Differential Effects of Dichlorodiphenyltrichloroethane Analogs, Chlordecone, and 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin on Establishment of Pregnancy in the Hypophysectomized Rat (43326)

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Abstract. **Many of the organochlorine pesticides have been shown to elicit estrogenic responses in laboratory animals. Two estrogenic actions, initiation of implantation and maintenance of pregnancy, were examined in progesterone-primed, delayed-implanting, hypophysectomized rats exposed to several polychlorinated hydrocarbons. The insecticide P,P′-dichlorodiphenyltrichloroethane (DDT) was nearly devoid of estrogenic activity for initiating implantation, as was a dichloro analog, 1,1-dichloro-2-[*p*-chlorophenyl],2-[*o*-chlorophenyl]ethane (O,P′-DDD), but another such analog, 1,1-dichloro-2-(*p*-chlorophenyl),2-(*o*-chlorophenyl)ethylene (O,P′-DDE), was nearly as estrogenic as the O,P′-DDT isomer of DDT and the methoxylated analog methoxychlor. The latter three compounds not only initiated implantation, but maintained pregnancy when given in large (200 mg/kg) and repeated doses. Another insecticide, chlordecone (Kepone) was more estrogenic than any of the DDT analogs and maintained pregnancy with a single dose of 50 mg/kg. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), a toxic contaminant of herbicide production, did not induce implantation at a dose of 125 μg/kg, but inhibited the implantation initiated by estrone in 35% of the animals. The mechanism of this antiestrogenicity is unknown but most probably does not involve direct action via the classical estrogen receptor. The possible interference with the normal blastocyst-uterine interactions of these polychlorinated xenobiotics may be an important factor in their being considered reproductive toxins.** [P.S.E.B.M. 1992, Vol 199]

Several polychlorinated hydrocarbons that are ingredients of pesticides or herbicides have proved to be reproductive toxins (1–3). The ability of these compounds to share or antagonize some actions of endogenous estrogens is the generally accepted basis for their toxicity. Although these xenobiotics are weakly estrogenic, their slow rate of metabolism and persistence in the environment provide threats for normal reproductive functions.

In the rat and mouse, estrogenic action is necessary for implantation of the blastocyst in the uterus (4). Therefore, removal of the ovaries or pituitary during the preimplantation period results in delayed implantation, whereby the blastocyst remains viable, but unattached, in the uterus. Exposure to a minute amount of estrogen promptly results in initiation of the implantation process (5). Studies of reciprocal transfer of blastocysts have indicated that the estrogen influences factors in the embryo as well as the uterus (6), and either could conceivably be affected by xenobiotics. However, the factors involved have not been determined.

The estrogenic activity of the polychlorinated hydrocarbons depends to a large extent upon the test

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system used. For example, although it is less efficient for interaction with the uterine estrogen receptor, chlordecone (Kepone; Allied Chemical Co., New York, NY) is a more potent *in vivo* estrogen than the most potent analog of dichlorodiphenyltrichloroethane (DDT). Even the estrogenic potency of the DDT analogs has been shown to be related to the end point of the test (1). O,P′-DDT, which has thus far proved to have the highest estrogenicity of the DDT analogs, and chlordecone (Kepone), have been shown to be effective agents for the initiation of implantation in the hypophysectomized, delayed-implanting rat model (7, 8); many of the other analogs have not been tested in this sensitive system. In the present study, the effects of five analogs of DDT are compared with those of chlordecone and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for initiating implantation and maintaining early pregnancy using the same model. TCDD is not considered an estrogen, but rather an antiestrogen (9). Many antiestrogens, however, are effective agents for the initiation of implantation (10), which suggested the testing of this potent toxin.

