Chapter

The Link between Environmental Toxicant Exposure and Endometriosis Re-Examined

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Abstract

Endometriosis is widely acknowledged to be an estrogen dependent disease or unknown etiology. Recognition that environmental toxicants can bind with and activate the estrogen receptor, dysregulate steroid metabolism and, in some cases, act as anti-androgenic substances (phthalate esters) has led to proposal that exposure to environmental toxicants are associated with increased risk of endometriosis. Since our last review of the subject in 2008, the literature has expanded with several epidemiological and biomonitoring studies suggesting a potential association, whereas others have been unable to demonstrate a link between exposure and enhanced risk. Therefore, we carried out a systematic review and critical appraisal of the literature published over the past decade (2009–2019). The majority of studies found dealt with exposure to polychlorinated biphenyls (PCBs), dioxins, dioxin-like and non-dioxin-like compounds, bisphenol A and phthalate esters. Several studies suggest a potential association between exposure to environmental toxicants; however, important weaknesses in study design, methodology, and analysis together with many contradictory studies weaken confidence in these associations. Consequently, we conclude that despite a growing literature, evidence for an association between exposure to environmental toxicants and risk of endometriosis remains weak.

Keywords: endometriosis, endocrine disrupters, phthalates, bisphenol A, dioxin, estrogenic

1. Introduction

Endometriosis an estrogen dependent disease characterized by ectopic growth of endometrial glands and stroma outside of the uterine cavity. It is estimated that endometriosis may affect anywhere from 5 to 45% of all women [1]. Although retrograde menstruation has become the most widely accepted theory for the development of endometriosis [2], it cannot account for endometriosis in distant organs such as the lung and brain. Therefore, alternative explanations are sought.

While the cause of endometriosis remains unknown, it most likely arises from a multifaceted origin involving the interaction of environment and genetics [3]. Among the different hypotheses advanced, a growing body of literature suggests that environmental factors including environmental toxicants may play a role in the pathophysiology of endometriosis. Lifestyle and medication use point to

a role for environmental factors in endometriosis. While alcohol consumption and cigarette smoking have been associated with lower endometriosis risk [4], developmental exposure to diethylstilbestrol and early life exposure to soy formula as well as alcohol consumption in adulthood was linked with an increased risk of endometriosis [4, 5]. Support for an environmental toxicant influence on the development of endometriosis surged with the report of endometriosis in rhesus monkeys treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) [6]. Evidence of estrogen mimicry, dysregulation of steroid signaling, and immune modulation by environmental toxicants such as the persistent organic pollutants including the polychlorinated biphenyls (PCBs), dioxins and dioxin like compounds, pesticides, plasticizers (phthalates and bisphenol A), and some metals has led some to hypothesize that human exposure to environmental toxicants may play an important role in reproductive health including endometriosis [3]. Although a recent systematic review and meta-analysis suggests a possible link between exposure to chlorinated organic chemicals and endometriosis [7], we postulate that the role of exposure to environmental toxicants in the pathophysiology of endometriosis remain uncertain.

Potential associations between exposure to environmental toxicants and women with endometriosis have been equivocal with several finding positive associations [8–10] whereas others were unable to document an association [11–13]. Since our last review of the subject [14–17] numerous studies have emerged suggesting a potential link between environmental toxicant exposure and endometriosis [7, 18]. Herein, we describe a systematic review and critical appraisal of the recent literature linking exposure to environmental toxicants and endometriosis using a modified weight-of-evidence approach to evaluate the strength of potential associations.

2. Approach

We conducted a systematic review of the literature between 2008 and present, to capture publications since our last review of the subject [14, 17]. An electronic search was performed using PubMed and web of science between October and November 2019. The following search terms were employed: endometriosis and environmental contaminants, environmental chemicals, environmental toxicants, endocrine disrupters, dioxins, polychlorinated biphenyls (PCBs), phthalates, bisphenol A, and metals. Inclusion criteria included biomonitoring, epidemiology studies reporting chemical concentrations in women with endometriosis compared to a reference population and associated risk. We also included articles describing experimental animal studies and *in vitro* experiments designed to explore the effect of chemical exposure on endometriotic lesion survival, growth, and to elucidate potential mechanisms relevant to human health. Review papers, meeting summaries, commentaries were excluded as were articles written in languages other than English. Article titles were downloaded into an Excel spreadsheet and duplicate titles excluded. Articles meeting inclusion and exclusion criteria were decided by review of article titles and abstracts by both authors. Disagreements were resolved through discussion. Articles meeting inclusion criteria were printed in full and read by both authors.

3. Results

Our electronic search of the literature revealed 67 articles from which four articles with duplicate titles were excluded (**Figure 1**). We further excluded six

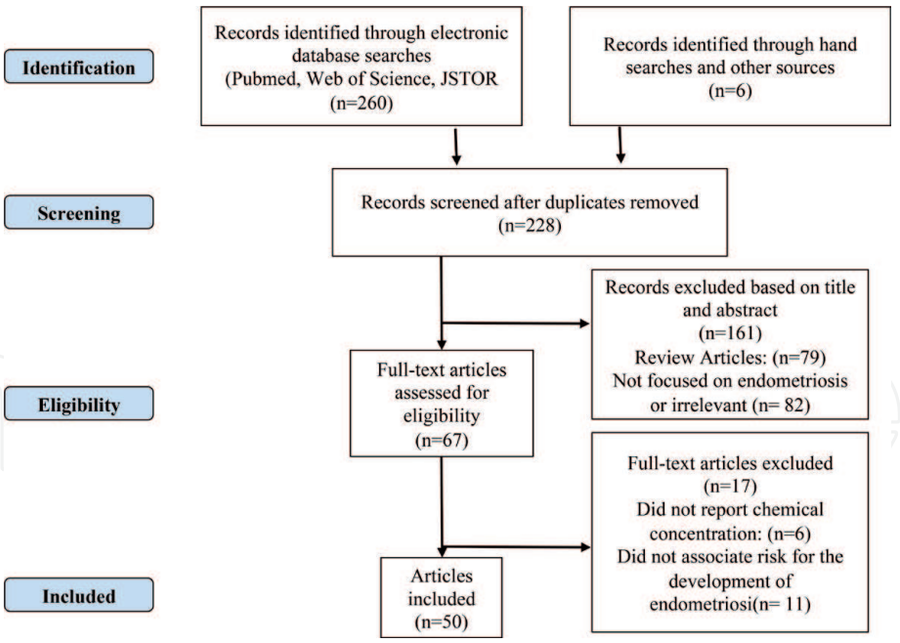

Figure 1.
Flow diagram summarizing the process of candidate article title identification in our electronic literature searches (PubMed and Web of Science) conducted between January 2018 and February 2019, screening, and article selection vs. exclusion. The number of articles included vs. excluded and reasons for exclusion are indicated.

review articles. An additional seven articles were excluded because they either did not report chemical concentrations or associated risk for the development of endometriosis. Consequently, 50 articles were retained for full assessment. The largest group of articles addressed the association between exposure to chlorinated organic compounds including polychlorinated biphenyls (PCBs), dioxins, and dioxin-like compounds with relatively few studies exploring the link between pesticide exposure and endometriosis. Of the chemicals with comparatively short half-lives relative to the chlorinated organic compounds and potential to disrupt endocrine signaling pathways, several reports linking phthalate esters and bisphenol A with endometriosis were found in our search whereas relatively few studies involving perfluoroalkyl compounds and metals studies were found.

3.1 Polychlorinated biphenyls (PCBs), dioxin and dioxin-like compounds

PCBs are one of the most widely produced chemicals worldwide, with millions of pounds being produced globally over the last decade alone [19] for use a coolants in electrical transformers. With 209 possible congeners, PCB toxicity is dependent on chemical structure. For example, non-ortho or mono-ortho PCBs are far more toxic due to the loss of chlorine atoms on the 2,2′,6,6′ of the benzene rings [20]. Due to their diverse structures, PCBs share similar characteristics to estrogen, allowing them to have both agonistic and anti-estrogenic activity [21, 22]. PCBs have been known to disrupt several organs and tissue types throughout the human body; with particular damage to the liver, kidney, and the endocrine system [19]. Our search revealed several studies primarily focused on PCB exposure and endometriosis and additional studies that explored the link between dioxin and dioxin-like compounds and endometriosis (**Table 1**). Since these compounds are frequently found together in human tissues, we will discuss them together.

