REPRODUCTION

Endocrine-disrupting chemicals in ovarian function: effects on steroidogenesis, metabolism and nuclear receptor signaling

Zelieann R Craig, Wei Wang and Jodi A Flaws

Department of Comparative Biosciences, University of Illinois, 2001 S. Lincoln Avenue, Urbana, Illinois 61802, USA

Correspondence should be addressed to J A Flaws; Email: jflaws@illinois.edu

Abstract

Endocrine-disrupting chemicals (EDCs) are exogenous agents with the ability to interfere with processes regulated by endogenous hormones. One such process is female reproductive function. The major reproductive organ in the female is the ovary. Disruptions in ovarian processes by EDCs can lead to adverse outcomes such as anovulation, infertility, estrogen deficiency, and premature ovarian failure among others. This review summarizes the effects of EDCs on ovarian function by describing how they interfere with hormone signaling via two mechanisms: altering the availability of ovarian hormones, and altering binding and activity of the hormone at the receptor level. Among the chemicals covered are pesticides (e.g. dichlorodiphenyltrichloroethane and methoxychlor), plasticizers (e.g. bisphenol A and phthalates), dioxins, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons (e.g. benzo[a]pyrene).

Introduction

According to the Environmental Protection Agency (EPA), an endocrine-disrupting chemical (EDC) is an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are responsible for homeostasis, reproduction, and developmental processes. Although previously thought to exert their effects only via nuclear receptors, such as those for sex steroids, today we know that EDCs may also act through membrane receptors, neurotransmitter receptors, orphan receptors, and enzymatic pathways involved in the synthesis of hormones. Reviews of existing literature on EDCs have been published previously and summarize basic and clinical research findings related to endocrine disruption in various organs (Diamanti-Kandarakis *et al.* 2009), including the ovary (Foster *et al.* 2004). The present review focuses on endocrine disruption in the ovary and is divided into three sections, two of which are major components of the EPA definition of EDC. First, an introductory section provides the reader with background related to the ovary and its physiology and an introduction to the most widely studied ovarian hormone receptors. Secondly, we discuss examples of chemicals that alter the availability of ovarian hormones (synthesis and metabolism). Finally, we discuss the most commonly studied ovarian hormone receptors and chemicals that alter their activation and signaling. A variety of studies and experimental approaches will be discussed while giving particular emphasis to the *in vitro* studies

that have contributed to expanding our understanding of the mechanisms of action of some of the most popular ovarian EDCs.

The ovary

The cycling ovary contains ovarian follicles at different stages of development and, following ovulation, one or more corpora lutea depending on the species. The process by which the most immature follicles (primordial) develop into preovulatory follicles is termed folliculogenesis (reviewed in Oktem & Urman (2010)). Primordial follicles exist in a finite number and consist of a single oocyte (egg) surrounded by a single layer of flattened somatic cells known as granulosa cells. During folliculogenesis, granulosa cells become activated, change into a cuboidal shape, and proliferate forming multiple layers around the oocyte. Also, during this process, the oocyte grows, and additional somatic cells known as theca cells are recruited to the follicular structure. Finally, toward the end of folliculogenesis, the follicle obtains a fluid-filled cavity known as the antrum. At this time, the follicle is now referred to as an antral follicle. Antral follicles are the major source of the ovarian steroids and are capable of ovulation following proper stimulation by LH. After ovulation or expulsion of the oocyte from the follicle, the remaining granulosa and theca cells undergo a process of differentiation known as luteinization. During luteinization, the granulosa and theca cells become luteal cells and the former follicular structure is now known as a corpus luteum. A functional

corpus luteum produces progesterone, the hormone necessary for successful implantation and maintenance of pregnancy. If fertilization does not occur, or a pregnancy is not accomplished, the corpus luteum will undergo a process of cell death known as luteolysis or corpus luteum regression. Disruption of the process of folliculogenesis and corpus luteum formation can lead to adverse reproductive outcomes such as anovulation, infertility, decreased fecundity, estrogen deficiency, and premature ovarian failure.

