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Review

Impact of endocrine-disrupting compounds (EDCs) on female reproductive health

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ABSTRACT

Evidence is accumulating that environmental chemicals (ECs) including endocrine-disrupting compounds (EDCs) can alter female reproductive development, fertility and onset of menopause. While not as clearly defined as in the male, this set of abnormalities may constitute an Ovarian Dysgenesis Syndrome with at least some origins of the syndrome arising during foetal development. ECs/EDCs have been shown to affect trophoblast and placental function, the female hypothalamo-pituitary–gonadal axis, onset of puberty and adult ovarian function. The effects of ECs/EDCs are complex, not least because it is emerging that low-level, 'real-life' mixtures of ECs/EDCs may carry significant biological potency. In addition, there is evidence that ECs/EDCs can alter the epigenome in a sexually dimorphic manner, which may lead to changes in the germ line and perhaps even to transgenerational effects. This review summarises the evidence for EC, including EDC, involvement in female reproductive dysfunction, it highlights potential mechanisms of EC action in the female and emphasises the need for further research into EC effects on female development and reproductive function.

Contents

1. Introduction

Environmental chemicals (ECs), including endocrine disrupting compounds (EDCs), comprise many different chemicals from a wide range of (primarily) anthropogenic, industrial, agricultural and domestic sources and in recent decades it has become

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increasingly clear that they have the capacity to interfere with female reproductive development and function in a wide range of species including humans (Woodruff and Walker, 2008).

2. Are EDCs implicated in Ovarian Dysgenesis Syndrome (ODS)?

The term testicular dysgenesis syndrome (TDS) is used to describe the phenotypic consequences of endocrine disruption in the human male (Skakkebaek et al., 2001). These include reduced adult sperm counts and increased incidences of hypospadias (malformation of the urogenital tract), cryptorchidism (testis maldescent) and testicular cancer. TDS has a developmental origin and the processes involved are being elucidated (Sharpe and Skakkebaek, 2008). There is, no evidence for such a tight set of inter-related developmental abnormalities inducing a comparable syndrome in females. One phenotype suggestive of endocrine disruption is the Mayer–Rokitansky–Kuster–Hauser (MRKH) syndrome (Sultan et al., 2009). Multiple abnormalities are associated with this syndrome whose main characteristic is Müllerian duct aplasia (including uterine and vaginal abnormalities) in XX individuals with a female phenotype and primary amenorrhoea after puberty. Attempts to identify a genetic basis for MRKH syndrome have not been successful. Anti-Mullerian hormone (*AMH*), the AMH receptor, *WT1*, *PAX2*, *CFTR* and *HOX* genes, for instance, do not associate strongly with MRKH (e.g. (Burel et al., 2006)) and the absence of a uterus and virilisation in women with WNT4 deficiency suggests that this is not a cause of MRKH but a distinct syndrome in itself (Biason-Lauber and Konrad, 2008). Nevertheless, despite the lack of evidence for a female equivalent of TDS, both oestrogenic and anti-androgenic signalling and chemicals can also disturb uterine and reproductive tract development in humans (e.g. (Smith and Taylor, 2007; Walters et al., 2009) and reviewed by (Crain et al., 2008)). So, what about the ovary and a unifying female syndrome? While effects of MRKH syndrome can include the ovary (e.g. (Al Omari et al., 2011)) this is rare, with the ovary normally remaining functional. Despite similarities between testis and ovary development, the differences, such as in genetic programming (Cederroth et al., 2007), have marked implications for potential mechanisms and consequences of EDC exposures. In their recent review Buck Louis et al. (2011) coined the term "Ovarian Dysgenesis Syndrome". They present a synthesis of the literature that supports the tentative use of the term, while highlighting some of the current knowledge deficits that need to be overcome. We support the use of this term, ODS, as a loose umbrella concept that may include various combinations of other syndromes, such as MRKH and polycystic ovarian syndrome all of which may have environmental components to their aetiology.