In a pilot case–control study [24], involving 17 women (10 cases; 7 controls), superficial endometriosis was present in 90% of the cases. Of the 29 congeners measured in this study, both polychlorinated dibenzofurans (PCDFs) and

Authors	Cases vs. controls	Exposure investigated	Tissue	Outcome
Porpora et al. [23]	80:78	PCBs 28, 52, 101, 105, 118, 138, 153, 156, 167, 170, and 180	Serum	Increased risk of endometriosis for DL-PCB-118 (OR = 3.79; 95% CI, 1.61–8.91), NDL-PCB-138 (OR = 3.78; 95% CI, 1.60–8.94), NDL-PCB-153 (OR = 4.88; 95% CI, 2.01–11.0), NDL-PCB-170 (OR = 3.52; 95% CI, 1.41–8.79), and the sum of DL-PCBs and NDL-PCBs (OR = 5.63; 95% CI, 2.25–14.10) were all significant in case versus controls.
Cai et al. [24]	10:7	PCBs 77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167, and 189	Serum	PCB concentrations were higher in peritoneal fluid than serum. However, the total TEQ LOD and dioxin-like PCBs were not significantly different between women with endometriosis and the controls.
Trabert et al. [25]	251:538	PCBs 18, 28, 44, 49, 52, 66, 74, 87, 99, 101, 118, 128, 138, 146, 149, 151, 153, 156, 157, 167, 170, 172, 177, 178, 180, 183, 187, 189, 194, 195, 196, 201, 206, and 209	Serum	Several PCB congeners were associated with significantly lower risk (PCB 170 3rd quartile vs. lowest: OR = 0.5; 95% CI, 0.3–0.9) PCN196 (3rd quartile vs. lowest: OR = 0.4; 95% CI, 0.2–0.7), PCB201 (2nd quartile vs. lowest: OR = 0.5; 95% CI, 0.3–0.8; and 3rd quartile vs. lowest: OR = 0.4; 95% CI, 0.2–0.7) but not summed values (PCBs 170, 196, 201; OR = 1.3, CI 0.8–2.2) and estrogenic PCBs (OR = 1.1; 95% CI, 0.8–1.4).
Ploteau et al. [26]	68:45	PCBs 77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167, 189, 28, 47, 99, 100, 153, 154, 183, 209	Serum, omental, and peritoneal adipose	Significant correlations for PCB concentrations within the three biological compartments omental versus peritoneal adipose tissue were found ($p < 0.0001$). 137.1 vs. 147.9 ng/g l.w. for sum of 6 NDL-PCB. Adipose vs. serum: WHO-TEQ2005 DL-PCB = 3.6 pg/g l.w., sum of 6 NDL-PCB = 81.1 ng/g l.w.
Buck-Louis et al. [27]	190:283 and 14:113	PCBs: 18, 28, 44, 49, 52, 66, 74, 87, 99, 101, 114, 118, 128, 138, 146, 149, 151, 153, 156, 157, 167, 170, 172, 177, 178, 180, 183, 187, 189, 194, 195, 196, 201, 206, and 209.	Serum and omental fat	Higher concentrations in omental fat than serum. PCB-74, and PCB-156 in fat were inversely associated with the odds of an endometriosis.

Authors	Cases vs. controls	Exposure investigated	Tissue	Outcome
Martínez-Zamora et al. [28]	30:30	2,3,7,8-TCDD, 1,2,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF,1,2,3,6,7,8-HxCDF,2,3,4,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, OCDF	Adipose tissue from the omentum	Dioxins and DL-PCBs were significantly higher in patients with deep infiltrating endometriosis; TCDD, PeCDD, PeCDF were the most significant $p < 0.01$ for each compound. PCB-126 (PCB-114 $p < 0.05$; PCB-156 $p < 0.05$; PCB-189 $p = 0.04$; PCB-126 $p < 0.01$).
Simsa et al. [29]	96:106	DLCs not specified	Plasma	DLC concentrations were marginally higher in patients with endometriosis (22.3±9.3 pg vs. 20.5±10.8 pg) and higher plasma levels of DLC were linked to a higher risk of endometriosis (aOR = 2.44; 95% CI 1.04–5.70; $p = 0.04$) adjusted for age. Moderate–severe endometriosis cases only (OR = 3.01; 95% CI 1.06–9.04; $p = 0.03$)
Vichi et al. [30]	181:162	PCBs 118, 153, 138, 170, 180, and total PCBs	Serum	With the presence of the GSTP1 wild type genotype, medium-high levels of PCB 153, high levels of PCB 180 and total PCBS were significantly associated with endometriosis risk (OR = 6.00; 95% CI, 1.88–19.18 and OR = 9.08; 95% CI, 2.14–44.4, respectively).

Table 1.
Summary of exposures and outcomes from biomonitoring studies designed to quantify the concentration of polychlorinated biphenyl congeners, dioxins, dioxin-like compounds (DLCs) and non-dioxin-like compounds (NDL) in women with endometriosis compared to healthy controls.

dioxin-like (DL) -PCBs showed no significant difference between the case and control [24]. However, both were elevated in peritoneal fluid relative to the serum, with the reverse seen in polychlorinated dibenzo-*p*-dioxins (PCDDs) [24]. Both PCDDs and PCDFs in peritoneal fluid were significantly associated with an increased risk of endometriosis [24]. Although a potential association was found, the small sample size, the authors did not adjust for other factors such as age that have previously been shown to affect endometriosis risk. Hence, confidence in the findings from this pilot study is low. In contrast, results of a case–control study [23] of 158 Italian women (80 cases; 78 controls), revealed that both non-dioxin-like (NDL)-PCBs and DL-PCBs levels were significantly elevated in women with laparoscopically and histologically confirmed endometriosis. An increased risk of endometriosis was found for DL-PCBs (PCB-118 [odds ratio (OR) = 3.79; 95% confidence interval (CI), 1.61–8.91], and NDL-PCBs including PCB-138 (OR = 3.78; 95% CI, 1.60–8.94), PCB-153 (OR = 4.88; 95% CI, 2.01–11.0), PCB-170 (OR = 3.52; 95% CI, 1.41–8.79), and the sum of DL-PCBs and NDL-PCBs (OR = 5.63; 95% CI, 2.25–14.10)). No

significant associations were observed with respect to hexachlorobenzene (HCB) or to the sum of polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and DL-PCBs expressed as total toxic equivalent quotients (TEQs). PCB-101, PCB-156, and PCB-170 were all shown to be statistically elevated, with PCB-52, PCB-118, PCB-138, PCB-153, and PCB-180 showing a highly significant difference. All four stages and endometriosis implant localizations (peritoneal, deep, or ovarian) were analyzed, with no significant differences detected. However, the lack of adjustment for potential confounding and failure to account for multiple comparisons are important limitations of this study. IN another study [29], DLC concentrations were quantified in plasma samples using the dioxin-responsive chemical-activated luciferase expression bioassay (CALUX). Blood samples were collected prior to laparoscopic surgery from women with endometriosis (n = 96) and control patients with a normal pelvis (n = 106). A marginal increase in DLC compound concentrations in endometriosis patients relative to controls (22.3 ± 9.3 pg, versus 20.5 ± 10.8 pg CALUX-TEQ/g lipid) was reported [29]. After adjusting plasma concentrations for age only, an increased risk for endometriosis was demonstrated for high concentrations of DLC (OR = 2.44; 95% CI 1.04–5.70, p = 0.04) and when considering moderate to severe endometriosis (OR = 3.01; 95% CI 1.06–9.04, p = 0.03). While the authors adjusted for age, adjustment for BMI, parity, and breast feeding was not undertaken. Thus, these results although suggestive must be interpreted with caution.

While several studies have provided evidence of a potential link significant associations between women with endometriosis and PCB levels could not be demonstrated by other investigators [25, 28, 31]. No significant association between PCBs and endometriosis risk was found in a study of 789 patients (251 cases; 538 controls); with 20 PCB congers measured in serum from surgically confirmed cases [25]. While the odds ratios (ORs) for several PCB congeners did show significant levels above and below the null; however, there was no specific pattern associated with endometriosis risk. Several PCBs were quantified in the serum of 473 women in an operative cohort (190 cases; 283 controls) and 127 patients from a general population cohort (14 cases; 113 controls), using omental fat in the operative cohort and serum in both [31]. Results were adjusted for confounding variables such as age, BMI, breast-feeding, cotinine, and lipids. Among the 35 PCB congers analyzed, geometric mean serum PCB levels were found to be inversely related in terms of risk in the operative cohort, with the opposite seen in the population cohort [31]. A similar relationship can be seen in omental fat, with sum PCB levels showing significantly higher levels in the non-endometriosis patients relative to the controls. Limitations of this study include the small number of women with endometriosis in the case population (only 11% of women had endometriosis), possible bias through the use of telephone directories, and use of controls without surgical confirmation of absence of disease suggest that results be interpreted with caution. The relationship between exposure to DLCs and deep infiltrating endometriosis (DIE) was explored in a case–control study of 30 cases and 30 controls [28]. Disease status was determined by clinical examination, magnetic resonance imaging (MRI), and transvaginal ultrasonography (TVUS), whereas the control population underwent laparoscopic surgery for adnexal benign gynecological disease. DLCs were analyzed omentum adipose tissue in both groups. The results suggest a significant increase of both dioxins and PCBs relative to the control, with the most toxic forms showing a significant difference (2,3,7,8-TCDD and 1,2,3,7,8-pentachlorodibenzo-p-dioxin [1,2,3,7,8-PeCDD]; $p < 0.01$) [28]. Furthermore, 2,3,4,7,8-PeCDF was also significantly higher and four of the most toxic PCB congeners (PCB 144, 156, 189, 126) had toxic equivalence values (TEQ) that were statistically higher in DIE patients [28]. However, no differences were seen when the data were adjusted for age, breast

feeding, and BMI. Limitations of this study include small sample size, and homogeneity of the sample population [28].

