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# Studies on the Estrogenic Activity of Chlordecone (Kepone) in the Rat: Effects on Uterine Estrogen Receptor

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SUMMARY

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The effects of chlordecone (Kepone) on rat uterine estrogen receptor were examined *in vitro* and *in vivo*. In cell free preparations, chlordecone was found to inhibit the binding of [$^3$H]estradiol to uterine cytosolic 8S estrogen receptor in a competitive manner, suggesting that chlordecone binds to the same site as estradiol. Incubation of isolated uteri *in vitro* in the presence of chlordecone resulted in an increrase in estrogen receptor in the nuclear fraction. This increase accompanied a decline in the amount of estrogen receptor in the cytosolic fraction, indicating that translocation of the estrogen receptor had occurred. Within one hour of the injection of immature rats with chlordecone there was an accumulation of estrogen receptor in the uterine nuclear fraction that was concomitant with a depletion of cytosolic estrogen receptor. The amount of nuclear receptors remained elevated up to 48 hours after chlordecone administration. Cytosolic receptors depletion persisted for 16 hours after injection, at which time replenishment of the cytosolic receptor began. Two hours after chlordecone injection, the ratio of uterine weight to body weight was elevated. Uterine cytosolic protein increased 16–48 hours after injection of chlordecone and the amount of uterine nuclear DNA was elevated 24–48 hours after chlordecone administration. The similarities between the effects of estradiol and chlordecone on uterine estrogen receptors and the effect of the long half life of chlordecone in the animal on the estrogenic action of chlordecone are discussed.

## INTRODUCTION

Chlordecone (Kepone) a chlorinated polycyclic ketone (Fig. 1) has been used as a pesticide in a variety of applications, particularly as an insecticide and fungicide (1). The illness among workers in a chlordecone production plant at Hopewell, Virginia, from high-level, multiroute exposure to the compound, and the contamination of the James River and its tributaries by the discharge of the compound from the plant are well documented (1). Workers exposed to chlordecone exhibited a wide spectrum of clinical symptoms including tremors and sterility, as evidenced by abnormal sperm morphology, decreased sperm mobility, and oligospermia (1, 2). Studies in both avian and mammalian systems also indicate that chlordecone can affect the reproductive system. In immature and adult Japanese quail fed chlordecone, the seminiferous tubules were dilated and both germinal epi-

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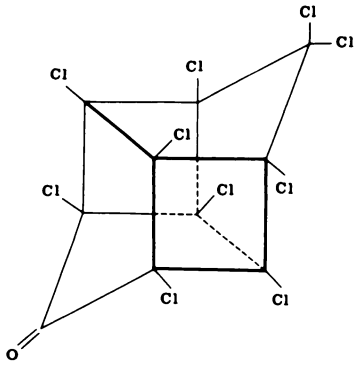


FIG. 1. *Chlordecone*
(Decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalene-2-one). Kepone is the trade name for chlordecone.

thelium and the number of spermatozoa were reduced in the testes (3). In female quail, chlordecone exerted a strong estrogenic effect on the oviduct. The effect was most noticeable in the immature animal (4). Most recently, Palmiter and Mulvihill (5) reported on the estrogenic activity of chlordecone on the chick oviduct. In this study, chlordecone induced oviduct synthesis of ovalbumin and conalbumin, both *in vivo* and *in vitro*, and inhibited the *in vitro* uptake of [$^3$H]estradiol into oviduct nuclei. Huber (6) described reduced reproduction, associated with the development of large follicles, the absence of corpora lutea, and constant estrus, in female mice fed chlordecone. Recently, Hammond *et al.* (7) reported that reproduction in female rats was markedly affected by chlordecone and that the effect was accompanied by depressed estradiol and LH[2] levels, constant estrus and elevated uterine weights. Although, other interpretations are possible, the above evidence strongly suggests that chlordecone is "estrogenic" in mammalian species. To further study this problem we investigated the *in vivo* and *in vitro* effect of chlordecone on the rat uterine estrogen receptor.

## MATERIALS AND METHODS

*Chemicals.* The following compounds were purchased: Tris and dextran, grade C (Schwarz/Mann); EDTA (Mallinckrodt); activated charcoal untreated powder, diethylstilbestrol, calf thymus DNA type I (Sigma Chemical Co.); dithiothreitol (Calbiochem); 17$\beta$-estradiol (Steraloids, Inc.); crystalized bovine serum albumin (Metrix, Armor Pharmaceutical Co.); USP grade corn oil (Matheson Coleman and Bell); diphenylamine (Aldrich); Liquiflor, Aquasol (New England Nuclear). Chlordecone (98% pure) was supplied by Chem. Service, Inc., West Chester, Pa. All other compounds utilized in this study were of reagent grade quality.

