The link between environmental toxicant exposure and endometriosis

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1. ABSTRACT

Endometriosis is an estrogen-dependent disease characterized by the growth of endometrial cells in ectopic locations. Although the etiology of endometriosis is unknown, several hypotheses have been proposed to explain its origin. Retrograde menstruation of endometrial cells into the peritoneum is the most widely accepted theory, however, this phenomenon occurs in approximately 90% of women while the prevalence of endometriosis is much lower. Hence, other factors are thought to contribute to the development of this disease, including exposure to environmental toxicants. Although the epidemiological evidence is equivocal, animal and experimental investigations provide a basis for the proposed association between dioxin and dioxin-like chemical exposure and endometriosis. However, the mechanism(s) underlying this potential association are poorly understood. Development of novel animal models that more reliably recapitulate the pathogenesis and pathophysiology of this disease provide exciting opportunities to further test the link between exposure to these chemicals and endometriosis. Moreover, differential expression of several novel genes that may be important in the disease provides new targets to test the actions of environmental toxicants in the pathobiology of endometriosis.

2. INTRODUCTION

Endometriosis is a common gynaecological disease of unknown aetiology occurring almost exclusively in women of reproductive age. Characterized by the growth of endometrial glands and stroma at ectopic sites, this condition is commonly associated with pelvic pain, menorrhagia, dyspareunia and infertility. The estrogen-dependent nature of endometriosis is illustrated by its resolution at menopause and its onset or return in women undergoing hormone replacement therapy (1-3). Although its prevalence is proposed to fall between 5 and 10% (4), it is suggested that endometriosis may affect up to 45% of women as many are asymptomatic and surgical confirmation is required for conclusive diagnosis (5).

Several different theories have been brought forward in the literature to explain the origin of endometriosis. These include retrograde menstruation (6), coelomic metaplasia, Müllerian remnant differentiation, vascular or lymphatic transplantation of endometrial fragments, iatrogenic transplantation during surgery (7), immunological dysfunction (8) and genetic predisposition (9). Of these theories, the most widely accepted is retrograde menstruation, the process by which menstrual debris is refluxed into the pelvis (6). However, this

phenomenon occurs in up to 90% of women (10) and thus cannot account for the relatively small percentage of women that develop endometriosis. It is therefore proposed that the eutopic endometrium is functionally different in women who develop endometriosis compared to healthy fertile women without the disease. Indeed, there is a plethora of literature documenting biochemical differences in the serum, peritoneal fluid and endometrium of women with endometriosis compared to those without it. Decreased expression of progesterone receptors (11;12) and alterations in cell adhesion molecule (13-16) and matrix metalloproteinase expression (17-19) have been documented in women with endometriosis compared to healthy controls. Furthermore, aberrant functioning of the immune system (reviewed in (20;21)) and angiogenic regulation (22-25) are suggested to play roles in the progression and maintenance of the disease. Increased expression of aromatase, the rate limiting enzyme responsible for the conversion of androgens to estrogen, has also been documented (26;27) and evidence suggests that endometriotic implants acquire the capacity for local estrogen production in order to support their growth. Unfortunately, these biochemical alterations do not adequately account for the development of endometriosis, and the complexity of this disease is enhanced further by the variety of presentations in which it may appear (e.g. cysts, deep infiltrating nodules), each demonstrating its own pathobiological phenotype (28-32).

A growing body of evidence suggests a role for biohazardous environmental toxicants in the pathobiology of endometriosis. Although the strength of this association has been contested in the literature (33-35), geographical differences in disease prevalence point to a potential environmental influence. Specifically, an elevated prevalence of endometriosis has been reported for areas with increased levels of contamination. For example, Belgium has the highest reported levels of dioxin worldwide, coinciding with an elevated prevalence of endometriosis in comparison to most other countries (36). Similarly, the incidence of endometriosis is greater in highly contaminated regions of New York State versus less polluted areas of the State (37). However, exposure measurements were not made in these studies and thus they only hint at a potential linkage between the prevalence of endometriosis and some undefined environmental factor.

Studies designed to investigate the association between environmental toxicant exposure and endometriosis focused initially on estrogenic contaminants, including members of the polychlorobiphenyl (PCB) family of chemicals, due to the inherent estrogen-dependency of the disease. Later, the estrogenic nature of phthalate esters also gained attention, prompting epidemiological investigations into their potential role in endometriosis. Several isolated studies have examined the link between endometriosis and exposure to pesticides (38;39), polybrominated biphenyls (40) and the metals cadmium and lead (41). Results of these studies have consistently failed to find a relationship between exposure to these toxicants and an increased risk of endometriosis.

Of all the biohazardous environmental toxicants studied, exposure to dioxin and dioxin-like chemicals has received the greatest attention (42;43) and is thought to be inculpated in the pathophysiology of endometriosis. While it is likely that a complex interaction of distinct biochemical pathways gives rise to endometriosis, the objective of this paper is to present and evaluate scientific literature describing the potential contribution of environmental contaminants to the development of endometriosis. Epidemiological, animal and experimental studies will be discussed regarding the potential contribution of three common classes of environmental contaminants, phthalates, PCBs, dioxin and dioxin-like chemicals, to the pathobiology of endometriosis.

3. EPIDEMIOLOGICAL EVIDENCE

3.1. Phthalate esters

Phthalate esters are a class of water-insoluble organic chemicals that are most commonly used as plasticizers in materials such as flexible polyvinylchloride formulations. Their ability to hold colour and fragrance or to provide a film or gloss has also contributed to their use in personal care products (e.g. perfume, lotions), lubricants, adhesives, safety glass and various types of medical equipment (44). Such broad-spectrum use has quickly caused phthalates to become ubiquitous environmental toxicants, as they do not bind to the molecules within these products but rather leak out over time. Accordingly, with their high level of production (18 billion pounds per year globally) and variety of uses, human exposure to phthalates occurs daily through ingestion, inhalation and dermal routes.

Di-(2-ethylhexyl) phthalate (DEHP; Figure 1) is the most common member of the phthalate family, approved for use in medical devices including blood bags, tubing, dialysis equipment and disposable gloves. This diester compound is metabolized by esterases in the blood, gut and liver into its monoester form, mono-(2-ethylhexyl) phthalate (MEHP; Figure 1), which is regarded as the *in vivo* toxicant.