A biomonitoring study conducted in France [26], measured the concentrations of PCBs in serum, peritoneal and omental adipose tissue of 113 adult French women with deep infiltrating endometriosis (DIE) (45 controls, 68 cases). There was a significant difference between omental versus peritoneal adipose tissue PCB concentrations ($p < 0.0001$). Similar trends were seen is peritoneal adipose tissue versus serum levels, with PCBs showing the highest level of significance in terms of concentration differences.

Potential gene–environment interaction among women with endometriosis was explored [30]. Specifically, the relationship between glutathione transferase (GST) gene polymorphisms PCB concentrations in a study of 343 Italian women (181 cases; 162 controls). Ability glutathione enzymes to regulate oxidative free radicals and thus oxidative stress and therefore genetic polymorphisms may influence tissue capacity to manage the damaging effects oxidative stress, in turn influencing disease susceptibility. No significant difference in genotype distribution (GSTM1, GSTA1, and GSTP1) between case and control patients could be elicited [30]. However, the GSTP1 wild-type with medium-high blood levels of PCB153, high levels of PCB180, or total PCB levels, showed a significant increase in potential risk, while GSTT1 null was negatively associated with the disease [30]. The potential association between five microsatellites and 28 single nucleotide polymorphisms among 10 dioxin detoxification genes (aryl hydrocarbon receptor (AhR), AHRR, ARNT, CYP1A1, CYP2E1, EPHX1, GSTM1, GSTP1, GSTT1, NAT2) was examined in 242 women (100 case; 143 control) from Japan [32]. Accounting for disease stages I-IV, BMI, and smoking, no significant association was seen between the polymorphisms and the contribution to the etiology of endometriosis. Taken together, these data suggest that genetic polymorphisms in detoxification enzymes do not modulate endometriosis risk.

Establishing a link between exposure to environmental toxicants and endometriosis using epidemiology and biomonitoring is difficult owing to challenges in diagnosis of endometriosis [33], lengthy diagnostic delays [34], and high prevalence of endometriosis in asymptomatic women [1] and thus the potential for misclassification error is high. Therefore, animal studies have been employed to better understand the potential hazard posed between toxicant exposure and endometriosis. Developmental exposure of mice to TCDD induced a progesterone-resistant phenotype in adult animals that persisted across generations [35]. Results of this study suggest that TCDD induced activation of the aryl hydrocarbon signaling pathway induces dysregulation of expression of tissue remodeling enzymes, and contributes to the inflammatory responses, cell migration, and proliferation seen in endometriosis patients. These data are supported by prior animal studies demonstrating PCB and dioxin effects in animal models of endometriosis [36–38].

Tissue culture studies have been employed to elucidate potentially important toxicant regulated mechanisms. PCBs have been linked to an increased estradiol synthesis and creating an inflammatory milieu through the production of interleukin (IL)-6 and IL-8 [39]. Primary cultures of endometrial stromal cells (ESCs) were treated with both DL-PCBs and NDL-PCBs. Dioxin-like CB126 treatment increased 17β-estradiol (E_2) biosynthesis in a dose dependent manner. CB126 exposure also increased 17β-hydroxysteroid dehydrogenase 7 (HSD17B7) as well as decreased methylation of the HSD17B7 promoter leading to an increase in expression. Inflammatory markers were also elevated in cultured endometrial stromal cells. Increased inflammation and E_2 synthesis were demonstrated in a mouse model of endometriosis [39]. Although PCB has shown to increase E_2 biosynthesis, combining 17β-Estradiol with TCDD showed a synergistic effect and induces M2

activation with macrophages co-cultured with ESCs. STAT3 and P38 phosphorylation in macrophages were also increased differentiation of M2 macrophages, leading to an inflammatory milieu [40]. Several studies also analyzed the impact of TCDD exposure on progesterone-dependent mechanisms. TCDD was found to induce cannabinoid receptor type 1 CB1-R mRNA expression in endometrial stromal cells and steroid-induced expression of the gene was inhibited. Through the use of tissue obtained from women with and without endometriosis, TCDD treatment-induced dysregulation of cannabinoid signaling, immune cell migration into the endometrium during embryo uterine attachment [41] and thus we propose could be an important mechanism in the pathophysiology of endometriosis. PCB was also seen to activate endogenous aryl hydrocarbon receptor (AhR) signaling pathway in immortalized human telomerase reverse transcriptase (hTERT) endometrial epithelial cell (hTERT-EEC), specific to time, concentration, and congener. The changes induced were modulated by changes in estrogen levels, in turn increasing cell migration by hTERT-EEC. Proteomic analysis also identified cell stress responses and metabolism markers (such as heat shock proteins (HSP) 27 and HSP 70) [42]. These proteins are both critical markers for the regulation of apoptosis and cellular stress response pathways. In another study [43] primary cultures of ESCs from both case and control patients showed that PCB-104 exposure affects cell migration, invasion and resultant gene expression. Treatments induced a significant increase in cell migration and invasion of ESCs. Enzyme-linked immunosorbent assays showed a time and dose dependent increase in matrix metalloproteinase 3 (MMP-3) and MMP-10 protein in ESCs, whereas MMP-2, MMP-9, TIMP-2, E-cadherin, Snail and Slug did not. MMP-3 contributes to the breakdown of the extracellular matrix and promotes tissue remodeling and migration [43]. The results from this study suggest that PCB-104 increased migration and invasion of ESCs through increasing MMP-3 and MMP-10 [43]. Taken together, results from tissue culture studies elucidate PCB and dioxin induced dysregulation of mechanisms potentially important in the pathophysiology of endometriosis.

In summary, several studies demonstrated a potential association between exposure to PCBs, dioxins, and dioxin-like compounds and increased risk of endometriosis; however, important study limitations decrease confidence in these study findings. Moreover, several studies were unable to evoke evidence of an association between exposure to these toxicants. While, animal studies are few, results from these studies provide evidence of biological plausibility. Results of tissue culture studies also provide evidence that PCBs and dioxins adversely affect mechanistic pathways important in the pathophysiology of endometriosis although the effective concentrations exceed human exposure. Consequently, we suggest that there is weak evidence linking exposure to PCBs and dioxin and DL-PCBs in the pathophysiology of endometriosis.

3.2 Pesticides

Chlorinated organic pesticides (COPs) resist degradation in the environment, are lipophilic and thus bioaccumulate in adipose tissues, and concentrations are biomagnified with increasing trophic level. Moreover, COPs are able to travel long distances and remain stable for several decades in the environment, and thus widespread human exposure to these chemicals has frequently been documented. Despite widespread human exposure, the relationship between pesticides and endometriosis risk in general are equivocal.

The concentrations of six COP levels were measured with gas chromatography and electron-capture, in blood samples of laparoscopically confirmed cases

of endometriosis [44]. Results showed that aromatic fungicides had a five-fold increase in risk (aOR = 5.3; 95% CI, 1.2–23.6) when comparing the highest and lowest tertile after adjusting for smoking and serum lipids [44]. Chlordane (t-nonachlor) (aOR = 4.6; 95% CI, 0.5–41.6) and HCB (aOR = 6.4; 95% CI, 1.0–42.8) showed a similar trend [44]. Aldrin, β-hexachlorocyclohexane (β-BHC) and mirex also had increased ORs; however, few women had concentrations above the limit of detection preventing further analysis. Two other studies yielded similar results. Specifically, hexachlorocyclohexane (HCH) was associated with an increased risk of endometriosis in a large study with 248 surgically confirmed endometriosis cases and 538 controls [45]. β-HCH concentrations were significantly elevated in the serum (third vs. lowest quartile: OR = 1.7; 95% CI: 1.0–2.8; highest vs. lowest quartile OR = 1.3; 95% CI: 0.8–2.4), as well as for mirex (highest vs. lowest category: OR = 1.5; 95% CI: 1.0–2.2). The results were adjusted for participant age, reference date year, serum lipids, education, race/ethnicity, smoking, and alcohol intake. Although trends were seen throughout multiple forms of endometriosis, the strongest association was seen in women with ovarian endometriosis. Similarly, γ-hexachlorocyclohexane (γ-HCH) had a significant association with endometriosis risk (adjusted OR (AOR) for age, body mass index, breast-feeding conditional on parity, cotinine, and lipids = 1.27; 95% CI: 1.01–1.59) [31]. Although these studies provide evidence for a link between exposure to different pesticides and increased risk of endometriosis, there are several limitations to note. In particular, while the authors adjusted their data for some potential confounding variables none appeared to adjust for BMI. Moreover, since multiple pesticides were quantified in each study, correction for multiple comparisons would add confidence to the findings and exclude the potential for type I error. Furthermore, the lack of a dose–response relationship [45] suggests that chance discovery cannot be excluded.