The effects of ECs on female fertility are mediated through diverse, physiological systems, reflecting equally diverse mechanisms of action amongst the EC classes. Consequently, if effects of ECs on fertility are to be identified, and associated mechanisms of action understood, it is imperative that the appropriate components of the reproductive system are fully characterised, including gene expression and developmental processes. Effects of exposure to ECs, which may encompass some EDCs, include direct effects on physiological mechanisms associated with gonadal development and function. Reproductive systems have been shown to be perturbed at multiple developmental stages; disrupted processes include hypothalamo-pituitary neuropeptide expression (Bellingham et al., 2009, 2010), ovarian follicle development and health (Fowler et al., 2008) in sheep exposed to pastures fertilised using sewage sludge, a by-product of waste water treatment containing thousands of ECs with known endocrine disrupting properties. Many additional effects have also been reported in studies involving a range of species; these include mitotic disorders, perturbed energy metabolism, reduced blastocyst development, delayed implantation and increased embryo loss (Rhind et al., 2010a). Many individual disruptions, often associated with exposure to a single chemical, by themselves may be of little biological consequence but it should be noted that humans and animals are typically exposed to a mixture of ECs. These may act additively, increasing the effect exerted on a single physiological process, or may initiate many, separate, disruptive effects in a single reproductive cycle, regardless of the mechanisms involved, the combined actions may have more severe reproductive consequences. Furthermore, there is evidence for xenochemical induction of epigenetic modification of genes involved in controlling female urogenital tract development, such as the HOX family, as shown in studies involving mice exposed to bisphenol A (BPA), in utero. (Smith and Taylor, 2007) (see Section 6). It should also be remembered that the effects of chemical exposure on reproductive development may be layered onto other programmed changes and a recent review has highlighted, in particular, the links between female reproductive deficits, early life events and nutrition in both females and males (Sloboda et al., 2011). Thus effects of EDCs have to be seen in combination with effects of other factors that can disrupt normal foetal development and adult health (Skinner et al., 2010).

3. EDC effects on trophoblast and placental function

During early embryonic development, potential targets of EDCs include cell cleavage and differentiation, cell lineage determination, methylation, implantation, maintenance of pregnancy and organogenesis (Table 1). Polychlorinated biphenyls (PCBs), dioxins (such as TCDD) and phthalates (such as DEHP) can affect cell lineage formation and trophoblast function in blastocysts and these chemicals are known to cross the placental barrier and to disturb embryonic development in humans (Myatt, 2006).

TCDD and coplanar PCBs are known to act on human endometrial cells, via the arylhydrocarbon receptor (AhR), (Willing et al., 2011). The AhR-arylhydrocarbon receptor nuclear translocator heterodimer (AhR-ARNT) is a typical transcription factor. AhR and ARNT are expressed in mouse preimplantation embryos (Peters and Wiley, 1995) and in 3 day-old (days post coitum, dpc) morulae and trophoblast cells of 4 dpc blastocysts in rabbits (Tscheudschilsuren et al., 1999). Exposures to coplanar (PCB 77, 126, and 169) and non-coplanar PCB (PCB 28, 52, 101, 118, 153, and 180) in low and high concentrations (0.1 ng or 1 μg/congener/mL medium) increase AhR signalling (AhR target genes include *CYP1A1, CYP1B1*) and expression of implantation-associated genes (*VEGFR2* and *COX2*) in rabbit 6 dpc blastocysts (Clausen et al., 2005), interfering with the cellular programmes that underlie early mammalian development. TCDD, via AhR activation, also decreases the expression of the glucose transporter 1 (GLUT1 in the plasma membrane in P19 mouse embryonic carcinoma cells (Tonack et al., 2007), disturbing glucose uptake in embryonic cells. Similarly, stress responses of human trophoblast-like JAR cells are affected by TCDD treatment. Specific responses include significantly increased levels of intracellular ROS and lipid peroxides, a reduction in ATP content and mtDNA copy numbers as well as higher rates of apoptosis (Chen et al., 2010).

Phthalates have also been shown to affect trophoblast cells in human placentae. For instance, higher maternal urinary concentrations of five phthalate metabolites (MEHP, MEOHP, MnBP, MiBO, MBzP) have been associated with a decreased expression of trophoblast differentiation markers (PPARγ, AhR, hCG), leading to alterations in human placental development and function (Adibi et al., 2010). Interestingly, in rat HRP-1 trophoblast cells exposure to DEHP, MEHP and 2-ethylhexanoic acid also results in significant increases in the concentrations of different lipid classes (Xu et al., 2006), leading to the conclusion that these chemicals might affect

Table 1
Overview of associations between EDCs and trophoblast and placenta function.

Chemical	Effect	Species	References
Polychlorinated biphenyls (PCBs)	Alteration in AhR signalling ↑CYP1A1, ↑CYP1B1 Induction of implantation associated genes ↑ VEGF, ↑ COX2 Induction of myometrial contractions Alteration in placental-embryonal interface ↓AQP1 expression, ↑ amniotic fluid	Rabbit blastocysts Bovine Mouse	(Clausen et al., 2005) (Wrobel et al., 2005) (Tewari et al., 2009)
Diethylhexylphthalate (DEHP) and its metabolites	Altered trophoblast differentiation: ↓PPARgamma, AhR, HCG Altered lipid profile ↑triacylglycerides, ↑ cholesterol ester, ↑phosphatidylcholine	Human Rat trophoblast cells	(Adibi et al., 2010) (Xu et al., 2006)
2,3,7,8-Tetrachlorodibenzodioxin (TCDD)	Induction of apoptosis ↑p53, ↑BAX, ↑ Caspase3 Alters glucose transport ↓GLUT1 (via AhR) AhR activation ↑CYP1A1, ↑CYP1B1	Cultured trophoblast-like cells Mouse embryonic carcinoma cells Human endometrial epithelial cells	(Chen et al., 2010) (Tonack et al., 2007) (Willing et al., 2011)
Cadmium	Induction of stress response ↑ HSP70, ↑MAPK ↓ leptin expression Retardation of trophoblast outgrowth	Human trophoblast cells Human trophoblast cells Chicken	(Valbonesi et al., 2008) (Stasenko et al. 2010)(Thompson and Bannigan, 2008)
Bisphenol A (BPA)	Positive correlation with miscarriages Induction of degenerative trophoblastic giant cells and spongiotrophoblast layers Altered oestrogen synthesis ↓ CYP19 expression	Human Mouse Human placental choriocarcinoma cells	(Sugiura-Ogasawara et al., 2005) (Tachibana et al., 2007) (Huang and Leung, 2009)