An association between phthalate exposure and endometriosis has been documented in three separate case-control studies (Table 1). In the first published investigation, concentrations of DEHP and MEHP were measured in the plasma and peritoneal fluid of reproductive-aged women with and without endometriosis using high performance liquid chromatography (45). Significantly higher plasma DEHP concentrations were found in cases (median = 0.57 μg/mL) compared to controls (0.18 μg/mL; P = 0.0047), while plasma MEHP levels were found to be comparable between the groups. Although DEHP and MEHP were also detected in the peritoneal fluid of endometriosis patients, no significant difference in concentration was noted. A subsequent study by Reddy *et al.* (46) examined the presence of phthalate esters in the plasma of infertile women with minimal to severe-stage endometriosis and women without the disease with proven fertility. A dose-dependent relationship between phthalate exposure and endometriosis severity was
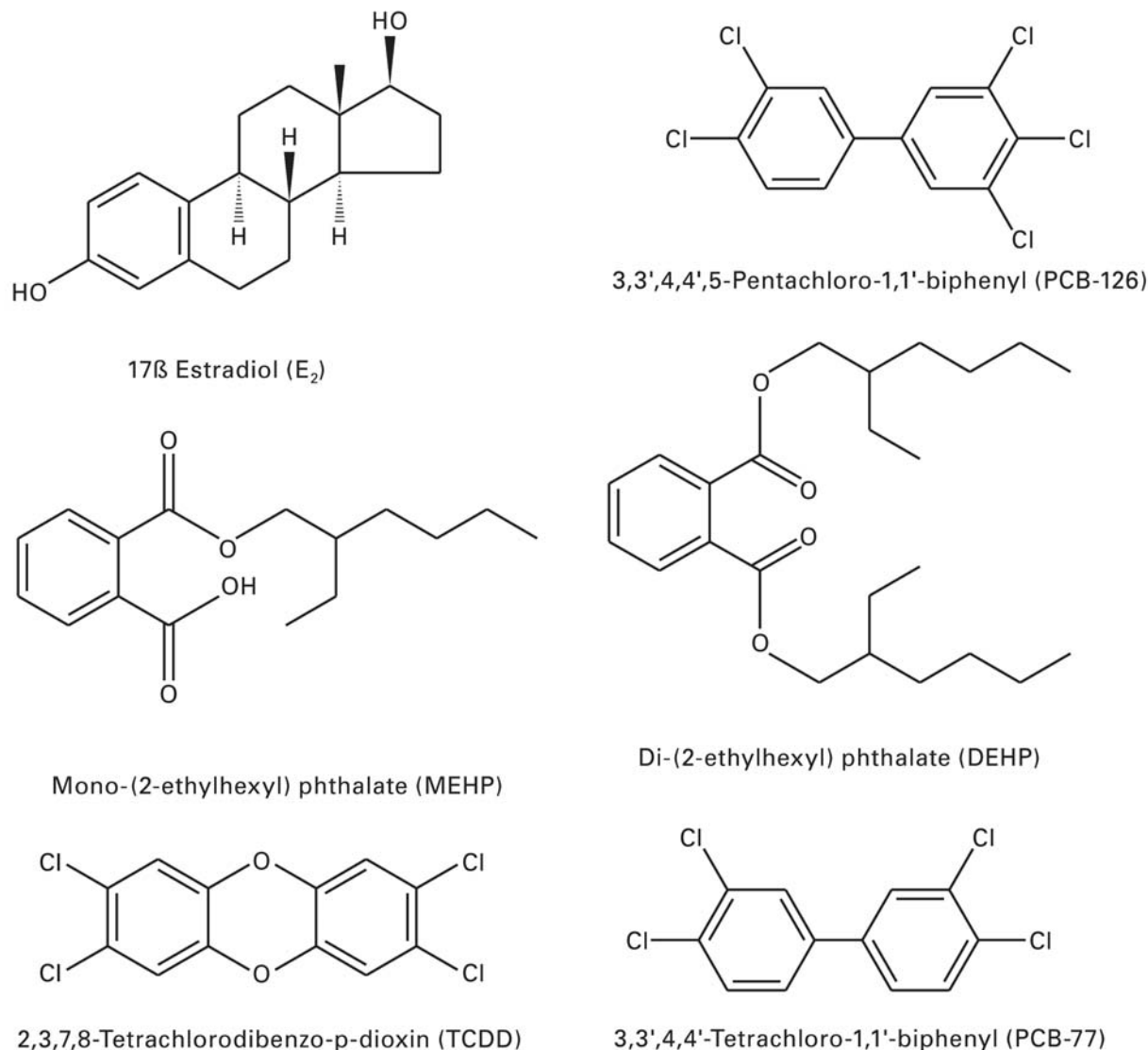
Figure 1. Chemical structures of estradiol and environmental toxicants implicated in the pathophysiology of endometriosis.

demonstrated for four phthalate congeners including DEHP (ranging from 0.48 to 4.39 μg/mL) but not MEHP. Similar results were found in a later study, in which infertile women with endometriosis had significantly elevated levels of plasma phthalates (P < 0.004 - 0.0001) compared to both infertile and fertile controls (47).

While the results of these investigations suggest a positive correlation between phthalate exposure and the presence of endometriosis, several questions arise. Examination of the pharmacokinetic properties of phthalates reveals that their rate of metabolism is relatively fast: DEHP has a biological half-life of less than six hours in humans as it is rapidly metabolized to MEHP (48). Therefore, one would have expected MEHP levels, particularly in the Cobellis *et al.* (45) study, to be elevated in comparison to those of its precursor. Thus, the serum DEHP concentrations reported in these studies (45;46;48) seem high

and may be more representative of acute exposure, such as that received during medical interventions. This may especially be the case in the study by Reddy *et al.* (46), where women with increased severity of disease demonstrated higher phthalate levels, possibly related to their increased need for medical treatment. Measurement of phthalate levels is well-known to be problematic given the risk of contamination from flexible vinyl used in many types of hospital and lab equipment (49). Overall, DEHP is metabolized to a number of metabolites which may serve as more appropriate markers of exposure (50) and should be measured in future studies.

