Endocrine disruption of oestrogen action and female reproductive tract cancers

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Abstract

Endocrine disrupting chemicals (EDC) are ubiquitous and persistent compounds that have the capacity to interfere with normal endocrine homoeostasis. The female reproductive tract is exquisitely sensitive to the action of sex steroids, and oestrogens play a key role in normal reproductive function. Malignancies of the female reproductive tract are the fourth most common cancer in women, with endometrial cancer accounting for most cases. Established risk factors for development of endometrial cancer include high BMI and exposure to oestrogens or synthetic compounds such as tamoxifen. Studies on cell and animal models have provided evidence that many EDC can bind oestrogen receptors and highlighted early life exposure as a window of risk for adverse lifelong effects on the reproductive system. The most robust evidence for a link between early life exposure to EDC and adverse reproductive health has come from studies on women who were exposed in utero to diethylstilbestrol. Demonstration that EDC can alter expression of members of the HOX gene cluster highlights one pathway that might be vulnerable to their actions. In summary, evidence for a direct link between EDC exposure and cancers of the reproductive system is currently incomplete. It will be challenging to attribute causality to any single EDC when exposure and development of malignancy may be separated by many years and influenced by lifestyle factors such as diet (a source of phytoestrogens) and adiposity. This review considers some of the evidence collected to date.

Key Words
- endocrine disruptor
- endocrine disrupting chemical
- oestrogen receptor
- reproductive cancer
- endometrial cancer
- obesity
- diethylstilbestrol
- bisphenol A
- dioxin
- phytoestrogen
- genistein
- HOX genes

Introduction

The tissues of the female reproductive tract (vagina, cervix, uterus and fallopian tube) are key targets for the action of oestrogens by virtue of their expression of oestrogen receptors (ERs). During a woman’s reproductive years, these tissues are subjected to cyclical variations in circulating concentrations of endogenous oestrogens that are synthesised and secreted by the ovarian follicles during natural menstrual cycles. Malignancies of the female reproductive tract are the fourth most common cancer in women (http://www.cancerresearchuk.org/cancer-info/cancerstats/incidence/commoncancers/uk-cancer-incidence-statistics-for-common-cancers#Top3; accessed August 2013). Endometrial cancer accounts for the majority of these cancers, although cervical and vaginal tissues are also susceptible to neoplastic transformation (Doll et al. 2008). Cancer of the fallopian tube is very rare (Alvarado-Cabrero et al. 2013). Recently, emerging evidence has indicated that cells located in the
fimbrial end of the fallopian tube may hold the key to development of some forms of cancers previously thought to be ovarian in origin (Dubeau 2008, Flesken-Nikitin et al. 2013).

Endocrine disrupting chemicals (EDC) are compounds that are known to interfere with normal hormone signalling and action. The most significant EDC are environmental pollutants or major constituents of preservatives and industrial plasticizers. Approximately 800 chemicals are known or suspected to have the potential to function as EDC (Bergman et al. 2013).

The effects of EDC on reproductive health of animals have been widely reported and animal models have been used to inform studies on the consequences of human exposure. For example, decreased fertility has been reported in fish and birds and feminisation of male fish, birds and mammals exposed to EDC in the environment documented (reviewed in Colborn et al. 1993). Notably, early life exposure has been identified as a key window of susceptibility for development of disorders of the female reproductive system in both animals and humans (see reviews by Miller et al. (2004) and Crain et al. (2008)).

EDC can have pleiotropic action throughout the body with effects being mediated via disruption of steriod-hormone-dependent signalling; in addition to effects on the reproductive system, some EDC are reported to have the potential to interfere with neuroendocrine regulation, adiposity, metabolism and the immune system (Newbold et al. 2005, Ponzo & Silvia 2013). A summary of the steroid receptor targets of a selection of widely studied EDC are presented in Table 1. It is notable that some EDC such as bisphenol A (BPA) are promiscuous in their action and have activity at several hormone receptors that may complicate interpretation of their precise role(s) in the development of malignancy.

Although exposure to EDCs has been suggested as a contributing factor to a range of women’s health disorders including infertility, polycystic ovaries, uterine fibroids and the early onset of puberty, considerable challenges remain in attributing cause and effect (reviewed in Crain et al. (2008)). In this review, we consider the evidence that exposure to EDCs can increase the lifetime risk of developing a reproductive tract cancer and the mechanisms that might be responsible for their effects.

### Table 1 Pleiotropic endocrine disrupting effects of major EDCs and their reported effect on nuclear hormone receptors

<table>
<thead>
<tr>
<th>EDC</th>
<th>ER</th>
<th>AR</th>
<th>Other nuclear receptors</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DES</td>
<td>Agonist</td>
<td>Weak binding affinity</td>
<td>Inverse agonist of ERRα, ERRβ and ERRγ</td>
<td>Kuiper et al. (1998) and Giguere (2002)</td>
</tr>
<tr>
<td>BPA</td>
<td>Agonist</td>
<td>Anti-androgen</td>
<td>Strong binding affinity to ERRγ</td>
<td>Kuiper et al. (1998), Morito et al. (2001), Moriyama et al. (2002), Lee et al. (2003), Takayanagi et al. (2006), Matsushima et al. (2007) and Sai et al. (2012)</td>
</tr>
<tr>
<td>Genistein (phytoestrogen)</td>
<td>RBA ERβ: ERα; activates transcription via ERE</td>
<td>Anti-androgen via ERβ action Very weak binding to AR (0.003 RBA DHT)</td>
<td>Agonist of PXR; Antagonist of ThR</td>
<td>Kuiper et al. (1998), Morito et al. (2001), Bektic et al. (2004) and Freyberger &amp; Ahr (2004)</td>
</tr>
<tr>
<td>Coumestrol (phytoestrogen)</td>
<td>RBA ERβ: ERα; activates transcription via ERE</td>
<td>No IC50 demonstrated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DDT and metabolites</td>
<td>Binds ERα and ERβ, transcriptional activation</td>
<td>Anti-androgen (p,p'-DDE)</td>
<td></td>
<td>Kelce et al. (1995), Klotz et al. (1996) and Kuiper et al. (1998)</td>
</tr>
<tr>
<td>Dioxins</td>
<td>TCDD modulates ERα activity through binding AhR</td>
<td>TCDD blocks androgen-dependent proliferation of LNCaP cells</td>
<td>Crosstalk with COUP-TF1 and ERRα via AhR</td>
<td>Klinge et al. (2000), Ohtake et al. (2003) and Barnes-Ellebe et al. (2004)</td>
</tr>
<tr>
<td>Polychlorinated biphenyls</td>
<td>Weak binding</td>
<td></td>
<td></td>
<td>Kuiper et al. (1998) and Fang et al. (2003)</td>
</tr>
</tbody>
</table>