Materials and Methods

Chemicals. 1,1-Bis(*p*-chlorophenyl)-2,2,2-trichloroethane (P,P-DDT; 99⁺% pure; batch CR36-6-1), 2,2 bis(*p*-methyoxyphenyl)-1,1,1-trichloroethane (methoxychlor; batch no. 20F-5059), and decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]-pentalen-2-one (chlordecone [Kepone]) were obtained from Radian Corp (Austin, TX) via the National Toxicology Program. 1,1-Dichloro-2-(*p*-chlorophenyl),2-(*o*-chlorophenyl)ethane (O,P′-DDD), 1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2,2-trichloroethane (O,P′-DDT), and 1,1-dichloro-2 (*p*-chlorophenyl),2-(*o*-chlorophenyl)-ethylene (O,P′-DDE), all 99⁺% pure, were purchased from Aldrich Chemical Co. (Milwaukee, WI). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was obtained from Dow Chemical Co. (Midland, MI). Progesterone and estrone were purchased from Sigma Chemical Co. (St. Louis, MO). Except for TCDD, all compounds were dissolved in benzyl benzoate and diluted with sesame seed oil (40/60 v/v). TCDD was dissolved in corn oil + acetone (95/5, v/v).

Animals. Young adult, virgin, female rats (225–250 g) of the Holtzman strain (Harlan-Holtzman Industries, Madison, WI) were maintained in temperature (22 ± 1°C)-controlled quarters with a 14:10-hr light:dark schedule (light 0600–2000 hr) and given free access to Purina Laboratory chow (No. 5001) and tap water. Examination of vaginal lavages was used to establish estrous cyclicity. Proestrus females were mated with fertile males of the same strain. The presence of vaginal spermatozoons the following morning established Day 1 of pregnancy.

The effect of the test compounds upon initiation of implantation was determined using the delayed-implanting, hypophysectomized rat model (5). The injection scheme is shown in Figure 1. Hypophysectomy was performed on Day 3 of pregnancy, using the parapharyngeal approach with diethyl ether anesthesia. Following this operation, a 5% aqueous solution of glucose was used as drinking water. A daily injection of 2 mg of progesterone was used to maintain rats on delayed implantation. Under these conditions, blastocysts survive and retain their capacity to implant for several weeks (11). Five days after hypophysectomy, initiation of implantation was attempted by an intraperitoneal injection of the test compound; additional doses were given by subcutaneous injection. In order to ensure sufficient hormone for decidualization, the dose of progesterone was increased to 4 mg daily after administration of the test compound. Two kinds of control animals were used: a negative-implanting control treated only with 4 mg of progesterone and a positive control treated with 4 mg of progesterone and 1 μg of estrone. Initiation of implantation was determined 24, 48, 72, or 120 hr after the initial exposure to the test compound by the use of a macromolecular dye (4). Fifteen minutes before autopsy (A in Fig. 1), 0.7 ml of a 1% solution of Chicago Blue B dissolved in 0.15 *M* NaCl was injected intravenously under light ether anesthesia. Because of increased capillary permeability, resulting in extravasation of the dye at the site of blastocyst attachment, each implantation site is indicated by a blue band around the uterus. In animals without implantation sites, the uterine lumen was flushed with 0.15 *M* NaCl. The failure to find blastocysts in the uterine flushings was taken as evidence that the animal was not pregnant. On the other hand, animals with incomplete hypophysectomy failed to undergo delayed implantation and had easily detected implantation sites that were at least 4 days more advanced in development than those of control rats; these animals were discarded from the study.

In several animals autopsied 5 days after the initial exposure to the test compounds, enlarged implantation sites were seen, i.e., the pregnancy was maintained. In these cases, the uterus was removed and freed from surrounding connective and fat tissues. The uterine swellings, containing the embryo, decidual tissue, and

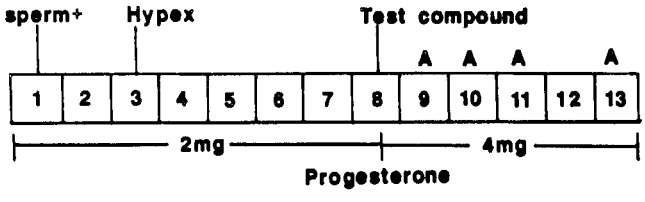

Figure 1. Treatment schedule for hypophysectomized (Hypex), delayed-implanting rats. (A) Groups autopsied at various intervals after the implantation-initiation compound was injected; details in Materials and Methods.

surrounding muscle layers, were cut from the uterus and weighed on a torsion balance to the nearest 0.1 mg (12). Representative implantation sites from each group were fixed in Bouin's fluid, sectioned in paraffin, and stained with hematoxylin and eosin to examine for the presence of an implanting blastocyst.