To date, there are no published reports of animal studies involving phthalate exposure and endometriosis, and the experimental evidence does not support a potential link between exposure and this disease. In particular, phthalates are weak estrogenic chemicals under in vitro and in vivo conditions and have failed to impact estrogen-dependent

Table 1. Case-control, prospective epidemiological studies designed to investigate the association between phthalate ester exposure and the presence of endometriosis

Cases vs. Controls	Exposure Investigated	Tissue	Outcome	Significance	Ref[1]
55:24	DEHP, MEHP	Plasma, Peritoneal fluid	Elevated DEHP plasma concentrations in endometriosis patients.	P = 0.0047	45
85:135	Di-n-butyl, butyl benzyl and di-n-octyl phthalate, DEHP	Plasma	Dose-dependent relationship observed between phthalate exposure and severity of endometriosis.	P < 0.05	46
49:38:21[2]	Di-n-butyl, butyl benzyl and di-n-octyl phthalate, DEHP	Plasma	Significantly elevated phthalate concentrations in women with endometriosis (P < 0.0001).	P < 0.0001	47

[1] Ref: Reference, [2]infertile women with endometriosis: infertile women without endometriosis: fertile women without endometriosis

sexual development and maturation in female rodents at exposures relevant to humans (51-54). Furthermore, studies reveal that DEHP decreases circulating estradiol levels in rats, and MEHP treatment causes a dose-dependent decrease in granulosa cell aromatase mRNA and protein expression (55). Taken together, the evidence suggests that phthalates are weak estrogenic contaminants that are not present in levels adequate to activate the estrogen receptor. Moreover, these compounds suppress aromatase expression, thereby decreasing circulating estrogen levels and potentially inhibiting the growth of endometriotic implants. Still, these findings do not dismiss the possibility that phthalates may contribute to endometriosis through a mechanism outside of an estrogen-dependent pathway, or through changes in steroidogenic enzyme expression or activity. Therefore, we conclude that while the link between phthalate exposure and endometriosis is weak, the widespread exposure of humans to these compounds and epidemiological evidence warrants that further investigations be carried out.

3.2. Polyhalogenated aromatic hydrocarbons

Polyhalogenated aromatic hydrocarbons (PHAHs) are a family of ubiquitous environmental contaminants that include the polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), diphenylethers (PCDEs), and napthalenes (PCNs). Results from a number of epidemiological and animal studies suggest a potential relationship between the occurrence and/or severity of endometriosis and exposure to these compounds. Of these contaminants, only the PCBs, PCDDs and PCDFs have received research attention for a potential role in the pathophysiology of endometriosis.

3.2.1. Polychlorinated biphenyls

Polychlorinated biphenyls are mixtures of up to 209 related chemicals that are found ubiquitously in our environment. Used most commonly as coolants and lubricants due to their resistance to combustion and insulating ability, U.S. manufacture of these compounds was discontinued in 1977 upon recognition of their broad-spectrum toxicity, including neurobehavioural, immunological and carcinogenic effects.

Over 90% of human exposure to PCBs occurs through dietary intake. The lipophilicity, resistance to degradation, and poor metabolism of these compounds promotes their persistence in the environment and food chain, accumulating in fatty tissues with age. 2,2',3,4,4',5-hexachlorobiphenyl (PCB-138), 2,2',4,4',5,5'-hexachloro-1,1'-biphenyl (PCB-153) and 2,2',3,4,4',5,5'-

heptachlorobiphenyl (PCB-180) are found in the highest concentrations in humans relative to all other congeners, accounting for 50-80% of total serum PCB content (56). While found in lower concentrations, 3,3',4,4'-tetrachloro-1,1'-biphenyl (PCB-77), 3,3',4,4',5-pentachlorobiphenyl (PCB-126) and 3,3',4,4',5,5'-hexachlorobiphenyl (PCB-169) are considered to be three of the most toxic congeners, able to assume coplanar conformations and elicit effects comparable to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (57) (Figure 1; Table 2).

Current epidemiological evidence of a causal relationship between PCB exposure and endometriosis is inconsistent. While a number of studies suggest a correlation between exposure and disease (46;58-63), several other investigations have failed to identify the existence of such a relationship (39;40;64-66) (Table 3). Comparison of the data from these studies, however, is made difficult due to the variety of study designs and methodologies employed. Most notably, the number and specific types of PCB congeners examined varies greatly between studies, an important factor due to the diverse structure-activity profile of each compound. Investigations proposing a correlation between exposure and disease commonly analyzed samples for a larger number of congeners, in particular, those with dioxin-like properties (e.g. those able to bind to and activate the aryl hydrocarbon receptor (AhR), and thus these studies will be discussed along with dioxin and other dioxin-like chemicals (Table 4).

Other limitations of the PCB studies include small sample sizes and the inability to confirm the presence of endometriosis (65) or verify its absence in women selected as controls. Moreover, while most investigations did employ laparoscopy to confirm disease, many failed to concurrently assess its severity and/or to determine whether a relationship existed between exposure level and stage of endometriosis (39;65). Further complicating matters, adjustment for various confounding variables including age, body mass index, indication for laparoscopy, serum lipids, parity, ovulatory dysfunction, smoking pattern, and diet occurred in some studies but not in others, and by different methods, thereby hindering one's ability to compare the results of these investigations. In studies that found no correlation between PCB exposure and endometriosis, it is possible that the organochlorine concentrations measured at laparoscopy were not indicative of exposure during a critical time point for induction of endometriosis. PCBs have a proposed half-life in human

Table 2. WHO (2005) toxic equivalency factors for 12 dioxin-like PCBs (humans).