RBA, relative binding affinity; ER, oestrogen receptor; AR, androgen receptor; DES, diethylstilbestrol; ERR, oestrogen-related receptor; BPA, bisphenol A; PXR, pregnane X receptor; ThR, thyroid hormone receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; AhR, aryl hydrocarbon receptor.
ER signalling

The most abundant endogenous oestrogens in normal premenopausal women, oestrone (E₁) and oestradiol (E₂) (Fig. 1), are lipophilic steroid hormones that activate cognate receptors in target tissues to alter cell function (Gruber et al. 2002). In women, there are two subtypes of ER encoded by separate genes, ERα and ERβ, both of which are widely expressed in cells throughout the reproductive system (reviewed in Gibson & Saunders 2012). Splice variant isoforms of both the ERα (ESR1) and ERβ (ESR2) genes have been described and their ability to participate in oestrogen-dependent signalling investigated using cell-based systems (Moore et al. 1998, Gibson & Saunders 2012). A number of SNPs associated with the same genes have also been documented, some of which are associated with the risk of developing endometrial cancer (Ashton 2012). A number of SNPs associated with the same genes have also been documented, some of which are associated with the risk of developing endometrial cancer (Ashton 2012).

Ligand-bound ERs can influence gene expression via several different pathways (reviewed in Hall et al. 2001, Nilsson et al. 2001 and Matthews & Gustafsson 2003). Briefly, ligand binding to ERα and ERβ causes a conformational change in the shape of the protein that is different for agonists and antagonists (Paige et al. 1999). An understanding of the 3D structure of both ERα and ERβ has allowed chemists to develop receptor-selective agonists and antagonists (Sun et al. 1999, Paruthiyil et al. 2009). Furthermore, modelling of putative ligand–receptor interactions is now used for in silico screening allowing investigators to predict whether compounds are likely to interact with ERs (Biesiada et al. 2011) and has been applied to environmental pollutants (Li et al. 2012a). Following ligand binding, ERs will form homo- or heterodimers, depending on whether one or both receptors are present within the cell; dimers interact with other co-regulatory proteins at binding sites in the promoter regions of genes either directly or in association with other transcription factors such as Jun/Fos and Sp1 (reviewed in Nilsson et al. 2001 and Heldring et al. 2007). In summary, prediction of the effects of an oestrogenic ligand, be it natural, synthetic, or an EDC, on cell function needs to consider whether it can bind to one or both ERs, and whether it can out-compete other ligands present within the tissue microenvironment, as well as the nature of the binding sites within the regulatory elements of the target genes.

ER expression and actions of oestrogens in reproductive tract tissues

During the first trimester of pregnancy (9.5–11.5 weeks of gestation), the female reproductive tract develops from Müllerian ducts of mesodermal origin. The vagina, cervix and uterus develop into distinct organs as a result of differentiation of the epithelium mediated by the underlying mesenchyme of each organ (Cunha 1976) and the activities of the distal Hox gene cluster (reviewed in Daftary & Taylor 2006). The fetal ovary has only a limited capacity for sex steroid biosynthesis (Fowler et al. 2011), but from puberty to menopause, a woman’s reproductive system is exposed to successive waves of oestrogen and progesterone secreted by the ovaries (Abraham 1974, Johannisson et al. 1987). Uterine endometrial gland formation starts in utero but is only completed at puberty when sex steroid levels rise. Steroids are also required for differentiation of the cervix (Hwang et al. 2009) that is completed during puberty when basal columnar epithelial cells transform into squamous epithelium.

ERα and ERβ are expressed in cells distributed throughout the female reproductive tract. In the adult human endometrium, their expression is both temporally and spatially regulated (Critchley et al. 2001). For example,
expression of ERz varies in a cycle-dependent manner within the functional layer; ERz is down-regulated in both epithelial and stromal cells during the secretory phase whereas expression is maintained in the same cell types within the basal layer (Critchley et al. 2001). Expression of ERβ exhibits little dynamic change across the cycle (Critchley et al. 2001). A truncated variant (ERβ2) that can form heterodimers with full-length ERs but does not contain a functional ligand binding pocket is also expressed (Critchley et al. 2002, Sierens et al. 2004) as are orphan receptors implicated in regulation of oestrogen responsiveness (Bombail et al. 2008, 2010). Notably, endothelial and immune cells within the human endometrium are immunopositive for ERβ but do not contain ERz protein (Henderson et al. 2003, Greaves et al. 2013). In the human fallopian tube, ER mRNAs are constitutively expressed during the menstrual cycle and have been immunolocalised to epithelial, stromal and smooth muscle cells (Horne et al. 2009). In the cervix, expression of ERz and ERβ has been documented in both stromal and epithelial cells (Taylor & Al-Azzawi 2000), whereas endothelial cells and leukocytes appear to express ERβ alone as is the case in the endometrium (Stygar et al. 2001). Both receptor subtypes are expressed in the myometrium with evidence for altered ratios between ERz and ERβ in tissue recovered from pre- and postmenopausal women (Sakaguchi et al. 2003), a reported increase in ERβ in myometrium at the end of pregnancy (Wu et al. 2000) and evidence that myometrial endothelial cells are ERβ-positive/ERα-negative (Greaves et al. 2013).

