# Dioxins and Endometriosis: A Plausible Hypothesis

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**A potential connection exists between exposure to organochlorine chemicals and the increasing prevalence of endometriosis. Evidence shows that dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) can increase the incidence and severity of the disease in monkeys and can promote the growth or survival of endometrial tissue implanted into rodents in a surgically induced model of endometriosis. The mechanism of the connection between organochlorine chemicals and endometriosis is not clear. Effects on growth factors, cytokines, and hormones (components of the immune and endocrine systems) are potential means of mediating the possible promotion of endometriosis by dioxins. Studies on epidemiology and on structure–activity relationships of organochlorine chemicals and endometriosis have been additional approaches to this problem. In this regard, toxic equivalence (TEQ) appears to be an important determinant of the effects of organochlorine chemicals on endometriosis. In this article, we review the literature related to endometriosis and dioxins and attempt to integrate the various sources of information that bolster the hypothesis connecting dioxins and endometriosis. *Key words*: dioxin, endometriosis, polychlorinated biphenyls, TCDD, toxic equivalence. *Environ Health Perspect* 110:15–21 (2002). [Online 10 December 2001]**
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Endometriosis affects millions of women in the United States. It can severely alter quality of life and leads to extensive problems with fertility and loss of work time. The incidence of endometriosis in the U.S. population is currently estimated at approximately 15% of reproductive-age women, but this estimate is far from certain (*1*,*2*). Significant complicating issues are diagnosis and ascertainment, as well as social and cultural mores. It has been suggested (*3*) that the incidence of endometriosis has increased over the past 50 years. There also appears to be a decrease in the age of onset, so whereas endometriosis often was considered a disorder associated with delayed childbearing, young teens are now being diagnosed with this condition (*4*). The severity of the disease is also hard to gauge, for although pain is a usual companion of the condition, in the most serious cases there is often no pain at all. The degree of pain is not correlated with the amount of ectopic material found. Immune suppression has been associated with severe endometriosis (*5*). In fact, the recognition that endometriosis is not only a reproductive and endocrine disorder but is also associated with dysfunction of the immune system has major implications not only for understanding the pathogenesis of this disorder but for potential therapies.

Endometriosis involves the inappropriate and uncontrolled growth of endometrial cells, normally confined to the lining of the uterus, outside the womb (*4*). Some scientists consider endometriosis a benign neoplastic disorder. As in nonmalignant tumors, cells have lost their normal control, often dedifferentiate, and grow in an inappropriate place. Although such a tumor is nonmetastatic, it can still cause a great deal of pain and suffering. Such is the case for endometriosis. Growing in their appropriate environment, endometrial cells are critical components of the reproductive tissue and function of women. However, when present outside the uterus, endometrial cells lack the normal growth control exerted by factors in their normal environment, but continue to respond to hormonal signals, causing noncyclical growth and regression of such tissue wherever it may have attached—the peritoneal cavity, the intestines, on the ovary or the outside of the uterus, the bladder, even on the lungs and in the pleural cavity.

Some of the most intriguing findings are that endometriosis can even be found in men who are highly exposed to antiandrogens and estrogens, such as in therapy for prostate cancer (*6*). Clearly, these men never had a uterus to produce the endometrial cells. Rather, dedifferentiation and then redifferentiation of other tissues has likely occurred under the influence of antiandrogen and estrogen therapy. In women, the major hypothesis for the origin of endometrial tissue outside of the uterus is retrograde menstruation (*7*). That is, a certain amount of endometrial cells escape from the uterus to the peritoneal cavity via the Fallopian tubes instead of exiting via the vagina during menstruation. Koninckx (*3*) postulated that either peritoneal leukocytes fail to remove these retrograde endometrial cells or peritoneal leukocytes and retrograde endometrial cells produce increased levels of cytokines and growth factors that facilitate ectopic endometrial growth. The endometrial cells may then be able to attach to organs and/or tissues on which they have landed. Once attached, the endometrial cells may be able to invade the underlying tissue, leading to deep lesions. The endometrial lesions often involve fluid-filled cysts, but they can also have different architecture, especially if they are highly invasive. Although the cysts are readily detected on the surface of the affected organ, the invasive lesions may be entirely below the surface and not readily detected, even by visual observation during surgery. Nevertheless, the clinical standard for diagnosing endometriosis involves surgery; without it, endometriosis is often missed. However, many efforts have been made to improve the detection of endometriosis via nonsurgical means, including careful patient history, medical examination, magnetic resonance imaging, and ultrasound methods (*4*).