Chemical Structure	IUPAC Number	TEF
3,3',4,4'-TeCB	PCB-77	0.0001
3,4,4',5-TeCB	PCB-81	0.00003
2,3,3',4,4'-PeCB	PCB-105	0.00003
2,3,4,4',5-PECB	PCB-114	0.00003
2,3',4,4',5-PeCB	PCB-118	0.00003
2',3,4,4',5-PeCB	PCB-123	0.00003
3,3',4,4',5-PeCB	PCB-126	0.1
2,3,3'4,4',5-HxCB	PCB-156	0.00003
2,3,3',4,4',5'-HxCB	PCB-157	0.00003
2,3',4,4',5,5'-HxCB	PCB-167	0.00003
3,3',4,4',5,5'-HxCB	PCB-169	0.03
2,3,3'4,4',5,5'-HpCB	PCB-189	0.00003

Based on ref 112

Table 3. Summary of epidemiological studies investigating the association between polychlorinated (or polybrominated) biphenyl exposure and endometriosis. Shading indicates studies that found a significant correlation between exposure and disease

Cases vs. Controls	Exposure Investigated	Tissue	Outcome	Significance	Ref[1]
28:441	PCB 138, 153 and 180	Serum	Women with endometriosis had significantly higher non-dioxin-like PCB levels.	PCB 138, P = 0.0441; PCB 153, P = 0.0078; PCB 180, P = 0.0307	58
86:70, matched indications for laparoscopy	14 PCB congeners (28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187).	Plasma	No significant correlation observed between exposure and disease.	No significance, all 95% CIs overlapped the null value.	39
34:27, all infertile	Co-planar and non-coplanar PCBs (118, 138, 153, 180).	Serum	No significant correlation observed between exposure and disease.	OR 4.33, CI 0.48 – 43.62.	64
10:132	Coplanar PCBs: 77, 81, 126 and 169; PCB markers: 3, 8, 28, 52, 101, 118, 138, 153, 180, 194, 206, 209.	Fasted serum fat	No significant correlation observed between exposure and disease.		65
10 AD: 7 PE; 10 controls	PCBs 3, 8, 28, 52, 101, 118, 153, 180, 194, 206 and 209.	Serum	Elevated concentrations of bulk PCBs observed in women with adenomyosis of the rectovaginal septum.	P = 0.024.	63
10:12 Italian women; 7:11 Belgian women; pooled samples	12 dioxin-like PCBs: 77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167, 189.	Serum	No significant correlation observed between exposure and disease.		66
32:52	62 individual PCB congeners including both estrogenic and anti-estrogenic compounds.	Serum	Three-fold increased risk of endometriosis observed for third tertile concentrations of anti-estrogenic PCBs; no association seen for estrogenic PCB congeners.	OR 3.77, 95% CI 1.12 – 12.68; after controlling for gravidity, cigarette smoking and lipids: OR 3.3, 95% CI 0.87-12.46.	59
Unknown; infertile women	Unknown.	Follicular fluid	Higher levels of PCBs 114, 153, 156, 157, 180 and 189 in patients with endometriosis versus controls.	Not stated in abstract.	60
25 PE; 25 DEN; 21 controls	TEQ levels calculated for 12 dioxin-like PCB's: 77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167, 189.	Fasted serum	Significantly elevated TEQ levels in women with DEN versus controls; slightly elevated levels in women with PE.	P = 0.16, OR 6.6, 95% CI 1.42-31.24 for DEN.	61
40:40	11 PCB congeners: 28, 52, 101, 105, 118, 138, 153, 156, 167, 170, 180.	Blood	Elevated concentrations of both dioxin-like and non-dioxin-like PCBs in women with endometriosis.	OR for upper tertile: 4.0, 95% CI 1.3-13; P = 0.0003.	62
85:135	PCB 1, 5, 29 and 98.	Plasma	Dose-dependent relationship observed between PCB exposure and severity of endometriosis.	P < 0.05.	46
79:864	PBB quantification based on 2,2',4,4',5,5'-hexabromobiphenyl; PCBs quantified as Aroclor 1254.	Serum	No correlation between PBB exposure and disease. Non-significant increased incidence of endometriosis in women exposed to moderate and high PCB levels.	Moderate PCBs: HR 1.67, 95% CI 0.91 – 3.10; high: HR 1.68, 95% CI 0.95 – 2.98.	40

[1] Ref: Reference, AD = adenomyosis; DEN = deep infiltrating endometriotic nodules; PBB: polybrominated biphenyl; PE = peritoneal endometriosis; TEQ = toxic equivalent

serum of approximately 10 years (67); thus, epidemiological studies investigating levels in women in their thirties and forties are representative of adult exposures. Should the critical window of exposure exist

Table 4. Summary of epidemiological studies designed to investigate the association between polychlorinated dioxin and dioxin-like chemical exposure and endometriosis. Shading indicates studies that found a significant correlation between exposure and disease

Cases vs. Controls	Exposure Investigated	Tissue	Outcome	Significance	Ref[1]
44:35, all infertile	2,3,7,8-TCDD	Blood	18% endometriosis patients were dioxin-positive, versus only 3% of control patients. Suggested association between TCDD and disease.	P = 0.04; OR 7.6, 95% CI 0.87 – 169.7.	68
42:27, all infertile	Dioxin and dioxin-like PCBs (compiled to give CALUX bioassay-based TEQ values).	Serum	No significant association.	OR 1.33, CI 0.49-38.19.	64
19:277	2,3,7,8-TCDD	Serum	Doubled, non-significant risk in women with TCDD levels greater than 100 ppt; no dose-response effect observed.	RR: serum 20.1-100 ppt TCDD, 1.2, 90% CI = 0.3-4.5; > 100 ppt, 2.1, 90% CI 0.5-8.0.	69
10:132	17 PCDDs and PCDFs (compiled with PCBs to give TEQ values).	Fasted serum fat	No significant association.		65
10:12 Italian women; 7:11 Belgian women; pooled samples	17 PCDD and PCDF 2,3,7,8-chlorosubstituted congeners.	Serum	No significant difference observed between cases and controls on a country basis.		66
25 PE; 25 DEN; 21 controls	17 PCDDs, PCDFs (and dioxin-like PCBs – expressed together as TEQ/g).	Fasted serum	Significantly elevated TEQ levels in women with DEN and PE versus controls; increased risk for PE with dioxin exposure alone.	DEN OR = 3.3, 95% CI =1.4-7.6; PE OR = 1.9, 95% CI 0.9-3.8; dioxin alone OR 3.2, 95% CI 1.0-9.9.	61
19 PE; 17 OE; 29 DEN	Examined correlation between serum dioxin-like compound levels (17 PCDDs/PCDFs and 12 PCBs) and aromatase expression in endometriotic tissues.	Serum for dioxin-like compounds; endometriotic tissue for aromatase.	Dioxin-like compound levels were not correlated with aromatase expression in endometriotic tissue.		113
31 early endometriosis; 48 advanced endometriosis; 59 controls, all infertile.	TEQ levels determined through measurement of 8 PCDDs, 10 PCDFs, 4 coplanar PCBs and 36 orthosubstituted PCBs; relationship to CYP450 polymorphisms investigated.	Serum	Presence of the CYP1A1 426Val allele was associated with a decreased risk of advanced endometriosis among women with high serum dioxin levels; significant interaction noted between advanced endometriosis CYP1B1 Leu432Val polymorphisms and PCB TEQ level.	OR = 0.13, 95% CI = 0.02 -.76, P interaction = 0.08 for CYP1A1 426Val allele; P = 0.05 for CYP1B1 Leu432Val interaction.	114