Ovarian steroidogenesis

Both major ovarian structures, the antral follicle and the corpus luteum, are steroidogenic glands. Antral follicle steroidogenesis has been described by the two-cell, two-gonadotropin theory of ovarian steroidogenesis. Specifically, this theory describes how granulosa and theca cells work together to make the ovarian steroids (Falck (1959); and others reviewed in Richards (1980); Fig. 1). Theca cells possess receptors for LH, which is released from the anterior pituitary (Channing & Kammerman (1974); and others reviewed in Richards (1980)). Upon binding to its receptor, LH signals theca cells to increase transcription of genes encoding the enzymes necessary for conversion of cholesterol to androgens (androstene-dione and testosterone). On the other hand, granulosa

cells possess receptors for FSH, which is also released from the anterior pituitary (Carson *et al.* (1979); and others reviewed in Richards (1980)). Upon binding to its receptor, FSH signals granulosa cells to increase transcription of genes encoding the enzymes necessary for conversion of theca-derived androgens into estrogens (17β-estradiol (E_2) and estrone). In the theca cell, cholesterol is either internalized by lipoprotein receptors or synthesized *de novo*. Once in the cytoplasm, cholesterol is transported into the mitochondria by STAR (Strauss *et al.* 1999, Christenson & Strauss 2000, Stocco 2001). In the mitochondria, cholesterol is converted to pregnenolone by cytochrome P450 cholesterol side-chain cleavage (CYP450scc; CYP11A1; Miller 1988, Hanukoglu 1992). Pregnenolone diffuses from the mitochondria into the smooth endoplasmic reticulum where it can be converted to progesterone by 3β-hydroxysteroid dehydrogenase (HSD3B; Penning 1997) or to DHEA by 17α-hydroxylase-17,20-desmolase (CYP45017α; CYP17A1). Both HSD3B and CYP17A1 will catalyze the conversion of DHEA and progesterone to androstenedione (Hanukoglu 1992) respectively. Androstenedione can either be converted to testosterone by 17β-hydroxysteroid dehydrogenase (HSD17B) within the theca cell or diffuse into the granulosa cell. In the granulosa cell, aromatase (CYP450arom; CYP19A1) can convert androstenedione to estrone and testosterone to
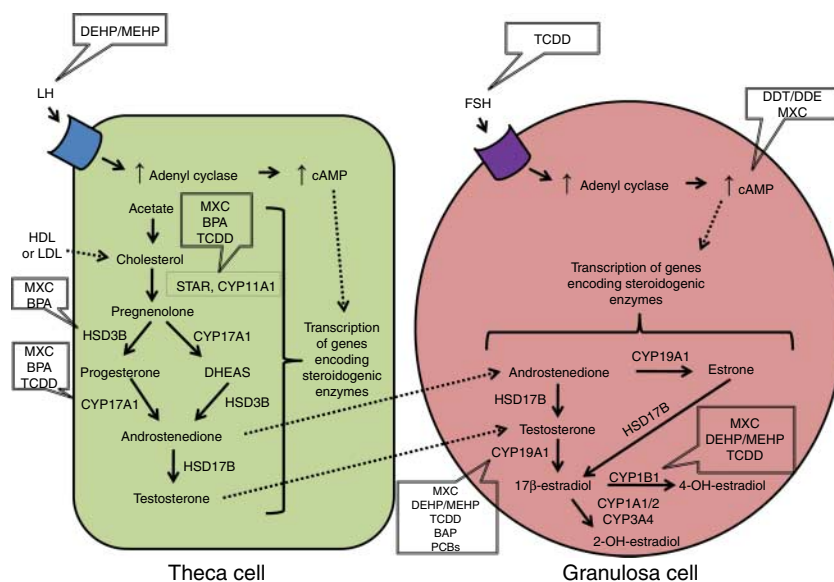

