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; *o,p'*-DDT, 1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)ethane; *p,p'*-DDT, 1,1,1-trichloro-2-bis(*p*-chlorophenyl)ethane; and DMSO, dimethylsulfoxide.

dextran/charcoal assay. Each sample contained 0.32 mg/ml of uterine cytosol protein 2×10^{-9} M [^{3}H]-estradiol (sp. act. 50 Ci/mmole, New England Nuclear, Boston, MA) 10 mM Tris buffer, pH 7.4, 1.5 mM EDTA, 16% (v/v) propylene glycol, 4% (v/v) ethanol plus the indicated concentrations of unlabeled competitors (17β-estradiol, *o,p'*-DDT or *p,p'*-DDT) in a final volume of 0.5 ml. Following a 3-hr incubation at 0°, 0.5 ml of 0.5% charcoal/0.05% dextran was added for 15 min to remove unbound [^{3}H]-estradiol, the samples were centrifuged and the radioactivity in the supernatant fraction was determined. Non-specific binding was determined by a parallel incubation with a 100-fold excess of unlabeled extradiol and subtracted from the values reported in the text, i.e. values reported represent specifically bound hormone.

RESULTS

Figure 1 illustrates the uterine response observed following the administration of 250 mg/kg body weight of *o,p'*-DDT to immature rats. As seen in Fig. 1, upper panel, DDT produced a doubling of uterine wet weight by 24 hr which began to decline toward control levels between 36 and 48 hr. This increase in wet weight was accompanied by a large increase in uterine DNA synthesis which was maximum at 24 hr (Fig. 1, lower panel). The time course of the uterine response to DDT is thus similar to the time course for uterine stimulation by 17β-estradiol, as observed in numerous reports [17–19].

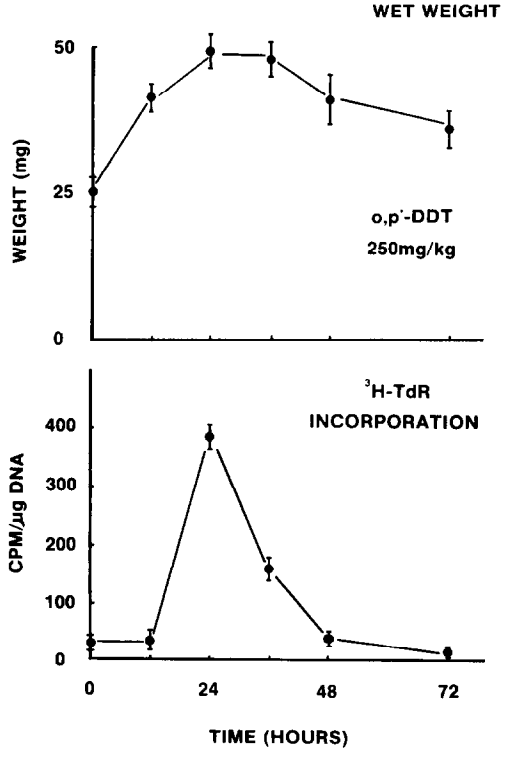

Fig. 1. Time course of uterine DNA synthesis following administration of 250 mg/kg of *o,p'*-DDT. Groups of eight animals were treated with *o,p'*-DDT for the indicated times prior to being killed. Uterine weight and DNA synthesis were then measured as described in Methods. Values are means with the indicated S.E.M.