Endometriosis requires estrogen (*8*,*9*). Growth of the endometrial cells, whether in the uterus or outside it, depends on estrogen. This dependency is the basis for the major therapeutic approach to endometriosis treatment with androgens or antiestrogens. Hysterectomy and ovariectomy is a treatment, but one of last resort. Even this may not solve the problem because the extrauterine implants can still respond to the estrogens that are produced by the adrenal glands and by peripheral tissues from adrenal-derived androgens. Recent data suggest that endometrial cysts have developed the ability to produce their own estrogens *in situ* from circulating androgens produced by the adrenals. Noble et al. (*10*) have shown that human endometrial explants have high levels of aromatase, the enzyme that converts testosterone to estrogen. Thus, in endometriosis, the lesions have achieved independence from the circulating levels of estrogens. Under this

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scenario, treatment with androgens would clearly not have the desired effect. In contrast, antiestrogens may still be effective if they can reach the site of the lesions. But not all endometrial lesions are highly vascularized, making serum therapies sometimes ineffective.

As mentioned above, some of the increase in reported incidence of endometriosis may derive from better ascertainment and reporting. However, other possibilities include higher estrogen levels in women caused by diet and medicines. One major recent hypothesis deserving attention is that environmental agents may play a role in the increased incidence of endometriosis. This hypothesis was first advanced for a nonchemical stressor, radiation. Studies in nonhuman primates, specifically rhesus monkeys, revealed that a single radiation treatment led to an increase in endometriosis in the colony after a latency of 7 years (*11*). To date, however, there have been no human studies to either support or refute this hypothesis.

As mentioned above, estrogen is required for endometriosis. Removal of estrogen (e.g., by ovariectomy) tends to reduce symptoms. In contrast, estrogen therapy restores and/or exacerbates the problem. Rodent models have been developed to examine the role of environmental estrogens in endometriosis. Because of a variety of differences in anatomy and physiology between the rodent and the primate, rodents do not develop endometriosis spontaneously. However, endometrial tissue from the same or genetically matched rodent can be surgically implanted in the peritoneum or mesentery of a recipient rat or mouse, and estrogen can then promote the growth of these implants. This approach was initially developed in rats by Vernon et al. (*12*) and used to develop antiendometriotic therapies. However, Cummings and Metcalf (*13*) extended the model to involve surgical induction of endometriosis in the mouse. They demonstrated that the implants required estrogen for growth. For example, although the implants had a mean diameter of 3.60 mm in the intact mouse, ovariectomy reduced the size of the lesions to 0.95 mm. Treatment with estrogen increased the lesion size to 5.28 mm, significantly greater than in the absence of endogenous estrogens. Using the rat model, they also showed that the environmental pesticide methoxychlor, which can be metabolized to a chemical with high affinity for the estrogen receptor, had the same ability as estrogen to promote the growth of the endometrial implant (*14*).

Methoxychlor is an example of a synthetic organochlorine. Organochlorines constitute a large and complex group of synthetic organic compounds that contain chlorine atoms. The presence of chlorine tends to make the chemicals more stable, both to environmental insult and to biologic breakdown. In fact, certain organochlorines are extremely persistent and bioaccumulative. These include pesticides such as DDT, mirex, and toxaphene, industrial chemicals such as polychlorinated biphenyls and pentachlorphenol, and unwanted byproducts, primarily of industrial processes, such as polychlorinated dibenzo-*p*-dioxins and dibenzofurans. These chemicals are of concern internationally and are the subject of international negotiations leading toward a regulatory treaty.

One of the compounds of greatest concern is TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin; dioxin). TCDD is an unwanted byproduct of many industrial and combustion processes (*15*). It is a prototype for a family of chemicals that have a common mechanism of action and a common spectrum of effects, are structurally related, and are persistent and bioaccumulative. Members of this group are often called the polyhalogenated aromatic hydrocarbons (PHAHs). They may contain multiple chlorine and/or bromine atoms, at three or more lateral positions on the multiaromatic ring structure. Well-characterized families include the polyhalogenated dibenzo-*p*-dioxins (PCDDs and PBDDs), dibenzofurans (PCDFs and PBDFs), biphenyls (PCBs/PBBs), naphthalenes (PCNs/PBNs), and azo-azoxybenzenes, among others. Certain PCBs are dioxin-like in their effects. In fact, PCBs are never present without dioxin-like PCBs. The toxicity of members of these classes can be assessed using a toxic equivalency factor (TEF) approach in which all chemicals that are dioxin-like are assigned a relative potency factor that expresses their toxicity in relation to that of TCDD (*16,17*). The TEF values are consensus estimates based on an evaluation of all the available data. The total toxic equivalency (TEQ) is the sum of the mass of each individual congener times its individual TEF: $[(PCDD_1) \times TEF_1] + [(PCDD_2) \times TEF_2] \ldots [(PCDD_x)] \times TEF_x]$.