[1] Ref: Reference, DEN = deep endometriotic nodules; PE = peritoneal endometriosis; OE = ovarian endometriosis; TEQ = toxic equivalency

earlier in life, for example, during infancy or puberty, these studies would have been unable to detect a relationship if one were to exist. Overall, measurement of dioxin-like chemicals was a consistent feature of studies that found an association between exposure to environmental toxicants and endometriosis; thus, this link may have more to do with dioxin-like activity than the estrogenic PCBs.

3.2.2. Dioxins and dioxin-like chemicals

The dioxin family comprises 210 chlorinated hydrocarbon compounds divided into 75 polychlorinated-dibenzo-dioxins and 135 polychlorinated-dibenzo-furans (PCDFs). The so-called Seveso dioxin, TCDD, has shown to be the most toxic congener in rodent studies, and is produced as a by-product of combustion in a number of industrial processes (Figure 1). Like PCBs, human exposure to dioxins occurs primarily through the consumption of foodstuffs, and their lipophilicity and chemical stability enable their prolonged persistence and accumulation in the environment and biological tissues.

The epidemiological evidence proposing an association between serum dioxin levels and endometriosis is equivocal. While several studies have reported increased odds ratios or relative risks for the disease, the confidence intervals for these data frequently include 1.0 (Table 4) (61;64;68;69). Other studies that report no interaction

between exposure and disease involved very small case populations (65;66), and most investigations failed todocument the American Fertility Society stage of endometriosis. Of interest was the report of a non-significant doubling of risk (TCDD levels > 100 ppt, RR = 2.1 (90% CI 0.5 – 8.0)) for endometriosis in women living in Seveso, Italy, who were exposed to TCDD as the result of an industrial accident in 1976 (69). Up to 30 kg of TCDD were deposited in an area approximately 18 km^2 in size, resulting in human exposures ranging from 2.5 – 17,300 ppt (69;70). An important limitation of this study was the inability to confirm the presence or absence of endometriosis by laparoscopy and thus the potential for asymptomatic women to be misclassified cannot be ruled out. Moreover, women with a clinical presentation of endometriosis were included as cases, however, may have been suffering from a distinct condition. Still, this study further highlights the potential role of TCDD exposure in this disease.

In contrast to the above reports, a number of epidemiological investigations have demonstrated a positive association between exposure to TCDD or dioxin-like PCBs and endometriosis. A greater than three-fold increase in the risk of endometriosis (3.77, 95% CI 1.12 - 12.68) was reported for women in the third tertile of anti-estrogenic PCB exposure (59). However, after controlling

Table 5. Summary of animal model studies designed to investigate the relationship between polychlorinated dioxin exposure and endometriosis. Shading indicates studies that found a significant correlation between exposure and disease

Animal Model	Treated vs. Controls	Exposure Route and Duration	Outcome	Significance	Ref[1]
Rhesus monkeys	24	0, 5, or 25 ppt TCDD	Significant dose-dependent association observed between TCDD exposure and disease incidence and severity.	$P < 0.001$	42
Sprague-Dawley rats B6C3F1 mice Autotransplantation of uterine sections to vessels of the intestinal mesentery.	8 rats and 8 mice per treatment.	Five treatments of 0, 3 or 10 µg/kg TCDD, once before surgery, at surgery and 3, 6 and 9 weeks following surgery.	Significant increases observed in rat (at 10 µg/kg) and mouse (3 and 10 µg/kg) endometriotic site diameters.	$P < 0.05$ and $P < 0.002$	79
B6C3F$_1$ mice Autotransplantation of uterine segments into peritoneal cavity.	10-12 animals per treatment.	Oral gavage of 0, 1, 3, or 10 µg/kg bw 2,3,7,8-TCDD, 1x every 3 weeks (5 doses).	Significant growth of endometrial lesions induced by 1 and 3 µg/kg bw TCDD.	$P < 0.05$	80
Sprague-Dawley rats and C57BL/6 mice Autotransplantation of endometrial strips to vessels of the intestinal mesentery.	25-20 dams per gestational treatment; n = 12 for postnatal treatments.	Gestation day 8: Rats – 0 µg or 1 µg TCDD/kg by gavage Mice – 0 µg or 3 µg TCDD/kg by gavage Postnatal treatments of offspring: 0, 3 or 10 µg TCDD/kg.	No effect on lesion diameter in rats. Perinatal plus adult exposure in mice resulted in a significant increase of endometriotic lesion diameter.	$P < 0.05$	78
Cynomolgus monkeys Autotransplantation of endometrial strips to multiple abdominal sites.	23 animals including 5 controls	Gelatin capsules containing 1, 5 and 25 ng/kg bw TCDD; 5 doses per week for 1 year.	3.57 and 17.86 ng/kg/day doses resulted in a significantly higher survival rate of implants and larger diameter implants in the latter dose group.	$P < 0.05$	81
Rhesus monkeys	9 TCDD exposed, 6 unexposed	0, 5 or 25 ppt TCDD by ingestion in animal feed 13 years earlier.	*Re-evaluation of 1993 study. Serum levels of TCDD were not significantly different in animals with endometriosis.		83
Mice Autotransplantation of strips to mesenteric blood vessels.	3 or 4 animals per treatment group	Sham, OVX, OVX + 10 µg/kg TCDD ± 100 µg/kg E2	TCDD + E2 treatment significantly reduced epithelial cell height, stromal thickness and proliferative activity of endometriotic lesions; TCDD increased epithelial expression of ERα	$P < 0.05$	82
C57BL6 mice	5-6 animals per treatment group	Unexposed, vehicle (corn oil) or TCDD (10 µg/kg) by gavage at different developmental time points	Progressive loss of both progesterone receptors A and B as duration of TCDD exposure increased; decrease of TGF-β2 expression also observed	No statistics reported.	87

[1] Ref: Reference

for confounding factors such as cigarette smoking, prior pregnancies and serum lipid levels, the odds ratio was reduced to 3.3 and the 95% confidence interval bracketed 1.0 (0.87 – 12.46). Another study found higher residue levels for dioxin-like and non-dioxin-like PCBs in women with endometriosis compared to controls (OR 4.0, 95% CI 1.3 – 13), although the contribution of the two groups of contaminants to this outcome is unknown (62). Perhaps the investigation by Heilier *et al* (61) stands alone in its observation of significantly elevated TEQ levels in women with deep endometriotic nodules (OR = 3.3, 95% CI = 1.4 – 7.6), although like the others, this study involved a small number of cases and controls.

