HHS Public Access

Author manuscript

Curr Opin Endocrinol Diabetes Obes. Author manuscript; available in PMC 2021 June 29.

Published in final edited form as:

Curr Opin Endocrinol Diabetes Obes. 2020 December ; 27(6): 380–387. doi:10.1097/MED.0000000000000578.

The role of endocrine-disrupting chemicals in uterine fibroid pathogenesis

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Abstract

Purpose of review—Uterine leiomyoma (fibroids) is a gynecologic disorder impacting the majority of women in the United States. When symptomatic, these noncancerous tumors can cause severe morbidity including pelvic pain, menorrhagia, and infertility. Endocrine-disrupting chemicals (EDCs) may represent a modifiable risk factor. The aim of this review is to summarize recent human and experimental evidence on EDCs exposures and fibroids.

Recent findings—Multiple EDCs are associated with fibroid outcomes and/or processes including phthalates, parabens, environmental phenols, alternate plasticizers, Diethylstilbestrol, organophosphate esters, and tributyltin. Epidemiologic studies suggest exposure to certain EDCs, such as di-(2-ethylhxyl)-phthalate (DEHP), are associated with increased fibroid risk and severity. Both human and experimental studies indicate that epigenetic processes may play an important role in linking EDCs to fibroid pathogenesis. In-vitro and in-vivo studies show that DEHP, bisphenol A, and diethylstilbestrol can impact biological pathways critical to fibroid pathogenesis.

Summary—While research on EDCs and fibroids is still evolving, recent evidence suggests EDC exposures may contribute to fibroid risk and progression. Further research is needed to examine the impacts of EDC mixtures and to identify critical biological pathways and windows of exposure. These results could open the door to new prevention strategies for fibroids.

Keywords

consumer product chemicals epigenetics; environmental phenols; parabens; phthalates; uterine leiomyoma; women’s health

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Conflicts of interest

There are no conflicts of interest.

INTRODUCTION

Uterine leiomyomas, more commonly known as fibroids, are noncancerous tumors that develop from smooth muscle tissue of the uterus. Despite the high prevalence of this gynecologic disorder and its profound social and economic impacts, the cause of fibroids remains elusive and there are few established risk factors. Endocrine disrupting chemicals (EDCs), or chemicals that interfere with hormone action, may represent a modifiable risk factor, as estrogen and progesterone play a critical role in fibroid growth [1] and EDC exposures are widespread among reproductive-aged women [2]. Prior reviews support a role for certain EDCs in fibroid pathogenesis, such as diethylstilbestrol (DES) that may act as estrogen agonists [3]. In this review, we extend this prior work by examining recent human and experimental evidence for a broader range of EDCs including phthalates, parabens, environmental phenols, alternate plasticizers, DES, organophosphate esters (OPEs), and tributyltin.

UTERINE FIBROID DISORDER OVERVIEW

The social and economic costs of fibroids in the United States is immense, with an annual estimated cost of up to $34 billion [4]. Although the majority of reproductive-aged women will develop fibroids, only approximately 25% will experience symptoms. Among symptomatic women, fibroids are associated with substantial morbidity including heavy menstrual bleeding, pain, subfertility, and pregnancy complications. The lack of consensus on uterine-preserving treatments leads fibroids to be the leading indication for hysterectomy [5]. Black women are disproportionately burdened by fibroids, often experiencing a higher risk of fibroids, an earlier age of onset, and more severe symptoms compared with nonblack women [6]. In addition to race/ethnicity, other risk factors for fibroids include age, family history, nulliparity, obesity, and hormonal factors [7]. Furthermore, there is evidence that hypovitaminosis D also contributes to the development of fibroids [8].

ESTROGEN AND PROGESTERONE

Fibroid development depends on the ovarian steroid hormones, estrogen and progesterone, which explains why they arise during women’s reproductive age and typically regress after menopause [1]. Estrogen exerts its genomic and nongenomic effects through the estrogen receptors (ERα and ERβ). Genomic pathways embroil direct binding of estrogen receptor complexes to specific sequences in gene promoters, whereas nongenomic signaling involves activation of signaling cascades that result in indirect changes in gene expression [9]. Similarly, progesterone binds progesterone receptors (PR-A and PR-B) and not only transduces its actions primarily via the genomic signaling pathways but also operates through nonclassical signaling pathways [10]. As a result of the hormonal dependence of fibroids, much of the pharmacological treatments suppress or modulate these hormones [11,12].