Figure 1 Ovarian steroidogenesis. Ovarian steroidogenesis requires the cooperative interactions of the theca and granulosa cells within the follicle. Theca cells respond to LH signaling by increasing the expression of enzymes necessary for the conversion of cholesterol to androgens (androstenedione and testosterone). Granulosa cells respond to FSH signaling by increasing the expression of enzymes necessary for the conversion of theca-derived androgens into estrogens (17β-estradiol and estrone). Finally, the major estrogen, 17β-estradiol can be further metabolized into hydroxylated metabolites and, thus, becomes inactive. Several chemicals have been shown to target various steps within this pathway and are shown in callout boxes. HDL, high-density lipoprotein; LDL, low-density lipoprotein; CYP11A1, cytochrome P450 11A1 or cholesterol side-chain cleavage; HSD3B, 3β-hydroxysteroid dehydrogenase; CYP17A1, cytochrome P450 17A1 or 17α-hydroxylase, 17,20-desmolase; HSD17B, 17β-hydroxysteroid dehydrogenase; CYP19A1, cytochrome P450 19A1 or aromatase; CYP1B1, cytochrome P450 1B1; CYP1A1/2, cytochrome P450 A1 and A2; CYP3A4, cytochrome P450 3A4; MXC, methoxychlor; BPA, bisphenol A; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; DDT, dichlorodiphenyltrichloroethane; DDE, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; DEHP, di-(2-ethylhexyl) phthalate; MEHP, mono-2-ethylhexyl phthalate; BAP, benzo[a]pyrene; PCBs, polychlorinated biphenyls.

E_2 (Hanukoglu 1992). Furthermore, granulosa cells can convert estrone to E_2 through the action of HSD17B (Penning 1997). E_2 is the most potent form of estrogen in female reproduction. However, following its synthesis, E_2 can be further metabolized into 2-hydroxyestradiol by CYP1A1/2 and CYP3A4, or to 4-hydroxyestradiol by CYP1B1 (Hayes *et al*. 1996, Tsuchiya *et al*. 2005). These modifications lead to inactivation of E_2 and further modifications are thought to lead to cell damage (Tsuchiya *et al*. 2005).

Several chemicals have the ability to alter the synthesis and metabolism of ovarian sex steroid hormones by altering the expression and catalytic activity of these enzymes. Therefore, endocrine disruption occurs when the hormone is no longer available to bind its receptor and fails to elicit its physiological function (Tables 1 and 2).

Ovarian hormone receptors

The ovarian hormone receptors include those for the sex steroid hormones estrogen, progestins, and androgens (reviewed in Drummond *et al*. (2002) and Brosens (2004)), as well as orphan receptors like the aryl hydrocarbon receptor (AHR; reviewed in Pocar *et al*. (2005)) and the gonadotropin receptors, which bind LH and FSH. The sex steroid hormone receptors and the AHR are ligand-dependent transcription factors that bind DNA and control expression of specific genes. On the other hand, LH and FSH receptors are G protein-coupled receptors that modulate cell function by activating second messenger signaling pathways upon binding to their peptide hormone ligands (Magoffin & Erickson 1982, Wang *et al*. 1982). A growing number of environmental contaminants, which are considered endocrine disruptors, can cause ovarian toxicity. In the ovary, most studies have focused on the effects of EDCs on estrogen receptors (ESRs), the AHR and the androgen receptor (AR).

Chemicals that alter the availability of ovarian hormones

Pesticides: dichlorodiphenyltrichloroethane and methoxychlor

Dichlorodiphenyltrichloroethane (DDT) is a broad-spectrum insecticide widely used in the 1940s, but presently banned in many countries. In mammals, DDT is mainly metabolized by dechlorination and subsequent dehydrochlorination to 1,1-dichloro-2,2-bis(4-chloro-phenyl)ethylene, referred to as DDE (Gold & Brunk 1982). The effects of DDT and DDE on the female reproductive system have been widely studied and have been reviewed elsewhere (Tiemann 2008). Early evidence supporting DDT and DDE as endocrine disruptors came from studies reporting altered sex steroid hormone concentrations in alligators hatched from eggs obtained