placental and/or foetal fatty acid/lipid homeostasis and lead to abnormal foetal development.

Amongst potentially toxic elements (PTEs), cadmium functions as a metallo-oestrogen (Guillette, 2006; McLachlan, 2001). A major source of gestational cadmium is tobacco smoke and cadmium accumulates in the human placenta (up to 2 times higher in female smokers than non-smokers). It disrupts the normal function of trophoblast cells by a down-regulation of leptin (Stasenko et al., 2010). This is important because leptin is an adipokine, which is an anti-apoptotic and cell proliferation-promoting factor, with a significant role in placental maintenance (Magarinos et al., 2007; Perez-Perez et al., 2008). Cadmium triggers different stress responses involving dose-dependent induction of HSP70 expression and phosphorylation of Stress Activated Protein Kinases (SAPK), such as ERK1/2, JNK1/2 and MAPK, in the human trophoblast cell line HTR-8/SVneo (Valbonesi et al., 2008). Taken together, maternal exposure to EDCs can lead to an inhibition of trophoblast differentiation and invasion and thereby affect normal placentation and placental function. This is clearly supported by the data on the negative effects of cigarette smoking on IVF outcomes in women (Neal et al., 2005).

Thus it is clear that multiple chemical classes act via multiple mechanisms to perturb normal early developmental processes. The effects may be additive and/or synergistic, adding to the degree of complexity when assessing the effects of chemical mixtures on trophoblast and placental interactions.

4. EDCs can disrupt the hypothalamo-pituitary–gonad (HPG) axis

While reproduction is dependent on gonadal activity, primary control is exerted via regulation of GnRH secretion from the hypothalamus and subsequent gonadotrophin release from the pituitary gland. Exposure to some ECs can have adverse effects on the hypothalamo-pituitary gland complex in a range of different adult, prepubertal (Adewale et al., 2009) and foetal animals. The activity of the GnRH neurosecretory system is sexually differentiated as the result of steroid exposure during foetal development, testosterone normally acting to 'masculinise' and 'defeminise' afferent inputs. Thus the hypothalamo-pituitary gland complex of foetal animals is particularly vulnerable due to programming effects of both androgenic and oestrogenic compounds. In female sheep and rats, the activity and structure of the hypothalamo-pituitary gland complex is sensitive to the effects of a wide variety of ECs (Bellingham et al., 2009, 2010; Savabieasfahani et al., 2006; Wright et al., 2002; Lewis et al., 2003) and phytoestrogens (Dickerson and Gore, 2007).

EDCs have the potential to perturb natural hormonal systems and processes, albeit by different mechanisms. Their effects are mostly exerted through their action on nuclear hormone receptors, such as oestrogen and androgen receptors, where they can have agonistic or antagonistic effects. Effects of exposure are compound and time specific. Thus, while in utero octylphenol (OP) exposure in sheep both directly suppresses gonadotrophin secretion (Sweeney et al., 2000) and affects LH secretion later in life (Wright et al., 2002), post-natal OP exposure is not always associated with an obvious phenotype (Evans et al., 2004). On the other hand, exposure of sheep to other oestrogenic compounds such as BPA and methoxychlor at later developmental time-points does result in altered gonadotrophin secretion (Savabieasfahani et al., 2006; Evans et al., 2004).

The effects on individuals of environmental EC exposure are complicated by the fact that under natural, rather than experimental situations, exposure involves a complex mixture of chemicals. Each of these chemicals may be present at very low concentrations, which are deemed 'safe' by regulatory bodies. Our recent work using a mixed-exposure, sheep model, has shown that such an "environmentally relevant" exposure scenario results in alterations to pituitary gland cell populations and the expression of hypothalamic GnRH (Bellingham et al., 2010). Galanin and kisspeptin are sexually dimorphic, oestrogen-sensitive neuropeptides associated with the regulation of GnRH and both are disturbed by exposure to a mix of ECs (Bellingham et al., 2009), despite tissue levels of individual chemicals in the affected animals being below specified no observed adverse effects levels (NOAEL) (Rhind et al., 2010b). The observed sensitivity of the kisspeptin system to

chemical exposure has been supported by studies in other species using other chemicals (Patisaul et al., 2009). In particular, neonatal exposure of the female rat to oestrogenic compounds, such as oestradiol benzoate and genistein, results in reduced kisspeptin immunoreactivity in the hypothalamic anteroventral periventricular nucleus (AVPV) at puberty. This is accompanied by premature anoestrous and impaired steroid feedback on GnRH neurons (Bateman and Patisaul, 2008). The kisspeptin system is well placed, therefore, as a putative target for EDC perturbation of the reproductive axis leading to female reproductive dysfunction (Tena-Sempere, 2010).