More is known about the mechanism of toxicity of TCDD than of almost any other chemical. The first step in its action involves binding to the Ah receptor, which functions as a ligand-activated transcription factor (*18*). In addition, recent studies suggest that the Ah receptor may act as a competitor for the PAS (Per-ARNT-SIM) family of nuclear regulatory proteins, which includes ARNT, a protein that controls hypoxic stress and circadian rhythms, and SIM, which plays a key role in neurodevelopment. The unliganded form of the Ah receptor also appears to be a negative regulator of tyrosine phosphorylation (e.g., *c-src*), apoptosis (e.g., *rel*), and cell cycling (e.g., *Rb*) (*19*). One of the key points about dioxins is that effects of the chemicals are seen in both sexes of multiple strains of a broad spectrum of laboratory animals, domestic animals, wildlife, and humans (*15,18*).

The effects of dioxin and related compounds range from the molecular and biochemical, through the cellular, to the tissue, organ, and systems level (*15,20,21*). Biochemical effects include induction of enzymes involved in metabolism of both endogenous substances as well as xenobiotics, oxidative stress, and induction of specific cytokines. At the cellular level, dioxins can induce either proliferation or differentiation, depending on the state and environment of the cells. The key role of the cellular environment is also seen in that apoptosis has been reported to be both induced or blocked by dioxins (*22*). Dioxins also perturb homeostasis at the systemic level. Dioxin is a known human carcinogen (*23,24*) and is toxic to multiple organ systems. Dioxins are reproductive and developmental toxicants, as well as being neurotoxic and immunotoxic (*22,25*). Dioxin is the prototypical endocrine disruptor, modulating essentially every hormone system investigated: at the level of the receptor, by altering metabolism, or by affecting serum transport. These hormonal effects involve both the steroid family (e.g., estrogen, androgen, thyroid, glucocorticoid) and peptide (e.g., prolactin, insulin) hormones and are tissue- and developmental-stage-specific. Dioxins induce multiple enzymes, and dioxins can lead to the generation of reactive oxygen species, leading to oxidative stress (*26*). Growth factors control proliferation, differentiation, and apoptosis. Dioxin effects on growth factors and their receptors have been seen on retinoic acid and its receptors, tumor growth factor α and β, the epidermal growth factor receptor, and the insulin growth factor receptor. Cytokines such as tumor necrosis factor and interleukins (IL) 1β and 6 have also been shown to be induced by dioxin exposure (*27,28*). Cytokines are key signaling molecules in the immune and nervous systems. TCDD has been shown to be an immune suppressant in multiple systems and was also recently suggested to cause autoimmunity in a mouse model (*29*). TCDD suppresses T-cell–mediated B-cell responses. It also causes a block in T-cell maturation and is associated with thymic atrophy at high doses in all species investigated. Dioxins cause an increase in IL-1β and tumor necrosis factor β1, and the region of DNA that recognizes the ligand-activated Ah receptor, the dioxin response element, is present in IL-1β, IL-2, IL-5, IL-6, IL-10, tumor growth factor β1, and interferon γ (*27,28*).

The hypothesis has been raised that dioxin, the most toxic of the organochlorines, is associated with the observed increase in endometriosis in the developed world. The association of endometriosis with organochlorines, specifically PCBs that are not dioxin-like, was first reported from Germany (*30*). A letter from Belgian epidemiologists (*31*) suggested that the higher prevalence of endometriosis at infertility clinics in Belgium could be caused by the relatively high TCDD concentration in the Belgian population. These epidemiologic findings were essentially coincident with the publication of a study (*32*) demonstrating an increase in both the incidence and severity of spontaneous endometriosis in a colony of rhesus monkeys that had been exposed to TCDD in their diet for 4 years and then held for up to 10 years longer. These were incidental findings; the TCDD treatment of the monkeys had occurred many years previously. The monkeys had been held because of their potential value for long-term effects of exposure to dioxin. The hypothesis that TCDD exposure was associated with endometriosis arose when three of the high-dose monkeys (out of seven) died from fulminating endometriosis, the first one 7 years after cessation of the 25-ppt TCDD diet. Laparoscopic observations were conducted on all surviving monkeys from this dose group, as well as the lower-dose (5 ppt) group and the controls 10 years after diet cessation. Whereas 33% of the dietary control animals had endometriosis, 70% of those receiving the low dose and more than 80% of the high-dose animals had either died of endometriosis or had been surgically diagnosed. The increased incidence was statistically significant at both dietary levels. In addition, the severity of the disease demonstrated a dose-related increase. Endometriosis in the two control monkeys (of six surviving monkeys at the time examined) was stage I according to the standard American Fertility Scale, as used to classify endometriosis in women. In the 5-ppt group, out of seven surviving monkeys, five with endometriosis, two were stage I, two were stage III, and one was the most severe, stage IV. Only one monkey in the high-dose (25 ppt TCDD in diet) group was free of endometriosis at this 10-year postexposure analysis. Of the six high-dose monkeys with endometriosis, none were stage I, one was stage II, one was stage III, and four had the most severe form of endometriosis, stage IV. Rier and co-workers (*32*) concluded that TCDD exposure was associated with an increase in both the incidence and severity of endometriosis in rhesus monkeys.