from contaminated lakes (Guillette *et al*. 1995, 1996). Also, evidence supporting exposure to DDT and DDE as a public concern has risen from studies reporting the presence of these chemicals in serum (range: 1.08–1.68 ng/ml) and follicular fluid (0.01–1.11 ng/ml) of women (Jarrell *et al*. 1993, De Felip *et al*. 2004, Al-Saleh *et al*. 2009, Meeker *et al*. 2009), farm animals (0.16–1.50 ng/ml; Kamarianos *et al*. 2003) and the fact that they can accumulate in the egg-forming tissues and egg yolk of laying hens (Furusawa 2002). Studies using porcine granulosa cells have demonstrated that DDE alters progesterone synthesis. Specifically, it has been shown that at low concentrations (10 ng/ml), DDE is able to increase progesterone synthesis by porcine granulosa cells (Crellin *et al*. 1999). Interestingly, higher concentrations of DDE (0.3–320 μg/ml) were shown to block progesterone synthesis in stable and primary cultures of porcine granulosa cells (Chedrese & Feyles 2001, Crellin *et al*. 2001). In fact, different effects on steroidogenesis have been reported for isomers of DDT isomers (p,p'- and o,p'-DDT) and isomers of its metabolite DDE (p,p'- and o,p'-DDE) in porcine follicular cells. Specifically, p,p'-DDT (0.4–4 μg/ml) was shown to decrease E_2 production, while o,p'-DDT and the DDE isomers at doses ranging from 0.004 to 4 μg/ml increased it. Also, progesterone secretion was decreased by addition of DDT isomers and p,p'-DDE (4 μg/ml), but not by o,p'-DDE (Wójtowicz *et al*. 2007). As far as the mechanism(s) by which DDE alters steroidogenesis, it has been observed that high doses of DDE ($\sim$3–32 μg/ml) lead to decreased cAMP synthesis (Chedrese & Feyles 2001) and decreased expression of *Cyp11a1* (Crellin *et al*. 2001) in porcine granulosa cells. Therefore, it is thought that DDE decreases progesterone synthesis in granulosa cells by impairing generation of cAMP, which leads to reduced transcription of *Cyp11a1* mRNA. In turn, decreased levels of *Cyp11a1* mRNA, which encodes the main rate-limiting enzyme in ovarian steroidogenesis, are responsible for decreased synthesis of ovarian sex steroids such as progesterone and E_2. However, the mechanism by which different doses of DDT and/or isomers cause opposite effects (e.g. increased/decreased progesterone) is not well understood. Perhaps this can be attributed to differences in affinity for receptors and/or enzymes due to different chemical structures, doses administered and length of exposure. Some studies also suggest that aromatase activity can also be a target of DDT because treatment with DDT and/or DDE (4 μg/ml) results in increased aromatase activity in porcine follicular cells (Wójtowicz *et al*. 2007) and human granulosa cells (100 ng/ml; Younglai *et al*. 2004a). Furthermore, another study demonstrated that DDE (100 ng/ml) can stimulate calcium (Ca^{2+}) mobilization in human granulosa-lutein cells (Younglai *et al*. 2004b). Therefore, DDT may not only interfere with gonadotropin receptor signaling second messengers such as cAMP, but also may directly

Table 1 Selected chemicals that alter availability of ovarian hormones.