Studies using cells, tissue explants and animal models have highlighted the importance of ERz-dependent signalling in E2-dependent cell proliferation and expression of progesterone receptors (Harris et al. 2002, Punyadeera et al. 2004). In ovariectomised (ovx) mice, injection of E2 is sufficient to stimulate a three- to fourfold increase in uterine wet weight (Lubahn et al. 1993), a finding that is the basis of the widely used uterotrophic assay (Table 2). Female ERz knockout (ERzKO) mice have hypoplastic uteri with sparse glands (Lubahn et al. 1993). Although the uterine weight in ERzKO mice is normal, they are hyper-responsive to E2-stimulated uterine proliferation (Dupont et al. 2000), a finding consistent with studies using cell lines that have demonstrated co-expression of ERβ with ERz can restrain the activities of the latter (Hall & McDonnell 1999). A study exploring the effects of oestrogens on endothelial cells derived from the endometrium and myometrium has recently reported that signalling via ERβ has opposite effects on network formation in the two cell types (Greaves et al. 2013). Thus, oestrogen action is dependent on ER isoform expression, which is cell-context-dependent.

Table 2  Models for assessing oestrogenicity and the responses of selected EDC

<table>
<thead>
<tr>
<th>Oestrogenic assay</th>
<th>EDC</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BPA; mixed agonist/antagonist concentration dependent</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Phytooestrogens: genistein, coumestrol; mixed agonist/antagonist concentration dependent</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PCBs; agonist</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>TCDD; antioestrogen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER binding affinity</td>
<td>Strong affinity: DES (ERz and ERβ), coumestrol (ERβ 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate affinity: genistein 1</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Weak affinity: DDT metabolites, PCBs, BPA 1,2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luciferase reporter assay (ERE)</td>
<td>Transcriptional activation: DES, BPA, genistein, coumestrol, PCBs, DDT</td>
<td>In vitro, both ER isoforms investigated</td>
<td>Kuiper et al. (1998), Frigo et al. (2002) and Li et al. (2013)</td>
</tr>
<tr>
<td>Luciferase reporter assay (AP1)</td>
<td>Transcriptional activation: BPA, DDT metabolites</td>
<td>In vitro, both ER isoforms investigated</td>
<td>Frigo et al. (2002) and Li et al. (2013)</td>
</tr>
<tr>
<td>Yeast β-galactosidase reporter assay</td>
<td>Transcriptional activation: DES, coumestrol, BPA, DDT metabolites</td>
<td>In vitro, Transient expression of human receptors E.g. PGR, CXCL12</td>
<td>Gaido et al. (1997)</td>
</tr>
<tr>
<td>Transcription of oestrogen-responsive genes</td>
<td>DES, BPA, genistein, coumestrol, benzophenones, PBDE</td>
<td></td>
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Oestrogen biosynthesis and ER expression in benign and malignant endometrial tract disorders

Altered expression of enzymes involved in steroid biosynthesis resulting in an increased capacity to produce oestrogens has been reported for both benign and malignant disorders of the reproductive system. Uterine fibroids (leiomyomas) are benign tumours that arise as a result of aberrant proliferation of smooth muscle cells within the myometrium (Cramer & Patel 1990). Tissue concentrations of oestrogens are elevated in fibroids (Pasqualini et al. 1990) and oestrogens are thought to drive cell proliferation.

In the European Prospective Investigation into Cancer and Nutrition (EPIC) study, the presence of endometrial cancer was positively associated with increased circulating levels of testosterone, E1 and E2 (Allen et al. 2008). An increase in expression of the CYP19A1 gene encoding the aromatase protein, which plays a critical regulatory role in biosynthesis of E1 and E2, has been documented in both endometrial and cervical cancers, consistent with a role for local E2 biosynthesis in disease progression (Bulun et al. 1994, Nair et al. 2005).

Expression of ERs has been documented in uterine smooth muscle (leiomyosarcomas) (Rodriguez et al. 2011), endometrial (Collins et al. 2009) and cervical (Nair et al. 2005) cancers. Studies examining expression of ERs in stage 1 endometrial adenocarcinomas have highlighted a reduction in expression of ERα and progesterone receptor in tissues graded as poorly differentiated and an inverse relationship between ERα and the COX-2 enzyme (Collins et al. 2009) consistent with a role for oestrogens in regulating expression of inflammatory mediators that can influence tumour progression (Wallace et al. 2010). In contrast to cells within the normal endometrium, proliferation of epithelial cells within endometrial adenocarcinomas does not appear to be ERα dependent, which may in part explain the mixed results obtained using aromatase inhibitors to treat endometrial cancer (Bulun et al. 2007).

Evidence that oestrogens increase the risk of developing a reproductive cancer

Oestrogens can act as potent mitogens in ER-positive cells with continual exposure to an oestrogenic stimulus having the potential to promote DNA instability, cellular hyperplasia and neoplastic transformation of epithelial cells into carcinomas (Key & Pike 1988). In normal menstrual cycles, the effects of oestrogen-driven proliferation on endometrial tissue are limited by progesterone produced by the corpus luteum following ovulation, with progesterone down-regulating expression of ERα during the secretory phase (Crichtley et al. 2001). Established risks of developing endometrial cancer such as high BMI (Arem et al. 2013), nulliparity, early onset of menses (Purdie & Green 2001) and the duration of the menopausal transition (Hale et al. 2002) are associated with increased lifetime exposure to oestrogens.

An increased risk of developing endometrial cancer is associated with exposure to tamoxifen, a selective ER modulator that exhibits mixed antagonist/agonist activity (Jordan 2003). Although tamoxifen is effective as a treatment for breast cancer by virtue of its antagonism of ERs expressed in breast cancer cells, in uterotrophic assays, it behaves as an agonist stimulating uterine weight, epithelial height, and the transcription of ER-responsive genes (Kwekel et al. 2009). The uterotrophic effects of tamoxifen are consistent with an increased risk of endometrial cancer observed in patients who have received tamoxifen treatment for breast cancer (Kedar et al. 1994, Bergman et al. 2000).