The publication of these experimental findings, along with the suggestive epidemiologic associations, led to additional animal research to test this hypothesis. Several groups of investigators used rodent models to explore this question. Bruner and co-workers (*33*) developed a hybrid model involving human endometrium implanted in nude mice. Cells from uterine endometrium were exposed *in vitro* to estrogen, estrogen and progesterone, estrogen plus TCDD, or estrogen and progesterone plus TCDD. The cultured cells were then injected into ovariectomized nude mice treated continuously with estrogen, and the numbers of endometrial lesions in the mouse peritoneum were counted after 10–12 days. Progesterone is well known to inhibit the growth of endometrial tissue, and while 20 lesions in eight mice were seen from the injected cells cultured with estrogen alone, these were totally blocked by the co-culture with progesterone. In contrast, TCDD more than doubled the number of estrogen-induced lesions to 42 in eight mice. This effect of dioxin could not be blocked by progesterone; in fact, 48 lesions were observed in co-treated mice.

Surgical induction of endometriosis by implantation of endometrial tissue into the mesentery was used to demonstrate the key role of estrogen and the ability of antiestrogens to block endometrial growth and has been used to test the promotion of endometrial growth by TCDD in both rats and mice. Cummings et al. (*34*) developed a protocol to be used in both species of rodents (see Figure 1) which involved five repeated doses of TCDD over 12 weeks, with the rodents held for 15 weeks. The surgical induction of endometriosis occurred 3 weeks after the first dose of dioxin. Repeated doses of 0, 3, or 10 µg/kg TCDD were given to $B6C3F_1$ mice or Sprague-Dawley rats. Rodents were killed 3, 6, 9, and 12 weeks after the endometrial implantation surgery, and the implants were measured. No significant increase in endometriosis could be detected in rats until 12 weeks after the surgery, which also involved five doses of 10 µg TCDD/kg per dose, for a total dose of 50 µg/kg. If all the treatment groups were pooled across time and within dose, the induction of endometriosis was significant at the 10 µg/kg dose of TCDD. In contrast, the induction of endometriosis in mice could be detected by 9 weeks after the surgery at both doses, 3 and 10 µg/kg, involving total doses of 12 and 40 µg/kg. This dioxin-induced promotion of surgical endometriosis was still detectable 12 weeks after surgery.

These studies demonstrated that the promotion of surgically induced endometriosis was both dose and time related. Yang and Foster (*35*) ovariectomized mice, implanted them with estrogen-containing pellets designed to supply a constant stream of estrogen into the blood, and surgically implanted endometrial tissues into the peritoneum. The mice were then exposed to up to 100 ng TCDD/kg body weight for 28 days. At the end of that time, the diameter of the implants was measured. Endometrial implants from the high-dose animals were significantly smaller than those in controls.

These data suggest that exposure to TCDD does not promote the growth of endometriotic lesions in mice. However, there are problems rendering this interpretation in doubt. The first is that Cummings et al. (*34*) demonstrated that there is a time requirement to see the dioxin-induced promotion of endometrial growth. At least 9 weeks are required for the difference in size of TCDD-treated versus control lesions to be observed. Yang and Foster (*35*) allowed only 4 weeks for this process to occur. In addition, it is likely that the exposure of animals to TCDD before surgery, as in Cummings et al.'s study, may predispose the ectopic sites to be receptive to endometriotic implantation. A recent report from Japan also showed not a promotion of the growth of implanted endometrial tissue by TCDD but a regression of the implants (*36*). Again, this group failed to allow sufficient time for