Chemical	Species	Effect	Reference(s)
DDT/DDE	Alligators	↓ Sex steroid levels	Guillette *et al.* (1995, 1996)
	Porcine granulosa cells	↑ Progesterone synthesis; low doses	Crellin *et al.* (1999)
		↓ Progesterone synthesis; high doses	Chedrese & Feyles (2001) and Crellin *et al.* (2001)
		↓ *Cyp11a1* mRNA	Chedrese & Feyles (2001) and Crellin *et al.* (2001)
	Porcine theca/granulosa cells	↑ E_2 and testosterone production	Wójtowicz *et al.* (2007)
		↑ Aromatase activity	Wójtowicz *et al.* (2007)
	Human granulosa cells	↑ Aromatase activity	Younglai *et al.* (2004a)
MXC	Pregnant rats	↓ Serum progesterone levels; ↓ *ex vivo* E_2 and testosterone production	Cummings & Laskey (1993)
	Xenopus ovaries	↓ Progesterone synthesis	Pickford & Morris (2003)
	Porcine granulosa cells	↑ Progesterone synthesis	Chedrese & Feyles (2001)
		↑ *Cyp11a1* mRNA	Crellin *et al.* (2001)
	Mouse antral follicles	↑ *Cyp1b1* mRNA	Basavarajappa *et al.* (2011)
		↓ *Star*, *Cyp11a1*, *Hsd3b1*, *Cyp17a1*, and *Cyp19a1* mRNAs	Basavarajappa *et al.* (2011)
Mono-OH MXC	Mouse antral follicles	↓ Progesterone, androstenedione, testosterone, E_2	Craig *et al.* (2010)
		↓ *Cyp11a1*, *Cyp17a1*, *Cyp19a1*, and mRNA	Craig *et al.* (2010)
HPTE	Rat granulosa cells	↓ FSH-stimulated progesterone and E_2 synthesis	Zachow & Uzumcu (2006)
		↓ FSH-induced *Cyp11a1* and *Cyp19a1* mRNAs	Zachow & Uzumcu (2006)
		↓ *Inha*, *Inhba*, *Kitl*, and *Igf1* mRNAs	Harvey *et al.* (2009)
		↔ *Cyp11a1* mRNA, protein, and catalytic activity	Akgul *et al.* (2008)
		↓ *Cyp11a1*, *Hsd3b1*, and *Cyp19a1* mRNAs	Harvey *et al.* (2009)
	Rat theca cells	↓ CYP11A1 catalytic activity; ↔ *Cyp11a1* mRNA and protein	Akgul *et al.* (2008)
BPA	Mouse antral follicles	↓ Progesterone, DHEA, androstenedione, testosterone, estrone, and E_2	Peretz *et al.* (2011)
		↓ *Star*, *Hsd3b1*, and *Cyp17a1* mRNAs	Peretz *et al.* (2011)
	Rat theca–interstitial cells	↑ Testosterone synthesis	Zhou *et al.* (2008)
		↑ *Star*, *Cyp11a1*, and *Cyp17a1* mRNAs	Zhou *et al.* (2008)
	Rat granulosa cells	↑ Progesterone production	Zhou *et al.* (2008)
		↑ *Cyp11a1* mRNA	Zhou *et al.* (2008)
	Porcine granulosa cells	↓ Progesterone production	Mlynarcíková *et al.* (2005) and Grasselli *et al.* (2010)
		↑ E_2 production; low doses	Grasselli *et al.* (2010)
		↓ E_2 production; high doses	Grasselli *et al.* (2010)
		↑ Progesterone levels; dependent on dose	Mlynarcíková *et al.* (2005)
		↓ E_2 production; dependent on dose	Mlynarcíková *et al.* (2005)
	Human granulosa cells	↓ *Cyp19a1* mRNA	Kwintkiewicz *et al.* (2010)
	Human granulosa-like tumor cells	↓ *Cyp19a1* mRNA	Kwintkiewicz *et al.* (2010)
	Caiman	↑ Serum E_2; ↓ serum testosterone	Stoker *et al.* (2008)
	Fish	↓ *Cyp19a1* mRNA	Wang *et al.* (2010)
DEHP/MEHP	Rats	↓ E_2 levels	Xu *et al.* (2010)
		↓ *Cyp19a1* mRNA and protein	Ma *et al.* (2006)
		↑ Serum LH; ↑ serum E_2	Ma *et al.* (2006)
	Mouse antral follicles	↓ E_2 production	Gupta *et al.* (2010)
		↓ *Cyp19a1* mRNA; prevented with E_2 co-treatment	Gupta *et al.* (2010)
	Mouse preantral follicles	↑ Conversion of E_2 to estrone	Lenie & Smitz (2009)
		Precocious increase in progesterone synthesis	Lenie & Smitz (2009)
	Human granulosa-lutein cells	↓ E_2 synthesis	Reinsberg *et al.* (2009)
		↓ *Cyp19a1* mRNA and activity	Reinsberg *et al.* (2009)
	Rat granulosa cells	↓ Serum progesterone levels	Svechnikova *et al.* (2007)
		↓ *Cyp19a1* mRNA and protein	Lovekamp & Davis (2001)
		↑ *Hsd17b4* and *Cyp1b1* mRNAs	Lovekamp-Swan *et al.* (2003)
TCDD	Rat; *in utero*	↓ *Star* and *Cyp17a1* mRNAs in ovaries	Takeda *et al.* (2009)
	Rat; *in utero*+lactational	↑ Plasma progesterone	Pesonen *et al.* (2006)
		↓ Plasma E_2 in dams and pups; ↑ plasma FSH in pups	Myllymäki *et al.* (2005b)
		↓ *Star* and *Cyp19a1* mRNAs in pup ovaries	Myllymäki *et al.* (2005b)
	Rat; whole ovary microarray	↑ *Cyp1b1*, ↓ *Cyp17a1*, and ↓ *Gdf9*	Valdez *et al.* (2009)