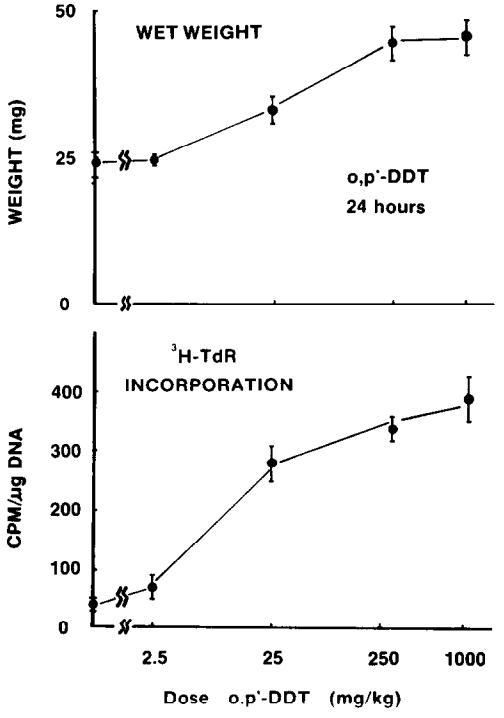

Fig. 2. Dose–response curves for the uterine response to *o,p'*-DDT. Groups of eight animals were treated with the indicated doses of *o,p'*-DDT for 24 hr prior to being killed. Uterine weight and DNA synthesis were then measured as described in Methods. Values are means with the indicated S.E.M.

Since the maximum response to DDT occurred 24 hr after administration, we examined the dose–response curve for the uterine response at this time. Figure 2 shows the results of these studies for uterine wet weight (upper panel) and uterine DNA synthesis (lower panel). In both cases, a maximal response was produced at a dose of 250 mg/kg body weight (the responses seen at 250 and 1000 mg/kg for DNA synthesis are not statistically different); half-maximal responses were observed in the range of 10–40 mg/kg body weight.

To determine the specificity of the uterine response to *o,p'*-DDT, we compared the responses of *o,p'*-DDT and *p,p'*-DDT. The *p,p'*-compound was chosen since other workers have reported previously that it binds poorly to the uterine estrogen receptor [4, 6]; we have confirmed these findings, as shown in Fig. 3. The data in this figure illustrate that *o,p'*-DDT was more effective than *p,p'*-DDT in competing for estradiol binding sites on the cytosolic uterine estrogen receptor. The results also indicate that unlabeled estradiol was about 3 orders of magnitude more effective than unlabeled *o,p'*-DDT in competing with labeled estradiol for receptor binding sites.

The uterine responses produced by *o,p'*-DDT and *p,p'*-DDT are shown in Fig. 4 for increases in uterine wet weight (upper panel) and increases in uterine DNA synthesis (lower panel) after administration of either 25 or 250 mg/kg doses of the two isomers.

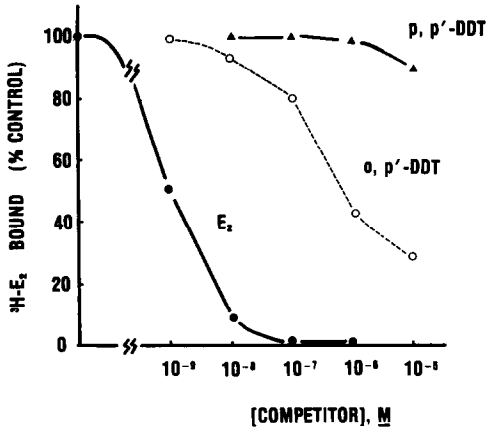

Fig. 3. Competitive binding of *o,p'*-DDT and *p,p'*-DDT to the cytosolic uterine estrogen receptor. Samples of uterine cytosol were incubated with 2×10^{-9} *M* [^{3}H]-estradiol and the indicated concentrations of unlabeled competitors as described in Methods. Values are means of duplicate determinations and represent specific binding (see Methods). The 100 per cent value for [^{3}H]-estradiol binding corresponds to 0.8 pmoles of hormone bound per mg of cytosol protein and represents 4800 c.p.m. in the assay used.