*Radiochemicals.* 17$\beta$-[2,4,6,7-$^3$H]Estradiol (85–110 Ci/mmole) was from Amersham Corporation. A radiochemical purity of 98%+ was established by thin-layer chromatography.

*Animals.* Immature female Sprague Dawley CD rats were obtained from Charles River Breeding Laboratories, Inc., and were maintained under conditions of controlled light and temperature. The animals were fed Charles River Rat Formula and allowed water *ad libitum*. The rats were 19–22 days old at the time of use. Ovariectomy was accomplished under ether anesthesia. Ovariectomized animals were used 7 days after surgery (27 days old). Sacrifice was by cervical dislocation and was always between 8:00 and 9:30 A.M.

*Sucrose gradient sedimentation analysis.* Experimentation was conducted according to the method of McGuire *et al.* (8), employing previously described minor modifications suited for investigation with chlorinated insecticides (9). The [$^{14}$C]BSA (4.6S marker) was prepared by the method of Rice and Means (10). The addition of chlordecone and [$^3$H]estradiol was carried out as described in the legend to Figure 2.

*Saturation analysis with dextran-coated charcoal.* The incubation of uterine cytosol with nonsaturating concentrations of [$^3$H]estradiol in the presence or absence of chlordecone was as previously described (9). Procedural details are described in the legend to Figure 3. Bound and free [$^3$H]-estradiol were separated by a DCC procedure described by McGuire (11) and the resulting data were analyzed by the method of Scatchard (12). Linear regression analy-

[2] The abbreviations used are: LH, luteinizing hormone; BSA, bovine serum albumin; DCC, dextran-coated charcoal; DDT, 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane.

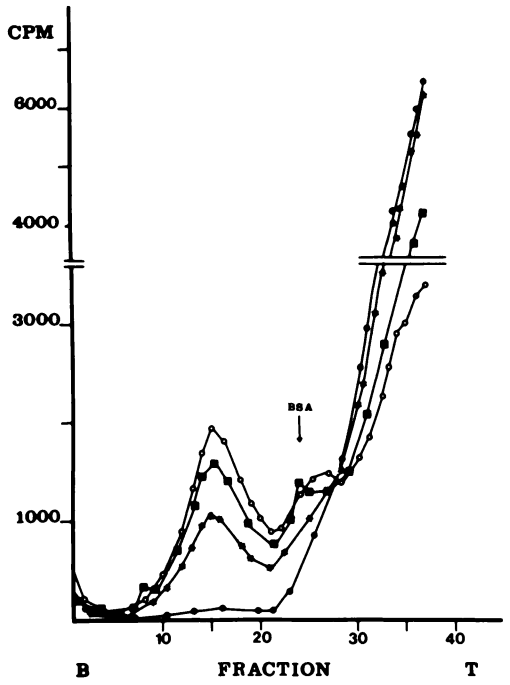


FIG. 2. *Sucrose gradient sedimentation analysis of [$^3$H]estradiol binding to immature rat uterine cytosol in the presence of various concentrations of chlordecone*

Cytosol (105,000 × *g*) was prepared from the uteri of 4 rats and 0.2 ml aliquots were incubated in the presence of chlordecone or diethylstilbestrol (in 2 μl ethanol) at 0°. Control cytosol received 2 μl ethanol only. After 15 min, [$^3$H]estradiol (4 nM) was added in 50 μl buffer and 4 hours later the cytosol was layered on a 10–30% sucrose gradient. Resolution of the 8S region was by centrifugation at 250,000 × *g* for 15 h, with subsequent determination of radioactivity in gradient fractions. Cytosol contained 1.6 mg protein per ml. (○) control, no chlordecone; (■) chlordecone, 2 μM; (*) chlordecone, 8 μM; (●) diethylstilbestrol, 0.4 μM. BSA (bovine serum albumin, 4.6S marker).

sis was employed to determine the $x$ and $y$ intercepts of the Scatchard plots. The $K_d$ for [$^3$H]estradiol was calculated by determining the reciprocal of the slope of the regression line. Assuming competitive inhibition, the $K_I$ for chlordecone was determined as follows:

$$K_I = \frac{K_d[I]}{K_d' - K_d}$$

where $K_d$ and $K_d'$ are affinity constants of [$^3$H]estradiol for uninhibited and inhibited estrogen-binding protein, respectively, and $[I]$ is the molar concentration of chlordecone.