DEVELOPMENTAL ORIGINS OF UTERINE FIBROIDS: ROLE OF THE MYOMETRIAL STEM CELLS

Fibroids are monoclonal tumors in which each fibroid nodule is derived from a distinct progenitor cell. Although the exact mechanisms of pathophysiology remain unclear, increasing research has supported the hypothesis that fibroids originate from pathologically transformed myometrial stem cell (MMSCs) [13,14]. Fibroid-forming MMSCs are referred to as tumor-initiating cells (TICs) (Fig. 1).

Smooth muscle cells of the uterus are under continuous proliferation and remodeling during a women's reproductive years placing them at increased risk of genomic instability and DNA mutations. The conversion of MMSCs to TICs is thought to be because of the acquisition of one or more gene mutations, such as the most prevalent in gene mediator complex subunit 12 (MED12) [15]. Moreover, several genomic studies have demonstrated the key role of microRNAs and epigenetic regulation of gene expression in fibroids development [16].

As MMSCs and TICs both express relatively low levels of estrogen receptors and progesterone receptors, it suggests that paracrine interactions between TICs and the surrounding myometrium are important for fibroid growth, which is dependent on steroid hormones [17].

SOMATIC MUTATIONS

MED12 somatic mutations have been consistently documented in approximately 70–80% of sporadic fibroids [18]. MED12 protein is involved in the transcriptional regulation of the RNA polymerase II initiation complex. Pathway analysis has shown that the Wnt/β-catenin signaling is increased in fibroids with mutated MED12, which regulates fibroid development mainly through the expression of proteins involved in cell proliferation, as well as extracellular matrix (ECM) components [19]. The second most common gene alteration found in fibroids is overexpression in HMGA2 with a prevalence of less than 10%. This mutation is associated with hypomethylation and epigenetic deregulation in the *HMGA2* gene body [13,20], which is a member of the high mobility group gene family that influences cellular processes like differentiation, death, growth, and proliferation. These somatic mutations occur independently and are mutually exclusive in fibroids.

ENDOCRINE-DISRUPTING CHEMICALS

There is growing interest in the possible reproductive consequences posed by EDCs, which are substances in our environment, food, and consumer products that interfere with hormone biosynthesis, metabolism, or action resulting in a deviation from normal homeostatic control or reproduction [21]. Many classes of EDCs, such as environmental phenols, phthalates and alternate plasticizers, parabens, and organophosphate esters are commonly found in consumer products. They can leach, migrate, or off-gas from products over time and can enter the human body through ingestion, inhalation, direct dermal application, or even transdermal exposure from air. Once ingested, inhaled, or absorbed, these chemicals are rapidly metabolized and excreted in urine and feces. Urinary concentrations of the parent compounds or their metabolites are commonly used as exposure biomarkers. National

biomonitoring data from the US Centers for Disease Control and Prevention demonstrate that the majority of reproductive-aged women in the United States are exposed to multiple EDCs [2]. Biomonitoring data also suggest that exposure to EDCs, such as phthalates and parabens that are commonly used in personal care products, are higher among black and Latina women compared to white women [22].

EDCs bind to nuclear receptors, which can alter hormone functions by mimicking endogenous hormones and blocking their binding to the receptors or interfering with their function and regulation [23]. Importantly, EDCs can demonstrate nonmonotonic dose response curve where even low doses of EDCs can result in pathologic effects, mainly when the exposure to these compounds is simultaneous [24,25]. Certain EDCs are reproductive toxicants in animal models [26,27] and are also associated with adverse reproductive outcomes in humans [28,29].