Postmenopausal oestrogen-only hormone replacement therapy has been associated with increased occurrence of endometrial, ovarian and breast cancers (Lacey et al. 2002, Beral et al. 2005b); however, inclusion of progestagens in the hormone replacement therapy (HRT) formulations is reported to reduce risk (Beral et al. 2005a), consistent with the physiological effect of progesterone in counteracting oestrogen-driven proliferation. Furthermore, the use of the combined oral contraceptive pill is apparently protective against endometrial cancer risk (Emons et al. 2000), an effect that persists even after discontinuation of use (La Vecchia et al. 1996). Thus, exposure to oestrogens unopposed by progestagens poses greater risk of endometrial cancer.

Clinically, endometrial cancers have classically been divided into oestrogen-dependent type I and the less common, but clinically more aggressive, oestrogen-independent type II. In women, the majority of endometrial cancers are oestrogen-dependent type I cancers with increased risk associated with exposure to excess oestrogens (Emons et al. 2000). However, in a recent examination of the risk factors for type I and type II endometrial cancer from 14 069 endometrial cancer cases, Setiawan et al. (2013) reported that type I and type II endometrial cancer share many common aetiological factors. Notably, parity, oral contraceptive use, cigarette smoking, age at menarche and diabetes were found to be
associated with both tumour types to a similar extent. However, BMI had a greater effect on risk of type I tumours than type II. Thus, while increased lifetime exposure to oestrogens is likely to have the greatest effect on the risk of developing type I endometrial cancers, there may also be some interaction with risk of developing type II endometrial cancers. To date, the majority of animal models assessing the effects of EDC on reproductive tract cancers have assessed development of oestrogen-dependent adenocarcinomas, i.e. modelling type I endometrial cancers. As such the investigation into the influence of EDC on endocrine-related gynaecological cancers has been largely restricted to this specific subset of reproductive tract cancers.

Persistent infection with human papillomavirus (HPV) is the predominant risk factor for invasive cervical carcinoma and precursor lesions (Bosch & de Sanjose 2007). Oestrogens are thought to modify HPV-associated risk. For example, in HPV16 transgenic mice (the HPV transgenic mouse model), oestrogen is required for the genesis and persistence of cervical cancer (Brake & Lambert 2005). In addition, studies in transgenic mice in which stromal ERα has been deleted have confirmed an essential requirement for ERα expression in oestrogen-dependent cervical cancer in the HPV mouse model (Chung et al. 2013). Interestingly, results from the EPIC study found that an increased risk of developing cervical cancer in premenopausal women was associated with increased concentrations of free testosterone and circulating levels of E2 (Rinaldi et al. 2011).

Models for assessing oestrogenicity of EDC
A summary of the assays that are most widely quoted in studies analysing the oestrogenicity of EDC is provided in Table 2. Primary methods for identifying oestrogenic effects of EDCs typically involve uterotrophic assays in laboratory animals such as mice or rats. This assay is based on the classical physiological response of the uterus following exposure to oestrogen: increased uterine weight due to fluid imbibition and increased epithelial proliferation. In this assay, EDC are considered oestrogenic if they induce increases in uterine dry or wet weight or affect other endpoints such as epithelial proliferation, epithelial cell height or the expression of oestrogen-responsive genes. Uterotrophic assays are reported to give variable responses depending on the specifics of the assay protocol such as dosing regimen, route of administration and whether immature or mature ovx animals are used (Kang et al. 2000). A limitation of this assay is that the uterotrophic response is mediated via ERα and therefore only EDC that are able to bind, activate or modulate ERα will elicit a positive response. To address this limitation, cell-based assays have been developed to investigate the capacity for EDC to promote ER-dependent transcription (Shelby et al. 1996), providing the opportunity for assessment of activity on ERβ or combinations of ERα/ERβ as well as other receptors (Table 2) that must be born in mind when assessing the risks associated with exposure/bioaccumulation.

Oestrogenic EDC
EDC from a variety of sources have been identified as having oestrogenic activity. These include industrial products and pollutants, plastic components, detergents, pesticides, a number of household cleaning products as well as personal care products such as sunscreens, cosmetics and hair dyes (reviewed in De Coster & van Larebeke (2012)). The oestrogenic activities of EDC such as diethylstilbestrol (DES) and BPA have been comprehensively studied and are described in the following sections. However, it is notable that several other EDC are recognised as having oestrogenic properties but their effect, particularly on the development of reproductive tract cancers, has not been extensively assessed. For example, polybrominated diphenyl ethers (PBDE) are flame-retardant additives found in several consumer products. PBDE is reported to alter the expression of oestrogen target genes in the rat uterus (Ceccatelli et al. 2006) and is reported to induce DNA damage in human
neuroblastoma cells (Pellacani et al. 2012). Alkylphenols such as 4-n-octylphenol, 4-tert-octylphenol and nonylphenol have weak oestrogenic activities (Kuiper et al. 1998). At high doses (50 mg/kg), microarray analysis has revealed that nonylphenol activates similar genes to E2 in the mouse uterus (Watanabe et al. 2004). Nonylphenol may also influence endometrial cancer development through activation of pregnane X receptor, which is highly expressed in human endometrial cancers (Masuyama et al. 2003, 2007). Chemicals that absorb u.v. (u.v. filters) are widely used in sunscreens and in a variety of cosmetic products for protection against u.v. radiation. Using a recombinant yeast reporter assay, Kunz & Fent (2006b) investigated hormonal activities of u.v. filters and reported that several of the compounds assessed exhibited agonist effects at ERα: benzophenone-1 (BP1), benzophe- none-2 (BP2), 4-hydroxybenzophenone (4HB), 4,4'-dihydroxybenzophenone (4DHB) and ethyl-4 amino benzoate (et-PABA); furthermore, several of the u.v. filters exhibited androgenic, anti-androgenic and anti-oestrogenic activity highlighting a diverse capacity for endocrine disruption. Notably, BP1, 4HB, 4DHB and et-PABA exhibited both oestrogenic and anti-androgenic activities (Kunz & Fent 2006b). In a further study, the capacity for mixtures of u.v. filters to activate ERα-mediated transcription was investigated in a recombinant yeast assay and it was found that low concentration mixtures of u.v. filters exhibited a synergistic increase in activity of an ERα-driven reporter gene (Kunz & Fent 2006a). In addition, BP2 is reported to increase uterine wet weight and induce expression of oestrogen target genes in the rat uterus (Schlecht et al. 2006). BP's are reported to increase proliferation of MCF-7 cells in an ER-dependent manner, stimulate reporter gene activity via oestrogen response elements (ERE) and Sp1 but not AP1 and increase mRNA expression of ER target genes such as CXCL12, amphiregulin, pS2 and progesterone receptor (Kerdivel et al. 2013).