Natural exposure to ECs and EDCs occurs over the whole lifetime of the animal and, thus, while exposure can result in changes in gene and or protein expression and change in hypothalamic activity, compensatory modifications may also be induced to restore normal reproductive function. Therefore, effects of long-term exposure on the reproductive neuroendocrine axis can sometimes be less apparent than those seen following more acute exposures. This possibility is supported by our data from female lambs exposed to OP (1000 μg/kg/day) either from Day 70 of gestation to birth, birth to weaning or D70 to weaning (Wright et al., 2002) and our current, unpublished, results of sheep studies which indicate that the effects of exposure to an environmental mixture of ECs via grazing on sewage sludge treated pastures has a greater effect on kisspeptin gene expression when exposure occurs over short developmental periods compared to when it is lifelong.

Increasing evidence suggests that EC exposure is linked to several disorders in female reproductive function (Table 2 and Fig. 1) in humans such as uterine fibroids (McLachlan et al., 2006); placental dysfunction, early pregnancy loss, recurrent abortion, foetal growth restriction (Gerhard et al., 1998; Korrick et al., 2001; Greenlee et al., 2003; Chiaffarino et al. 2006) breast cancer, reduced duration of lactation (Olaya-Contreras et al., 1998; Romieu et al., 2000; Crain et al., 2008) and finally to pubertal timing (Sorensen et al., 2010; Colon et al., 2000; Krstevska-Konstantinova et al., 2001), although this is still a controversial point. It is accepted that exposure to toxicants in early life, while hormone sensitive systems are still forming, particularly in the hypothalamus and pituitary gland, can impact on reproductive health. Therefore it is not unreasonable to suggest that chemical effects during hypothalamus and pituitary gland development may play a role in such disorders of female reproductive function by programming later reproductive health outcomes since it is established that the foetal environment can determine health outcomes in adulthood for other systems (foetal basis of adult disease hypothesis, (Barker, 1997)).

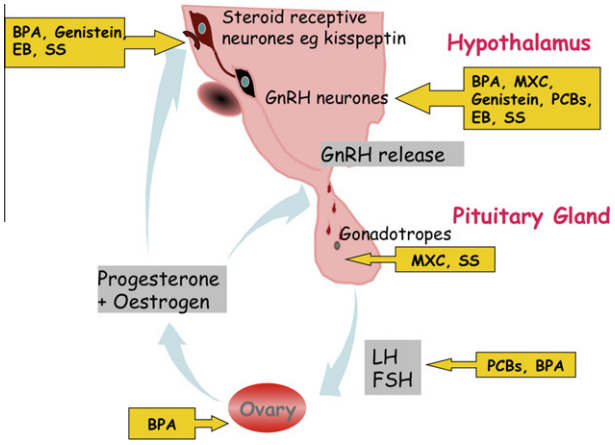

Fig. 1. Summary of major reported effects of EDCs at different levels of the hypothalamo-pituitary–ovary axis. Chemicals have been shown to act at different, sometimes multiple, levels of the axis, e.g. BPA appears to affect expression of steroid sensitive GnRH afferent systems such as kisspeptin, as well as GnRH expression in the hypothalamus. This may lead to alteration of GnRH signalling onto to the pituitary, which, in turn, may perturb, gonadotrophin release and action at the level of the gonad. Action of individual chemicals at different levels, via different mechanisms, adds to the level of complexity in determining how such chemicals may perturb reproductive function. Note, however, that in sheep low-level environmental exposure to chemical mixtures in the form of sewage sludge alters similar hypothalamic and pituitary gland target systems, as individual chemical exposures.

Table 2
Overview of associations between EDCs and disturbance of the hypothalamo-pituitary–ovary axis.