RECENT EVIDENCE FROM ENVIRONMENTAL EPIDEMIOLOGY STUDIES

Since 2018, there have been four additional epidemiologic studies on exposures to EDCs and fibroid outcomes (Table 1). Two of the studies used data collected on women with symptomatic fibroids in the Washington DC metropolitan area (United States) undergoing invasive surgery for fibroid treatment as part of the Fibroids, Observational Research on Genes and the Environment (FORGE) study. The cross-sectional studies from FORGE examined associations between EDCs exposures and: measures of fibroid severity; and microRNA expression in fibroid tumors and adjacent myometrium. The other two studies were case–control studies that examined imaging-based fibroid prevalence as the main outcome among reproductive-aged women in South Korea. Collectively, these studies suggest associations between various EDCs and measures of fibroid prevalence and severity. Moreover, the preliminary results for the environmental epigenetics study suggest that microRNA regulation may be involved in biological pathways linking phthalates to fibroid pathogenesis.

Zota *et al.* in 2019 conducted a preliminary, cross-sectional study of 57 premenopausal women undergoing a hysterectomy or myomectomy for their fibroids to evaluate associations between phthalates exposures and measures of fibroid burden. Most women were black, overweight or obese, college-educated, and exposed to multiple phthalates (9 out of the 14 urinary phthalate metabolites were detected in >90% of participants). The geometric mean of three phthalate metabolites were greater than 30% higher in black women compared with white or Latina women. In multivariable models, higher urinary concentrations of several phthalate biomarkers were significantly associated with greater uterine volume [30▪].

The objective of the second study (2020) was to examine the associations between phthalate exposures and miRNA expression levels in fibroid tumors and myometrium among a subset of the FORGE study population ($N = 45$). As part of the study design, expression levels of 754 miRNAs were quantified in tissue samples, and all analyses were adjusted for multiple comparisons testing. Mono-hydroxybutyl phthalate and mono(2-ethyl-5-hydroxyhexyl) phthalate were positively associated with miR-10a-5p and miR-577, respectively. A total of eight phthalate-miRNA associations varied by race/ethnicity. Pathway analysis revealed that

mRNA gene targets of phthalate-associated miRNAs were significantly associated with multiple fibroid-related processes including angiogenesis, apoptosis, and proliferation of connective tissues. Although these results are preliminary, validation of these findings may provide insight into mechanisms underlying associations between phthalates and fibroids and contribute to novel hypotheses regarding racial/ethnic disparities in fibroids [31▪].

The third study, by Lee *et al.* in 2020, examined associations between exposures to nonpersistent EDCs and odds of fibroid prevalence among reproductive-aged women in South Korea. A total of 484 women were analyzed, with 95 uterine fibroid cases and 336 controls, and presence of fibroids was determined through transvaginal ultrasound. They observed that certain parabens and phthalates, including ΣDEHP, were associated with increased risk of fibroids. Associations between chemical exposures and fibroid risk was stronger among more frequent users of personal care products [32].

A fourth study out of South Korea used a similar case-control study design as Lee *et al.* to examine fibroid prevalence among reproductive-aged women but with a much smaller sample size (32 cases and 79 controls). They examined an expanded list of EDCs that included OPEs and alternate plasticizers. They observed that certain phthalates, OPEs, and alternative plasticizers are associated with increased risk of fibroids. Mixtures of certain phthalates, such as DEHP, and OPEs are also associated with increased fibroid risk [33].

Although the existing epidemiologic studies are relatively small in scope and lack temporality between exposure and outcome, all of the studies indicate a positive association between DEHP metabolites and fibroid outcomes. This is likely because of the suspected influence on signaling pathways, which ultimately impact cell proliferation as well as apoptosis.

RECENT EVIDENCE FROM EXPERIMENTAL STUDIES

A variety of experimental models have been developed for the study of fibroids [34,35]. However, they have not all been employed to evaluate the impact of EDCs on the pathogenesis of fibroids. Studies included in this review relied on human fibroid sample-derived primary cell cultures, human fibroid cell line cultures, and rat models (Table 2).

Most of the in-vitro studies indicate that EDCs, such as DEHP and bisphenol A increased the proliferation of human fibroid cells, contributing to fibroid growth [36–38]. Several of the fibroid mechanistic studies suggest an association between EDCs and inflammation-related pathways [37,39].