In vitro *translocation of estrogen receptor.* Rats were sacrificed and their uteri excised. A pool of six uteri was used in each determination. Each uterus was divided in half by transsection through the fused portion and the two halves were divided between two incubation vials. Each vial contained tissue equivalent to three uteri and 3 ml of Krebs-Ringer solution (13). The control vial received 5 μl of ethanol and the other vial received a given concentration of chlordecone in 5 μl of ethanol. Incubation was at 37° for 1 hour. At the end of the incubation period the uteri were removed and washed in Krebs-Ringer solution at 4°. The uteri were homogenized and the amount of estrogen receptor in the nuclear and cytosolic fractions was determined by

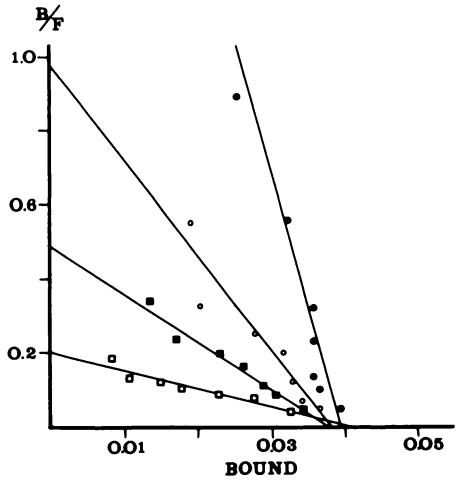


FIG. 3. *Scatchard plot analysis of inhibition by chlordecone of [$^3$H]estradiol binding to immature rat uterine cytosol*

Uterine cytosol (105,000 × *g*) was prepared from 4 uteri. To 0.2 ml aliquots of cytosol was added various concentrations of chlordecone in 2 μl of ethanol. Control cytosol received 2 μl ethanol only. Incubation was at 0° for 1 hour at which time various concentrations of [$^3$H]estradiol (0.06–0.80 nM) were added in 50 μl buffer and the incubation continued for 18 hours. The separation of bound from free [$^3$H]estradiol was by dextran-coated charcoal (See METHODS). (●) control, no chlordecone; (○) chlordecone, 0.10 μM; (■) chlordecone, 0.20 μM; (□) chlordecone, 0.40 μM. Bound (pmoles [$^3$H]estradiol per ml uterine cytosol). B/F (ratio of bound to free [$^3$H]estradiol).

exchange with [$^3$H]estradiol (see below). Four determinations were performed for each concentration of chlordecone and the results were expressed as the mean value. The Student's *t*-test was employed for comparing control preparations with those containing added chlordecone.

In vivo *translocation of estrogen receptor.* The rats employed in the *in vivo* experiments received an i.p. dose of chlordecone (40 mg/kg) in 0.2 ml of corn oil. Each determination employed four rats (3–6 determinations per time point). Most of the animals developed tremors within three hours of injection. However, none of the chlordecone treated rats died during the experimental period (0–48 h). No difference in the compartmentalization of estrogen receptor was observed between zero time animals (treated with chlordecone) and sham injected ones. Furthermore, we previously observed that 0.2 ml of USP grade corn oil was not responsible for the estrogenic activity of certain DDT analogues. Animals were sacrificed at various times after injection (0–48 h). The uteri were excised, washed in 0.9% NaCl and homogenized. The amount of estrogen receptor in the cytosolic and nuclear fractions was determined by exchange with [$^3$H]estradiol. Statistical analysis was carried out by the Student's *t*-test for groups run at different times. Namely, within each experiment a difference ($\Delta$) between control and experimental (e.g., receiving chlordecone) was calculated and these differences were analyzed for inter-experiment variations.

$$\sigma^2 = \frac{\Sigma\Delta^2 - \frac{[\Sigma\Delta]^2}{n}}{n-1}$$

$$t = \frac{\bar{\Delta}}{\sigma/\sqrt{n}}$$

where $\sigma$ = standard deviation; $\Delta$ = difference between control and experimental within a single experiment; $\Sigma\Delta$ = sum of differences; $n$ = number of experiments and $\bar{\Delta}$ = mean of the differences.