Chemical	Effect	Species/dose/route	References
Bisphenol A (BPA)	Alters brain sexual differentiation	Rat/250 μg/rat/s.c.	(Patisaul et al., 2006)
	↓Kisspeptin fibre density (hypothalamus)	Rat/50 mg/kg or 500 mg/rat/s.c.	(Patisaul et al., 2009; Navarro et al., 2009)
	↓GnRH expression (hypothalamus)	Sheep/5 mg/kg/i.m.	(Mahoney and Padmanabhan, 2010)
	↓oestrogen receptor expression (hypothalamus)	Sheep/5 mg/kg/i.m.	(Mahoney and Padmanabhan, 2010)
	↓GnRH signalling	Rat/1 or 10 μg/μl/s.c.	(Fernandez et al., 2009)
	↓LH	Rat/0.1 or 1.2 mg/kg/oral, Sheep/	(Rubin et al., 2001; Fernandez et al., 2009) Evans
	Effects on female reproductive tract	3.5 mg/kg/i.m.	et al., 2004
		Mice/10,100 or 1000 μg/kg/s.c.	(Newbold et., al 2007)
Methoxychlor (MXC)	Altered pituitary cell populations	Rat/1200 ppm/oral	(Masutomi et al., 2004)
	↓GnRH expression	Sheep/5 mg/kg/i.m.	(Mahoney and Padmanabhan, 2010)
	↓Eostrogen receptor expression (hypothalamus)	Sheep/5 mg/kg/i.m.	(Mahoney and Padmanabhan, 2010)
	↓GnRH signalling	Sheep/5 mg/kg/i.m.	(Mahoney and Padmanabhan, 2010)
Genistein	↓ Kisspeptin fibre density (hypothalamus)	Rat/1 or 10 mg/kg/s.c	(Losa et al., 2011)
	↓GnRH expression	Rat/1200 ppm/oral	Masutomi et al., 2004)
	Alters brain sexual differentiation	Rat/250 μg/rat/s.c.	(Patisaul et al., 2006)
	↓GnRH activation	Rat/10 mg/kg/s.c	(Bateman and Patisaul, 2008)
	↓kisspeptin fibre density	Rat/10 mg/kg/s.c.	(Bateman and Patisaul, 2008)
Polychlorinated biphenyls	↓GnRH expression	GT1–7 cells/see reference	(Gore et al., 2002)
(PCBs)	Enhanced gonadotrophin response	Cultured pituitary cells/0.1–50 ppm	(Jansen et al., 1993)
	Disrupts neuroendocrine development	Rat/1 mg/kg/i.p.	(Dickerson et al., 2011a)
Oestadiol Benzoate (EB)	Altered kisspeptin receptor expression (POA	Rat/50 μgkg/i.p.	(Dickerson et al., 2011b)
	hypothalamus)	Rat/50 μgkg/i.p.	(Dickerson et al., 2011b)
	↑ERα immunoreactivity in MNM hypothalamus	Rat/50 μg/s.c.	(Bateman and Patisaul, 2008)
	↓GnRH activation	Rat/50 μg/s.c.	(Bateman and Patisaul, 2008)
	↓kisspeptin fibre density		
Sewage sludge mixture	↓KiSS1 expression (hypothalamus + pituitary)	Sheep/unknown mixed/multiple	(Bellingham et al., 2009)
(SS)	↓GnRH expression (hypothalamus)	Sheep/unknown mixed/multiple	(Bellingham et. al., 2010)
	↓GnRH receptor (hypothalamus + pituitary)	Sheep/unknown mixed/multiple	(Bellingham et al., 2010)

5. EDCs and the timing of puberty

Puberty is a multifaceted developmental process that is under the control of different hormonal regulatory mechanisms and, because there can be significant social consequences of premature puberty, this section will focus on the human. Puberty is characterised by activation of the hypothalamic-pituitary–gonadal axis, the appearance of secondary sexual characteristics and a growth spurt (Kakarla and Bradshaw, 2003). The onset of puberty begins late in childhood and results in the individual's transition period from a non-reproductive to a reproductive state. In girls, puberty is initiated by changes in the expression of hypothalamic neurotransmitters, which awaken the ovaries leading to oestradiol secretion. Age at onset of puberty is determined by multiple genetic and environmental factors, including psychosocial and socio-economic conditions, nutrition and ethnicity (Parent et al., 2003). During the past decades, secular trends of earlier age at onset of puberty have been reported, especially in girls, in both the US and Europe (Parent et al., 2003; Mul et al., 2001). One prominent hypothesis, developed to explain the recent changes in puberty timing, is that exposure to EDCs during the prepubertal period, but also in early perinatal programming windows, may cause an early age of puberty (Den Hond et al., 2002; Herman-Giddens, 2006; Sharpe and Skakkebaek, 1993). Currently, most of the evidence implicating EDCs in the dysregulation of pubertal development stems from animal experiments and in vitro studies. In humans, a large number of cohort studies also suggest that exogenous compounds may have pronounced effects on pubertal timing although a conclusive relationship between precocious puberty and environmental agents has not yet been established, as summarised in Table 3.