Animal models have been used to investigate possible links between early-life exposure to EDCs and relevant disease [39,40,41▪]. The Eker rat is one of the most used animal models to evaluate the role of early developmental exposure to EDCs in uterine fibroid etiology. These rats possess a germline heterozygous mutation on tumor suppressor gene *Tsc2* and spontaneously develop fibroids between the ages of 12 and 16 months with ~65% of penetrance. However, when Eker rats are developmentally exposed to DES during early life, fibroids appear later in adult life at higher frequency (100% tumor penetrance), increased size, number, and severity versus unexposed counterparts [42]. Importantly, fibroids that

develop in Eker rats are hormone-responsive, and express ERα and PR. This animal model was used to demonstrate the increased risk of genomic instability because of early-life EDCs exposure. Developmental exposition to DES can alter the MMSC's ability to repair and reverse DNA damage. It have been shown that DES-MMSCs accumulated more DNA damage than vehicle (VEH)-MMSCs, and presented less capacity to repair it [40,41▪].

An advantage of using animal models is that they are powerful tools to investigate new treatment options for fibroids induced by EDC exposure. Elkafas *et al.* [41▪] in 2020 have shown that vitamin D3 treatment attenuated the DNA damage load in MMSCs exposed to DES and restores the DNA repair signaling network in the Eker rat Model. Furthermore, a recent study has demonstrated that the therapy with the traditional herb pair *Curcumae rhizoma–Sparganii rhizoma* markedly reduced uterine growth and attenuated the process of ECM deposition provoked by the exposure to DES and progesterone in rats [43].

Moreover, several rat leiomyomas-derived cell lines have been established from these tumors (ELT lines), being ELT-3 cell line the most used as an in-vivo and in-vitro models for preclinical studies and for studying fibroid pathogenesis [44–46].

EPIGENETIC REPROGRAMMING

Accumulating evidence demonstrates that environmental exposures to EDCs can reprogram the epigenome of developing tissues in such a way as to increase susceptibility to disease later in life [47,48]. Previous studies suggest that developmental exposure to EDCs causes uterine diseases and increased the risk of fibroids via epigenomic reprogramming [49].

Epigenetic reprogramming at DNA methylation and histone modification levels, in response to exposure to EDCs have been demonstrated in hormone-dependent tumor development [49,50]. The highly plastic state of the stem cells during development and tissue maintenance provides an opportunity for aberrant cellular reprogramming via epigenetic mechanisms because of inappropriate exposures to EDCs and toxins. Although MMSCs have been identified as the cells from which fibroids originate [14], the epigenetic mechanism of MMSC programming because of developmental exposure to EDCs has not been characterized. In this regard, RNA-seq and CHIP-seq studies in rat vehicle- and DES-exposed MMSCs have demonstrated that early-life exposure to DES reprogrammed several biological pathways including estrogen responsive signaling [49] and inflammatory pathways [51] in MMSCs in early adult stage. The increased expression of estrogen responsive genes significantly correlated with the enrichment of H3K4me3, an active epigenetic mark. In addition, bisulfite next-generation sequencing demonstrated that reprogrammed estrogen-responsive genes with increase in RNA expression also exhibited hypomethylation within their CpG islands in DES-exposed MMSCs compared with VEH-exposed MMSCs. These studies suggested that developmental exposure to EDCs reprogrammed the estrogen responsive genes via histone modification and DNA methylation in MMSCs, from which the fibroids originate [52]. Moreover, additional genes involved in the pathogenesis of fibroids were also identified through multiomic analysis [53]. By ChIP-seq analysis, inflammatory responsive genes have been identified with enrichment of H3K4Me3 at their promoter regions in DES-MMSCs compared with VEH-MMSCs. The increased expression of inflammatory responsive genes was positively correlated with

elevated H3K4me3 mark. These studies provide compelling evidence that MMSCs are the direct epigenetic targets of xenoestrogenic actions and illustrate the strength of epigenomic profiling in revealing novel information about mechanisms that modulate the transcriptional landscape of MMSCs leading to increased risk of fibroid development.

