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

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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

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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 steroid-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

EDC	ER	AR	Other nuclear receptors	References
DES	Agonist	Weak binding affinity	Inverse agonist of ERRα, ERRβ and ERRγ	Kuiper *et al*. (1998) and Giguere (2002)
BPA	Agonist	Anti-androgen	Strong binding affinity to ERRγ Agonist of PXR Antagonist of ThR	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 Sui *et al*. (2012)
Genistein (phytoestrogen)	RBA ER$\beta \gg$ ERα Activates transcription via ERE	Anti-androgen via ERβ action Very weak binding to AR (0.003 RBA DHT)		Kuiper *et al*. (1998), Morito *et al*. (2001), Bektic *et al*. (2004) and Freyberger & Ahr (2004)
Coumestrol (phytoestrogen)	RBA ER$\beta \gg$ ERα Activates transcription via ERE	No IC50 demonstrated		Kuiper *et al*. (1998) and Freyberger & Ahr (2004)
DDT and metabolites	Binds ERα and ERβ, transcriptional activation	Anti-androgen (p,p′-DDE)		Kelce *et al*. (1995), Klotz *et al*. (1996) and Kuiper *et al*. (1998)
Dioxins	TCDD modulates ERα activity through binding AhR	TCDD blocks androgen-dependent proliferation of LNCaP cells	Crosstalk with COUPTF1 and ERRα via AhR	Klinge *et al*. (2000), Ohtake *et al*. (2003) and Barnes-Ellerbe *et al*. (2004)
Polychlorinated biphenyls	Weak binding	Weak binding		Kuiper *et al*. (1998) and Fang *et al*. (2003)

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.

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ER signalling

The most abundant endogenous oestrogens in normal premenopausal women, oestrone (E_1) and oestradiol (E_2) (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 *et al.* 2009).

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.* 2012*a*). 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.

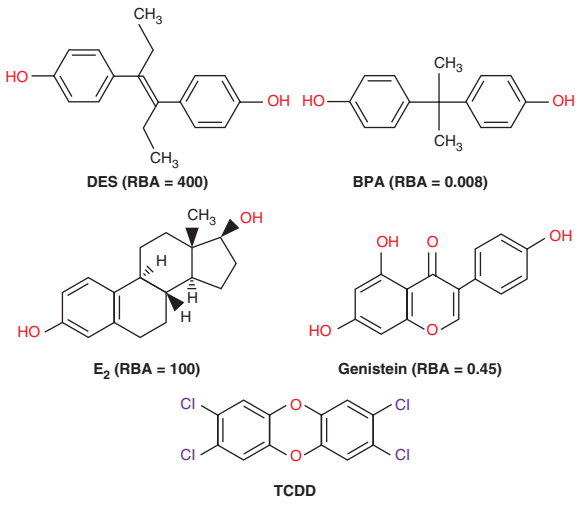

Figure 1
Two-dimensional (2D) chemical structure of ligands diethylstilbestrol (DES), oestradiol (E_2), genistein, bisphenol A (BPA) and their relative binding affinity (RBA) for oestrogen receptor α (ERα) and the 2D chemical structure of the aryl hydrocarbon receptor (AhR) ligand 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). DES, E_2 and genistein contain key structural features critical to ER binding such as phenolic ring and 17β-OH group. DES has the greatest affinity for ERα (RBA = 400) due to the presence of ethyl groups, which increase the hydrophobicity of DES and increase interaction with the ERα binding pocket site. BPA has a phenolic ring but lacks a 17β-OH group and only binds ERα weakly (RBA = 0.008 of E_2). TCDD lacks the characteristic structural features of an ER ligand and does not bind directly to ERs. RBA and structural analysis based on observations reported in structure–activity relationship study by Fang *et al.* (2001). Chemical structures obtained from http://www.chemspider.com/(accessed Sept. 2013).