A temporal trend toward premature thelarche in Puerto Rico was noted during the early 1980s (Bongiovanni, 1983; Freni-Titulaer et al., 1986; Saenz de Rodriguez et al., 1985). About 68% of the girls with premature breast development had measurable circulating levels of the phthalates [dimethyl, diethyl, dibutyl and di-(2-ethylhexyl)], compared with 17% of girls with normal thelarche timing (Colon et al., 2000). The association between phthalate exposure and precocious puberty in female proposed by Colon is very suggestive. However, interpretation of these results is still controversial due to the very high levels of serum phthalates reported which may imply major analytical problems and possibly laboratory contamination (for comments see: McKee, 2004). In more recent studies, association between increased serum levels of DBP and DEHP and precocious puberty, in Chinese and Korean girls, was also reported (Qiao et al., 2007; Lee et al., 2006), whereas other investigators failed to demonstrate a link between phthalate exposure and age of sexual maturity (Lomenick et al., 2010). Thus more studies are necessary to elucidate the possible links between phthalate exposure and the risk of early puberty. Detectable plasma levels of the DDT metabolite p,p′-DDE in children with precocious puberty immigrating from developing countries to Belgium, were strikingly different to the undetectable levels in Belgian-born girls with idiopathic or organic precocious puberty. Since DDT is still used in a number of developing countries, but banned in Europe, the data suggest a possible relationship between early exposure to this pesticide and premature menarche (Krstevska-Konstantinova et al., 2001). In-utero exposure to EDCs can also alter the growth of the mammary gland and the age of onset of puberty of the offspring many years after exposure (Wang et al., 2005). The effect of in utero exposure to polybrominated biphenyls (PBBs) on sexual maturation has been evaluated in US girls whose mothers were accidentally exposed through diet to a PBB flame retardant (FireMaster) (Blanck et al., 2000). Menarche and pubertal hair growth were significantly advanced in breastfed girls exposed to high levels of PBB in utero compared to breastfed girls exposed to lower levels of PBB in utero or girls who were not breastfed.

Precocious puberty has several physical, psychological, and social consequences (reviewed in Golub et al., 2008). The early onset of thelarche and/or menarche is positively associated with an early diagnosis of breast cancer in susceptible populations (Hamilton and Mack, 2003) and adult obesity (Biro et al., 2003). From a social point of view, changes in the timing of pubertal development may influence the risk for substances abuse, antisocial behaviour, eating disorders and emotional stress (Patton and Viner, 2007).

6. Factors leading to gender differences in EDC exposure

The processes that determine tissue exposure to EDCs appear, at first sight, to be both simple and independent of gender. However, gender-related factors can affect exposure to EDCs, indirectly, through differences in the ecology of the two sexes. e.g. in many deer species, males and females inhabit different geographic areas and/or exploit different feed resources and may therefore be exposed to subtly different EDC burdens. In mammals, gender differences in physiology, especially those associated with the nutrient demands of pregnancy and lactation may result in increased food (and possibly EC) consumption in some species (e.g. sheep; Foot and Russel, 1979) and result in the mobilisation of fat reserves and associated release of stored pollutants (Bigsby et al., 1997);

Table 3
Human cohort studies investigating the potential relationship between endocrine disrupting agents and early onset of puberty in girls.

Compound	Study area	Exposure	Subjects	Main findings	References
Main studies supporting associations between early puberty and EDCs					
DDE	Michigan (USA)	Prenatal	151 Girls	Reduced age of menarche by 1 year	(Vasiliu et al., 2004)
DDE	Belgium	Pubertal	41 Girls	Serum DDE was significantly elevated in girls with precocious puberty	(Krstevska-Konstantinova et al., 2001)
PBBs	Michigan (USA)	Prenatal & lactational	327 Girls	High PBBs were associated with precocious menarche and earlier pubic hair stage	(Blanck et al. 2000)
Phthalate	Puerto Rico	Pubertal	76 Girls	Elevated phthalate levels were associated with earlier thelarche	(Colon et al., 2000)
Phyto-estrogens	Turkey	Pubertal	4 Girls	Use of Foeniculum vulgare was associated with premature thelarche	(Turkyilmaz et al., 2008)
Zearalenone	Tuscany (Italy)	Prepubertal	32 Girls	Serum zearalenone was significantly elevated in girls with precocious puberty	(Massart et al. 2008)
DDT/DDE	Shanghai	Prepubertal	466 Girls	Higher DDT/DDE associated with earlier age at menarche	(Ouyang et al., 2005)
Main studies finding no association between early puberty and EDCs					
DDE PCBs	North Carolina (USA)	Prenatal and lactational	316 Girls	No effect on pubertal stages	(Gladen et al., 2000)
Dioxin	Seveso (Italy)	Prepubertal	282 Girls	No effect on age at menarche	(Warner et al., 2004)
PCBs	Michigan (USA)	Prenatal and lactational	327 Girls	No effect on pubertal stages	(Blanck et al., 2000)
DDE	Mohawk nation (USA)	Prepubertal	138 Girls	No association with age of menarche	(Denham et al., 2005)

both effects may contribute to different chemical exposures between males and females.