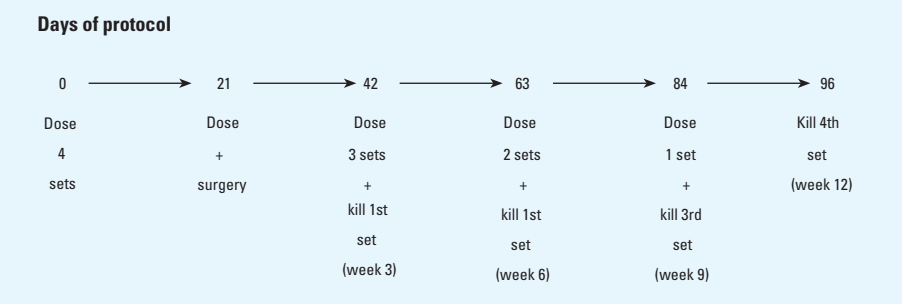


**Figure 1.** Protocol for the evaluation of the effect of 2,3,7,8-TCDD on surgically induced endometriosis in rodents. Animals were dosed 3 weeks before the induction surgery, again on the day of surgery, and three more times at 3-week intervals ending on the 9th week after surgery. Groups of animals were killed at 3-week intervals beginning 3 weeks after surgery and ending 12 weeks after surgery at which time evaluations included the size and/or weight of the induced endometriotic lesions. [Based on Yang and Foster (*35*).]

the growth differential to be observed. It should be noted that Matsui et al. (*36*) used Ah receptor knock-out mice. The study can thus be considered to have examined the role of the Ah receptor in endometriosis, and it is apparent that the Ah receptor is required for the promotion of endometriosis by TCDD.

Another problem is that the ovariectomy of the animals plus treatment with estrogen confounds the physiologic basis of the model. A comparison of the ovariectomized model with the intact rodent model of TCDD-promoted surgically induced endometriosis suggests that ovarian factors are involved that are not yet defined. The presence of the ovary may be necessary for the secretion of hormones or factors that might affect the availability of Ah receptors or that otherwise might affect the immunocompetency of the animals or the growth of endometriotic tissue independent of TCDD. The model of TCDD-promoted surgically induced rodent endometriosis in which the ovaries are removed (*35*) differs from the ovariectomized nude mouse model of endometriosis (*33*) in that human endometrial or endometriotic tissue is used for the implants in the latter, whereas rodent endometrial tissue is autotransplanted in the former. The two ovariectomized rodent models are limited by the absence of the influence of the ovary, which supplies a complex physiologic milieu that may be required for the response. The intact model is somewhat limited by the hormonal effects of the rodent estrous cycles, which can complicate the collection of data on endometriotic lesions due to the effects of the hormones on the size of the lesions.

Thus, the promotion of endometriosis in rodents by TCDD is both dose and time related, increases the size of the implants, requires estrogen, and is not blocked by progesterone. The mechanism of this response appears to involve the Ah receptor, the specific basic helix-loop–helix PAS protein required for essentially all of the activities of TCDD. Evidence for the role of the Ah receptor usually comes from studies demonstrating structure–activity relationships and/or genetics (*18*). To date, only the first category of studies have been conducted. Johnson and co-workers (*37*) used surgically implanted endometrial lesions in mice according to the model of Cummings et al. (*34*). Mice were exposed once before and four times after surgery, at 3-week intervals, to TCDD or four other polyhalogenated aromatic hydrocarbons, two of which are high-affinity agonists for the Ah receptor, 2,3,4,7,8-pentachlorodibenzofuran (4PeCDF) and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126); and two of which do not bind, 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153) and 1,3,6,8,-tetrachlorodibenzo-*p*-dioxin (1,3,6,8-TCDD). Doses were selected on the basis of the relative potency of these congeners to TCDD. The study by Johnson et al. (*37*), which predated the World Health Organization publication of relative TEF values, used enzyme data obtained in Birnbaum's laboratory to determine relative potency. TCDD, 4PeCDF, and PCB 126 all promoted the growth of the endometrial implants (Figure 2). In contrast, the non-Ah receptor ligands PCB 153 and 1,3,6,8-TCDD failed to alter the growth properties as compared to controls. Although not definitive, these results support the role of the AhR in endometriosis.