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 ERα varies in a cycle-dependent manner within the functional layer; ERα 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 ERα 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 ERα 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 ERα 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 ERα-dependent signalling in E_2-dependent cell proliferation and expression of progesterone receptors (Harris *et al.* 2002, Punyadeera *et al.* 2004). In ovariectomised (ovx) mice, injection of E_2 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 ERα knockout (ERαKO) mice have hypoplastic uteri with sparse glands (Lubahn *et al.* 1993). Although the uterine weight in ERβKO mice is normal, they are hyper-responsive to E_2-stimulated uterine proliferation (Dupont *et al.* 2000), a finding consistent with studies using cell lines that have demonstrated co-expression of ERβ with ERα 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

Oestrogenic assay	EDC	Comments	References
Uterotrophic assay	DES; agonist BPA; mixed agonist/antagonist concentration dependent Phytoestrogens: genistein, coumestrol; mixed agonist/antagonist concentration dependent PCBs; agonist TCDD; antioestrogen	*In vivo*, ERα-mediated. Model organisms; mice, rat. Immature animals (prepubertal) or ovariectomy of adult animals Primary endpoint; wet or dry uterine weight	Faqi & Chahoud (1998), Arcaro *et al.* (1999), Markey *et al.* (2001), Jefferson *et al.* (2002), Kim *et al.* (2002) and Ohta *et al.* (2012)
ER binding affinity	Strong affinity: DES (ERα and ERβ), coumestrol (ERβ[1]) Moderate affinity: genistein[1] Weak affinity: DDT metabolites, PCBs, BPA[1,2]	[1]RBA for both ER isoforms assessed [2]RBA for total rat uterine cytosol ER	[1]Kuiper *et al.* (1998) [2]Blair *et al.* (2000)
Luciferase reporter assay (ERE)	Transcriptional activation: DES, BPA, genistein, coumestrol, PCBs, DDT	*In vitro*, both ER isoforms investigated	Kuiper *et al.* (1998), Frigo *et al.* (2002) and Li *et al.* (2013)
Luciferase reporter assay (AP1)	Transcriptional activation: BPA, DDT	*In vitro*, both ER isoforms investigated	Frigo *et al.* (2002) and Li *et al.* (2013)
Yeast β-galactosidase reporter assay	Transcriptional activation: DES, coumestrol, BPA, DDT metabolites	*In vitro*. Transient expression of human receptors	Gaido *et al.* (1997)
Transcription of oestrogen-responsive genes	DES, BPA, genistein, coumestrol, benzophenones, PBDE	E.g. *PGR*, *CXCL12*	Ceccatelli *et al.* (2006), Newbold *et al.* (2007), Kerdivel *et al.* (2013) and Li *et al.* (2013)

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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, E_1 and E_2 (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 E_1 and E_2, has been documented in both endometrial and cervical cancers, consistent with a role for local E_2 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 (Critchley *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*. 2005*b*); however, inclusion of progestagens in the hormone replacement therapy (HRT) formulations is reported to reduce risk (Beral *et al*. 2005*a*), 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

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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 E_2 (Rinaldi *et al.* 2011).

Endocrine disruptors implicated in reproductive tract disorders and cancer

As the female reproductive tract is exquisitely sensitive to changes in bioavailable oestrogens, exposure to substances that mimic the action of oestrogens or disrupt normal oestrogen homoeostasis is likely to pose an increased risk to developing cancers of the reproductive tract. Due to the ubiquitous nature of EDC exposure and the pleiotropic action(s) of EDC, assessing the link between lifetime exposure and the associated risk of reproductive cancer for any single EDC or pathology is difficult and epidemiological evidence is often contradictory. Important evidence that EDC can alter oestrogen-dependent signalling pathways and thereby contribute to cancer risk has come from studies using *in vitro* and animal model systems that are outlined below; less attention has been paid to the influence of EDC on morbidity and mortality of cancer sufferers.

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

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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 E_2 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 (2006*b*) investigated hormonal activities of u.v. filters and reported that several of the compounds assessed exhibited agonist effects at ERα: benzophenone-1 (BP1), benzopehnone-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 2006*b*). 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 2006*a*). 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). BPs 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 overexpressing 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

cell growth, differentiation and adhesion (Newbold *et al.* 2007). Kabbarah *et al.* (2006) collected uterine cancer tissue RNA from DES-exposed mice by laser capture microdissection to minimise contamination with other cell types and performed targeted transcriptional profiling. Interestingly, the tumour suppressor PTEN was down-regulated in the majority of tumours, analogous to loss of PTEN expression in human tumours (Mutter *et al.* 2000). In addition, genes associated with cell adhesion, such as Decorin, were down-regulated in DES-induced tumours while suppressor of cytokine signalling 3 (*Socs3*) was over-expressed (Kabbarah *et al.* 2006). Other studies have also identified molecular similarities between DES-induced tumours in mice (Kabbarah *et al.* 2003) and endometrial cancer in humans, such as microsatellite instability brought about by defects in expression of DNA mismatch repair genes such as *MSH2* (Cederquist *et al.* 2004) and *MSH6* (Goodfellow *et al.* 2003).