**Diethylstilbestrol**

DES, a synthetic non-steroidal oestrogen, is often regarded as the archetypal endocrine disruptor (Fig. 1). From about 1940 to 1970, DES was given to pregnant women in the mistaken belief that it would reduce the risk of pregnancy complications. Herbst et al. (1971) reported a probable link between DES and vaginal clear cell adenocarcinoma in girls and young women who had been exposed to this drug. It is estimated that five to ten million people were exposed to DES, including the pregnant mothers who received treatment and their offspring. Of the several million women exposed to DES in utero, a cohort of 4653 DES-exposed women have been followed up to investigate the long-term consequences of exposure (Hoover et al. 2011). Patient data stratified to account for the extent of exposure or dose effects of DES identified an association between treatment of mothers earlier during their pregnancy and adverse vaginal epithelial changes at a younger age in their offspring (Hoover et al. 2011). While DES exposure is associated with increased risk of breast and cervical/vaginal clear cell adenocarcinoma, several studies have indicated that there is no associated risk of endometrial or ovarian cancer (Troisi et al. 2007, Hoover et al. 2011). As endometrial cancer is most likely to present after menopause, many of the DES-exposed women may not yet be old enough to determine whether they are at excess risk, as in the 2011 report only 27% were older than 50 years (Hoover et al. 2011). Although to date epidemiological data indicate that DES-exposed women may not be at increased risk of developing endometrial cancer, studies in animal models provide evidence to the contrary.

In the early 1990s, Newbold et al. (1990) developed a mouse model for investigating hormonal carcinogenesis in mice by investigating the effects of neonatal exposure to oestrogens on cancer development. Treatment of CD1 neonatal mice with DES on postnatal days 1–5, which correspond to late prenatal human development, resulted in 90% of DES-exposed mice developing uterine adenocarcinomas after 18 months while none of the control animals had neoplastic lesions (Newbold et al. 1990). Crucially, while administration of DES increased the risk of uterine adenocarcinoma, endogenous oestrogen was required for tumour development with prepubertal ovariectomy preventing tumour development (Newbold et al. 1990). In DES-exposed women, vaginal and cervical carcinomas were only detected post-menarche consistent with a requirement for endogenous oestrogen in tumour development (Hoover et al. 2011). ERα knockout mice (ERKO) did not develop tumours following neonatal DES exposure (Couse & Korach 2004); transgenic mice over-expressing ERα displayed accelerated tumour development (Couse et al. 1997) but mice with a dominant negative isoform of ERα (ERΔ3) were not protected (Davis et al. 2012), highlighting the complexity of the molecular signalling mechanisms involved.

Interestingly, gene expression analysis indicates that developmental DES exposure results in persistent altered gene expression of oestrogen-responsive genes in the uterus that may explain the increased susceptibility to tumour development. Gene ontology analysis of microarray data revealed altered expression of genes involved in...
Bisphenol A

BPA is an industrial chemical primarily used to make plastics and is also found in epoxy resins that line many canned foods and beverages (Brotons et al. 1995). BPA was identified as an oestrogenic substance by Krishnan et al. (1993) who reported that it had weak binding affinity for ER (1/2000th that of E2), was able to induce proliferation of MCF7 cells (ERα-positive, breast epithelial carcinoma cell line) and expression of progesterone receptor. BPA has structural similarities to E2 (Fig. 1) and has been shown to interact with both ERα and ERβ (Kwekel et al. 2009). In vitro assays have confirmed that BPA can activate ERs in a luciferase reporter assay (Table 2). The activity of BPA has also been investigated using the ER action indicator (ERIN) mouse, which has been engineered to express a transgene with an oestrogen-responsive promoter linked to a β-galactosidase reporter gene (Nagel et al. 2001). Interestingly, BPA was reported to be a potent agonist of ER transcriptional activity in the uterus of the ERIN mouse (Nagel et al. 2001) but other studies report that BPA only weakly stimulated uterine weight gain in mice (Papacostantinou et al. 2000, Tinwell et al. 2000) and rats (Ashby & Tinwell 1998, Yamasaki et al. 2000). BPA also weakly activated the IGF signalling pathway via ERα in the uterus of ovx adult mice, leading to an increase in mitotic cells, indicating that BPA may also promote epithelial proliferation via alternative signalling pathways (Klotz et al. 2000). In a series of in vitro assays, Li et al. (2012b, 2013) demonstrated that BPA and the fluorinated derivative bisphenol AF (BPAF) may activate both ERα and ERβ but that activation was both cell-type- and concentration-dependent. For example, in Ishikawa cells (endometrial adenocarcinoma cell line), BPA at concentrations lower than 10 nM antagonised E2-mediated ERα activation of luciferase activity while in HeLa cells (cervical adenocarcinoma cell line) similar concentrations of BPAF antagonised E2-mediated ERβ activation of luciferase activity (Li et al. 2012b). BPA and BPAF at concentrations greater than 10 nM and up to 100 nM were reported to act as agonists through both ERα and ERβ (Li et al. 2012b, 2013).