We found no recent animal studies and only one *in vitro* study was found. HCB treatment enhanced MMP-2 and MMP-9 activities in human endometrial stromal cell line T-HESC, primary cultures of Human Uterine Fibroblast (HUF), and ESCs [46]. Specifically, MMP-2 was only elevated in ESCs, whereas MMP-9 was elevated in all models. An increase in COX-2 and prostaglandin receptor-4 expression, prostaglandin E_2 secretion and the c-Src kinase activation in T-HESC was also seen after HCB exposure. The results suggest that HCB may promote inflammation and invasion parameters through regulation of the AhR pathway.

In summary, the epidemiological and biomonitoring studies suggest a potential association between exposure to chlorinated organic pesticides and increased risk of endometriosis; however, study limitations cannot exclude chance discovery owing to multiple comparisons, failure to adequately adjust for important confounders and lack of a dose–response relationship all weaken confidence in the link between COP exposure and endometriosis risk. A single tissue culture animal experiment conducted within the search window suggests that it is biologically plausible for COPs to promote endometriosis risk. Consequently, we suggest weak evidence linking exposure to COPs and endometriosis.

3.3 Perfluoroalkyl and polyfluoroalkyl substances

Perfluoroalkyl substances are a rather unique group of compounds due to their seemingly harmless properties. However, over the last decade, perfluoroalkyl and polyfluoroalkyl substances have been detected in blood and urine across the globe [47, 48]. Compromised of carbon-fluorine atoms, this extremely strong bond forms stable compounds that are used in clothing, cookware, carpets, and other common household items. Exposure to these compounds has been linked to adverse effects on metabolism, immune function, and fertility [49–51].

In a case–control study [27], nine perfluorochemicals (PFCs) were measured in the blood of study participants by liquid chromatography–tandem mass spectrometry. Surgical visualization was used to confirm endometriosis in the operative population and MRI was used to confirm the absence of endometriosis in the control population. Both perfluorooctanoic acid (PFOA; OR = 1.89 [95% CI = 1.17–3.06]) and perfluorononanoic acid (PFNA) (2.20 [1.02–4.75]) were seen to be associated with endometriosis risk, where results were only moderately changed when adjusted for fecundity [27]. Patients with more severe stages of endometriosis (Stages III and IV) showed a higher concentration of perfluorooctane sulfonic acid (1.86 [1.05–3.30]) and PFOA (2.58 [1.18–5.64]) in their blood compared to controls [27]. Although this study shows a significant association between PFC exposure with an apparent dose response, there are a number of limitations to consider. First assignments of healthy study participants to the control population using MRI alone to exclude asymptomatic endometriosis cannot exclude women with endometriosis. Undiagnosed endometriosis was found in 45.3% of asymptomatic women undergoing laparoscopies for benign conditions [1] and thus the potential for misclassification error in this study weakens confidence in the purported association. Finally, circulating concentration of PFCs from the NHANES (2003–2006) study was compared in 753 women with self-reported diagnosis compared to healthy women without a diagnosis of endometriosis [52]. Results from this study showed that PFNA, PFOA, and perfluorooctane sulfonate (PFOS) were significantly higher among women with endometriosis compared to the control population. Women in the referent population of this study were significantly younger, non-Hispanic white, had more than one menstrual period in the last year and reported to be pregnant at the time of the exam. Furthermore, use of self-reported diagnosis of endometriosis may introduce group assignment bias and thus, these data must be interpreted with caution.

The data linking exposure to Perfluoroalkyl substances and endometriosis are limited to the results of two biomonitoring studies. Although the results suggest that women with endometriosis have exposure to Perfluoroalkyl substances, any potential association with endometriosis is weak owing to limitations of these studies and absence of experimental animal studies or mechanistic experiments.

3.4 Bisphenol A (BPA)

A monomeric compound, bisphenol A (BPA) is used to polymerize plastics and can be found in common household items such as toilet paper, water bottles, the lining of tin cans, cash register receipts, dental sealants, and building supplies [53]. With over a million tons of BPA being used in the United States alone, BPA has become ubiquitous in the environment leading to widespread human exposure. BPA is able to bind to both estrogen receptors (Esr1 and Esr2), activate the estrogen signaling cascade and thus is considered a xenoestrogen [54]. Estrogenic capacity has led some to postulate that BPA exposure may play a role in the pathophysiology of endometriosis (**Table 2**).

A population-based case–control study [56], analyzed the urine from 143 women with confirmed or suspected endometriosis (cases) and 287 healthy controls. Urinary creatinine concentrations, age, reference year, as well as both ovarian and non-ovarian pelvic endometriosis were taken into account. Overall, the urinary BPA concentrations in cases did not differ from the control group. However, unconditional logistic regression analysis revealed that the second versus lowest quartile and third versus lowest quartile had increased adjusted odds ratio (aOR 3.0; 95% CI: 1.2–7.3 and aOR 3.0; 95% CI: 1.1–7.6) for higher BPA concentrations in women with non-ovarian pelvic endometriosis; however, there was no association

Authors	Cases vs. controls	Exposure Investigated	Tissue	Outcome
Simonelli et al. [55]	68:60	BPA	Urine and peritoneal fluid	Urinary BPA levels were found in all analyzed samples; with a statistically significant difference between patients and controls. Urinary BPA concentrations were significantly greater (p = 0.001) in women with endometriosis compared to the control group.
Upson et al. [56]	143:287	BPA	Urine	No statistically significant association between total urinary BPA concentrations and endometriosis overall. However, significant results were seen in urine in relation to non-ovarian pelvic endometriosis (2nd quartile vs. lowest quartile: OR = 3.0; 95% CI: 1.2–7.3 and 3rd vs. lowest quartile: OR = 3.0; 95% CI: 1.1–7.6), but not ovarian endometriosis.
Cobellis et al. [57]	58:11	BPA and BPB	Serum	BPA was found in 51.7% and BPB was found in sera 27.6% but either could not be detected in all the control cases. Suggests an association between at least one of the compound endometriosis risk.
Itoh et al. [58]	166 infertile women	BPA	Urine	No significant (p = 0.24) association of endometriosis with urinary BPA concentration.

Table 2.
Summary of exposures and outcomes in epidemiological studies designed to investigate the association between Bisphenol A (BPA) exposure and endometriosis.

between urine BPA concentrations and ovarian endometriosis. Moreover, there was no relationship between the highest urine concentrations of BPA and endometriosis overall as well as for non-ovarian pelvic endometriosis and ovarian endometriosis. Furthermore, the lack of a dose–response relationship with increasing urine concentrations of BPA weakens confidence in the potential link between BPA exposure and endometriosis risk.

Results of biomonitoring studies revealed that mean BPA concentrations in the plasma of infertile women with endometriosis (n = 11), polycystic ovarian syndrome (PCOS, n = 31) and PCOS plus endometriosis (n = 3) combined (4.66 ± 3.52, 95% CI; 3.60–5.72 ng/ml) were significantly greater than in a control population (n = 34) of healthy fertile women (2.64 ± 3.99, 95% CI; 1.24–4.03 ng/ml) [59]. In women who reported a diagnosis of endometriosis, the mean ± (SD) concentration of BPA was 4.59 ± 1.22 ng/ml (range < LOQ – 5.31 ng/ml). Moreover, BPA concentrations were quantifiable in only 3% of study participants and comparisons with the fertile controls was not reported. Given the ubiquitous nature of BPA, the low detection frequency in this study is rather surprising and thus we interpret these findings with caution. The small sample size, self-reported diagnosis of endometriosis and associated potential for misclassification error are important limitations of this study. Results of a much larger cross-sectional study of 166 Japanese women [58], showed no significant difference in BPA levels in the urine. BPA concentrations were non-significantly (p = 0.24) greater in women with endometriosis stage 0–I (median = 0.74 μg/g after adjusting to creatinine levels), whereas women with stages II-IV endometriosis had a median concentration of 0.93 μg/g creatinine [58]. BPA levels measured in the sera from healthy fertile (n = 11) and endometriotic women (n = 58) found that both BPA and bisphenol B (BPB) levels were detectable

in 51.7% and 27.6% of cases, respectively whereas the control patients showed a complete absence of both compounds [57]. Recently, urinary concentrations of BPA were significantly greater in women (n = 68) with endometriosis (1.17–12.68 pg/μl) compared to a control population (n = 60) (1.28–2.35 pg/μl) [55]. Finally, BPA has a short half-life and the measures in women with a diagnosis of endometriosis are temporally disconnected from the onset of disease which may have originated years earlier in time. The interval between onset of symptoms and diagnosis ranges from 6 to 12 years [34] and thus exposure measurements made after diagnosis are difficult to link with the development of endometriosis. Therefore, reverse causation cannot be excluded as a potential explanation for differences in circulating concentrations of the toxicants measured.