*Fractionation of uteri.* The cytosolic and nuclear fractions were prepared from the uteri obtained from the *in vitro* and *in vivo* experiments as follows. Two buffer systems were used: Buffer A; Tris-HCl (10 mM), EDTA (1.5 mM), pH 7.4. Buffer B; Tris-HCl (10 mM), EDTA (1.5 mM), dithiothreitol (0.5 mM), pH 7.4. Tissue equivalent to 3–4 uteri was homogenized in 2 ml of buffer A employing a motor driven Kontes Duall all-glass tissue grinder. The resulting homogenate was centrifuged for 20 min at 800 × *g*. The supernatant was saved and the resulting nuclear pellet was washed three times employing buffer A and centrifugation at 800 × *g* for 10 min. The washed pellet was suspended in buffer B (1 ml per uterus) and employed in the exchange assay. Concentrated dithiothreitol (in buffer A) was added to the supernatant so that the final buffer concentration was equivalent to buffer B and that the final tissue concentration was equivalent to 1 uterus per ml. Cytosol was prepared by centrifugation at 20,000 × *g* for 30 min and was employed in the exchange assay (see below).

*Exchange assay with [$^3$H]estradiol.* The precedure was essentially as described by Anderson *et al.* (14) and Clark *et al.* (15). The amount of nuclear estrogen receptor was determined by allowing an exchange to occur between the chlordecone or estradiol-receptor complex and [$^3$H]estradiol. The exchange was carried out in the presence of 14.1 nM [$^3$H]estradiol in 0.7 ml of buffer A containing uterine nuclei equivalent to one half uterus, at 37° for 1 hour. The exchange was stopped by adding 2 ml of buffer A at 4°. The nuclei were washed three times by vortexing with 2 ml buffer A at 4° and centrifugation at 800 × *g* for 10 min. [$^3$H]Estradiol was extracted from the nuclei by incubation with 2 ml ethanol at 30° for 15 min followed by vortexing and centrifugation at 800 × *g* for 10 min. The radioactivity in the ethanol extract was determined by counting in 10 ml of Liquiflor. The results were corrected for nonspecific [$^3$H]estradiol binding by subtracting the value obtained from a similar incubation containing a concentration of diethylstilbestrol 100 times greater than the [$^3$H]estradiol concentration.

The amount of cytosolic estrogen receptor was determined by allowing the ex-

change to occur at 30° for 30 min in the presence of 16.7 nM [$^3$H]estradiol in 0.3 ml of buffer B which contained uterine cytosol equivalent to one-fourth uterus. The exchange was terminated by immersion in a 4° water bath. The free [$^3$H]estradiol was removed by shaking for 15 min at 4° in the presence of DCC (1% charcoal, 0.05% dextran, 10 mM Tris-HCl, at pH 8.0). The DCC was removed by centrifugation at 800 × *g* for 10 min and the amount of [$^3$H]estradiol in 0.5 ml of the supernatant was determined by counting in 10 ml of Aquasol. Nonspecific binding of [$^3$H]estradiol was corrected for by subtracting the value from a similar incubation performed in which the concentration of diethylstilbestrol was 100 times that of the [$^3$H]estradiol.

*Determination of protein.* Protein was determined by the method of Lowry *et al.* (16) employing the modifications recommended by Stauffer (17) with bovine serum albumin as a standard.

*DNA determination.* The DNA in the nuclei equivalent to one quarter uterus was determined by the diphenylamine method of Dische (18), employing the modifications described by Burton (19). Calf thymus DNA, type I, was employed as a standard.

## RESULTS AND DISCUSSION

The results depicted in Figure 2 demonstrate that chlordecone inhibits the binding of [$^3$H]estradiol to the 8S uterine cytosolic receptor under saturating conditions with respect to [$^3$H]estradiol concentration (4 nM). Chlordecone concentrations of 2 and 8 μM suppressed [$^3$H]estradiol binding by 13.9% and 44.2%, respectively. A concentration of 0.8 μM chlordecone failed to suppress [$^3$H]estradiol binding (not depicted), whereas 0.4 μM diethylstilbestrol caused almost total suppression of [$^3$H]estradiol binding in the 8S region. There was no apparent chlordecone-produced aggregation of the 8S receptor, which if it had occurred would be expected to sediment to the bottom region of the gradient. The decrease in bound radioactivity ([$^3$H]estradiol) in the suppressed gradients was accounted for by a shift in the amount of radioactivity toward the top of the gradients, further indicating that the effect of chlordecone was to inhibit [$^3$H]estradiol binding as opposed to causing receptor aggregation. These results, however, did not permit the evaluation of whether inactivation of the receptor may have occurred and merely escaped detection. To explore this possibility, we performed the experiments shown in Figure 3 and Table 1. The binding of various nonsaturating concentrations of [$^3$H]estradiol (0.06–0.80 nM) to uterine cytosolic protein was determined in the presence of three concentrations of chlordecone (0.10, 0.20, and 0.40 μM) and a Scatchard plot analysis was performed (Fig. 3). As can be seen from the convergence of the lines on the abscissa, the effect of chlordecone was to inhibit the binding of [$^3$H]estradiol