DDT, dichlorodiphenyltrichloroethane; DDE, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene; E_2, 17β-estradiol; MXC, methoxychlor; Mono-OH MXC, mono-hydroxy methoxychlor; HPTE, bis-hydroxy methoxychlor; BPA, bisphenol A; DEHP, di-(2-ethylhexyl) phthalate; MEHP, mono-(2-ethylhexyl)phthalate; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

alter enzymatic activity, and modulate Ca^{2+}-dependent pathways. DDT and DDE ($\sim$108 and 98 mg/kg, respectively) have also been shown to increase expression and activity of male rat hepatic cytochrome P450 enzymes such as those in the CYP2 and CYP3 families (Nims *et al.* 1998). Interestingly, enzymes within the CYP3A sub-family of metabolic enzymes are involved in the metabolism of estrogens in humans (reviewed in Tsuchiya *et al.* (2005)). Therefore, although no studies have described this phenomenon in females or in ovarian cells, it is important to consider that perhaps DDT may affect hepatic metabolism and increase breakdown of ovarian estrogens. Thus, it is possible that DDT causes endocrine disruption by altering both synthesis and metabolic breakdown of the ovarian steroids.

Methoxychlor (MXC) is an organochlorine pesticide introduced as a less persistent alternative to DDT. Unfortunately, many studies have demonstrated that MXC is also an endocrine disruptor and its effects on female reproduction have been reviewed previously (Tiemann 2008). Evidence for the ability of MXC to alter levels of ovarian hormones has been provided by various groups. Experiments by Cummings & Laskey (1993) showed that MXC (250–500 mg/kg) reduced serum progesterone levels, impaired implantation and decreased *ex vivo* production of E_2 and testosterone in rats. Studies in amphibians have shown that MXC (0.5–25 ng/ml) inhibits progesterone synthesis and interferes with progesterone-induced oocyte maturation (Pickford & Morris 1999, 2003). In cultured porcine granulosa cells, MXC (0.1–3.5 ng/ml) reduced basal and FSH- and