In addition to differences in exposure associated with ecology or physiological state, there is also potential for gender differences in post-exposure metabolism, which in turn affects target organ exposure. Sex-based differences in the pharmacokinetics of EDCs can be attributable to sex differences in hormone profiles and associated effects on gastrointestinal motility and body composition, organ blood flow and renal clearance rates (Waxman and Holloway, 2009). However, differences in metabolism are considered to be the primary determinants of sex differences in the processing of drugs and xenobiotics. These differences are a function of the activities of many phase 1 and phase 2 liver enzymes, including cytochrome P450s, sulfotransferases, glutathione transferases and UDP-glucuronosyltransferase and of complex interactions between the relevant genes, hormonal signals and associated transcription factors (Waxman and Holloway, 2009). While many of the principles underlying sex differences in metabolism have been established through studies of pharmaceuticals, it has also been shown in rats that sexually dimorphic metabolic responses occur in response to well known disrupting chemicals such as DDT (Sierra-Santoyo et al., 2000). Similarly, the dimorphic neuronal responses to the herbicide atrazine shown in mice (Giusi et al., 2006), and dimorphic behavioural responses to PCBs observed in humans (Ibanez et al., 2000), may be a function of underlying metabolic differences.

In humans, foetal exposure to environmental toxicants is a major concern (Mattison, 2010). During development in the human, the basic structure of the liver is present by the end of the first trimester and several enzymes involved in drug metabolism are already expressed at this stage (Koukouritaki et al., 2002; Stevens et al., 2003). About 70% of the blood going to the foetal liver comes directly from the placenta and the feto–maternal interface through the umbilical vein and the foetal liver is, therefore, exposed to high concentrations of maternally-derived xenochemicals. It is likely, therefore, that the liver is both affected by xenobiotics and is important for their metabolism/activation from early in foetal development through to adult life. In foetal and neonatal development there is growing evidence that sex-dependent differences exist in hepatic function. In rats, for example, foetal hepatic transcript levels can be affected by changes in maternal diet in a sexually dimorphic manner (Kwong et al., 2007). More recently, in a study of 55 normally-progressing, electively terminated, human foetuses, we have shown that there are significant sex-differences in the levels of mRNA transcripts encoding a number of hepatic enzymes in the human foetal liver (O'Shaughnessy et al., 2011). The most likely cause of such foetal gender effects is the action of testicular androgen in males although differences in foetal growth rates cannot be excluded. The contribution of foetal metabolism to overall pharmacokinetics of xenobiotics and pharmaceuticals during pregnancy in the human remains unclear. However, there is evidence from other primates that foetal levels of some metabolites exceed maternal levels (Garland et al., 2005) suggesting that for some ECs and EDCs, foetal toxicant burden are probably higher than those in the mother.

Once puberty is reached, differences in the pattern of pituitary GH release induce a clear sexual dimorphism in hepatic metabolic activity. This is most apparent in rodents although less pronounced GH-dependent sex differences in hepatic function are also seen in humans (Waxman and Holloway, 2009). These endogenous sex differences can interact with ECs and EDCs in the human to alter patterns of liver metabolism. For example, CYP3A4 is the major catalyst of cytochrome P450 oxidative metabolism in the liver (responsible for metabolism of more than 60% of all drugs). It is expressed at higher levels in women than in men and is induced in both sexes by xenobiotics through action on the orphan nuclear pregnane X receptor (PXR). PXR is a member of a larger group of nuclear receptors which act to detect and activate a defensive response to xenobiotics and they can themselves show sex differences in expression (Jalouli et al., 2003). Overall hepatic responses to EDC exposure, therefore, are complex and likely to depend on age and sex as well as level and type of exposure.

7. Evidence for epigenetic effects of EDCs

Given the level of interest in epigenetic programming of early development over the past decade, the paucity of primary data on the effects of EDCs on epigenetic programming through the germline, particularly the female germline, is perhaps surprising (Zama and Uzumcu, 2009, 2010; Bernal and Jirtle, 2010). This is a critical deficiency since such epigenetic changes may induce deficits or alterations in the developmental trajectory of unexposed generations, i.e. true transgenerational effects (Fig. 2). Studies to date, understandably, have largely been conducted in rodents and have tended to focus on DNA methylation at specific regions (usually promoter associated CpG islands) in specific genes, or have employed global DNA methylation assays that can provide quantitative estimates of changes to DNA methylation but little in the way of a mechanistic insight. Consequently, these data tend to be correlative in nature. That said, that there is sufficient evidence to support the notion that at least some of the actions of EDCs on developmentally-important genes and associated processes are epigenetic in nature and there is also evidence of epigenetic transgenerational transmission of these effects.