The data were analyzed by one-way analysis of variance and, when significant differences were indicated ($P < 0.05$), Student's t test was used for comparison of means.

Results

None of the rats treated only with progesterone (Group 1, Table I), but all of those given estrone (about 4 μg/kg), had implantation sites when examined at 48 or 120 hr (Groups 2, 3, and 4, Table I). Furthermore, all of the available blastocysts implanted; i.e., compare the blastocyst number in Group 1 with the implantation sites in Groups 2, 3, and 4. Injecting estrone three times, 48 hr apart, did not significantly alter the number of implantation sites nor their weights 120 hr later (Group 4, Table I). In contrast to the response with estrone, only one of three rats given 200 mg/kg of methoxychlor had four implantation sites within 48 hr (Group 5, Table I).When the uterus of this animal was flushed with saline, six blastocysts were recovered; i.e., not all of the embryos had initiated implantation. Even three doses of methoxychlor initiated implantation in only half of the rats autopsied at 120 hr (Group 6, Table I). In one animal, the implantation sites were smaller than those of controls treated with estrone, and histological examination failed to detect any embryos in sections of these sites. That is, decidualization was initiated, but the embryos did not survive. Interestingly, the animal with normal-sized uterine swellings had a nonimplanted blastocyst located between two well-implanted, developing embryos. Daily administration of 200 mg/kg of methoxychlor for five days (Group 7, Table I) resulted in a full complement of implantation sites in three of four animals. The weights of the implantation sites were not different from those in controls and all contained a developing embryo.

We reported previously (7) that 67% of hypophysectomized, delayed-implanting rats exposed to 200 mg/kg of O,P′-DDT had implantation sites within 48 hr (shown as Group 8, Table I). Animals that did not have implantation sites had a reduced number of blastocysts recovered, indicating some loss during the 2 days after treatment. In the present study, injection of 200 mg/kg daily for 5 days (Group 9, Table I) induced

Table I. Implantation and Maintenance of Pregnancy in Hypophysectomized, Delayed-Implanting Rats Exposed to Analogs of DDT[a]

Group	Test compound	Dose	No. of doses	Time to autopsy[b] (hr)	No. of animals with implant sites/total no. (%)	Sites per rat	Blastocysts recovered[c]	Weight of sites[d] (mg)
1	Control (none)	—	—	48	0/4 (0)	—	14.2 ± 2[*]	—
2	Control estrone	1 μg	1	48	9/9 (100)	12.2 ± 1	—	—
3	Control estrone	1 μg	1	120	11/11 (100)	14.3 ± 0.1	—	43.3 ± 2.5[§]
4	Control estrone	1 μg	3	120	3/3 (100)	13.0 ± 1.1	—	54.4 ± 4.6[§]
5	Methoxychlor	200 mg/kg	1	48	1/3 (33)	4	9, 12	—
6	Methoxychlor	200 mg/kg	3	120	2/4 (50)	11, 12	10, 11	29.7 ± 3.3[‖]
7	Methoxychlor	200 mg/kg	5	120	3/4 (75)	11.0 ± 2	8	41.8 ± 4.1[§]
8	O,P′-DDT	200 mg/kg	1	48	10/15 (67)	13.1 ± 0.7	7.4 ± 1.6[†,‡]	—
9	O,P′-DDT	200 mg/kg	5	120	5/5 (100)	11.5 ± 0.5	—	48.6 ± 1.7[§]
10	P,P′-DDT	200 mg/kg	2	48	0/4 (0)	—	8.5 ± 0.3[†,‡]	—
11	P,P′-DDT	200 mg/kg	3	120	1/6 (17)	6	7.0 ± 1.9[†,‡]	—
12	O,P′-DDE	200 mg/kg	1	48	4/12 (33)	12 ± 2.4	10.9 ± 0.8[*,†]	—
13	O,P′-DDE	200 mg/kg	3	120	3/5 (60)	2, 9, 14	7, 7	41.6 ± 3.8[§]
14	O,P′-DDE	300 mg/kg	1	48	6/8 (75)	12 ± 2	12, 4	—
15	O,P′-DDD	200 mg/kg	1	48	2/9 (22)	8, 10	8.2 ± 1.7[†,‡]	—
16	O,P′-DDD	200 mg/kg	3	120	0/5 (0)	—	8.6 ± 1.6[*,†,‡]	—
17	O,P′-DDD	200 mg/kg	5	120	0/5 (0)	—	6.6 ± 1.2[‡]	—
18	O,P′-DDD	300 mg/kg	1	48	1/7 (14)	9	9.7 ± 0.4[*,†]	—