It could be argued that the apparent trans-generational effect of endocrine disruption is of greater significance. Following neonatal DES exposure in mice, the F1 generation of DES daughters have an increased incidence of uterine adenocarcinoma. Newbold *et al.* (1998) found that 31% of F1 females from the maternal germ cell lineage developed tumours after 18 months despite there being no exogenous endocrine exposure in these animals, highlighting the potential for future risk to the daughters of DES-exposed women. DES is reported to induce epigenetic changes. Altered methylation patterns have been reported for several uterine genes that are permanently dysregulated after developmental DES exposure; lactoferrin and c-Fos are permanently up-regulated following neonatal DES exposure to due to hypomethylation of the promoter region (Li *et al.* 1997, 2003).

DES has been reported to promote hypermethylation of the homeobox gene *Hoxa10* in mice exposed *in utero* to DES. DES exposure was also associated with increased expression of DNA methyltransferases 1 and 3b leading to long-term altered expression of *Hoxa10* (Bromer *et al.* 2009). Contrary to the reported action of DES on *Hoxa10*, exposure to BPA in mice *in utero* results in hypomethylation of the *Hoxa10* promoter, which leads to enhanced binding of ERα to EREs in the promoter region and an increase in an ERE-driven reporter gene *in vitro* (Bromer *et al.* 2010).

Thus, epigenetic changes in uterine genes may indicate a possible mechanism for trans-generational effects of DES because altered expression of genes is reported to persist in DES-lineage females.

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 E_2), 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 E_2 (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 EREs 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 (Papaconstantinou *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.* (2012*b*, 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 E_2-mediated ERα activation of luciferase activity while in HeLa cells (cervical adenocarcinoma cell line) similar concentrations of BPAF antagonised E_2-mediated ERβ activation of luciferase activity (Li *et al.* 2012*b*). 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.* 2012*b*, 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

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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 ERE 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′-dichlorodiphenyldichloroethylene (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 (Grochowalski *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 E_2-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 E_2, 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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higher exposure to isoflavones in postmenopausal women was associated with lower plasma E_2 (Horn-Ross *et al.* 2003). However, in a randomised, double-blind, placebo-controlled study of 376 healthy postmenopausal women, the individuals who received 150 mg isoflavones per day showed a significant increase in simple endometrial hyperplasia when compared with women given a placebo for 5 years (Unfer *et al.* 2004). In a recent prospective study conducted on 70 000 low-risk individuals, vegan diets were found to offer statistically significant protection from female-specific cancers, although the authors commented that this may reflect, in part, effects on BMI (Tantamango-Bartley *et al.* 2013).

Standard oestrogen response assays have revealed that phytoestrogens can induce increased uterine wet weight, bind ER and induce ER-dependent transcription (Jefferson *et al.* 2002). In mature ovx female rats, 3 days of oral administration of genistein produced a dose-dependent increase in uterine wet weight and increased mRNA expression of complement component 3 (C3), an oestrogen-regulated gene (Diel *et al.* 2001). Interestingly, in the same study, the authors also examined the uterine and vaginal epithelia and observed dose-dependent increases in epithelial height (Diel *et al.* 2001). While genistein stimulated the uterus and vagina, in another assay using transplanted RUCA-1 endometrial adenocarcinoma cells in ovx rats, genistein did not affect tumour growth (Diel *et al.* 2001). Results from *in vitro* assays indicated that several phytoestrogens including genistein, daidzein and coumestrol can activate ERα- and ERβ-dependent ERE luciferase activity (Li *et al.* 2013). Phytoestrogens may also promote activation of ER transcription via tethered mechanisms as cell-based assays have reported increased expression of luciferase reporter genes via AP-1 (but not Sp1) in HeLa cells expressing ERα (kaempferol, apigenin and coumestrol) or ERβ (daidzein) (Li *et al.* 2013).

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 E_2 for ERβ (Kuiper *et al.* 1998, Morito *et al.* 2001). It has also been reported that phytoestrogens promote distinct receptor confirmations 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). EREs 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 E_2-induced activity at one of these EREs 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

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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 E_2 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 E_2 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, chlordanes 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

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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 E_2 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).

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 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 reduced 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

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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 *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 on the aetiology of different reproductive disorders and their relationships with whole-body physiology throughout the life course.

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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