By way of example, (Alworth et al., 2002) screened CpG island loci in uteri of CD-1 mice exposed during foetal life to varying doses of diethylstilbestrol (DES). Their analysis showed that in response to both low and high doses of DES there was an increase in methylation of a number of ribosomal genes, a state frequently observed in breast cancer. While the long term effects of hypermethylation were not characterised in that study, Tang et al. (2008) reported that in aged CD-1 mice neonatally exposed to DES (1 or 1000 μg/kg) and the isoflavonoid phytoestrogen genistein (50 mg/kg), *Nsbp1*, which is involved in chromatin remodelling, was hypomethylated and over-expressed in the uteri of DES/GEN

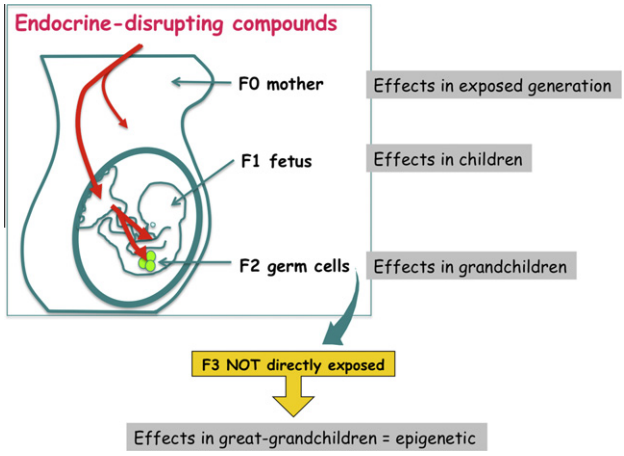

Fig. 2. Schematic summarising EDC actions across generations. If the mother is exposed to EDCs directly her fertility may be affected and, if she is pregnant, her foetus will be directly exposed (F1, children). However, the germ cells in the foetus will also be exposed and this may result in disturbance of both the F1 directly and of the F2 in the form of the F1 germ cells. For a true transgenerational effect however, the F3 generation (great-grandchildren) would have to show disruption without direct exposure of the F1 and F2 generations, post-natally. Of course the F3 generation is likely to be exposed to a different panel of chemicals and pollutants since the profiles of different chemical classes change with time, production and use.

neonatally exposed mice. Thus early life epigenetic programming of gene expression following EDC exposure is possible and such changes may lead to altered function; however, the effects may change with age or be influenced differentially by the prevailing endocrine milieu.

Transgenerational epigenetic actions of EDCs were first reported in the rat following exposure to the antiandrogenic compound vinclozolin and the oestrogenic compound methoxychlor, during gonadal sex determination (Anway et al., 2005). Such studies have demonstrated that male-germline transmission of both testicular epigenetic and phenotypic effects of EDC exposure are evident until at least the F3 generation and that at some of the genes epigenetically modified by vinclozolin are imprinted (Stouder and Paoloni-Giacobino, 2010). In contrast, in the females, no major detrimental epigenetic effects of EDC exposure were reported with regard to reproductive development (Guerrero-Bosagna and Skinner, 2009). A relatively low, but significant, incidence of pregnancy abnormalities, including uterine haemorrhage (~9% incidence) and mammary-gland tumours (~2% incidence) have more recently been reported in F2 and F3 offspring, but an epigenetic basis for these abnormalities has not been established (Nilsson et al., 2008). The basis of this sexual dimorphism in effect and epigenetic germline transmission is not currently understood, but may relate to the nature and/or timing of the initial insult.

8. Conclusions and perspective

The issue of the nature and extent of disturbance of female reproductive development and subsequent adult reproductive health remains complex and, in some cases, confused. There is a considerable need for additional research, especially into the effects of low-dose exposures, pre- and post-natally, to complex chemical cocktails which include EDCs, in other words, "real-life" exposures. We propose a series of take-home messages from recent findings in the field:

- The effects of chemicals on female reproductive development and subsequent adult heath and fertility can be loosely grouped under the heading "Ovarian Dysgenesis Syndrome", incorporating various components of a range of abnormalities, from ovary to Mullerian duct development and reproductive tract cancers.
- Maternal exposure to EDCs can lead to an inhibition of trophoblast differentiation and invasion, thereby affecting normal placentation and placental function. Thus, chemical exposure can impair post-fertilisation reproductive success in the female mammal.
- Given the critical role of the neuroendocrine systems within the hypothalamus and pituitary gland, perturbation of this system by EDCs will have knock-on effects, including disruption of gonadal function.
- Currently available human data are inadequate to fully support a rigorous conclusion that puberty onset in girls is affected by exposure to EDCs. However, the weight of the evidence strongly indicates the need for further studies on the effects of defined environmental substances on human pubertal development and for prompt precautionary actions against excessive exposure to chemicals, especially those known to affect hormonal homeostasis.
- The foetus is likely to play a significant role in the metabolism of xenobiotics with potential consequences for both the foetus itself and for the mother. This would suggest that more studies are urgently needed to determine the extent of sex-differences in human foetal hepatic function, which may have potential implications for chemical safety screening programmes.
- Sexual dimorphism in the extent and consequences of epigenetic changes in the germline pose an additional level of complexity and additional research is essential in order to better understand potential transgenerational threats posed by all chemicals.

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