As seen previously, comparisons between these investigations are made difficult due to the divergent methods used to measure and report dioxin-like PCB and dioxin levels in patient samples. In most cases, residue analysis was performed using gas chromatography and mass spectrometry, and data were reported on a lipid-adjusted basis. Lipid-adjustment is required due to the apolar and lipophilic nature of these toxicants; however, variation in the methods employed to assess their presence (including solvent selection and calculation type) may introduce uncertainty into the results obtained (71;72). The

reporting of toxicant levels varies, with exposures presented as either tissue concentrations or toxic equivalency factors (TEF; (73;74)). TEFs permit the conversion of PHAH chemical data sets into AhR-related toxic potencies, calculated as the sum of individual PHAH concentrations that have been multiplied by their respective TEF value to give the total TCDD toxic equivalency (TEQ). Alternatively, the CALUX (chemical-activated luciferase gene expression) bioassay has been used to assess sample toxicant levels using cells transfected with an AhR-regulated luciferase reporter gene (64). Light produced in this bioassay corresponds to receptor activation by coplanar PCBs and thus reflects the biological activity of toxicants present in the sample. This assay has the advantage of assessing the strength of the AhR signal delivered to target cells; however, competitive inhibition by other contaminants present in the sample may lead to underestimation of the dioxin-like chemical level present.

Moreover, the assay also ignores competition for transcription factors that are operative in the target cells of intact tissues (75).

In summary, the epidemiological evidence does not provide an overwhelmingly positive link between

Table 6. Animal model studies examining the relationship between polychlorinated biphenyl exposure and development and/or maintenance of endometriosis. Shading indicates studies that found a significant correlation between exposure and disease

Animal Model	Study Objective	Treatment	N values	Outcome	Significance	Ref[1]
Rhesus monkey	To assess the ability of treatment to cause disease. *not the primary study endpoint	0, 5, 20, 40 or 80 µg of Aroclor 1254 per kg bw; dosed daily for 6 years.	16 animals per treatment group.	No change in incidence or severity of endometriosis.		84
B6C3F$_1$ mouse	To examine the effect of treatment on growth of sutured autologous uterine segments in the peritoneal cavity.	0, 3, 30 mg/kg bw PCB 153 or 100, 300, 1000 µg/kg bw PCB 126; 5 oral gavage treatments, 3 week interval between each.	10-12 animals per treatment group.	No observable effect on endometriotic lesion size.		80
Rhesus monkey	To measure serum levels of PCBs and identify any relationships to the presence and/or severity of endometriosis.	0, 5, 25 ppt TCDD for 4 years through ingestion in animal feed.	9 TCDD exposed versus 6 unexposed	Higher prevalence of endometriosis observed in animals with elevated serum levels of PCB-77 and PCB-126. Correlation between endometriosis severity and PCB-77 serum concentrations.	$P < 0.05$ for comparisons between endometriosis and endometriosis-free; $P = 0.02$ for correlation between disease severity and PCB-77.	83

[1] Ref: Reference

TCDD and dioxin-like chemical exposure and endometriosis. As observed with the PCB investigations, however, sample sizes, methodological issues, and the diverse characteristics of endometriosis may account for this weak association. Care should be taken when designing future studies to address these issues, particularly through the development of stricter subject-selection strategies and the incorporation of both environmental and genetic factors (76;77)

4. EXPERIMENTAL EVIDENCE

4.1. Animal Investigations

Unlike the epidemiological evidence, results derived from animal experiments have demonstrated a significant interaction between TCDD or dioxin-like PCB treatment and endometriosis (Tables 5 and 6). Non-human primate and rodent models have revealed an effect of TCDD on both the incidence of endometriosis (42) and the survival and growth of autotransplanted endometrial tissue (78-82).

Rier *et al.* (42) were the first to report a dose-dependent increase in the incidence and severity of spontaneous endometriosis in a colony of rhesus monkeys that was chronically exposed to TCDD. This serendipitous observation - endometriosis was not a prospective endpoint of the study - led to a multitude of investigations into this possible relationship between dioxins with endometriosis (Table 5), including a second look at the original results (Table 6) (83). Interestingly, this subsequent analysis of the monkey serum for TCDD and PCB specific congeners found that the incidence and severity of endometriosis was not correlated with serum TCDD levels, but rather with elevated serum levels of two coplanar or dioxin-like PCBs (PCB-77 and PCB-126). Another study undertaken by Arnold *et al.* (84) retrospectively examined the impact of Aroclor 1254 treatment on endometriosis in the Rhesus monkey (Table 6). While this investigation found no association between exposure to this PCB and the prevalence of endometriosis six years following treatment, the possibility remains that the study was terminated before

the disease was apparent in all of the toxicant-treated animals. Several reports on endometriosis in the Rhesus monkey suggest that the disease commonly develops after a latency period of about 7 years (42;85;86).

In contrast to the above studies, only one investigation has been specifically designed to determine the effect of TCDD exposure on endometriotic implants in a monkey model (81). In this study, TCDD treatment was observed to promote the survival and growth of endometriotic tissue autotransplanted to the pelvic cavity of cynomolgus monkeys. While the findings of this study were significant, this type of endometriosis model is unlikely to be representative of the natural disease. Still, the observations derived from this work further highlighted the enigmatic role of TCDD in endometriosis. A critique of the primate data suggests that while there is evidence supporting the hypothesis that dioxin exposure may enable the short-term survival of endometrial implants, there is no credible evidence that dioxin facilitates or leads to the development of endometriosis (35). However, we propose that recent epidemiological investigations and results of mechanistic studies provide increasing support that dioxin and dioxin-like chemicals may be important in the pathophysiology of this disease.