TABLE 1

*Effect of chlordecone on the binding of [$^3$H]-estradiol to rat uterine cytosolic receptor*

Values were determined by Scatchard plot analysis employing the procedure described under METHODS and Fig. 3.

| Experiment No. | Concentration of chlordecone | $r^b$ | $K_d$ | [$^3$H]estradiol bound |
|---|---|---|---|---|
| | (μM) | | (M) | (fmoles/mg protein[c]) |
| 1. | Control[a] | −0.94 | $2.59 \times 10^{-11}$ | 516.67 |
| | 0.04 | −0.89 | $3.67 \times 10^{-11}$ | 469.44 |
| | 0.20 | −0.90 | $1.48 \times 10^{-10}$ | 516.68 |
| | 0.40 | −0.88 | $3.28 \times 10^{-10}$ | 552.78 |
| 2. | Control | −0.84 | $4.56 \times 10^{-11}$ | 866.67 |
| | 0.04 | −0.98 | $4.54 \times 10^{-11}$ | 827.16 |
| | 0.20 | −0.94 | $9.51 \times 10^{-11}$ | 818.52 |
| | 0.40 | −0.89 | $2.60 \times 10^{-10}$ | 972.84 |
| 3. | Control | −0.95 | $1.74 \times 10^{-11}$ | 237.16 |
| | 0.10 | −0.91 | $4.95 \times 10^{-11}$ | 230.21 |
| | 0.20 | −0.82 | $1.84 \times 10^{-10}$ | 280.99 |
| | 0.40 | −0.89 | $1.86 \times 10^{-10}$ | 214.18 |
| 4.[d,e] | Control | −0.96 | $1.50 \times 10^{-11}$ | 305.70 |
| | 0.10 | −0.96 | $3.89 \times 10^{-11}$ | 296.80 |
| | 0.20 | −0.99 | $7.63 \times 10^{-11}$ | 291.56 |
| | 0.40 | −0.95 | $2.03 \times 10^{-10}$ | 318.75 |

[a] Control preparations received vehicle (2 μl ethanol) only.

[b] Correlation coefficients for linear regression routine.

[c] Cytosolic protein concentration (mg/ml): experiment No. 1 (0.036); No. 2 (0.081); No. 3 (0.075); No. 4 (0.128).

[d] Calculations from data shown in Figure 3.

[e] Rats in this experiment were 26 days old.

to uterine cytosol without changing the number of high affinity binding sites of receptor protein(s). The diminished ratio of bound to free [$^3$H]estradiol (see intersecting lines on the ordinate) indicates an apparent decrease in the affinity of estradiol to its receptor. In additional experiments (Table 1) the $K_d$ of [$^3$H]estradiol (15–46 pM) was found to increase in the presence of 0.10–0.40 μM chlordecone while the amount of bound [$^3$H]estradiol remained constant within a given experiment, indicating that chlordecone inhibited [$^3$H]estradiol binding in a competitive manner. Data from Scatchard plot analyses was employed to determine the $K_I$ of chlordecone, which was 48 ± 7 nM (mean ± S.E. for 9 determinations). The relatively low $K_I$ value indicates that chlordecone is a potent inhibitor of estradiol binding to the receptor and suggests a high affinity binding of chlordecone to the cytosolic estrogen receptor.

To examine whether the binding of chlordecone to cytosolic estrogen receptor could produce a translocation of the receptor from the uterine cytosol into the nuclear fraction, we incubated isolated uteri *in vitro* in the presence of various concentrations of chlordecone. Results (Table 2) demonstrate that chlordecone (4, 8, and 16 μM) significantly alters the compartmentalization of estrogen receptor in the cytosolic and nuclear fractions by increasing the nuclear uptake of estrogen receptor while diminishing the amount of receptor in the cytosolic compartment. Similar results were achieved with 20 nM estradiol (Table 2); however even at this concentration, estradiol appeared to be much more potent than chlordecone. It was concluded that estrogen receptor translocation occurred *in vitro*, presumably by the formation of a chlordecone-receptor complex prior to the translocation of the receptor.