In a recent study investigating the effect of BPA on human endometrial stromal cell (ESC) differentiation, BPA was found to decrease proliferation of ESC and decrease expression of mRNAs encoding CYP11A1, HSD17B1 and HSD17B2; however, this effect was only observed with high (50–100 μM) doses (Aghajanova & Giudice 2011). Serum concentrations of BPA measured in both pre- and postmenopausal women were significantly lower in patients with complex endometrial...
hyperplasia or endometrial cancer than in healthy controls (Hiroi et al. 2004).

BPA has been reported to alter expression/activity of enzymes involved in steroid synthesis and metabolism in the murine ovary (Peretz et al. 2011). Although the receptor involved in mediating this effect was not identified, it is notable that BPA can also bind with high affinity to the orphan receptor oestrogen-related receptor γ (Takayanagi et al. 2006) and may also bind androgen receptors (Lee et al. 2003) both of which are expressed in the ovary. While BPA has been shown to affect murine ovarian function, an analysis of human urinary BPA levels found no significant association with expression of CYP19 in human granulosa cells (Ehrlich et al. 2013) leaving the question of whether effects of BPA in women could be mediated by alterations in ovarian function unanswered.

**Dichlorodiphenyltrichloroethane**

Dichlorodiphenyltrichloroethane (DDT) and its metabolites bind ERs and have oestrogenic activity (Klotz et al. 1996). Interestingly, DDT has been shown to mediate ER-dependent changes in gene expression by binding either ERα or AP-1 binding sites in promoters. In addition, DDT has been shown to activate ER-independent transcription in Ishikawa cells (Frigo et al. 2002). DDT and its metabolites have weak binding affinity for ERα and ERβ, although o,p'-DDT has been reported to activate ERα (Kuiper et al. 1998). Despite the reported oestrogenic activity of DDT, epidemiological evidence indicates that there is no significant association between organochlorine exposure and endometrial cancer risk (Sturgeon et al. 1998). In another study serum concentrations of chlorinated pesticides were measured in 154 endometrial cancer patients and 205 healthy controls. While the study found that concentrations of p,p'-DDT, p,p'-dichlorodiphenyl-dichloroethylene (p,p'-DDE), β-HCH and oxychlordane were significantly increased in endometrial cancer patients, when concentrations were adjusted for age and BMI, no significant increased risk was found (Weiderpass et al. 2000).

**Dioxins**

Polychlorinated dioxins, furans and polychlorinated benzene are a group of industrial chemicals that are persistent toxic environmental pollutants. Tetrachlorodibenzo-p-dioxin (TCDD) is a designated carcinogen and the most potent dioxin (Fig. 1). Human exposure is prevalent, largely through diet; dioxins are slowly metabolised and due to their lipophilic nature tend to bioaccumulate (Kogevinas 2001). Dioxins target the endocrine system and have been reported to alter metabolism of oestrogens and androgens (Cooke et al. 1998, van Duursen et al. 2003).

The potential risk of dioxins to human health has been evident from series of industrial exposures, the most informative of which involved accidental exposure of industrial workers to dioxins in Seveso in 1976. Epidemiological studies have revealed an increased overall risk for all cancers and increased risk of breast and endometrial cancer from dioxin exposure in adults (Kogevinas et al. 1997, Bertazzi et al. 1999, Kogevinas 2001).

Dioxins mediate cellular effects through binding of the aryl hydrocarbon receptor (AhR), which heterodimerises with AhR nuclear translocator (ARNT) to mediate transcription through xenobiotic response elements in promoter regions of target genes (Mimura & Fujii-Kuriyama 2003). TCDD can mediate cell-specific oestrogenic and antioestrogenic effects (Grochowska et al. 2001) and the action of TCDD seems to involve crosstalk between AhR and ER (Gierthy et al. 1996). In Ishikawa cells derived from an endometrial adenocarcinoma expressing Erα and AhR, TCDD treatment reduced Erα-mediated increases in cell proliferation and ER-mediated transcription (Wormke et al. 2000). Although this study indicates that stimulation of AhR by dioxins may be protective against oestrogenic stimulation and development of endometrial cancers, Ohtake et al. (2003) suggested that oestrogen signalling could be modulated through association of unliganded ER with AhR. Specifically, following activation of AhR and heterodimerisation with ARNT, the receptor complex is able to recruit unliganded ER and the co-activator p-300 to oestrogen-responsive gene promoters resulting in transcriptional activation (Ohtake et al. 2003), an affect that was attenuated by ligand-bound ER. Thus, low-level exposure to dioxins may only mediate stimulatory effects in the absence/low bioavailability of Erα, indicating that premenarche and postmenopause the reproductive tract might be more vulnerable to adverse stimulation by dioxins.

**Phytoestrogens**

Phytoestrogens are non-steroidal compounds present in dietary foodstuffs and include isoflavones such as genistein, daidzein and coumestrol (Fig. 1). Phytoestrogens are found in a range of legumes and soya-based products, and individuals with diets that are predominantly based on consumption of these foodstuffs have been the focus of several epidemiological studies. It has been reported that
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Phytoestrogens have varying affinities for ERs and this affects their ability to promote ER-dependent ligand-activated transcription. In an elegant study by Kuiper et al. (1998), analysis of binding affinities of various phytoestrogens for ERα and ERβ revealed that several phytoestrogens, including coumestrol and genistein, have greater binding affinity for ERβ than ERα. Interestingly, while most xenoestrogens have weak affinity for ERs, coumestrol and genistein have been reported to have a similar affinity to E2 for ERβ (Kuiper et al. 1998, Morito et al. 2001). It has also been reported that phytoestrogens promote distinct receptor conformations when bound to ERs; 3D modelling based on X-ray crystallography of receptor structure has revealed that genistein binds to ERβ and promotes a distinct confirmation of the AF-2 domain helix 12 in the ligand binding domain, resulting in an orientation that is more similar to that induced by ER antagonists (Pike et al. 1999). This distinct interaction with the ER ligand binding domain may in part explain the partial agonist characteristics of this ligand–receptor interaction. Thus, it is important to consider that individual phytoestrogens can have different effects on ERs, that their affinity for ER isoforms also differs, that the resultant transcriptional response may be variable and that their effects on the endometrium could vary during the menstrual cycle and be different depending on the grade of endometrial cancer.