In an animal study [60], BPA and bisphenol AF (BPAF) affected endometriosis lesion development in ovariectomized and hormonally intact mice specific to dose and hormonal status of the host mouse. Minced uterine tissue was injected into the peritoneal cavity of host mice. In this study, BPA treatment disrupted ovarian steroidogenic pathways resulting in lower progesterone levels and higher atretic oocyte numbers [60]. BPAF and BPA had higher epithelial proliferation scores, although this was only significant in the highest dose of 900 ppm. Both compounds mimicked estrogen, with BPAF having a stronger effect than estrogen [60]. Taken together, these data suggest that BPA and related compounds can affect mechanisms important in the pathophysiology of endometriosis. However, the concentrations of BPA needed to achieve these effects are higher than human exposure and thus are unlikely to be relevant at the concentrations of BPA measured in the general human population in contemporary studies.

Results of a tissue culture experiments demonstrated that BPA treatment arrested human ESCs at the G2/M phase of the cell cycle, allowing for cell migration. Progesterone amplifying receptors such as insulin growth factor binding protein 1 and prolactin were also increased in response to BPA treatment [61]. These results suggest that BPA exposure could modulate endometrial stromal cells function; however, the effective concentrations exceed human exposure. Consequently, ambiguous study results from biomonitoring studies and lack of animal studies suggests a lack of association between BPA exposure and risk of endometriosis.

3.5 Phthalate esters

Phthalate esters are used as a softener in polyvinyl chloride plastics to make plastics flexible and can be found in products such as cosmetics, building materials, and in medical equipment such as intravenous bags, tubing and rubber stoppers in syringes and blood collection tubes. Phthalates leach from finished products leading to ubiquitous human exposure [62, 63]. Exposure to phthalate esters has been linked with decreased circulating testosterone [64] and animal experiments have shown that phthalates are competitive antagonists of the androgen receptor that displace testosterone from the receptor increasing its availability for conversion to estrogens via aromatase [65]. Therefore, it is postulated that exposure to phthalates could be associated with increased risk of endometriosis (**Table 3**).

A large case–control study [67], examined 626 women (495 cases; 131 controls) from 14 clinical centers. Study participants in both groups had a laparoscopy or a pelvic MRI to diagnose the presence of endometriosis. Among the 14 phthalate metabolites, mono-n-butyl phthalate, mono-[(2-carboxymethyl) hexyl] phthalate, mono (2-ethyl-5-carboxyphentyl) phthalate, mono (2-ethylhexyl) phthalate, mono (2-ethyl-5-hydroxyhexyl) phthalate, and mono (2-ethyl-5-oxohexyl), all showed two-fold significant increase in the odds of diagnosis. Results were adjusted for age, BMI, and creatinine. Depending on the method of diagnosis, monooctyl phthalate

Authors	Cases vs. controls	Exposure investigated	Tissue	Outcome
Pednekar et al. [59]	34:45	BPA, MMP, MBzP, MEHP, MEHHP, MiBP-d4 and BPA-d6	Plasma	Significantly higher plasma concentrations of MBzP (95% CI; 11.69–28.12 versus 3.34–8.10), BPA (95% CI; 3.60–5.72 versus 1.24–4.03), and MEHHP (95% CI; 5.10–8.43 versus 0.58–2.85).
Nazir et al. [66]	50: 50	DEHP	Serum	The mean (± SD) DEHP concentration in cases was 65.29 ± 21.69 ng/ml and undetectable in controls. An increasing trend was seen across stages (I-IV).
Buck Louis et al. [67]	495:131	DEHP, mECPP, mCMHP, mEOHP, mEHHP, mEHP, mCPP, mMP, miBP, mBP, mCHP, mBzP, mNP, and mOP,	Urine	MBP, mCMHP, mECPP, mEHP, mEHHP, mEOHP, all showed a two-fold significant increase in the odds of diagnosis.
Huang et al. [68]	28: 29	MBP	Urine	Increase in urinary mono-n-butyl phthalate (94.1 versus 58.0 microg/g creatinine, $p < 0.05$) in women with endometriosis compared to controls.
Itoh et al. (2009)	57:80	MEP, MBP, MBzP, MEHP, mEOHP, and MEHHP	Urine	No significant ($p = 0.23$–0.90) association between endometriosis and any urinary creatinine-adjusted phthalate measured.
Weuve et al. [69]	n = 1227	MEHP, BMP, MEP, and MBzP	Urine	Positive associations for MBP (OR = 1.36; 95% CI, 0.77–2.41) for the highest versus lowest three quartiles, and inverse associations for MEHP in relation to endometriosis (OR = 0.44; 95% CI, 0.19–1.02)
Huang et al. [70]	44:69	MMP, MEP, MnBP, MBzP, MEHP, 5oxo-MEHP, 5OH-MEHP	Urine	Marginally increased level of urinary MEHP only.
Upson et al. [45, 71]	92:195	MEHP, MEHHP, MEOHP, MECPP, MBzP, MEP, MiBP, MnBP	Urine	Greater urinary concentrations of MBzP and MEP in the urine of women with endometriosis compared to controls. Strong inverse association between urinary MEHP and endometriosis risk (aOR 0.3, 95% CI: 0.1–0.7).

Table 3.
Summary of exposures and outcomes in epidemiological studies designed to investigate the association between phthalate exposure and endometriosis.

was restricted to surgical diagnosis of endometriosis with histological confirmation, whereas mono (2-ethylhexyl) phthalate was restricted to surgical diagnosis alone. However potential limitations may arise through adding concentrations as mECPP, mEHHP, mEOHP where all are metabolites of DEHP that were elevated in the operative cohort. Yet when summing DEHP metabolites (mECPP, mCMHP, mEHHP, mEOHP, and mEHP), there is a higher odds of endometriosis in the control population cohort. A further limitation is the lack of adjustment for multiple comparisons and thus chance discovery cannot be excluded. A large study from the National Health and Nutrition Examination Survey (NHANES, 1999–2004), examined phthalate levels in 1227 women, with a self-reported history of endometriosis

and uterine leiomyomata. MEHP, monobutyl phthalate (MBP), monoethyl phthalate (MEP), and MBzP levels were measured in patients with each disease as well as patients that reported both [69]. Comparing the highest versus lowest three quartiles of urinary phthalate levels, MBP had an OR of 1.36 (95% CI, 0.77–2.41), MEHP was 0.44 (95% CI, 0.19–1.02), with no association for MEP and MBzP in endometriosis patients. Significantly higher plasma concentration of DBP which is broken down into MBP was also seen [69]. However, the use of self-reported cases may be unreliable. Contrary to the NHANES study, an increased endometriosis risk with an increase in urinary MBzP and MEP was described although the results were not significant [71]. Moreover, an inverse relationship between endometriosis risk and urinary MEHP was found (OR = 0.3; 95% CI = 0.1–0.7) and an inverse relationship was also suggested for DEHP, MEHHP, mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and ΣDEHP. Therefore, a compelling link between phthalate exposure and endometriosis has not been established.

Results of several biomonitoring studies have documented higher concentrations of phthalate metabolites in the urine of women with endometriosis compared to a reference population. Plasma concentrations of mono-methyl phthalate (MMP), mono-benzyl phthalate (MBzP), mono-2-ethylhexyl phthalate (MEHP) and mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) were recently quantified by gas chromatography–mass spectrometry in infertile women with endometriosis (n = 11), polycystic ovarian syndrome (PCOS, n = 31) and PCOS plus endometriosis (n = 3) and 34 fertile women without evidence of gynecological disorders [59]. Overall, the mean (± SD) concentrations (ng/ml) of MBzP (19.9 ± 27.3 95% CI;11.69–28.12) and MEHHP (6.76 ± 5.54, 95% CI; 5.10–8.43) were significantly higher in infertile women compared to fertile women (5.72 ± 6.82, 95% CI; 3.34–8.10 and 1.71 ± 3.24, 0.58–2.85; respectively), whereas no differences were detected between groups for MMP and MEHP. The mean concentrations of MBzP and MEHHP in women with endometriosis were 40.9 ± 51.4 (range < LOQ – 116.5) and 5.43 ± 5.53 ng/ml (range < LOQ – 14.76), respectively. However, only 4–5% of women with endometriosis had concentrations of MBzP and MEHHP above the LOQ. Study participants were assigned to groups based upon self-reports of gynecological diagnoses which is open to misclassification error. In addition, the small sample size overall together with the limited number of study participants with quantifiable concentrations of phthalates are important limitations of this study.

Recently, differences in serum DEHP concentrations were found between women with endometriosis and control patients using high-performance liquid chromatography [66]. The mean ± SD concentration of DEHP in cases (n = 50) was 65.3 ± 21.7 ng/ml, whereas it was undetectable in the controls. Among the four stages of the disease, women with endometriosis showed a linear increase in DEHP concentration with more advanced stages, although the sample size for stage I was n = 1. Age groups did not impact DEHP serum levels. Controversy remains, as DEHP is broken down by glutathione S-transferase and P450 enzyme, which has been reported to be compromised in endometriosis patients [72]. This may explain the difference in serum concentration, as the control patients are able to metabolize DEHP into metabolites which were not recorded. A further weakness of this study is the measurement of DEHP in the serum rather than metabolites in either the serum or urine and thus the potential for sample contamination cannot be discounted.