CONCLUSION

This review highlights recent publications showing the current knowledge of the physiological and molecular effects of EDCs on uterine fibroids. In summary, exposure to several EDCs is linked with the development and progression of fibroids (Fig. 1). Epidemiologic studies suggest that exposure to environmental EDCs, such as DEHP is associated with increased fibroid risk and severity. In-vitro and in-vivo experimental studies have found that EDCs can promote antiapoptotic events and stimulate the proliferation of fibroids cells, leading to tumor growth. Inflammation, DNA damage, and epigenetic processes play a crucial role in linking EDCs to fibroid origin and evolution. Although the investigation on epigenetic reprogramming by EDCs and its influence on fibroids development has been conducted, the information in this field is still quite limited. Additional mechanistic studies to decipher the epigenetic biomarkers/signatures to specific EDCs will hold promise in precision medicine. Moreover, further analysis of the effects of EDCs mixtures and exposure window need to be addressed to develop prevention programs for fibroids.

Financial support and sponsorship

This work was supported by the National Institutes of Health (R21HD096248, R01HD094378, R01ES028615, and U54MD007602), National Center for Advancing Translational Sciences (UL1TR001876, KL2TR001877), The George Washington University Milken School of Public Health, and The George Washington University Office of the Vice President for Research (Cross-disciplinary Research Fund). The content is solely the responsibility of the authors and does not necessarily represent the official views of any of the funding agencies.

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KEY POINTS

- Human exposure to EDCs is associated with increased fibroid risk and severity in reproductive-aged women.
- Effects of EDCs on fibroid development are related to inflammation, DNA damage, and epigenetic processes.
- EDCs can impact cell proliferation as well as apoptosis signaling, leading to fibroid growth.

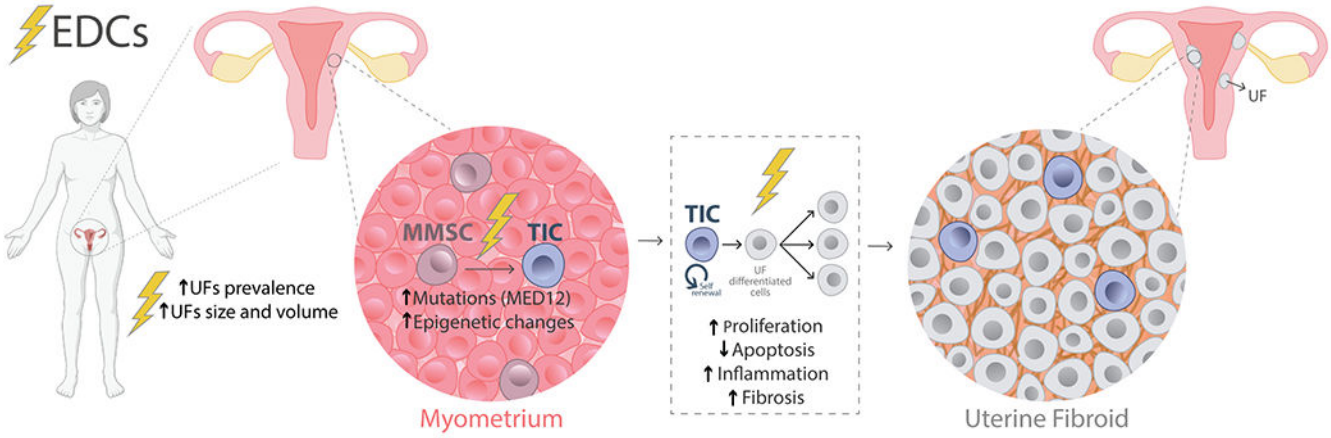

FIGURE 1.

Impact of endocrine-disrupting chemicals on uterine fibroid pathogenesis. Environmental exposure to endocrine-disrupting chemicals (EDCs) is associated with increased fibroid prevalence and severity in women. EDCs can induce mutations, such as MED12 and epigenetic changes in myometrial stem cells (MMSCs), leading to the conversion of these into tumor-initiating cells (TICs). Moreover, EDCs can act on uterine fibroid differentiated cells stimulating their proliferation and triggering antiapoptotic events, eventually driving the tumor growth.

Curr Opin Endocrinol Diabetes Obes. Author manuscript; available in PMC 2021 June 29.

Table 1.

Summary of the evidence on environmental EDC exposures and uterine fibroid outcomes from human studies.