Evidence that expression of HOX genes can be altered by exposure to EDC

It is apparent from a number of studies highlighted in this review and others (Crain et al. 2008) that EDCs can act as agonists of ERs to promote transcriptional activation of tissues in the reproductive tract. Results from epidemiological and animal studies are indicative of exposure during fetal and/or neonatal life (childhood) being particularly deleterious. A potential mechanism by which EDC may have lifelong effects on reproductive competence has been revealed by studies on members of the HOX gene cluster (HOX-A9, -A10, -A11 and -A13), which demonstrates that spatial and temporal expression of these genes is critical to formation and function of the female reproductive system (Taylor et al. 1997). Several studies have also provided robust evidence that expression of HOXA10 and A11 is steroid-regulated in the normal adult endometrium and that the products of these genes play a key role in preparation of the uterus for implantation (reviewed in Daftary & Taylor 2006). Alterations in Hox gene expression in the female reproductive tract have been reported in mice exposed to DES in utero (Block et al. 2000); mice with targeted deletion of Erα appear to be unaffected by DES treatment (Couse et al. 2001). ERs have been identified in the human HOXA10 promoter that were capable of binding both Erα and Erβ when tested in cell-based reporter systems (Akbas et al. 2004). Importantly, the authors of the study reported that E2-induced activity at one of these ERs was approximately fivefold greater than DES-induced activity. This differential ERE activation was unrelated to ER or co-regulatory protein binding, indicating distinct transcription of HOXA10 in response to different specific ligands. This may offer a plausible explanation for the molecular mechanism by which an EDC such as DES can drive altered HOX gene expression.
resulting in malformations in the reproductive system (reviewed in Daftary & Taylor (2006)). Results from studies using DES are also complemented by those on other EDC, indicating that the Hox gene-dependent signalling pathway may be vulnerable to modification by any agent that can alter ER activation. For example, in utero exposure to methoxychlor (Fei et al. 2005) or BPA (Smith & Taylor 2007) can disrupt Hoxa10 gene expression in the uterus. Rats exposed to BPA during neonatal life are also reported to have impaired fertility characterised by reduced numbers of implantation sites (Varayoud et al. 2011).

**Evidence that early life exposure to EDC can alter onset of puberty or timing of menopause**

The proven association between lifetime oestrogen exposure and the risk of developing a gynaecological cancer means that onset of puberty and timing of menopause are both important risk factors. Precocious puberty, i.e. early menarche, has been reported to be associated with exposure to EDC (reviewed in Schoeters et al. (2008)), although the evidence is contradictory. Studies in women have reported an association of early puberty with exposure to DDT, DDE and polybrominated biphenyls (PBB) (Blanck et al. 2000, Vasiliu et al. 2004, Ouyang et al. 2005). However, women exposed to DES in utero do not differ in their age at menarche compared with unexposed women (Hoover et al. 2011) and other studies have reported that exposure to dioxins has no association with early puberty (Warner et al. 2004). A recent report from the Centers for Disease Control and Prevention’s National Health and Nutrition Examination Survey (NHANES, 2003–2008) measured selected environmental chemicals and metabolites in urine in 440 young women (12–16 years of age). The authors reported an association between concentrations of 2,5-DCP, the major metabolite of dichlorobenzene, and earlier age of menarche (Buttke et al. 2012).

Early onset of menopause has been reported to increase the risk of endometrial cancer, particularly in obese women (Thomas et al. 2009), and DES-exposed women are reported to be at increased risk of early menopause (Hoover et al. 2011). Exposure to methoxychlor, a commonly used pesticide, advanced reproductive senescence in female rats (Gore et al. 2011), and exposure to the PCB mixture A1221 in utero resulted in elongated oestrous cycles, which is a characteristic of reproductive ageing (Walker et al. 2013). Knox et al. (2011) examined E2 levels and onset of menopause in 25 000 women who were part of the C8 Health Project, a cohort of individuals who were exposed to perfluorocarbons due to contamination of drinking water in 2005/6. They reported an inverse relationship between perfluorooctane sulfonate and E2 in both peri-menopausal and menopausal groups (Knox et al. 2011), indicating a potential link to early menopause.

**Evidence that lifestyle factors can increase the influence of EDC on lifetime risk of developing reproductive tract cancers**

Obesity has been estimated to account for 39% of endometrial cancer cases in European women (Bergstrom et al. 2001) and a strong linear association of BMI and endometrial cancer risk has been described (Lindemann et al. 2008). Increased BMI is also associated with increased risk of cancers of the ovary and cervix (Calle et al. 2003). Obesity is associated with increased inflammation, increased levels of oestrogens and abdominal adiposity is most strongly correlated with an increased risk of endometrial cancer (Canchola et al. 2010, Wallace et al. 2010). Obesity is also associated with increased risk of developing insulin resistance and type 2 diabetes (Kahn et al. 2006), which are associated with a threefold increased risk of endometrial cancer (Lindemann et al. 2008). Studies in mice based on neonatal administration of DES or genistein indicated that EDC exposure may also contribute to development of higher BMI, highlighting the potential for complex interactions between different risk factors (Newbold et al. 2005).