A group from Taiwan investigated the association between GSTM1 polymorphisms and phthalates in adenomyosis, leiomyoma and endometriosis [68]. Although no relationship between the gene and the disease was found, there was an increase in urinary mono-n-butyl phthalate (94.1 versus 58.0 microg/g creatinine, $p < 0.05$) among the 28 women with endometriosis relative to the 29 controls.

In a subsequent study [70], the potential relationship between polymorphisms of CYP17A1 and phthalate exposure was explored in women with leiomyoma (fibroids, n = 36), endometriosis or adenomyosis (n = 44) and healthy controls (n = 69). However, only a marginally increased level of urinary MEHP was found in patients with endometriosis or adenomyosis [70].

Our search failed to identify any experimental animal studies and only two mechanistic studies were located. MMP-2 and 9 activities, cellular invasiveness, Erk phosphorylation, and p21-activated kinase 4 expression (PAK4) were increased in endometrial stromal cell cultures exposed to DEHP [73]. All five significantly elevated markers play a role in cellular division, actin cytoskeletal dynamics, motility, cell survival, and immune defense [73, 74]. Another study found that DEHP treatment increased ESC reactive oxygen species (ROS) generation and decreased expression of superoxide dismutase (SOD), glutathione peroxidase (GPX), heme oxygenase (HO), and catalase (CAT). p-ERK/p-p38 and NF-κB were also increased [75]. This provides a potential explanation for the decreased expression of antioxidant enzymes and increased ROS. Lastly, Esr1 expression was also increase proportional to dose [75].

In summary, while several studies revealed higher phthalate esters concentrations in women with endometriosis compared to controls the results of epidemiological studies remain equivocal. Moreover, the short half-life of 5–6 h for these chemicals suggests that higher concentrations detected in women with endometriosis compared to controls may be a consequence of the disease rather than a causal factor and thus reverse causation cannot be excluded. While *in vitro* studies suggest that phthalate esters can adversely affect mechanisms relevant to the pathophysiology of endometriosis, the effective concentrations are beyond human exposure and thus are unlikely to be clinically important.

3.6 Metals

Trace metals are nearly impossible to avoid in one's lifetime, as they are found both naturally in our bodies and are produced during industrial processes. Exposure to metals has been reported to interfere with cell proliferation, migration, cell degeneration, oxidative stress, and apoptosis, nearly all of which are properties of endometriosis [76]. Therefore, a link between circulating concentrations of metals and endometriosis has been explored by several groups.

A positive relationship between lead and endometriosis (adjusted OR = 2.59, 95% CI = 1.11–6.06) was found in Asian women whereas zinc levels were inversely associated with the disease (adjusted OR = 0.39, 95% CI = 0.18–0.88) [77]. While cadmium (Cd) levels were greater in women with endometriosis, the adjusted odds ratio was not significant [77]. Furthermore, no significant relationship was found between 20 trace elements quantified in the urine and three in blood [76]. Cases were surgically confirmed, whereas the controls were confirmed for the absence of endometriosis through MRI. Contrary to the findings by [24], Cd was inversely related to endometriosis risk, while urinary chromium and copper were marginally associated with endometriosis (aOR = 1.97; 95% CI: 1.21–3.19; aOR = 2.66; 95% CI: 1.26–5.64) [76]. Comparisons for each of the metals increase the probability of chance discovery and thus any association is considered suspect.

Our search of the literature failed to reveal any recent animal studies; however, a tissue culture study revealed that Cd treatment-induced higher ESC proliferation ($p = 0.02$) in cultures derived from eutopic endometrium of women with endometriosis compared to controls [78]. Although the mechanism was not identified, it is suggested that Cd at 10^{-5} M is the toxic threshold for ESCs [78], a concentration that is orders of magnitude above typical human exposure.

In summary, biomonitoring studies offer weak support for a potential link between metals exposure and endometriosis. Moreover, results from a tissue culture experiment suggest that Cd can adversely affect ESC proliferation but only at concentrations far in excess of human exposure. Consequently, we consider the evidence of a link between exposure to metals and risk of endometriosis to be speculative at best.

4. Future directions

The current literature fails to provide compelling evidence for an association between exposure to environmental toxicants and endometriosis risk. Although current evidence is weak, involvement of environmental toxicants in the pathophysiology of endometriosis cannot be excluded. However, we propose that establishing a link between exposure to environmental toxicants and endometriosis is particularly challenging. Endometriosis is a heterogeneous disease in which peritoneal and ovarian endometriomas may arise by mechanisms that differ from DIE [79] and thus environmental interactions may be different from other forms of the disease.

Absence of diagnostic tools such as a blood test for endometriosis together with normalization of pelvic pain and use of oral contraceptives among other factors leads to lengthy delays in diagnosis. Importantly, the interval between the onset and symptoms and definitive diagnosis of disease can be lengthy varying between 6 and 12 years [34]. Thus, there is a temporal disconnection between collection of biological samples for analysis and the onset of disease. Hence, the use of case–control studies may not permit convincing evidence of an association and the potential for reverse causation cannot be excluded.

Identification of appropriate control groups poses an additional challenge since the prevalence of endometriosis in asymptomatic women can be high [1]. Furthermore, the hallmarks of endometriosis include chronic pelvic pain and infertility. Women dealing with chronic pain and or infertility may adopt activities or behaviors to reduce their pain or improve their chances of conceiving that diverge from the healthy fertile population and thus their exposures may be a function of disease status rather than factors contributing to the pathophysiology of endometriosis. Consequently, in the absence of clinical tools to diagnosis endometriosis, the most appropriate control group in the future may be symptomatic women undergoing laparoscopy with careful inspection of the pelvic cavity to exclude the presence of endometriosis, even though this step is admittedly imperfect [80].

Epidemiological studies that adjust for potential confounders (e.g. age, BMI, parity, breast feeding, cigarette smoking, and alcohol consumption) and account for multiple comparisons could prove valuable in elucidating the role of exposure to environmental toxicants in the pathophysiology of endometriosis. Finally, it is unlikely that any group of women are exposed to a singly chemical or group of chemicals and thus quantification of chemicals from different chemical groups in a single study with an appropriate control, control for confounds and correction for multiple comparisons could prove informative.

In the absences of robust epidemiological data experimental animal studies take on greater importance for establishing biological plausibility of a potential association. In general, there is a paucity of literature addressing the potential hazards of environmental toxicants in the survival and growth of endometriotic implants in animal models of endometriosis. While spontaneous endometriosis is predominately limited to humans and some non-human primates, animal xenotransplant models using dispersed cells from ectopic implants in women with endometriosis

can provide valuable insight into potential chemical hazards relevant to endometriosis and mechanisms. However, dose levels used should include a concentration representative of human exposure. Similarly, tissue culture studies are essential for mechanistic insight; however, we propose that test concentrations should cover a range of doses that include concentrations below and representative of human exposure as well as high doses through to toxic levels.

5. Summary and conclusions

While in general, the epidemiological studies are judged to provide weak evidence of an association between exposure to environmental toxicants and endometriosis, a potential link cannot be excluded. Animal and cell culture models suggest biologically plausible mechanisms between the environmental toxicant exposures and endometriosis risk; however, the effective concentrations exceed human exposure levels. Consequently, we conclude that a causal relationship between exposure to any environmental toxicant and endometriosis does not currently exist, but the evidence does not allow us to exclude a potential link.

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References

[1] Rawson JM. Prevalence of endometriosis in asymptomatic women. The Journal of Reproductive Medicine. 1991;**36**:513-515

[2] Sampson J. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. American Journal of Obstetrics and Gynecology. 1927;**14**:422-469

[3] Matta K, Ploteau S, Coumoul X, Koual M, Le Bizec B, Antignac JP. et al. Associations between exposure to organochlorine chemicals and endometriosis in experimental studies: A systematic review protocol. Environment International. 2019;**124**:400-407

[4] Missmer SA, Hankinson SE, Spiegelman D, Barbieri RL, Marshall LM, Hunter DJ. Incidence of laparoscopically confirmed endometriosis by demographic, anthropometric, and lifestyle factors. American Journal of Epidemiology. 2004;**160**:784-796

[5] Upson K, Sathyanarayana S, Scholes D, Holt VL. Early-life factors and endometriosis risk. Fertility and Sterility. 2015;**104**:964.e965-971.e965

[6] Rier SE, Martin DC, Bowman RE, Dmowski WP, Becker JL. Endometriosis in rhesus monkeys (*Macaca mulatta*) following chronic exposure to tetrachlorodibenzo-p-dioxin. Fundamental and Applied Toxicology. 1993;**21**:433-441

[7] Cano-Sancho G, Ploteau S, Matta K, Adoamnei E, Louis GB, Mendiola J, et al. Human epidemiological evidence about the associations between exposure to organochlorine chemicals and endometriosis: Systematic review and meta-analysis. Environment International. 2019;**123**:209-223