Study	Sample Size	Country	Study Design	Outcome Measurements	Chemicals Measured in Urine	Chemicals Associated	Outcome
Zota et. al, 2019 [30*]	57	U.S.	Cross-Sectional Study	Fibroid size and uterine volume from MRI or ultrasound imaging	Phthalate metabolites 14: MEP, MnBP, MiBP, MHiBP, MBzP, MCPP, MCOP, MCNP, MEHP, MEHHP, MEOHP, MECPP, ΣDEHP, ΣAA	Phthalates 9: MEHHP, MEOHP, MECPP, ΣDEHP, ΣAA, MCOP, MCNP, MEHP, MHiBPs	Association between most of the phthalate biomarkers and uterine volume, a clinical marker of fibroid severity, were positive and significant.
Zota et. al, 2020 [31*]	45	U.S.	Cross-Sectional Study	MicroRNA expression from fibroids and myometrium tissue	Phthalate metabolites 19: MEP, DnBP, MnBP, MHBP, DiBP, MiBP, MHiBP, BBzP, MBzP, DnOP, MCPP, DiNP, MCOP, DiDP, MCNP, DEHP, MEHP, MEHHP, MEOHP, MECPP, ΣDEHP, ΣAA	miRNAs 2: miR-10a-5p and miR-577 Phthalates 2: MHBP and MEHHP	In fibroid tumors, but not myometrium, biomarkers of certain phthalates were associated with miRNA expression. The association varies by race/ethnicity.
Lee et. al, 2020a [32]	484 95 cases/336 controls	South Korea	Cross-Sectional Study	Fibroid prevalence from transvaginal ultrasound	Phthalate metabolites (18: MMP, MCPP, MEP, MiPrP, MECPP, MCMHP, MEHHP, MEOHP, MiBP, MnBP, MBzP, MCHP, MPeP, MEHP, MOP, MiNP, MiDP, ΣDEHP) Bisphenols (8: BPS, BPAF, BPF, BPA, BPB, BPAP, BPZ, BPP), Parabens (10: MeP, EtP, PrP, BzP, BuP, HeP, OH-MeP, OH- EtP, 4-HB, 3,4-DHB), Benzophenones (4: BP1, BP2, BP3 4OH-BP), and Antimicrobials (2: TCC, TCS)	Phthalates: 6: MMP, MECPP, MEOHP, MEHHP, MCMHP, ΣDEHP, Parabens: 1: DHB	Certain parabens and phthalates were associated with increased risk of fibroids. Associations between chemical exposures and fibroid risk was stronger among more frequent users of personal care products.
Lee et al., 2020b [33]	111 32 cases/79 controls	South Korea	Cross-Sectional Study	Fibroid prevalence from transvaginal ultrasound	Phthalate metabolites (15: MMP, MEP, MiPP, MiBP, MBP, MPeP, MBzP, MCHP, MHxP, MEOHP, MEHHP, MECPP, MCMHP, MEHP, MCPP), OPEs (12: 4-HO-DPHP, DPHP, 5-HO-MEHP, EHPHP, BCIPHIPP, BCIPP, TCEP, BBOEP, BBOEHEP, 3-HO-TBOEP, BDCIPP, DNBP), AP metabolites (13: OH-MINP, cxMINP, OH-MEHTP, MEHTP, OH-MEHA, oxoMEHA, MEHA, cxMPrHpP, OH-MPrHpP, oxoMPrHpP, cxMINCH, OH-MINCH, MINCH)	Phthalates: MBzP, OH-MINP; MEOHP, MEHHP, MECPP, ΣDEHP OPEs: BDCIPP, BBOEP, BBOEHEP APs: OH-MEHTP, OH-MPrHpP, OH-MINCH	Certain phthalates, OPEs, and APs are associated with increased risk of fibroids. Mixtures of phthalates and OPEs are also associated with increased fibroid risk.