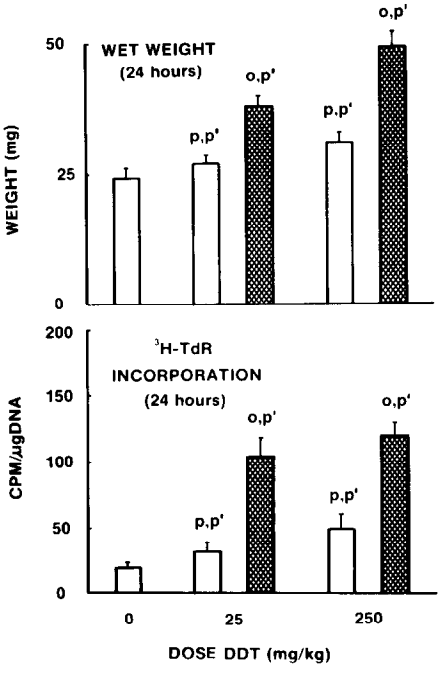

Fig. 4. Specificity of the uterine response to *o,p'*-DDT. Groups of eight animals were treated with 0, 25 or 250 mg/kg of either *p,p'*-DDT or *o,p'*-DDT for 24 hr prior to being killed. Uterine weight and DNA synthesis were then measured as described in Methods. Values are the means with the indicated S.E.M. For both variables and both dose levels, the responses to *o,p'*-DDT are significantly higher ($P < 0.01$) than the responses to *p,p'*-DDT.

It is clear that *o,p'*-DDT was much more potent than *p,p'*-DDT at producing either uterine response. Previous reports [4, 6, 8] have also noted that the *o,p'*-compound is more effective at producing various uterine responses after *in vivo* administration.

Having investigated the time course, dose–response relationships and specificity of the uterine response to *o,p'*-DDT, we next compared the efficacy of *o,p'*-DDT and of the naturally occurring hormone 17β-estradiol. The results of a series of such experiments are illustrated in Table 1. In all experiments, estradiol and *o,p'*-DDT produced virtually identical increases in uterine wet weight. The stimulation of uterine DNA synthesis by *o,p'*-DDT was 65, 67, 86, 71 and 80 per cent of that observed with estradiol for experiments 1–5, respectively. The dose of estradiol used in these studies is known to produce maximum increases in uterine weight and DNA synthesis [11, 13, 20–22].

Table 1. Effects of estradiol and *o,p'*-DDT on uterine weight and DNA synthesis 24 hr after treatment*

Expt. No.	Control	E_2	*o,p'*-DDT
	Uterine wet weight (mg)		
1	23.3 ± 0.5 (9)	48.0 ± 3.3 (9)	46.0 ± 1.3 (10)
2	24.6 ± 1.4 (7)	45.9 ± 2.2 (8)	45.2 ± 2.3 (8)
3	25.8 ± 1.9 (8)	47.3 ± 1.7 (8)	49.5 ± 1.9 (8)
4	24.3 ± 1.4 (7)	49.5 ± 2.5 (7)	49.0 ± 3.4 (8)
5	22.9 ± 0.9 (9)	45.3 ± 1.2 (10)	47.7 ± 3.0 (10)
	Uterine DNA synthesis (c.p.m. [^{3}H]-thymidine incorporated/μg DNA)		
1	92 ± 25 (9)	974 ± 78 (10)	660 ± 36 (10)
2	43 ± 7 (7)	487 ± 19 (8)	340 ± 15 (8)
3	33 ± 6 (8)	447 ± 31 (8)	338 ± 17 (8)
4	20 ± 4 (7)	159 ± 9 (8)	119 ± 8 (8)
5	30 ± 4 (9)	249 ± 7 (10)	201 ± 16 (10)

* Animals were treated with the vehicle alone (control), 40 μg/kg 17β-estradiol (E_2) or 250 mg/kg *o,p'*-DDT 24 hr before being killed. Uterine DNA synthesis was then measured as described in Methods. Values represent means ± S.E.M.; N values are in parentheses. All values for the E_2 and *o,p'*-DDT groups are significantly different from control values ($P < 0.01$). For DNA synthesis, values for the E_2 and *o,p'*-DDT groups are significantly different ($P < 0.01$) in experiments 1, 2 and 4, and ($P < 0.05$) in experiment 5.