[8] Louis GM, Weiner JM, Whitcomb BW, Sperrazza R, Schisterman EF, Lobdell DT, et al. Environmental PCB exposure and risk of endometriosis. Human Reproduction. 2005;**20**:279-285

[9] Porpora MG, Ingelido AM, Domenico AD, Ferro A, Crobu M, Pallante D, et al. Increased levels of polychlorobiphenyls in Italian women with endometriosis. Chemosphere. 2005;**63**(8):1361-1367

[10] Reddy BS, Rozati R, Reddy S, Kodampur S, Reddy P, Reddy R. High plasma concentrations of poly-chlorinated biphenyls and phthalate esters in women with endometriosis: A prospective case control study. Fertility and Sterility. 2006;**85**:775-779

[11] Lebel G, Dodin S, Ayotte P, Marcoux S, Ferron LA, Dewailly E. Organochlorine exposure and the risk of endometriosis. Fertility and Sterility. 1998;**69**:221-228

[12] Pauwels A, Schepens PJC, Hooghe TD, Delbeke L, Dhont M, Brouwer A, et al. The risk if endometriosis and exposure to dioxins and polychlorinated biphenyls: A case-control study of infertile women. Human Reproduction. 2001;**16**:2050-2055

[13] Tsukino H, Hanaoka T, Sasaki H, Motoyama H, Hiroshima M, Tanaka T, et al. Associations between serum levels of selected organochlorine compounds and endometriosis in infertile Japanese women. Environmental Research. 2005;**99**:118-125

[14] Anger DL, Foster WG. The link between environmental toxicant exposure and endometriosis. Frontiers in Bioscience. 2008;**13**:1578-1593

[15] Foster WG, Agarwal SK. Environmental contaminants and dietary factors in endometriosis. Annals of the New York Academy of Sciences. 2002;**955**:213-229

[16] Foster WG. Do environmental contaminants adversely affect human reproductive physiology? Journal of Obstetrics and Gynaecology Canada. 2003;**25**:33-44

[17] Foster WG. Endocrine toxicants including 2,3,7,8-terachlorodibenzo-p-dioxin (TCDD) and dioxin-like chemicals and endometriosis: Is there a link? Journal of Toxicology and Environmental Health. Part B, Critical Reviews. 2008;**11**:177-187

[18] Vabre P, Gatimel N, Moreau J, Gayrard V, Picard-Hagen N, Parinaud J, et al. Environmental pollutants, a possible etiology for premature ovarian insufficiency: A narrative review of animal and human data. Environmental Health. 2017;**16**:37

[19] Faroon O, Ruiz P. Polychlorinated biphenyls: New evidence from the last decade. Toxicology and Industrial Health. 2016;**32**:1825-1847

[20] Bruner-Tran KL, Osteen KG. Dioxin-like PCBs and endometriosis. Systems Biology in Reproductive Medicine. 2010;**56**:132-146

[21] Korach KS, Sarver P, Chae K, McLachlan JA, McKinney JD. Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: Conformationally restricted structural probes. Molecular Pharmacology. 1988;**33**:120-126

[22] Moore M, Mustain M, Daniel K, Chen I, Safe S, Zacharewski T, et al. Antiestrogenic activity of hydroxylated polychlorinated biphenyl congeners identified in human serum. Toxicology and Applied Pharmacology. 1997;**142**:160-168

[23] Porpora MG, Medda E, Abballe A, Bolli S, De Angelis I, di Domenico A, et al. Endometriosis and organochlorinated environmental pollutants: A case-control study on Italian women of reproductive age. Environmental Health Perspectives. 2009;**117**:1070-1075

[24] Cai LY, Izumi S, Suzuki T, Goya K, Nakamura E, Sugiyama T, et al. Dioxins in ascites and serum of women with endometriosis: A pilot study. Human Reproduction. 2010;**26**(1):117-126

[25] Trabert B, De Roos AJ, Schwartz SM, Peters U, Scholes D, Barr DB, et al. Non-dioxin-like polychlorinated biphenyls and risk of endometriosis. Environmental Health Perspectives. 2010;**118**:1280-1285

[26] Ploteau S, Antignac JP, Volteau C, Marchand P, Vénisseau A, Vacher V, et al. Distribution of persistent organic pollutants in serum, omental, and parietal adipose tissue of French women with deep infiltrating endometriosis and circulating versus stored ratio as new marker of exposure. Environment International. 2016;**97**:125-136

[27] Louis GM, Peterson CM, Chen Z, Hediger ML, Croughan MS, Sundaram R, et al. Perfluorochemicals and endometriosis: The ENDO study. Epidemiology. 2012;**23**:799-805

[28] Martínez-Zamora MA, Mattioli L, Parera J, Abad E, Coloma JL, van Babel B, et al. Increased levels of dioxin-like substances in adipose tissue in patients with deep infiltrating endometriosis. Human Reproduction. 2015;**30**:1059-1068

[29] Simsa P, Mihalyi A, Schoeters G, Koppen G, Kyama CM, Den Hond EM, et al. Increased exposure to dioxin-like compounds is associated with endometriosis in a case-control study in women. Reproductive Biomedicine Online. 2010;**20**:681-688

[30] Vichi S, Medda E, Ingelido AM, Ferro A, Resta S, Porpora MG, et al. Glutathione transferase polymorphisms

and risk of endometriosis associated with polychlorinated biphenyls exposure in Italian women: A gene-environment interaction. Fertility and Sterility. 2012;**97**:1143-1151

[31] Buck Louis GM, Chen Z, Peterson CM, Hediger ML, Croughan MS, Sundaram R, et al. Persistent lipophilic environmental chemicals and endometriosis: The ENDO study. Environmental Health Perspectives. 2012;**120**:811-816

[32] Matsuzaka Y, Kikuti YY, Goya K, Suzuki T, Cai LY, Oka A, et al. Lack of an association human dioxin detoxification gene polymorphisms with endometriosis in Japanese women: Results of a pilot study. Environmental Health and Preventive Medicine. 2012;**17**:512-517

[33] Sinaii N, Cleary SD, Younes N, Ballweg ML, Stratton P. Treatment utilization for endometriosis symptoms: A cross-sectional survey study of lifetime experience. Fertility and Sterility. 2007;**87**:1277-1286

[34] Greene R, Stratton P, Cleary SD, Ballweg ML, Sinaii N. Diagnostic experience among 4,334 women reporting surgically diagnosed endometriosis. Fertility and Sterility. 2009;**91**:32-39

[35] Bruner-Tran KL, Ding T, Osteen KG. Dioxin and endometrial progesterone resistance. Seminars in Reproductive Medicine. 2010;**28**:59-68

[36] Bruner-Tran KL, Rier SE, Eisenberg E, Osteen KG. The potential role of environmental toxins in the pathophysiology of endometriosis. Gynecologic and Obstetric Investigation. 1999;**48**(Suppl 1):45-56

[37] Rier SE, Tuner WE, Martin DC, Morris R, Lucier GW, Clark GC. Serum levels of TCDD and dioxin-like chemicals in rhesus monkeys chronically exposed to dioxin: Correlation of increased serum PCB levels with endometriosis. Toxicological Sciences. 2001;**59**:147-159

[38] Yang JZ, Agarwal SK, Foster WG. Subchronic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin modulates the pathophysiology of endometriosis in the Cynomolgus monkey. Toxicological Sciences. 2000;**56**:374-381

[39] Huang Q, Chen Y, Chen Q, Zhang H, Lin Y, Zhu M, et al. Dioxin-like rather than non-dioxin-like PCBs promote the development of endometriosis through stimulation of endocrine-inflammation interactions. Archives of Toxicology. 2017;**91**:1915-1924

[40] Wang Y, Chen H, Wang N, Guo H, Fu Y, Xue S, et al. Combined 17β-estradiol with TCDD promotes M2 polarization of macrophages in the endometriotic milieu with aid of the interaction between endometrial stromal cells and macrophages. PLoS One. 2015;**10**:e0125559

[41] Resuehr D, Glore DR, Taylor HS, Bruner-Tran KL, Osteen KG. Progesterone-dependent regulation of endometrial cannabinoid receptor type 1 (CB1-R) expression is disrupted in women with endometriosis and in isolated stromal cells exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Fertility and Sterility. 2012;**98**:948.e941-956.e941

[42] Willing C, Peich M, Danescu A, Kehlen A, Fowler PA, Hombach-KlonischS.Estrogen-independent actions of environmentally relevant AHR-agonists in human endometrial epithelial cells. Molecular Human Reproduction. 2011;**17**:115-126

[43] Hu T, Yao M, Fu X, Chen C, Wu R. Polychlorinated biphenyl 104 promotes migration of endometrial

stromal cells in endometriosis. Toxicology Letters. 2018;**290**:19-28

[44] Cooney MA, Buck Louis GM, Hediger ML, Vexler A, Kostyniak PJ. Organochlorine pesticides and endometriosis. Reproductive Toxicology. 2010;**30**:365-369