Notes: 3-HO-TBOEP, bis(2-butoxyethyl) 3'-hydroxy-2-butoxyethyl phosphate; 3,4- DHB, 3,4-hydroxybenzoic acid; 4-HB, 4-hydroxybenzoic acid; 4-HO-DPHP, 4-hydroxyphenyl phenyl phosphate; 5-HO-MEHTP, 2-ethyl-5-hydroxyhexyl diphenyl phosphate; AA, anti-androgenic; AP, alternative plasticizers BBOEHEP, 2-hydroxyethyl bis(2-butoxyethyl) phosphate; BBOEP, bis(2-butoxyethyl) phosphate; BBzP, butylbenzyl phthalate; BCIPHIPP, 1-hydroxy-2-propyl bis)1-chloro-2-propyl) phosphate; BCIPP, bis(1-chloro-2-propyl) phosphate; BDCIPP, bis (1,3-dichloro-2-propyl phosphate; BuP, butyl paraben; BzP, benzyl paraben; cxMINCH, cyclohexane-1,2-dicarboxylic mono carboxyisooctyl ester; cxMINP, mono(4-methyl-7-carboxyheptyl phthalate; cxMPrHpP, mono(2-propyl-6-carboxyhexyl) phthalate; DEHP, Σdi-(2oehtyl hexyl) phthalate; DiBP, diisobutyl phthalate; DiNP, diisodecyl phthalate; DNBP, di-n-butyl phosphate; DnBP, di-n-butyl phthalate; DnOP, di-n-octyl phthalate; DPHP, diphenyl phosphate; EHPHP, 2-ethylhexyl phenyl phosphate; EtP, ethyl paraben; HeP, heptyl paraben; MBP, mono-n-butyl phthalate; MBzP, mono-benzyl phthalate; MBzP, monobenxyl phthalate; MCHP, mono-cyclohexyl phthalate; MCMHP, mono- (2-carboxylmethylhexyl) phthalate; MCNP, monocarboxynoyl phthalate; MCOP, monocarboxyoctyl phthalate; MCPP, mono- (3-carboxypropyl) phthalate; MECPP, mono- (2-ethyl-5-carboxypentyl) phthalate; MEHA, mono(2-ethylhexyl) adipate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono-2-ethylhexyl phthalate; MEHTP, mono(2-ethylhexyl) terephthalate; MEOHP, mono- (2-methyl-5-oxyhexyl) phthalate; MeP, methyl paraben; MEP, mono-ethyl phthalate; MHBP, mono-hydroxybutyl phthalate; MHiBP; mono-hydroyisobutyl phthalate; MHxP, monohexyl phthalate; MiBP, mono-isobutyl phthalate; MiBP, monoisobutyl pthphthalate; MiDP, mono-isodecyl phthalate; MINCH, cyclohexane-1,2- dicarboxylic mono isononyl ester; MiNP, mono-isononyl phthalate; MiPP, mono-isopropyl phthalate; MiPrP, mono- isopropyl phthalate; MMP, mono-methyl phthalate; MnBP, mono-n-butyl phthalate; MOP, mono-n-octyl phthalate; MPeP,

mono- n-pentyl phthalate; OH- EtP hydroxylated ethyl paraben; OH- MEHA, mono(2-rthyl-5-hydroxyhexyl) adipat; OH- MEHTP, mono(2-ethyl-5-hydroxyhexyl) terephthalate; OH- MeP, hydroxylated methyl paraben; OH-MINCH, cyclohexane-1,2-dicarboxylic mono hydroxyisonomyl ester; OH-MINP, mono(4-methyl-7-hydroxyoctyl) phthalate; OPE, organophosphate esters; oxoMEHA, mono(2-ethyl-5-oxohexyl adipate; PrP, propyl paraben; TCEP, tris(chloroethyl) phosphate.

Table 2.

Summary of the evidence of environmental EDC exposure in human and animal uterine fibroid experimental models.