The aromatase gene is expressed in adipose tissue under the control of a promoter regulated by class 1 cytokines and TNFα (Simpson 2003). It has been reported that the capacity of adipose tissue, particularly in gluteal rather than abdominal depots, to produce oestrogen shows an age-dependent increase, being higher after menopause (Misso et al. 2005). Adipose tissue can act as a depot for EDCs and might therefore enhance exposure and risk of developing endometrial cancer in obese individuals due to bioaccumulation of lipophilic EDCs. In a study by Hardell et al. (2004) adipose tissue concentrations of polychlorinated biphenyls, hexachlorobenzene, p,p′-DDE, chlordane and PBBS were evaluated in a case–control study for endometrial cancer. The authors reported that while most odds ratios (OR) were close to unity, an increased OR was found for p,p′-DDE, and OR was further increased in association with use of oestrogen-replacement therapy (Hardell et al. 2004). Researchers in Argentina recorded high levels of organochlorines including p,p′-DDE in breast adipose tissue noting a significant correlation with BMI (Munoz-de-Toro et al. 2006). Interestingly, a previous study assessing serum
The female reproductive tract is exquisitely sensitive to the actions of oestrogens but other sex steroids including progestins and androgens also play critical roles, in part by modulating the potentially deleterious effects of oestrogens but other sex steroids including excess proliferation (see above). It is notable that EDC such as BPA (Table 1) may act via more than one steroid receptor pathway (Lee et al. 2003). Recently, Teng et al. (2013) used a systematic screening approach to investigate the effects of BPA on ten different nuclear receptors including ERα, AR, GR, LXRβ, VDR and TRβ, and demonstrated that BPA is an agonist of ERα and an antagonist of AR but had no effect on other nuclear receptors (Teng et al. 2013). Our recent study reported that apoptosis or cell migration may be regulated by androgen-receptor-dependent mechanisms in the endometrium (Marshall et al. 2011). We speculate that EDC such as BPA that exhibit anti-androgen activity could alter processes known to play a role in cancer metastases. An array study conducted in rats identified a subset of genes that could respond to both androgens and oestrogens, with distinct but overlapping gene sets (Nantermet et al. 2005), emphasising the potential importance of EDC in mediating crosstalk between these signalling pathways. Studies in breast tissues indicated that AR-dependent signalling may have opposite effects in normal vs cancer tissue (Hickey et al. 2012), something that merits further investigation in the context of reproductive tract cancers. There has been interest in using androgens to treat a range of disorders in postmenopausal women and inclusion of androgens in HRT has been reported to have beneficial effects on endometrial tissue (Zang et al. 2007), although there is an ongoing concern about negative side effects and long-term studies are needed.

The expression of ER subtypes within target tissues is also likely to influence the effects of EDC. For example, co-expression of ERβ with ERα and consequent formation of ERα-ERβ heterodimers reduces ERα-driven transcription of some genes by competing for shared response elements (Charn et al. 2010). Furthermore, EDC such as phytoestrogens that demonstrate high affinity binding to ERβ may have a disproportionate effect on the function of endothelial and immune cells (Greaves et al. 2013), providing a potential link between EDC and the inflammatory processes known to contribute to the aetiology of endometrial cancers (Wallace et al. 2010).

Historically, the approach for investigating the effects of EDC on the reproductive tract has relied heavily on the ‘one EDC’, ‘one disease’ paradigm; however, focusing on the action of single EDC may be underestimating the risk from total exposure (Bergman et al. 2013). It has concentrations of organochlorine compounds found no increased risk for endometrial cancer (Sturgeon et al. 1998), highlighting the importance of assessing concentrations of EDCs within the adipose tissue itself.

Smoking is inversely associated with endometrial cancer risk (Weiderpass & Baron 2001, Lindemann et al. 2008). This is thought to be due to the influence on metabolism of oestrogens. Cigarette smoking has been reported to promote 2-hydroxylation of E2 and diminish 16α-hydroxylation (Michnovicz et al. 1986), leading to formation of oestrogen metabolites that are less oestrogenic and do not stimulate the uterus (Martucci & Fishman 1977).

Data related to the effects of alcohol on risk of endometrial cancers appear to be conflicting, with the EPIC study reporting no increased risk (Fedirko et al. 2013) while another using 26 years of follow-up data from the Nurses’ Health Study indicated that moderate alcohol intake might be protective (Liu et al. 2013). To date, the effects of alcohol consumption on metabolism, bioaccumulation or activity of EDC have not been investigated.

Summary and future perspectives

Many hundreds of compounds with the potential to alter oestrogen-dependent signalling have been identified in our environment; ingestion via breast milk, food or water are key routes of exposure (Knox et al. 2011, Govarts et al. 2012). To date, the best evidence that EDC can have an adverse effect on reproductive health in women has come from follow-up on women exposed in utero to DES (DES daughters). Evidence from animal- and cell-based studies reporting that DES can alter expression of HOX genes that play a key role in regulation of Müllerian duct development as well as fertility in adulthood has offered one mechanism by which EDC may act to alter reproductive function. There has been intense interest in the potential for EDC to increase the risk of a range of cancers but establishing a causal link between exposure and disease has proved challenging. In utero exposure to EDC has also been linked with an increased likelihood of reduced birth weight as well as other features of the metabolic syndrome, including increased incidences of obesity and diabetes, which are risk factors for endometrial cancer (Govarts et al. 2012, Dossus et al. 2013). Epidemiological studies recording clear associations between breast and other cancers with occupational exposures are now offering new and exciting data sets with which to address the question of how exposure in adulthood can affect development or progression of malignancy (Brophy et al. 2012).

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previously been reported that mixtures of EDCs that individually have no effect on ER act simultaneously to promote an agonist response when tested as a mixture (Tinwell & Ashby 2004). Future studies will need to make more accurate measurements of body-wide concentrations of EDC and endogenous steroids using up-to-date methodology based on mass spectrometry (Tamae et al. 2013). One option being explored is the use of \textit{in silico} methods for elucidating potential EDCs, including pharmacophore modelling and docking models (reviewed in Vuorinen et al. (2013)).

In summary, although the prevalence of female reproductive cancers is increasing, the evidence that EDC exposure in humans has a direct effect on the development of endometrial cancer is incomplete and confounded by factors such as increased rates of obesity. Evidence from animal models indicates that early developmental exposure to EDC increases the occurrence of reproductive cancers and that the trans-generational effects of EDC exposure (as described in DES animal models) may pose a risk for future generations. New insights await carefully controlled prospective data from EDC-exposed women and greater use of multidisciplinary systems modelling the effects of mixtures of putative EDC ligands.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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