[45] Upson K, De Roos AJ, Thompson ML, Sathyanarayana S, Scholes D, Barr DB, et al. Organochlorine pesticides and risk of endometriosis: Findings from a population-based case-control study. Environmental Health Perspectives. 2013;**121**:1319-1324

[46] Chiappini F, Bastón JI, Vaccarezza A, Singla JJ, Pontillo C, Miret N, et al. Enhanced cyclooxygenase-2 expression levels and metalloproteinase 2 and 9 activation by hexachlorobenzene in human endometrial stromal cells. Biochemical Pharmacology. 2016;**109**:91-104

[47] Calafat AM, Kuklenyik Z, Caudill SP, Reidy JA, Needham LL. Perfluorochemicals in pooled serum samples from United States residents in 2001 and 2002. Environmental Science & Technology. 2006;**40**:2128-2134

[48] Guruge KS, Taniyasu S, Yamashita N, Wijeratna S, Mohotti KM, Seneviratne HR, et al. Perfluorinated organic compounds in human blood serum and seminal plasma: A study of urban and rural tea worker populations in Sri Lanka. Journal of Environmental Monitoring. 2005;**7**:371-377

[49] Bach CC, Vested A, Jørgensen KT, Bonde JP, Henriksen TB, Toft G. Perfluoroalkyl and polyfluoroalkyl substances and measures of human fertility: A systematic review. Critical Reviews in Toxicology. 2016;**46**:735-755

[50] Kielsen K, Shamim Z, Ryder LP, Nielsen F, Grandjean P, Budtz-Jørgensen E, et al. Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. Journal of Immunotoxicology. 2016;**13**:270-273

[51] Liu G, Dhana K, Furtado JD, Rood J, Zong G, Liang L, et al. Perfluoroalkyl substances and changes in body weight and resting metabolic rate in response to weight-loss diets: A prospective study. PLoS Medicine. 2018;**15**:e1002502

[52] Campbell S, Raza M, Pollack AZ. Perfluoroalkyl substances and endometriosis in us women in NHANES 2003-2006. Reproductive Toxicology. 2016;**65**:230-235

[53] Rashtian J, Chavkin DE, Merhi Z. Water and soil pollution as determinant of water and food quality/contamination and its impact on female fertility. Reproductive Biology and Endocrinology. 2019;**17**:5

[54] Rochester JR. Bisphenol a and human health: A review of the literature. Reproductive Toxicology. 2013;**42**:132-155

[55] Itoh H, Iwasaki M, Hanaoka T, Sasaki H, Tanaka T, Tsugane S. Urinary phthalate monoesters and endometriosis in infertile Japanese women. Science of the Total Environment. 2009;408:37-42

[56] Upson K, Sathyanarayana S, De Roos AJ, Koch HM, Scholes D, Holt VL. A population-based case-control study of urinary bisphenol A concentrations and risk of endometriosis. Human Reproduction. 2014;**29**:2457-2464

[57] Cobellis L, Colacurci N, Trabucco E, Carpentiero C, Grumetto L. Measurement of bisphenol A and bisphenol B levels in human blood sera from healthy and endometriotic women. Biomedical Chromatography. 2009;**23**:1186-1190

[58] Itoh H, Iwasaki M, Hanaoka T, Sasaki H, Tanaka T, Tsugane S. Urinary

bisphenol-A concentration in infertile Japanese women and its association with endometriosis: A cross-sectional study. Environmental Health and Preventive Medicine. 2007;**12**:258-264

[59] Pednekar PP, Gajbhiye RK, Patil AD, Surve SV, Datar AG, Balsarkar GD, et al. Estimation of plasma levels of bisphenol-A & phthalates in fertile & infertile women by gas chromatography-mass spectrometry. The Indian Journal of Medical Research. 2018;**148**:734-742

[60] Jones RL, Lang SA, Kendziorski JA, Greene AD, Burns KA. Use of a mouse model of experimentally induced endometriosis to evaluate and compare the effects of bisphenol A and bisphenol AF exposure. Environmental Health Perspectives. 2018;**126**:127004

[61] Forte M, Mita L, Cobellis L, Merafina V, Specchio R, Rossi S, et al. Triclosan and bisphenol A affect decidualization of human endometrial stromal cells. Molecular and Cellular Endocrinology. 2016;**422**:74-83

[62] Silva MJ, Barr DB, Reidy JA, Malek NA, Hodge CC, Caudill SP, et al. Urinary levels of seven phthalate metabolites in the U.S. population from the national health and nutrition examination survey (NHANES) 1999-2000. Environmental Health Perspectives. 2004;**112**:331-338

[63] Tyrrell J, Melzer D, Henley W, Galloway TS, Osborne NJ. Associations between socioeconomic status and environmental toxicant concentrations in adults in the USA: NHANES 2001-2010. Environment International. 2013;**59**:328-335

[64] Meeker JD, Ferguson KK. Urinary phthalate metabolites are associated with decreased serum testosterone in men, women, and children from NHANES 2011-2012. The Journal of Clinical Endocrinology and Metabolism. 2014;**99**(11):4346-4352

[65] Lague E, Tremblay JJ. Antagonistic effects of testosterone and the endocrine disruptor mono-(2-ethylhexyl) phthalate on INSL3 transcription in Leydig cells. Endocrinology. 2008;**149**:4688-4694

[66] Nazir S, Usman Z, Imran M, Lone KP, Ahmad G. Women diagnosed with endometriosis show high serum levels of diethyl hexyl phthalate. Journal of Human Reproductive Sciences. 2018;**11**:131-136

[67] Buck Louis GM, Peterson CM, Chen Z, Croughan M, Sundaram R, Stanford J, et al. Bisphenol A and phthalates and endometriosis: The endometriosis: Natural history, diagnosis and outcomes study. Fertility and Sterility. 2013;**100**:162-169

[68] Huang PC, Tsai EM, Li WF, Liao PC, Chung MC, Wang YH, et al. Association between phthalate exposure and glutathione S-transferase M1 polymorphism in adenomyosis, leiomyoma and endometriosis. Human Reproduction. 2010;**25**:986-994

[69] Weuve J, Hauser R, Calafat AM, Missmer SA, Wise LA. Association of exposure to phthalates with endometriosis and uterine leiomyomata: Findings from NHANES, 1999-2004. Environmental Health Perspectives. 2010;**118**:825-832

[70] Huang PC, Li WF, Liao PC, Sun CW, Tsai EM, Wang SL. Risk for estrogen-dependent diseases in relation to phthalate exposure and polymorphisms of CYP17A1 and estrogen receptor genes. Environmental Science and Pollution Research International. 2014;**21**:13964-13973

[71] Upson K, Sathyanarayana S, De Roos AJ, Thompson ML, Scholes D, Dills R, et al. Phthalates and risk of endometriosis. Environmental Research. 2013;**126**:91-97

[72] Kubiszeski EH, de Medeiros SF, da Silva Seidel JA, Barbosa JS, Galera MF, Galera BB. Glutathione s-transferase M1 and T1 gene polymorphisms in Brazilian women with endometriosis. Journal of Assisted Reproduction and Genetics. 2015;**32**:1531-1535

[73] Kim SH, Cho S, Ihm HJ, Oh YS, Heo SH, Chun S, et al. Possible role of phthalate in the pathogenesis of endometriosis: In vitro, animal, and human data. The Journal of Clinical Endocrinology and Metabolism. 2015;**100**:E1502-E1511

[74] Dart AE, Wells CM. P21-activated kinase 4—Not just one of the PAK. European Journal of Cell Biology. 2013;**92**:129-138

[75] Cho YJ, Park SB, Han M. Di-(2-ethylhexyl)-phthalate induces oxidative stress in human endometrial stromal cells in vitro. Molecular and Cellular Endocrinology. 2015;**407**:9-17

[76] Pollack AZ, Louis GM, Chen Z, Peterson CM, Sundaram R, Croughan MS, et al. Trace elements and endometriosis: The ENDO study. Reproductive Toxicology. 2013;**42**:41-48

[77] Lai GL, Yeh CC, Yeh CY, Chen RY, Fu CL, Chen CH, et al. Decreased zinc and increased lead blood levels are associated with endometriosis in Asian women. Reproductive Toxicology. 2017;**74**:77-84

[78] Silva N, Tennekoon K, Senanayake H, Samarakoon S. Metalloestrogen cadmium stimulates proliferation of stromal cells derived from the eutopic endometrium of women with endometriosis. Taiwanese Journal of Obstetrics & Gynecology. 2013;**52**:540-545

[79] Gordts S, Koninckx P, Brosens I. Pathogenesis of deep endometriosis. Fertility and Sterility. 2017;**108**:872. e871-885.e871

[80] Wykes CB, Clark TJ, Khan KS. Accuracy of laparoscopy in the diagnosis of endometriosis: A systematic quantitative review. BJOG. 2004;**111**: 1204-1212