The mouse model has also been used to address the issue of whether exposure early in life can alter the susceptibility of the adult to dioxin-promoted endometriosis. Both rats and mice were exposed on gestation day 8 to doses of 1, 3, or 10 µg TCDD/kg (*38*). At 9 weeks of age, the prenatally exposed pups were treated with 1, 3, or 10 µg TCDD/kg, held for 3 weeks, and then had endometrial tissue surgically implanted according to Cummings et al. (*34*). This was followed by four additional doses of TCDD at 3-week intervals. Although no enhanced sensitivity to endometriosis could be detected in the rat, which appears less susceptible to this response overall than the mouse—possibly because of the decreased sensitivity of the adult immune system of the rat to dioxin-induced immune suppression—prenatal exposure to a low dose of TCDD resulted in greater sensitivity of the mouse pup to surgically induced endometriosis as an adult (*38*). This ability of TCDD to induce delayed developmental effects on endometriosis is in line with other reports demonstrating that prenatal exposure to dioxins is associated with altered breast and vaginal development in female pups, decreased sperm counts and altered seminal vesicle and prostate morphology in male offspring, deficits in learning and memory and behavioral alterations, permanent immune suppression, and decreases in core body temperature (*39–47*).

Several other investigations have followed up on the initial association with endometriosis and TCDD in rhesus monkeys (*32*). A preliminary report had appeared earlier from a Canadian study with rhesus monkeys exposed to PCBs (*48*). Early deaths were associated with endometriosis in monkeys exposed to a high dose of Aroclor 1254. (Aroclor 1254 is a commercial mixture of PCBs which is 54% chlorine by weight.) However, following the Rier et al. (*32*) study, Arnold and co-workers (*49*) conducted a thorough review of the pathology reports from the PCB monkey study and reported no significant association between exposure to this complex mixture of PCBs and endometriosis (*49*). There are several potential explanations for the apparent discrepancy between the Canadian results and those of Rier et al. The most obvious one is that the monkeys in the Canadian study were exposed to PCBs, not to TCDD. Although there are some dioxin-like PCBs in Aroclor 1254, most of the PCBs have no dioxin-like activity. Therefore, in agreement with the studies of Johnson et al. (*37*), one would not expect endometriosis in this cohort. Another explanation may be that the Canadian monkeys were not held long enough for the endometriosis to become apparent. Studies with radiation demonstrated that at least 7 years were required for endometriosis to appear. In the Rier cohort, endometriosis was not detected until at least 11 years after the initiation of exposure to TCDD (7 years after the feeding stopped). The total time of the PCB exposure and postexposure part of the study was only 6 years, possibly inadequate for appearance of endometriosis in the monkeys.

In contrast, Rier has followed up on analysis of the original cohort of rhesus monkeys exposed to TCDD for 4 years in their diet. After a total of 17 years from the onset of exposure, endometriosis was assessed in all of the monkeys (*50*). There was a clear association between TCDD exposure and endometriosis. However, in addition, Rier et al. (*50*) observed an association between endometriosis and TEQ, in many cases determined not entirely by the historical TCDD dietary exposure, but also by PCBs, especially 3,3′,4,4′-tetrachlorobiphenyl (PCB 77), PCB 126, as well as 4PeCDF in serum. The source of these PCBs in the monkeys has not been determined, but the presence of PCBs was also observed in another cohort of monkeys maintained in the same facility which had been exposed to lead (*50*). In these lead- (and, apparently, PCB-) exposed monkeys,

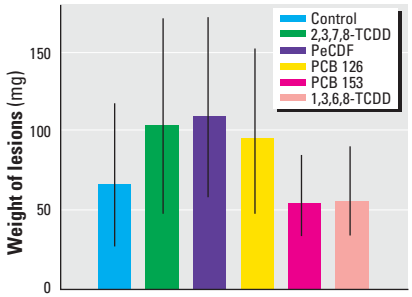


**Figure 2.** A comparison of the effect of selected polyhalogenated aromatic hydrocarbons on the growth of surgically induced endometriotic lesions in rats. The method used for treatment of the animals was identical to that depicted in Figure 1. Data represent the mean and SE. [Data from Cummings et al. (*38*).]

there was also an association between TEQ and endometriosis that is a function of the dioxin-like PCB levels and not the lead. This suggests that it is the activation of the Ah receptor by both dioxins and other Ah receptor ligands that is associated with endometriosis.

Recently, Yang et al. (*51*) demonstrated that surgically induced endometriosis in cynomolgus monkeys could be promoted by TCDD. After the implantation of endometrial tissue in the peritoneum, the monkeys were treated with TCDD 5 days per week for 1 year, at which time the survival and size of the implants were measured. There was a dose-related increase in the percentage of surviving implants (Figure 3). This increase in survival was significant at both the middle (3.57) and high doses (17.86 ng/kg/day). There was also a significant decrease in the size of the implants at the lowest dose (0.71 ng/kg/day) compared to the control monkeys. However, the size of the implants was significantly larger than in the controls at the high dose. An interesting aspect of these studies is the examination of the cytokine signaling pathway involving IL-6, a key mediator in the immune system. Yang et al. (*51*) observed a dose-related decrease in the circulating levels of IL-6 after 6 months of TCDD treatment. However, after 1 year, the decrease was only evident in the high-dose group. These results were compatible with analysis of the levels of IL-6 receptor, which is normally regulated in a negative feedback loop with its ligand, Il-6. Yang et al. (*51*) observed a dose-related increase in the concentration of IL-6 receptor at 6 months, but there was an increase only in the high-dose group after 1 year. Nevertheless, these studies clearly indicate that TCDD has affected the immune system of these monkeys, which may play a role in the enhanced survival of the endometrial implants.