A number of rodent models have also demonstrated an association between dioxin exposure and endometriosis, reporting dose-dependent increases in the survival rate and growth of autotransplanted uterine tissue (78-80). In further support of these findings, Kitajima *et al.* demonstrated an influence of steroid hormones on the activity of TCDD, suggesting that interactions between this toxicant and estradiol are required for alterations in endometrial cell properties to occur (82). Only one study to date has specifically set out to investigate the impact of dioxin-like and non-dioxin-like PCB treatment on surgically induced endometriosis in a mouse model; however, no significant difference in lesion size was found (80) (Table 6). Finally, Nayyar *et al.* recently observed that TCDD treatment induced a uterine phenotype in mice akin to that seen in humans with endometriosis, reporting
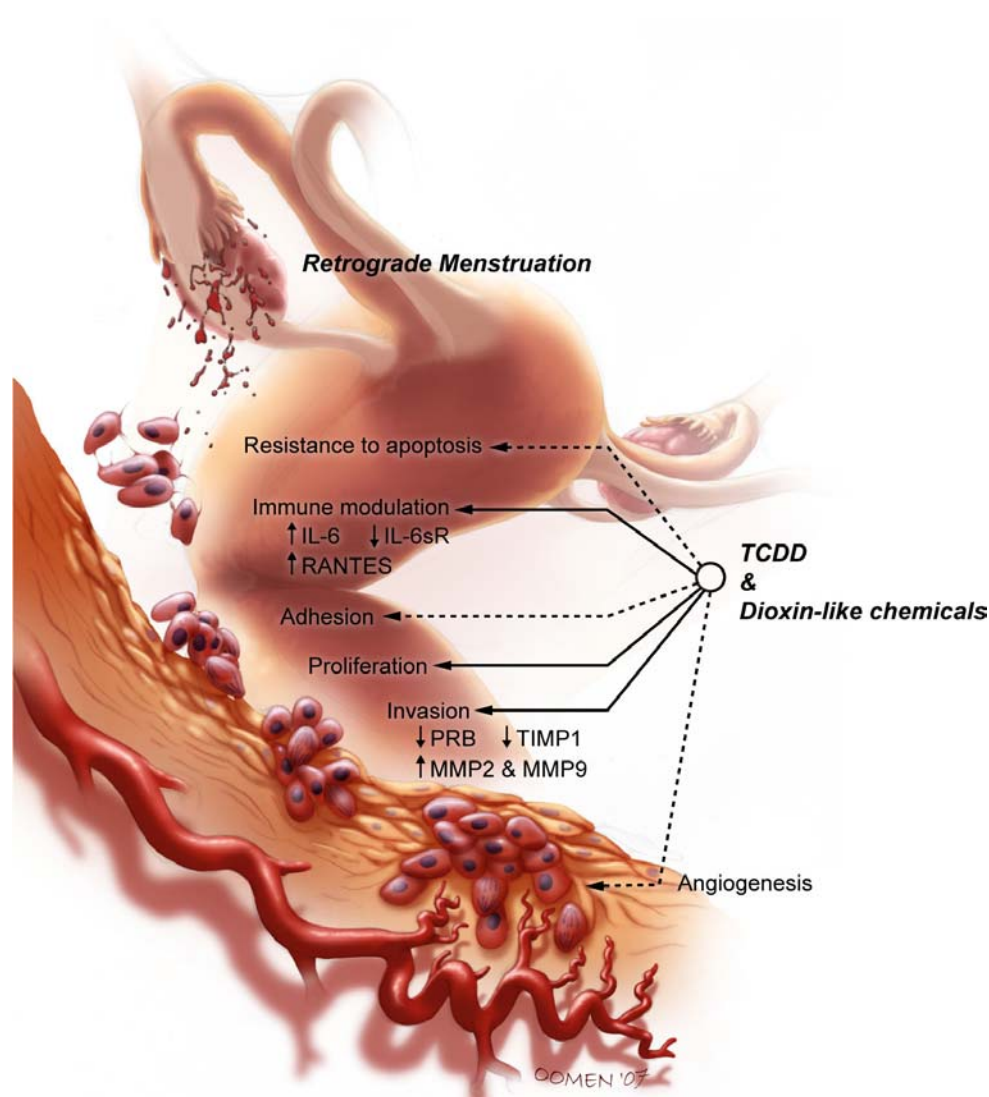
Figure 2. Endometriosis is thought to arise from retrograde menstruation of endometrial cells and fragments into the pelvic cavity where they adhere, attach, invade, proliferate and ultimately establish a blood supply. TCDD and dioxin-like chemicals have been shown to modify multiple steps important in the pathophysiology of endometriosis (solid lines) whereas the effect on other steps are unknown (dashed lines) but probable based on results from studies using non-endometrial tissues.

decreased expression of progesterone receptors and transforming growth factor β2 (87). These findings strongly suggest that TCDD exposure is relevant to the pathophysiology of endometriosis, possibly affecting both its incidence and progression. However, although the rodent model of endometriosis has been used with some success, it is far from ideal due to the following: (a) rodents do not spontaneously develop endometriosis, therefore auto- or xenografted endometrial tissue is required to establish endometriotic implants; (b) the rodent estrous cycle, lacking a true luteal phase, is distinct from the primate menstrual cycle; and (c) in cases where endometrial tissue is xeno- rather than autotransplanted, use of an immune compromised animal is required, further limiting the translation of experimental results to humans. Thus, although experimentally induced endometriosis in rodents does not accurately recapitulate the pathogenesis of

the disease, and may not reflect the pathophysiology of established endometriosis, at the current time it is one of the most convenient means of investigating the *in vivo* impact of toxicant exposure. Overall, despite the obvious limitations of the animal studies, the data suggest that environmental toxicants acting through the AhR are important in the pathophysiology of endometriosis.