[a] Values are expressed as mean ± SE in last two columns. Animals were hypophysectomized on Day 3 of pregnancy and injected subcutaneously, daily, with 2 mg of progesterone. Five days after hypophysectomy, the test compound was injected intraperitoneally; subsequent doses were given subcutaneously at 24 (5 doses)- or 48 (3 doses)-hr intervals. Each animal also received 4 mg of progesterone daily until autopsy. Means with same superscript symbol (*, †, ‡, §, ‖) are not different ($P > 0.05$).
[b] Interval between first dose and autopsy.
[c] Number of blastocysts flushed from uteruses of animals lacking implantation sites.
[d] Weight of the implantation sites containing embryo, decidual tissue, and myometrium.

the normal number of implantation sites in all five animals. Furthermore, the weights of the implantation sites were not different from those obtained with estrone treatment. Although the O,P′-isomer of DDT was quite effective at initiating implantation, the P,P′-isomer was not. With two doses of 200 mg/kg, given 24 hr apart, none of the rats had implantation sites (Group 10, Table I). Even with three doses, only one of six rats had six faintly staining sites (Group 11, Table I) and five blastocysts were flushed from the uterus, indicating incomplete attachment of the embryo to the uterus. During the 5 days from the first dose of P,P′-DDT until autopsy, embryos were lost, as indicated by the reduced number of blastocysts recovered. Larger dosing was not attempted due to the toxicity of the compound.

An analog of O,P′-DDT, O,P′-DDE, at a dose of 200 mg/kg induced a normal number of implantation sites in a third of the animals (Group 12, Table I). Increasing the dose to 300 mg/kg increased the implantation rate to 75% (Group 14). Three injections of 200 mg/kg produced implantation sites in 60% of the animals, but, in one animal, only two embryos implanted and developed (Group 13, Table I). The weights of the implantation sites were, however, not different from those of estrone-treated rats and contained a developing embryo.

In contrast to the strongly positive results with O,P′-DDE, a closely related analog, O,P′-DDD, produced only weakly staining sites in two of nine rats injected with 200 mg/kg (Group 15, Table I). The effects of this analog appear to be paradoxical, because frequent doses (three or five doses) failed to induce implantation (Groups 16 and 17, Table I). The daily dosing had a profound effect upon loss of blastocysts in the uterus and less than half of the expected number were recovered. A single dose of 300 mg/kg did, however, induce nine sites in one of seven animals within 48 hr (Group 18, Table I).