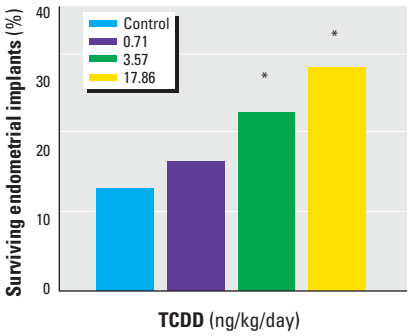


**Figure 3.** Promotion by 2,3,7,8-TCDD on the survival rate of surgically induced endometriotic implants in monkeys. Animals were treated with TCDD for 1 year after the implantation of endometrial tissue in the peritoneum, and an evaluation of the size and survival rate of the implants was made. Data from Yang et al. (*51*).
*$p < 0.05$.

Thus, to date the weight of the evidence seems to support an association between TCDD and related chemicals and endometriosis in experimental animals. TCDD and TEQ are associated with both spontaneous and surgically induced endometriosis in two species of nonhuman primates. TCDD and TEQ promote the growth of surgically induced endometriosis in both rats and mice. And prenatal exposure to TCDD may increase the sensitivity to endometriosis during adulthood.

The situation in humans is less clear. The ecologic epidemiology reports in the early 1990s of an association between organochlorines and endometriosis gained prominence with the publication of Rier et al.'s (*32*) monkey study. This led to a cohort study in Israel in which the plasma concentrations of TCDD were compared in 44 women with surgically confirmed endometriosis to 35 women with no surgical evidence of this condition (*52*). The women were matched for socioeconomic status and age but not ethnic backgrounds. The percentage of women with dioxin above the limits of detection in this study (2 ppt) increased with the incidence of endometriosis. Only 1 of 35 women without any endometriosis had dioxin > 2 ppt. In contrast, 3 of 24 women with stage I and II endometriosis had detectable dioxin, and 25% of the women with moderate and severe endometriosis (stages III and IV) had concentrations of TCDD > 2 ppt. This study supports the hypothesis, but the high limits of analytic detection of TCDD and the failure to measure all of the TEQ introduce some questions into the interpretation of this study. However, it does encourage further investigation.

Pauwels et al. (*53*) conducted a small study in Belgium in which the total TEQ was measured in plasma samples. Again, as in the study by Mayani et al. (*52*), the presence or absence of endometriosis was surgically confirmed. In a report that did not present the endometriosis data per se but presented statistical evaluations, Pauwels et al. found a high relative risk for endometriosis in association with elevated TEQ, based on a bioassay for all of the dioxin-like activity (PCDDs, PCDFs, and dioxin-like PCBs). However, because the number of women in these studies is small, the increased risk is not statistically significant at the $p = 0.05$ level. There was no association with total PCB measurements, and this is in agreement with the animal data. Significantly, in both of these studies, the blood levels are within the range of the background population.

In contrast to these studies suggesting an association between dioxins and endometriosis, two other reports do not. Boyd et al. (*54*) presented a preliminary report on 30 women, 15 with apparent endometriosis, based on symptomology, and 15 with no evidence of this condition. There was no surgical ascertainment. These investigators failed to observe any association between the clinical diagnosis of endometriosis and the dioxin equivalency due to polychlorinated dibenzo-*p*-dioxins and dibenzofurans. They did not measure the dioxin-like PCBs; therefore, they could not examine an association with the total TEQ, as done by Pauwels et al. (*53*). The lack of surgical ascertainment of endometriosis is also an issue because some of the controls may in fact have had the condition, and some of those identified as having endometriosis may have had other diseases.