To determine whether the translocation of the estrogen receptor could also occur *in vivo*, chlordecone (40 mg/kg) was administered to immature rats and the amount of estrogen receptor present in the uterine cytosolic and nuclear fractions was determined at various time points after injection (Fig. 4). Thirty to sixty minutes after chlordecone administration there was an accumulation of estrogen receptor in the nuclear fraction, paralleled by a concomitant depletion of cytosolic receptor. The amount of receptor in the nuclear compartment remained elevated and was markedly in ex-

TABLE 2

*Amount of [$^3$H]estradiol bound in the nuclear and cytosolic fractions prepared from rat uteri incubated* in vitro *in the presence of various concentrations of chlordecone or estradiol*

The uteri from 6 immature rats were used in each determination. Each uterus was transsected through the fused portion and divided between two incubation vials (each vial contained 3 ml Krebs-Ringer solution), so that one half of each uterus was placed in each vial. Chlordecone or estradiol was added in 5 μl ethanol to one vial. The second vial served as a control and received 5 μl ethanol only. Vials were incubated for 1 hour at 37°, the uteri were washed, nuclear and cytosolic fractions prepared and the exchange with [$^3$H]estradiol was performed. Detailed procedures are described under METHODS. Values represent the means ± the standard errors for the number of determinations (*n* values) given in parenthesis. Student's *t*-test was employed for comparing control preparations with those containing added chlordecone or estradiol.

| Compound added | Bound [$^3$H]estradiol | |
|---|---|---|
| | Cytosolic fraction | Nuclear fraction |
| | *(fmoles/uterus)* | |
| Chlordecone, 4 μM | 332.9 ± 19.7 (4)** | 176.9 ± 18.9 (4)* |
| Control | 486.6 ± 35.1 (4) | 121.9 ± 6.9 (4) |
| Chlordecone, 8 μM | 293.4 ± 28.0 (4)*** | 209.1 ± 20.0 (4)** |
| Control | 446.9 ± 10.1 (4) | 114.9 ± 9.9 (4) |
| Chlordecone, 16 μM | 230.6 ± 13.7 (4)*** | 244.5 ± 11.2 (4)**** |
| Control | 443.9 ± 31.9 (4) | 120.1 ± 7.5 (4) |
| Estradiol, 20 nM | 40.0 ± 0.8 (3)*** | 445.3 ± 19.0 (3)**** |
| Control | 416.7 ± 46.2 (3) | 101.9 ± 13.1 (3) |

*p* values with respect to control: * ≤0.050; ** ≤0.010; *** ≤0.005; **** ≤0.001.

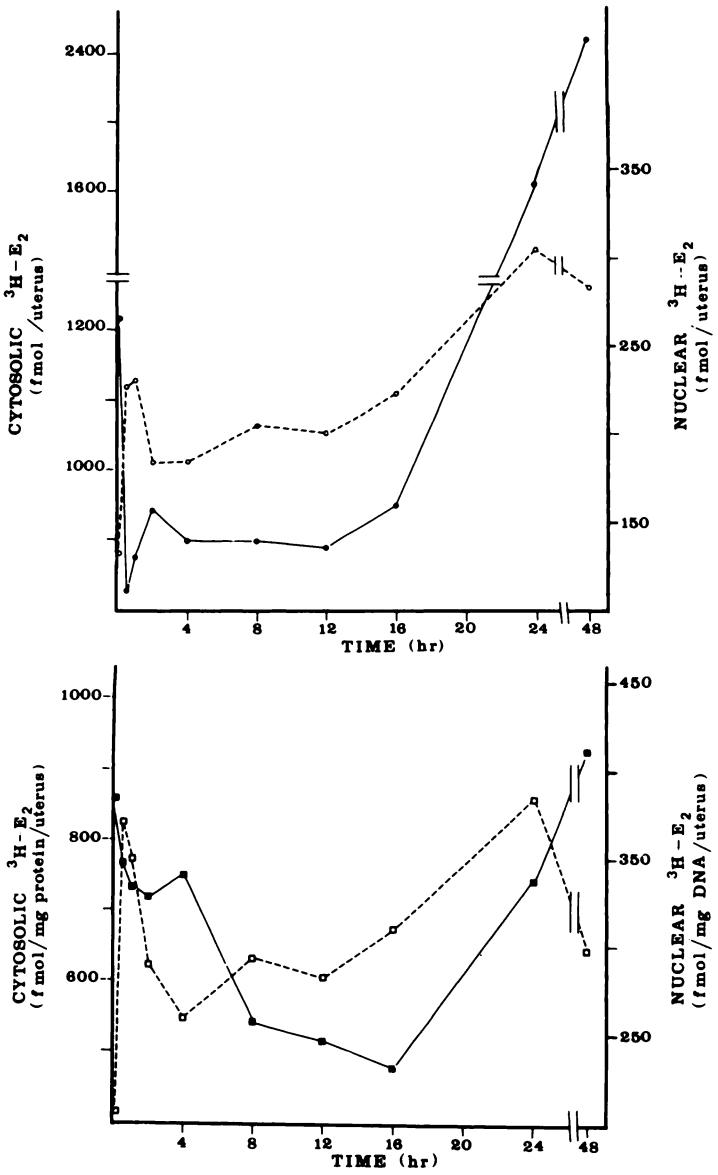


FIG. 4. *Binding of [$^3$H]estradiol ($^3H$-$E_2$) to the cytosolic and nuclear fractions prepared from the uteri of immature rats injected with chlordecone*