4.2. *In Vitro* investigations

While the precise mechanism by which these toxicants impact the pathobiology of endometriosis is unknown, experimental evidence suggests that dioxin and dioxin-like chemicals can change the expression of proteins involved in metastasis, adhesion, invasion, and proliferation, and thus play a role in the development of endometriosis (Figure 2). TCDD signals through the AhR, a cytosolic receptor that belongs to the helix-loop-helix

family of DNA-binding proteins (88) and is expressed in human endometrium (89). Receptor-ligand interaction results in cleavage of hsp90 from the receptor, homodimerization, and binding with the AhR nuclear translocator protein (Arnt). Once in the nucleus, this complex binds with dioxin response elements in dioxin-responsive genes including the cytochrome P450 enzymes 1A1 and 1B1 (90) and those required for inflammation, differentiation and proliferation (91). A number of other environmental toxicants are ligands for the AhR, including the polychlorinated biphenyls, chlorinated pesticides, furans and polynuclear aromatic hydrocarbons (88). AhR ligands have been shown to induce a number of biochemical changes which are important in the pathophysiology of endometriosis, including but not limited to: (1) altered expression of EGF, IL-1β, IL-6, TGF-α, and TGF-β; (2) decreased endometrial sensitivity to progestins, (3) decreased expression of progesterone receptor B; and (4) increased matrix metalloproteinase expression (12;92-98); these have been reviewed previously (99). TCDD-induced changes in immune function have also been documented, including the dysregulation in expression of IL-6 and its soluble receptor (ILs6-R) in the Rhesus monkey (81). In addition, TCDD treatment of endometrial stromal cells has shown to increase the expression of RANTES in culture (100). Hence, dioxin and dioxin-like chemicals are able to decrease endometrial responsiveness to progesterone, enhance the proliferative and invasive characteristics of endometrial implants, and impair immune function, all of which have the potential to facilitate the development of endometriotic implants.

Despite the experimental evidence, the role of TCDD and dioxin-like chemicals in the pathobiology of endometriosis remains controversial. Not all women with exposure to these chemicals develop endometriosis, and a dose-response relationship between exposure and disease severity has not been documented. Finally, while headway is being made, the precise mechanisms of dioxin and dioxin-like chemical action in the development and progression of endometriosis remain to be determined.

5. FUTURE DIRECTIONS

Establishing the role of environmental toxicants in the pathophysiology of endometriosis presents numerous challenges. The epidemiological studies conducted to date underscore the need for statistically robust investigations coupled together with thorough exposure assessments. Furthermore, considerations must be made regarding recognized genetic risk factors, such as mutations in the plasminogen activator inhibitor gene (101), as this finding could have important implications for future investigations. Moreover, it is recognized that endometriosis has many different clinical presentations including but not limited to appearance (white, blue, red, and chocolate cysts) and location (deep infiltrating, ovarian etc.). These may arise from different causes and thus may respond differently to environmental toxicants. Consequently, the role of environmental toxicants in the pathogenesis and biology of these different lesions could be different

and merits study. Similarly, it is unclear as to whether critical windows for exposure exist, and this may be important in deciphering the role of environmental toxicants in the pathophysiology of this disease. Developmental exposure has been shown to have important implications in programming that persists into subsequent generations, and thus early life exposures could be critical in the pathogenesis of endometriosis. Another important limitation in studying the relationship between environmental toxicants and endometriosis is the lack of an appropriate animal model. While there are rodent models of the disease, including immune compromised mice (102-107), rodents do not spontaneously develop endometriosis and thus do not fully recapitulate the pathogenesis and pathophysiology of the disease. Moreover, the absence of a functional immune system in the immune compromised mouse imposes a further obstacle in the translation of experimental results to humans. Thus, due to their limitations, there is a pressing need to develop novel animal models for the study of endometriosis. Improved models that allow for investigation of the early events in the pathogenesis of endometriosis would be of great benefit and would expand our understanding of the role of toxicants in this disease. Alternatively, gene array studies have identified changes in the expression of several genes that could be important in the pathogenesis of endometriosis and may be targets for toxicant-induced modulation (30-32). Finally, while aromatase expression and activity has been induced by pesticide treatment in several different cell lines and endometrial stromal cell cultures (108-111), the implications of these changes to endometriosis remains to be demonstrated. We propose that if dioxin and dioxin-like chemicals are important factors in the pathobiology of endometriosis, exposure to these toxicants on a background of susceptibility would be expected to increase the risk of developing the disease. There are a number of potentially promising avenues to be explored to better define the role of dioxin and dioxin-like chemicals in the pathophysiology of endometriosis.

6. PERSPECTIVE

Risk factors important in the pathogenesis of endometriosis are poorly understood and known risk factors do not adequately account for the prevalence of this disease. While several epidemiological studies have been unable to find an association between exposure to environmental toxicants and endometriosis, these studies are commonly underpowered and suffer from methodological issues that bias the results towards the null. Other investigations have reported a positive association, providing support for the hypothesis that these chemicals play a role in the pathobiology of this disease, yet the evidence is not overwhelming. While rodent models of endometriosis have demonstrated that environmental toxicants promote the survival and growth of autografted uterine tissue, these models remain of limited value. Therefore, we conclude that animal studies provide modest support for the association between environmental toxicant

exposure and endometriosis. Tissue culture investigations provide experimental evidence that further supports a potential role for environmental toxicants in endometriosis. Taking together the epidemiological and experimental data, we conclude that there is only a weak association; however, we acknowledge that it is biologically plausible that environmental toxicants are important in the pathophysiology of endometriosis. Finally, although the relationship between dioxin and dioxin-like chemical exposure and endometriosis is not fully understood, emerging research tools are providing exciting avenues through which the link and underlying mechanisms can be established.

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Abbreviations: AhR: aryl hydrocarbon receptor, Arnt: AhR nuclear translocator protein, CALUX: chemical-activated luciferase gene expression, CI: confidence interval, DEHP: diethyl-(2-hexyl) phthalate, EGF: epidermal growth factor, IL: interleukin, MEHP: mono-(2-ethylhexyl) phthalate, PCB: polychlorobiphenyl, PCDD:

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dibenzo-p-dioxins, PCDF: dibenzofuran, PCDE: diphenylether, PCN: naphthalene, PHAH: polyhalogenated aryl hydrocarbon, TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin, TEF: toxic equivalency factor, TEQ: TCDD toxic equivalency, TGF: transforming growth factor.

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