Two polychlorinated hydrocarbons that are chemically quite distinct from DDT and its analogs were also examined in this study. The estrogenicity of one of these, chlordecone (Kepone), is well known (3). As reported previously (8), three doses of 20 mg/kg of chlordecone initiated a full complement of implantation sites in all animals (Group 2, Table II). A single dose of 50 mg/kg of chlordecone also induced a normal number of sites in 78% of the animals within 48 hr (Group 3, Table II). Animals treated with this dose plus a daily injection of 4 mg of progesterone had implanted embryos that were increasing in size in three of four rats. The implantation sites weighed 41.8 ± 4.1 mg, which was not different from the weights in animals treated with a single dose of estrone (Group 2, Table I). Not included in Table II are data from five animals that received two doses of 50 mg/kg of chlordecone 96 hr apart: pregnancy continued in these animals for 10 days. Some of the embryos, equivalent to Day 15 of pregnancy, were the same size and developmental stage as those of control animals treated daily with estrone and progesterone, but others showed delayed development. In every case, however, the embryos were living, as evidenced by beating hearts.

A single dose of 125 μg/kg of TCDD, which is more than double the LD_{50} dose (13), did not produce implantation sites in any of the rats within 48 or 72 hr (Groups 5 and 6, Table II). Because of its presumed antiestrogenic action, we attempted to interfere with the initiation of implantation induced by estrone. When 125 μg/kg of TCDD was given intraperitoneally 24 hr before injection of estrone (1 μg), only 65% of the animals had implantation sites 24 hr later (Group 7, Table II), and there were three animals that had only two implantation sites each. Three animals were treated with TCDD 48 hr before receiving estrone, and all had implantation sites 24 hr later (Group 8, Table II). The corn oil-acetone vehicle used for TCDD had no effect on the action of estrone (Group 9, Table II).

Discussion

The initiation of pregnancy in mammals requires cell type-specific interactions between the uterus and embryo under the influence of estrogen and progesterone (5). Although estrogen is required for the initiation of implantation and subsequent decidualization in a progesterone-primed uterus, it can be highly detrimental if present in supernormal amounts or at an inappropriate time prior to arrival of the blastocysts into the uterus (14). Acute exposure of rats to O,P′-DDT, the most estrogenic of the DDT analogs, at the time of mating or shortly thereafter resulted in the loss of implantable embryos a few days later (7). This is probably an important factor for considering the compound a reproductive toxin. O,P′-DDT was quite effective for initiating implantation and maintaining embryo development for at least 5 days in progesterone-treated animals (7; Table I). Although this early embryo development appeared to be normal, the effect of the compound upon later development could be detrimental. The uterotropic effect of O,P′-DDT has been associated with its interaction with intracellular estrogen receptors (15). We do not know, however, if the same mechanism is responsible for initiating implantation. Part of the latter question relates to the inadequacy of our information concerning mechanisms of implantation. Estrogenic action has been classified into an early (Phase I) and a late (Phase II) response (discussion in Ref. 10). The initiation of implantation is presumably a Phase I response, while the maintenance of pregnancy is clearly a Phase II response; O,P′-DDT action appears to involve both of these phases.

Methoxychlor, an analog of DDT that is a commonly used pesticide, has been shown to : (i) produce

Table II. Pregnancy Initiation by Chlordecone and TCDD in Hypophysectomized, Delayed-Implanting Rats[a]

Group	Treatment	Dose	Time to autopsy (hr)[b]	No. of animals with sites/ total no. (%)	Sites per rat	Blastocysts recovered[c]
1	Estrone	1 μg	24	5/5 (100)	14.0 ± 1.0	—
2	Chlordecone	20 mg/kg ×3[d]	72	4/4 (100)	14.0 ± 2.5	—
3	Chlordecone	50 mg/kg	48	7/9 (78)	11.2 ± 1.0	9, 10
4	Chlordecone	50 mg/kg	120	3/4 (75)	14.7 ± 1.1	10
5	TCDD	125 μg/kg	48	0/6 (0)	—	13.5 ± 2.0
6	TCDD	125 μg/kg	72	0/6 (0)	—	10.3 ± 0.8
7	TCDD + estrone	(24 hr)[e]	48	11/17 (65)	10.4 ± 1.8	9.0 ± 1.1
8	TCDD + estrone	(48 hr)[f]	72	3/3 (100)	10.7 ± 2.4	
9	Vehicle + estrone	(24 hr)[e]	48	7/7 (100)	15.5 ± 1.1	