Another study failed to demonstrate an association between total PCBs and endometriosis. Lebel et al. (*55*) examined a cohort of women in Quebec who were surgically diagnosed. The authors measured 14 of the most common PCBs, but they did not measure any of the dioxin-like PCBs, the PCDDS, or PCDFs. Given the animal data, as well as the other epidemiology studies, the lack of an association with total PCBs is not a surprise. Thus, to date, the data are not definitive, but are suggestive because the few negative studies are confounded by disease ascertainment and by comparison to the wrong metric (i.e., to PCBs and not to TEQ). A study is being conducted involving women in Seveso, the Italian town poisoned by dioxin in 1976 (*56*). Women in the two most highly contaminated zones are being examined for clinical diagnosis of endometrioisis. Blood levels of dioxin from samples taken shortly after the incident are being measured. However, surgical evaluation is not being conducted, leading to the possibility of misclassification of disease status. Also, the total TEQ is not being determined, and although this was primarily a TCDD poisoning, the levels of other dioxins may contribute to the risk as well. A meta-analysis involving surgical diagnosis of disease status as well as measurement of total TEQ might allow greater confidence in confirming or refuting the association between endometriosis and dioxins. To date, none of these studies has detected clear differences in exposure to dioxins between women with and without endometriosis.

The etiology of endometriosis is often thought to involve hormonal alterations as well as changes in the immune system. Recent studies aimed at developing a mechanistic understanding of how dioxins could promote endometriosis have focused on the effects of dioxins on the endocrine and immune systems. Dioxin is the prototypical endocrine disruptor (*15*), leading to alterations in every hormone system investigated to date, both of

the steroid family and peptide. Dioxins have been shown in a cell, tissue, developmental stage, and species-specific manner to modulate hormone signaling via changes in the number of receptors (up- vs. down-regulation), alteration of the metabolism of the hormones (increased/decreased synthesis/degradation), and alteration of serum transport via competition for plasma-binding proteins. Endometriosis requires estrogen, both in clinical situations and in experimental animal models. However, even ovariectomy does not always block the growth of endometrial lesions. Bulun et al. (*57*) have demonstrated that testosterone can be converted *in situ* in endometrial implants to estrogen via aromatase action. The adrenal glands produce androstenedione, which is converted by aromatase in peripheral tissues to estrone, and which can be further metabolized to estradiol, the most active form of estrogen, by 17β-hydroxysteroid dehydrogenase. Thus, the adrenal production of androgens can lead to local elevation of estrogen. Also potentially significant about these findings is that aromatase activity is enhanced by prostaglandins whose production is increased when inflammatory cytokines such as IL-1β and tumor necrosis factor α are produced during an immune reaction. In several animal models, TCDD induced the synthesis of these cytokines (*58*,*59*).

Another key requirement for the ability of dioxin to promote the growth of endometriosis is the presence of the required signaling pathway for dioxin's effects. Essentially all of the effects of dioxin require binding to the Ah receptor. Several laboratories have demonstrated the presence of the Ah receptor in uterine endometrium. Recently, Igarashi et al. (*60*) demonstrated the presence of mRNAs, not only for the Ah receptor but for its cognate dimerization partner, ARNT, as well as genes that are under the direct transcriptional control of the Ah receptor/ARNT heterodimer, CYP1B1. These investigators showed that these genes were expressed not only in normal endometrium but in endometriosis as well, demonstrating the ability of the diseased tissue to respond to dioxins. However, Bulun et al. (*61*) could find no evidence that the presence of endometriosis significantly altered the expression of the Ah receptor or ARNT, although another dioxin-responsive gene, CYP1A1, was induced in endometriotic tissues. Induction of CYP1A1 is often considered a biomarker for dioxin responsiveness. These data are consistent with the data of Bruner et al. (*33*), which suggest a direct effect of TCDD on endometrial tissue independent of other potential mechanisms such as those affecting the immune or endocrine systems.

Thus, TCDD and related compounds, well known to alter proliferation and differentiation in multiple cells and tissues, as well as to modulate hormones and the immune system, clearly have the potential to be associated with endometriosis. Dioxin is also a known human carcinogen (*23*). Recent studies have suggested that endometriosis may be a type of benign neoplasm and that women with endometriosis may be at elevated risk for cancer as well (*62*). Animal studies, both in nonhuman primates and rodents, have shown that exposure to dioxins can promote the growth of spontaneous as well as surgically induced endometriosis. Dioxin-like PCBs, but not nondioxin-like PCBs, are associated with endometriosis. Therefore, studies should look at the association between the total TEQ, not the total PCBs, and this disease. Surgical ascertainment of endometriosis appears essential to avoid misdiagnosis. Clear evidence of a role for dioxins in the increased incidence of endometriosis will require careful cohort studies of women with and without endometriosis coupled with adequate exposure determination. If such an association is confirmed, we speculate that the decrease in exposure to dioxins that has been occurring over the past 10–20 years might eventually reverse the increase of this debilitating condition.

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