# TCDD Promotes the Growth of Endometriosis in Rodents

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## ABSTRACT

The causes of endometriosis, a disease of women where endometrial tissue is found outside the uterus, are unknown. An alteration in immune competence may be one mechanism. TCDD can alter immune system function. The chemical also increases the incidence of endometriosis in primates. Our studies were designed to examine the effect of TCDD on endometriosis in rodents. Rats and mice were dosed, by gavage, three times at 30 day intervals with 0, 3, or 10 μg/kg TCDD, starting with day 1. Endometriosis was surgically induced on day 30. On day 90, the size of each endometriotic site was measured. TCDD had no significant effect on endometrial site size in rats, although a trend toward increased growth may exist. In mice, TCDD promoted the growth of endometrial tissue outside the uterus. Thus, TCDD promotes endometriotic growth in rodents. Mechanisms involved may include immunological changes in the animals.

## INTRODUCTION

The increase in endometriosis has provoked the study of potential etiologies of the disease. Characterized by the presence of endometrial tissue outside the uterus, endometriosis is a source of pain and infertility for approximately 20% of subfertile women[1]. Retrograde menstruation provides the endometrial tissue, and one factor that may be involved in the development of endometriosis is an alteration in the immune system[2] that enables subsequent growth. Hormones such as estradiol also play a role in the growth and maintenance of endometriotic tissue[3].

TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) is known to produce changes in immunocompetence[4]. There is now evidence that exposure to TCDD can enhance the incidence and severity of spontaneous endometriosis in primates[5]. The mechanisms through which TCDD produces this effect are unknown.

The effect of TCDD exposure on surgically-induced endometriosis was evaluated in rats and mice in order to test whether TCDD affects endometriosis in these species as it does in primates. If this proved to be true, then investigations of the mechanisms of TCDD-induced endometriosis could be pursued using a rodent model.

## METHODS

Animals: Adult, female mice of the B6C3F1 strain and Sprague Dawley CD rats were purchased from Charles River at 60 days of age. Animals received food and water ad libitum; photoperiod was 12:12.

Surgery: Endometriosis was induced in mice by a newly developed surgical method[6] which is similar to that used in rats[7]. Briefly, part (in rats) or all (in mice) of one uterine horn was removed, split, and cut into equal pieces. Each piece, 6 for rats and 3 for mice, was sutured to a mesenteric vessel of the small intestine.

Experimental: Endometriosis was induced in 30 rats and in 36 mice. Groups of 10 rats received 0, 3, or 10 μg/kg TCDD, and groups of 8, 9, and 11, respectively, surviving (from surgery) mice received the same doses. Both received TCDD in corn oil, by gavage, at 2 (rats) or 4 (mice) ml/kg-b.w. TCDD was administered on day 1, day 30, and day 60 of the experiment. This dosing regimen was selected in order to maintain relatively high tissue levels over time. Surgical induction of endometriosis was performed on day 30. On day 60 the rats, but not mice, underwent laparotomy to measure the endometriotic sites. Animals were killed on day 90. In rats, the diameter of each site was measured with calipers and body weight was assessed at necropsy. In mice, parameters measured at necropsy included body weight, endometriotic site diameter, and thymus, liver, ovarian, and uterine weights. Blood was collected from mice for the radioimmunoassay of estradiol.

Statistics: The mean of the diameter of all the sites in each animal was initially computed. The mean and standard error was calculated for each treatment group. Data was analyzed by ANOVA, using the General Linear Models (GLM) procedure. When significant effects on the overall ANOVA were detected ($p < 0.05$), post hoc comparisons among treatments were made with t-tests.

## RESULTS

When rats were exposed to TCDD, the data revealed no significant difference between the size of endometriotic sites in TCDD treated versus control rats (Table 1). There was no significant difference in the size of sites between the time of laparotomy on day 60 and the measures at necropsy on day 90. However, a trend toward the promotion of growth by TCDD is evident for measures at both times (Table 1).

Table 1. Effect of TCDD on Endometriosis in Rats

| Dose of TCDD | Diameter of sites (mm)[a] | |
|---|---|---|
| μg/kg | At Laparotomy | At Necropsy |
| 0 | 3.17 ± 0.340 | 2.70 ± 0.355 |
| 3 | 3.51 ± 0.538 | 3.52 ± 0.571 |
| 10 | 3.77 ± 0.483 | 3.94 ± 0.452 |

[a]*No significant effect of TCDD was found on site diameter at either time. Data are presented as the mean ± S.E.*

Exposure of mice to a similar regimen of TCDD produced different effects. Doses of 3 μg/kg TCDD produced a significant increase in the size of the endometriotic sites, measured at necropsy (Table 2.). TCDD at 10 μg/kg did not affect the diameter of endometriotic sites, but ovarian weight was significantly decreased (Table 2.). No

significant effect on any other measured parameter was observed.

Table 2. Effect of TCDD on Endometriosis in Mice

| Dose of TCDD (μg/kg) | Diameter of sites (mm) | Ovarian Weight (mg) | Serum Estradiol pg/ml |
|---|---|---|---|
| 0 | 4.94 ± 0.204 | 8.13 ± 0.403 | 32.0 ± 3.9 |
| 3 | 6.13 ± 0.274** | 7.17 ± 0.401 | 41.8 ± 4.8 |
| 10 | 4.80 ± 0.241 | 6.59 ± 0.345** | 38.5 ± 3.7 |

***Significantly different from vehicle controls at $p < 0.001$. Data are presented as the mean ± S.E.*

## DISCUSSION

A variety of mechanisms may mediate of the TCDD-induced promotion of endometriotic growth. Estrogen is required for the development and maintenance of endometriosis in women[3], primates[10], and rodents[6]. Thus it is unlikely that TCDD is acting like an antiestrogen[8] when it promotes the growth of endometriosis. There may, in fact, be an interaction between endogenous estradiol and TCDD, much like that seen in the promotion of liver tumors by ovarian hormones and TCDD[11]. Another possibility is that immunotoxicity is involved in the TCDD-enhanced endometriotic growth in mice. TCDD can affect cell mediated, humoral, and innate immunity[4]. It is notable that mice responded to TCDD with enhanced endometriotic growth and rats did not, because mice have an immune system that is more sensitive to TCDD-induced suppression than the rat[9]. The determination of exactly what changes in immunocompetence produced by TCDD might produce enhanced growth of endometriosis will require further study. The finding of a significant effect at 3 but not at 10 μg/kg TCDD in mice may reflect the previously reported finding[12] that TCDD down-regulates cytosolic estrogen receptor levels in mouse uterus at 10 but not 3 μg/kg. Then, the resulting antiestrogenicity would prevent the stimulation of growth at 10 mg/kg, and the lack of antiestrogenicity at 3 μg/kg would permit if not promote the stimulation of endometriotic growth via hormonal or immunological mechanisms.

The finding of decreased ovarian weight following exposure of mice to 10 but not 3 μg/kg TCDD was apparently not related to ovarian estrogen secretion, since no difference in serum estradiol levels was found across doses. The significance of this decrease in ovarian weight remains to be determined.

In summary, the data show that TCDD promotes endometriotic growth in mice but not rats. Mechanisms involved in the TCDD-stimulated promotion of the growth of surgically induced endometriotic sites may include alterations in immunological competence but not the antiestrogenicity of TCDD.

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