[a] Values are expressed as mean ± SE in the last two columns.
[b] Interval between injection of test compound and autopsy.
[c] Number of blastocysts flushed from uteruses of animals without implantation sites.
[d] Injected at 24-hr intervals.
[e] Animals were injected intraperitoneally with TCDD, dissolved in corn oil + acetone (95/5 v/v), or vehicle 24 hr before being injected with estrone.
[f] TCDD given 48 hr before estrone.

loss of embryos by accelerating tubal transport of embryos (16); (ii) reduce implantations and cause fetal resorption soon after implantation (17); and (iii) reduce the uterine decidual response to trauma (18). All of these effects can be explained on the basis of the estrogenicity of the compound. Therefore, the finding that multiple doses are capable of initiating implantation should not be surprising. In contrast to embryo loss reported with oral dosing (16), we have not found that acute exposure to methoxychlor by systemic injection causes loss of embryos. Six rats given 200 mg/kg of methoxychlor ip a few hours before mating implanted the normal number (14.2 ± 1.2) of embryos on Day 6. Furthermore, three rats given 200 mg/kg before mating and at 0900 hr on Days 1, 2, and 3 of pregnancy also did not show loss of embryos; i.e., 15 ± 2 implantation sites/rat were recorded. Kupfer and Bulger (1) have summarized the evidence for pure methoxychlor being a proestrogen, which must be at least partially demethylated to produce a compound that competes with estradiol for binding to the classical estrogen receptor. The increased efficacy for initiating implantation, and sustaining the pregnancy, with daily dosing may involve the accumulation of an active metabolite of methoxychlor.

The failure of P,P′-DDT to effectively initiate implantation correlates with its weak binding to the estrogen receptor (19). In a previous trial (7) using the same model, 400 mg/kg of P′P-DDT as a single dose did not induce implantation; this amount proved toxic and a third of the animals died within 48 hr. Even with three doses of 200 mg/kg each, in the present study, blastocytes could be flushed from the uterus of the single animal with six sites, suggesting that only the initial reaction of implantation had been initiated within 120 hr.

The efficacy of O,P′-DDE at initiating implantation, and maintaining pregnancy (Table I) with multiple doses, was unexpected. Although this analog has been shown to inhibit estradiol binding (19), it is much less estrogenic than O,P′-DDT in all of the biologic assays that have been used (1). O,P′-DDD is structurally very similar to O,P′-DDE, lacking only a double bond between the two chlorinated carbons that connect the two chlorinated phenols. O,P′-DDD inhibited estradiol binding to its receptor at slightly lower doses than did O,P′-DDE (19), which could be interpreted as indicative of greater estrogenicity, but several other factors are probably involved. The compound has been shown to be a little less uterotropic than O,P′-DDE (19). O,P′-DDD was almost totally ineffective at initiating implantation (Table I). Partial success with a single dose of 200 mg/kg (Group 13, Table I) suggested that multiple doses would increase the implantation rate. This did not prove to be the case, suggesting that the initial success was due to animal variation in response. Even with a dose of 300 mg/kg, only one animal responded. The difference in response seen with O,P′-DDE and O,P′-DDD underscores the profound difference in estrogenicity afforded by a slight change in a structure that is usually not associated with estrogen-receptor interaction (20).