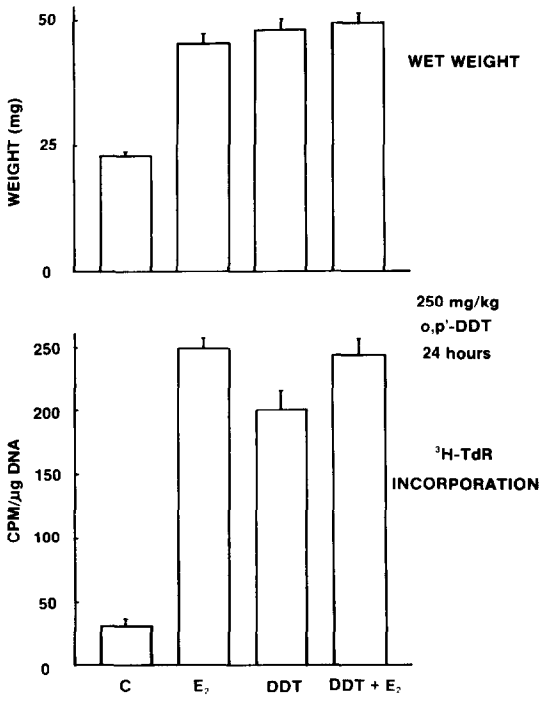

Fig. 5. Effects of o,p'-DDT and 17β-estradiol administered simultaneously. Groups of nine to ten animals were treated with the vehicle (C), 250 mg/kg of o,p'-DDT, 40 μg/kg of 17β-estradiol (E_2) or the same doses of o,p'-DDT + E_2 24 hr prior to being killed. Uterine weight and DNA synthesis were then measured as described in Methods. Values are the means with the indicated S.E.M. For both responses, all groups are significantly higher ($P < 0.01$) than controls, and for DNA synthesis the o,p'-DDT group is significantly less ($P < 0.05$) than either the E_2 or E_2 + DDT group.

Since DDT is thought to elicit an estrogenic response via its interaction with the uterine estrogen receptor, we determined if the effects of the pesticide and the hormone were additive. Figure 5 illustrates an experiment in which these compounds were administered separately and together. It is clearly seen that the effects were not additive; the response following administration of both compounds was virtually identical to that seen after the hormone alone and only slightly higher than that seen with DDT alone.

Table 2. Effects of estradiol and o,p'-DDT on uterine DNA content 48 hr after administration*

Treatment	DNA (μg/uterus)
Control	271 ± 7 (7)
E_2	495 ± 17 (8)
o,p'-DDT	418 ± 22 (8)

* Animals were treated with the vehicle alone (control), 40 μg/kg 17β-estradiol (E_2) or 250 mg/kg o,p'-DDT 48 hr before being killed. Uterine DNA content was measured as described in Methods. Values are means ±S.E.M.; N values are in parentheses. Values for E_2 and o,p'-DDT are both significantly greater than controls ($P < 0.01$) and values for E_2 and o,p'-DDT are significantly different from each other ($P < 0.05$).

Table 3. Effects of estradiol and o,p'-DDT on total uterine protein content 24 hr after administration*

Treatment	Protein (mg/uterus)
Control	1.04 ± 0.04 (9)
E_2	2.36 ± 0.13 (10)
o,p'-DDT	2.84 ± 0.15 (9)

* Animals were treated with the vehicle alone (control), 40 μg/kg 17β-estradiol (E_2) or 250 mg/kg o,p'-DDT 24 hr before being killed. Total uterine protein content was measured as described in Methods. Values are means ± S.E.M.; N values are in parentheses. Values for E_2 and o,p'-DDT are both significantly greater than controls ($P < 0.01$) and values E_2 and o,p'-DDT are significantly different from each other ($P < 0.05$).