Model	Chemicals and dose	Design	Main results/ conclusions	Reference
Primary culture of human uterine fibroid cells	BPA: 10 μM for 24, 48 and 72 h	*In-vitro* Fibroid primary cell culture from tissues extracted from premenopausal women undergoing hysterectomy (n=15)	BPA promoted proliferation of human fibroid cells by GPR30-EGFR-dependent pathway by essentially activating MAPK/ERK/c-fos signaling pathway.	Li et al., 2019 [36]
Culture of human uterine fibroid cell line	DEHP: Low dose: 0.01 μM High dose: 1 μM for 24 and 48 h	*In vitro* GM10964 human fibroid cell line	DEHP-treated fibroid cells presented increased viability and PCNA protein levels, and higher protein levels of Bcl-2, an anti-apoptotic protein, as well as lower apoptosis rates (TUNEL, Annexin V-PI) compared with controls when treated with 0.01μM (48 h) or 1μM (24 and 48 h). Also, low and high dose DEHP treatment promoted cell viability and anti-apoptotic protein expression and induced the expression of inflammatory proteins such as HIF-1α and COX-2 in human fibroid cells after 48h of treatment.	Kim, 2018 [37]
	BPA: Dose range: 10^{-6} - 200 μM for 24, 48, and 72 h	*In vitro* ht-UtLM human fibroid cell line	BPA at low concentrations triggered the entry of cells into S phase and increased proliferation whereas, higher concentrations of BPA (100 μM–200 μM) decreased growth. Moreover, low doses of BPA significantly induced gene and protein expression of ERα36 which is involved in the proliferative effects on fibroid cells induced by this EDC, through activation of Src, EGFR, Ras, ERK nongenomic signaling.	Yu et al., 2019 [38]
Rat	TBT: 10 and 100 ng/kg/day BPA: 50 μg/kg/day (positive control) VEH:10% ethanol-90% sesame oil/day SC injection on PND 1–16	*In vivo* treatment Sprague-Dawley rats (n=13 each group) *Ex vivo* Uteri from TBT- and BPA-treated rats at 6-month-old	TBT led to uterine dysplasia of endometrial epithelial cells and glands, in part through changes on Wnt-β-catenin signaling. TBT activated TNFα and NF-κB signaling pathways causing inflammation and upregulated the TGF-β1/SMADs signaling pathway which trigged uterine fibrosis.	Chen et al., 2020 [39]
	DES: 10 μg/day VEH: 50 μl of sesame oil/day SC injection on PND 10–12	*In vivo* treatment Long Evans Eker rats (Tsc2 Ek/+) (n=5 each group) *In vitro* Isolation of VEH and DES-exposed MMSC using Stro-1/CD44 surface markers from 5-month-old rats and culture	DES-MMSC showed decreased DNA end-joining ability, higher levels of DNA damage, and impaired ability to repair DNA double-strand breaks relative to VEH-MMSC, leading to acquisition of mutations that may promote the origin and progress of tumors in adult life. Early-life developmental DES exposure increases DNA damage and alters MMSC's ability to repair and reverse DNA damage	Prusinski Fernung et al., 2018 [40]
	DES: 10 μg/day VEH: 50 μl of sesame oil/day SC injection on PND 10–12	*In vivo* treatment Long Evans Eker rats (Tsc2 Ek/+) (n=5 each group) *In vitro* Isolation of VEH and DES-exposed MMSC using Stro-1/CD44 surface markers from 5-month-old rats and culture	Early-life DES exposure increased DNA damage and altered MMSC–s ability to repair DNA damage, through the downregulation of RAD50 and MRE11, which are critical DNA double-strand breaks sensors on homologous recombination DNA repair pathway, causing genomic instability.	Elkafas et al., 2020 [41*]

Notes: BPA: Bisphenol A; COX-2: Cyclooxygenase-2; DEHP: Di-(2-ethylhxyl)-phthalate; DES: Diethylstilbestrol; GFR: Epidermal growth factor receptor; ERK: extracellular signal-regulated kinase; ERα: Estrogen receptor alpha; ERα36: Estrogen receptor alpha variant 36; GPR30: G protein-coupled receptor for estrogen; h: hours; HIF-1α: hypoxia inducible factor 1α; MMSCs: Myometrial Stem Cells; n: sample size; NP: Nonylphenol; OP: Octylphenol; PCNA: Proliferating cell nuclear antigen; PI: Propidium iodide; PND: postnatal day; SC: subcutaneous; TBT: Tributyltin; TGB-β: Transforming growth factor beta; Tsc2: Tuberous Sclerosis Complex 2 gene; VEH: Vehicle.