At various time points (0–48 h) after injection of chlordecone (40 mg/kg body wt.) rats were sacrificed and the uteri excised. The uteri from 4 rats were employed per determination. Each time point represents the mean of 3–6 such determinations (15 determinations for zero time). For detailed procedures and statistical treatment of data see METHODS. Upper diagram: Binding of [$^3$H]estradiol per uterus; (---○---) nuclear fraction, (——●——) cytosolic fraction. Lower diagram: Binding of [$^3$H]estradiol per nuclear DNA per uterus (-□-); binding of [$^3$H]estradiol per cytosolic protein, per uterus (——■——). All time points differ significantly from the zero times ($p \leqq 0.05$–$0.001$).

cess of the control value at 24–48 hours (Fig. 4 upper diagram). When the quantity of nuclear receptor was expressed in terms of nuclear DNA per uterus (lower diagram), a similar pattern was observed. The depletion of cytosolic receptor persisted for 16

hours after chlordecone injection, at which time replenishment of the receptor began (Fig. 4). The replenishment process was concomitant with an increase in total uterine cytosolic protein (Table 3). The amount of cytosolic receptor was markedly elevated above control value 24 and 48 hours after administration of chlordecone (Fig. 4, upper diagram). However, this increase (48 h) was less dramatic when the receptor level was expressed in terms of [$^3$H]estradiol binding per uterine cytosolic protein (Fig. 4 lower diagram). A secondary rise in the level of nuclear receptor occurred at 4 hours and was accompanied by a further decline in cytosolic receptor. This secondary response was especially evident when receptor levels were expressed in terms of uterine nuclear DNA or cytosolic protein (Fig. 4, lower diagram).

Two hours after chlordecone administration the ratio of uterine weight to body weight was significantly elevated (Table 3). This was followed by a second increase in uterine weight at 24 and 48 h, which was accompanied by increases in uterine cytosolic protein at 16–48 hours and uterine nuclear DNA at 24–48 hours (Table 3). This evidence indicates that true uterine growth occurred about 16 h after chlordecone administration and that earlier increases in uterine weight to body weight ratios probably reflected chlordecone-induced uterine water imbibition. The initiation of uterine growth also accounts for the differences observed between the receptor levels, at 24 and 48 hours, in the upper and lower diagrams of Figure 4. The increase in receptor at 24 and 48 hours (Fig. 4, upper diagram) and the increase in uterine nuclear DNA (Table 3) indicates that chlordecone caused an increase in the amount of uterine estrogen receptor. This increase was concomitant with an increase in the number of uterine cells.

Similarly, the administration of chlordecone (40 mg/kg, i.p.) to ovariectomized rats elevated within 8 hours uterine weight to body weight ratios, diminished levels of cytosolic receptor and elevated the amount of nuclear receptor (not shown). This finding suggests that chlordecone did not function by affecting the release of endogenous estradiol from the ovary. The remote possibility of chlordecone-mediated elevation of estradiol levels by other mechanisms has not been ruled out, however.

The effect of chlordecone on uterine cytoplasmic and nuclear estrogen receptors in immature intact rats (Fig. 4) can be char-

TABLE 3

*Effect of chlordecone injection on uterine weight, uterine cytosolic protein and nuclear DNA in the immature rat*

Immature (19–22 day old) rats were injected i.p. with chlordecone (40 mg/kg) in corn oil. At various time points after injection (0–48 h) animals were weighed, sacrificed, and the uteri excised and weighed. The ratio of uterine weight (mg) per 100 g body weight was determined. Measurement of total cytosolic protein per uterus and total nuclear DNA per uterus is described under METHODS. Values represent the means ± standard error for the number of determinations given in parenthesis. Significance of increases in values with respect to zero time was determined by Student's *t*-test.