The results of an experiment to determine if o,p'-DDT actually caused an increase in total uterine DNA content, as well as an increase in the incorporation of tritiated thymidine, are shown in Table 2 and illustrate that both estradiol and o,p'-DDT produced significant increases in the total DNA content of the organ.The increase in total DNA produced by o,p'-DDT administration was 66 per cent of that observed after estradiol treatment.

We also examined the effect of the pesticide on the total uterine protein content as another indicator of tissue growth. Both o,p'-DDT and the naturally occurring hormone estradiol produced large increases in the total uterine protein content (Table 3). In this particular experiment, the increase in total protein produced by o,p'-DDT was, in fact, larger than that produced by estradiol. Since we have not routinely measured uterine protein content in other experiments, we are not certain whether the administration of the pesticide would routinely lead to increases in uterine protein content larger than the administration of estradiol produces.

Finally, we determined if the effects of DDT on uterine growth and DNA synthesis also occur in adrenalectomized/ovariectomized animals in case the observed effects of the pesticide had been caused by stimulating adrenal estrogen or progesterone production. As shown in Table 4, the administration of o,p'-DDT to adrenalectomized/ovariectomized rats produced increases in uterine weight comparable to

Table 4. Effects of estradiol and o,p'-DDT on uterine weight and DNA synthesis in ovariectomized/adrenalectomized rats*

Treatment	Uterine wet wt (mg)	Uterine DNA synthesis (c.p.m. [^{3}H] thymidine incorporated/μg DNA)
Control	21.1 ± 1.1 (10)	35 ± 4 (10)
E_2	42.4 ± 1.8 (10)	415 ± 36 (10)
o,p'-DDT	39.2 ± 3.4 (6)	190 ± 71 (6)

* Animals were treated with the vehicle alone (control), 40 μg/kg 17β-estradiol (E_2) or 250 mg/kg o,p'-DDT 24 hr before being killed. Uterine DNA synthesis was then measured as described in Methods. Values are means ± S.E.M.; N values are in parentheses. All values for E_2 and o,p'-DDT are significantly greater than control values ($P < 0.01$). Values of DNA synthesis for the E_2 and o,p'-DDT groups are significantly different ($P < 0.01$).

those seen after estradiol treatment. Both the pesticide and the hormone stimulated DNA synthesis in these animals; the level of DNA synthesis observed after pesticide treatment was 46 per cent of that seen after estradiol. This is less than the values of 60–80 per cent seen with ovariectomized animals (Table 1), but 40 per cent of the adrenalectomized animals receiving DDT died in the 24-hr period before DNA synthesis was measured. This obvious increase in the toxicity of DDT in the adrenalectomized animals (no other fatalities were observed in this work at any dose levels) may be the reason that the response to DDT seen in adrenalectomized/ovariectomized animals was less than that seen in ovariectomized animals. It is clear, nevertheless, that o,p'-DDT produced a 5.5-fold increase in DNA synthesis in adrenalectomized/ovariectomized animals relative to vehicle-treated controls.

DISCUSSION

These studies demonstrate that o,p'-DDT is capable of producing long-term (i.e. 24–48 hr after treatment) increases in wet weight, DNA synthesis, total DNA content and protein content in the rat uterus. It appears that this compound produces the same overall uterine response as the naturally occurring hormone 17β-estradiol, following acute administration. Whether or not o,p'-DDT produces the same overall uterine responses as 17β-estradiol under other dosage and treatment regimens remains to be elucidated.