The estrogenic activity of the pesticide chlordecone (Kepone) has been studied in several species (3, 21). This compound lacks a phenolic group and has posed difficulties for understanding how it could react with the estrogen receptor. Our previous studies (8) showed that its ability to initiate implantation exceeded its

ability to maintain embryo development. That is, three doses of 20 mg/kg each induced a normal number of implantation sites in all animals, but embryos did not develop. Apparently, the Phase I response was induced with this dose, but the Phase II response was not. On the other hand, a single dose of 50 mg/kg initiated implantation and maintained embryo development to the same degree as did a single dose of estrone (Table II). The lack of correlation between its ability to bind the estrogen receptor and manifest estrogenic activities suggests that metabolic changes in the molecule may occur *in vivo*. Meyers *et al.* (22) suggested that either carboxylic, or a derivative, acid formed from chlordecone or present as a contaminant could be responsible for the estrogenicity of chlordecone.

TCDD binds an intracellular aryl hydrocarbon receptor protein, but not the classical estrogen receptor (23). In some responses, TCDD mimics certain effects of steroid hormones, but recent attention has focused upon its antiestrogenic activity (24). The latter activity may involve its reduction of the amount of the uterine estrogen receptors (23) or of estrogen-induced epidermal growth factor (EGF) receptor (9, 25). Astroff *et al.* (9) reported a reduction in transcription as the cause for reduced EGF receptor levels in the uterus; affinity of the EGF receptor for EGF was not reduced. Although TCDD has been shown to reduce the EGF receptor level in several tissues, it has the seemingly paradoxical action of mimicking EGF activity, particularly in fetal development (26). With the current interest in EGF as a mediator of estrogen action (25, 27) and the presence of EGF receptors in the mouse preimplantation embryo, as well as the beneficial effects of EGF on preimplantation embryo development (28), the effect of TCDD upon uterine functions gains additional importance.

The results obtained in the present study indicate that TCDD has no estrogenic activity for initiating implantation (Table II). Perhaps this should have been anticipated, considering that TCDD is presumably an antiestrogen but not an estrogen. However, several antiestrogens, such as tamoxifen, CI-628, clomiphene citrate, Ly-117018, and Nafoxidine, have estrogenic activity. Their ability to induce the Phase I estrogenic response for initiation of implantation has been demonstrated (10). The lack of response with large doses of TCDD suggests that its antiestrogenic activity is probably quite different in action from that provided by the typical triphenylethylene antiestrogens, which presumably compete for occupancy on the estrogen receptor. This remains an important area for investigation.

An antiestrogenic action of TCDD was seen in animals given the toxin 24 hr before an implantation-inducing dose of estrone (Table II); 35% of the animals failed to have implantation sites. The mean number of sites per rat was reduced by about 33%, but this was statistically insignificant ($P > 0.05$). However, three of the 11 animals with implantation sites had only two sites each, and this may add to the biologic significance of TCDD exposure. To establish that the toxin was not directly affecting the embryo, we transferred 76 blastocysts from the nonimplanting animals into eight pseudopregnant, progesterone-primed, hypophysectomized rats (6). Implantation was induced in these animals by estrone and pregnancy was maintained at least 3 days when given estrone and progesterone. All of the animals had implanted embryos and the total number recovered was 38 (50%). Five rats that received a total of 49 blastocysts recovered from animals treated with vehicle implanted 23 (47%). Thus, it appears that the effect of TCDD on early pregnancy is mediated via the uterus and not on the embryo. Whether the effects of TCDD on reproduction are due to its effects on the expression and regulation of growth factors or on the expression of their receptors is not clear, but remains an important question for study.

Several polychlorinated hydrocarbons act as estrogens for the initiation of implantation in the hypophysectomized, progesterone-primed rat. The major component of commercial DDT, P,P′-DDT, does not appear to be estrogenic for this response, but two analogs, O,P′-DDT and O,P′-DDE, are essentially equipotent for establishing early pregnancy when given in large and repeated doses. The chemically nonrelated pesticide chlordecone (Kepone) is considerably more potent than any of the DDT analogs as an estrogen and, therefore, as a reproductive toxin. Highly toxic TCDD is not estrogenic for initiating implantation, but rather antiestrogenic; the mechanism of this antiestrogenicity is unclear, but would not appear to involve inhibition of estrogen binding to the estrogen receptor.

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