| Time point | Uterus | Cytosolic protein | Nuclear DNA |
|---|---|---|---|
| *(h)* | *(mg/100 g body wt)* | *(mg/uterus)* | *(μg/uterus)* |
| 0 | 77.6 ± 1.7 (52) | 1.45 ± 0.09 (17) | 303 ± 8 (15) |
| ½ | 73.6 ± 2.8 (16) | 1.08 ± 0.03 (4) | 290 ± 24 (3) |
| 1 | 76.8 ± 2.4 (18) | 1.21 ± 0.10 (5) | 312 ± 13 (3) |
| 2 | 85.8 ± 3.7 (19)* | 1.32 ± 0.12 (5) | 313 ± 30 (4) |
| 4 | 88.5 ± 2.5 (30)**** | 1.19 ± 0.06 (5) | 340 ± 16 (4) |
| 8 | 108.5 ± 3.5 (16)**** | 1.67 ± 0.14 (5) | 311 ± 45 (5) |
| 12 | 110.4 ± 3.2 (22)**** | 1.75 ± 0.17 (5) | 341 ± 35 (4) |
| 16 | 117.1 ± 5.4 (18)**** | 2.07 ± 0.19 (7)*** | 350 ± 42 (4) |
| 24 | 143.4 ± 5.8 (17)**** | 2.18 ± 0.10 (6)**** | 373 ± 27 (6)** |
| 48 | 148.5 ± 6.4 (16)**** | 2.66 ± 0.08 (6)**** | 444 ± 27 (6)**** |

*p* values with respect to zero time: * ≦ 0.050; ** ≦ 0.025; *** ≦ 0.010; **** ≦ 0.001.

acterized by two phases. The first phase occurs within four hours following chlordecone administration and resembles the uterine events described by Clark *et al.* (20, 21) following a single injection of estradiol. Both chlordecone and estradiol caused a rapid rise in nuclear receptor, which peaked within two hours and was followed by a rapid drop in nuclear receptor levels within four hours. With estradiol, a concomitant decline and subsequent rise in the amount of cytosolic receptor also occurred during this period. However, in the case of chlordecone, cytosolic receptor levels were replenished much later. The second phase (4–48 hours) was similar to the effect of estrogen implants on the compartmentalization of rat uterine cytosolic and nuclear estrogen receptors (21). During this phase, chlordecone or estradiol *implants* increased the amount of estrogen receptor in the nuclear compartments. This was accompanied by a prolonged decline in cytosolic receptor. Receptor replenishment of the cytosolic compartments of uteri exposed to chlordecone or estrogen implants occurred at 24 hours. The prolonged depression of the amounts of cytosolic receptor and elevation of nuclear receptor levels caused by chlordecone is suggestive of the action of an estrogen antagonist such as nafoxidine (21, 22). However, replenishment of cytosolic receptor levels within 24 hours after chlordecone exposure suggests that this is not the case, since the cytosolic compartment is not replenished within this period after treatment with nafoxidine (21, 22). The prolonged effect on the compartmentalization of uterine estrogen receptors probably reflects the persistence of the pesticide in the animal. In the rat, the half-life of chlordecone in the blood after a single dose was 8.5 days for the first 4 weeks, 24 days for the next 8 weeks and 45 days for the final 14 weeks, Egle *et al.* (23). Cohn *et al.* (24) reported a half-life of 165 days in the blood, and 125 days in the fat of industrial workers exposed to chlordecone. The bile was the primary means of excretion in the rat and man, with reabsorption from the intestinal tract prolonging the half-life of chlordecone (23–25).

Although chlordecone induces hepatic mixed function oxidase activity (26–28), there appears to be little evidence for its biotransformation. Therefore, chlordecone is probably active *per se* and does not owe its estrogenic properties to a metabolite. However, this has not been established with certainty.

The binding of chlordecone to uterine cytosolic estrogen receptors in cell free preparations and the translocation of estrogen receptors from the cytosolic into the nuclear compartment *in vitro* in isolated uteri and *in vivo* in the immature rat indicates that chlordecone acts directly on the uterus. It was concluded in the rat, at the receptor level, that chlordecone was best described as an estrogen acting directly on the uterus, with an activity resembling the sustained effect of estradiol. The estrogenic activity of a chlorinated hydrocarbon insecticide is not surprising given the fact that such activity has been well established by a number of workers for certain DDT analogues (see Kupfer (29) and Nelson (30) for reviews). The estrogenic activity of certain DDT analogues might be related to the formation of the corresponding *p*-phenolic metabolites (31). However, what is highly surprising is that chlordecone, given its cage-like structure (Fig. 1) which is unlikely to form phenolic metabolites, is in fact "estrogenic."

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