Dose–response studies (Fig. 2) of increase in uterine weight and DNA synthesis indicate that maximal, or near maximal, responses occur at dose levels of 250–1000 mg/kg, and half-maximal responses occur in the range of 10–40 mg/kg. These doses are generally in the range reported by other workers measuring different uterine responses. Kupfer and Bulger [5], for example, noted increases in uterine ornithine decarboxylase activity at doses of 5–250 mg/kg, Welch *et al.* [8] noted increases in uterine wet weight 6 hr after treatment with 5–50 mg/kg of o,p'-DDT, and Singhal *et al.* [9] used a dose of 100 mg/kg o,p'-DDT to produce increases in uterine weight and in the activity levels of several uterine enzymes. We have determined in previous work that a dose of 17β-estradiol in the range of 2–4 μg/kg produces maximum increases in uterine weight and DNA synthesis 24 hr after treatment [21]; comparable results have been obtained in many other laboratories. Based on these values for maximum responses, a rough approximation would be that estradiol is about 1000 to 10,000 times as potent as o,p'-DDT in producing uterine responses. This is in keeping with the findings of Cecil *et al.* [23] that 10^4-fold more o,p'-DDT than estradiol is required to produce similar increases in uterine wet weight, water, RNA and glycogen content.

The values for the relative potencies of estradiol and o,p'-DDT are generally in line with the relative affinities of the estrogen receptor for these two compounds. Nelson [4] originally reported that the affinity of the rat uterine estrogen receptor for o,p'-DDT was 1/2,000 of that for diethylstilbestrol, which is generally thought to be bound to the estrogen receptor with about the same affinity as estradiol. Our data (Fig. 3) also indicate that the affinity of the uterine receptor for o,p'-DDT is about 3 orders of magnitude different than that of estradiol. Other workers [24, 25] also noted that estrogen receptors from other target tissues bind o,p'-DDT about 1/10,000 as well as estradiol.

Our data support the hypothesis that o,p'-DDT elicits uterine responses by interacting with the uterine estrogen receptor. These observations are: p,p'-DDT, which binds poorly to the estrogen receptor (Fig. 3), was relatively ineffective in producing uterine responses (Fig. 4); the responses to maximum doses of estradiol and o,p'-DDT were not additive (Fig. 5), suggesting that both compounds elicited responses via the same mechanism; o,p'-DDT produced responses in ovariectomized/adrenalectomized animals, ruling out the possibility that the effects of the pesticide resulted secondarily from stimulation of steroidogenic tissues; and the relative *in vivo* potencies of estradiol and o,p'-DDT are generally in line with their relative affinities for the uterine estrogen receptor.

One question in studies of this nature is whether the effects observed resulted entirely from the interaction of the administered compound (i.e. o,p'-DDT) with the uterine estrogen receptor, or whether a metabolite(s) was involved. Clearly, we cannot provide a definitive answer to this question from the studies reported here. We observed recently, however, that o,p'-DDT can induce the synthesis of a specific estrogen inducible uterine protein [26, 27] in a completely *in vitro* system*. It seems reasonable to suggest that the *in vivo* effects observed after o,p'-DDT administration are at least partially due to the interaction of the unmetabolized pesticide with the estrogen receptor.

The results in Table 1 indicate that a dose of 250 mg/kg of o,p'-DDT produced the same increase in uterine weight as did 17β-estradiol but only about 74 per cent (the average of the five experiments shown) of the hormone stimulation of DNA synthesis. It is not known at present if measurements with slightly higher doses of o,p'-DDT or at slightly different times after treatment would yield identical results for the two compounds.

We feel it is more important to note that o,p'-DDT produces substantial increases in uterine DNA synthesis and DNA content which at least approach those seen after estradiol treatment. As far as we are aware, this is the first report that o,p'-DDT produces these effects in an estrogen target tissue.

The studies performed in this work certainly cannot be directly extrapolated to human or animal exposures to pesticides in the environment. Our results suggest, however, that hyperplastic responses of estrogen target tissues, both normal and malignant, should be considered as one possible toxicity of o,p'-DDT and related compounds.

* J. S. Ireland, V. R. Mukku, A. K. Robison and G. M. Stancel, unpublished observation.

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