cholera toxin-stimulated progesterone production (Chedrese & Feyles 2001, Crellin *et al.* 2001), and increased levels of *Cyp11a1* transcript without altering levels of cAMP (Crellin *et al.* 2001). Therefore, it has been proposed that MXC may affect sex steroid levels by blocking a step in the steroid synthesis pathway that is downstream of generation of cAMP, but upstream of conversion of cholesterol to pregnenolone (Crellin *et al.* 2001). The fact that MXC has also been shown to increase intracellular Ca^{2+} concentrations at low doses ($\sim$0.01–0.5 μg/ml; Wu *et al.* 2006), suggests that MXC may interact with membrane receptors and parallel pathways that modulate ovarian steroidogenesis. In mouse antral follicles, MXC (1–100 μg/ml) was shown to decrease expression of mRNAs encoding the main steroidogenic enzymes with subsequent decreases in the main sex steroids (Basavarajappa *et al.* 2011). Studies in rodents have determined that upon entering the body, MXC is absorbed and sequentially demethylated by hepatic CYP450 enzymes to form a mono-hydroxylated metabolite commonly known as mono-OH and a bis-hydroxylated metabolite known as HPTE (Imai & Coulston 1968, Kapoor *et al.* 1970). It has been proposed that effects of MXC are due to these metabolites. In fact, several studies have shown that these metabolites disrupt ovarian steroidogenesis *in vitro*. For example, mono-OH (10 μg/ml) decreases synthesis of progesterone, androstenedione, testosterone, and E_2 in isolated mouse antral follicles (Craig *et al.* 2010). In that study, decreased steroidogenesis was accompanied by decreased expression of *Cyp11a1*, *Cyp17a1*, and *Cyp19a1*. HPTE (1–10 μM) has been shown to reduce FSH-stimulated

Table 2 Chemicals that alter binding and activity of ovarian receptors.

Receptor	Chemical	Species	Effect	Reference(s)
ESR	DES	Mouse (neonatal)	Delayed folliculogenesis (ESR1)	Kim *et al.* (2009a, 2009b)
			Polyovular follicles (ESR2)	Kirigaya *et al.* (2009)
	Genistein	Mouse (neonatal)	↓ Fertility	Jefferson *et al.* (2002, 2009) and Cimafranca *et al.* (2010)
			Polyovular follicles (ESR2)	
			Altered estrous cycles	
			Delayed parturition	
	MXC	Rat (prenatal and neonatal)	Hypermethylation on ESR2	Zama & Uzumcu (2009)
	BPA	Mouse (prenatal)	Disrupting early oogenesis (ESR2)	Susiarjo *et al.* (2007)
		Mouse (prenatal)	↓ Ovulation	Maffini *et al.* (2006)
AHR	TCDD	Rat	Premature reproductive senescence	Shi *et al.* (2007)
			↓ E_2 levels	
		Porcine	↑ The expression of AHR in granulosa cells	Wojtowicz *et al.* (2005)
		Rat	↓ Ovulation by ↓ the expression of PTGS2	Mizuyachi *et al.* (2002)
	PAH	Rat (prenatal)	↓ Ovarian reserve of female offspring	Jurisicova *et al.* (2007)
		Mouse	↑ The expression of pro-apoptotic genes	Pru *et al.* (2009)
			↑ Germ cell depletion	
AR	CNP	*In vitro* (hamster ovary cell)	Binds to AR as antagonist in ovary	Kojima *et al.* (2003)
PGR	HPTE	Rat	↓ PGR expression	Harvey *et al.* (2009)
LHR	HPTE	Rat	↓ LHR expression	Harvey *et al.* (2009)
	TCDD	Rat	↓ Expression and mRNA stability of LHR	Minegishi *et al.* (2003)

ESR, estrogen receptor; DES, diethylstilbestrol; ESR1, estrogen receptor α; ESR2, estrogen receptor β; MXC, methoxychlor; BPA, bisphenol A; AHR, aryl hydrocarbon receptor; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; E_2, 17β-estradiol; PTGS2, prostaglandin-endoperoxide synthase 2 (also known as cyclooxygenase-2 (COX-2)); PAH, polycyclic aromatic hydrocarbon; AR, androgen receptor; CNP, chlornitrofen; PGR, progesterone receptor; HPTE, bis-hydroxy methoxychlor; LHR, LH receptor.

synthesis of progesterone and E_2 in cultured rat granulosa cells (Zachow & Uzumcu 2006). In that same study, HPTE decreased FSH-induced expression of *Cyp11a1* and *Cyp19a1*. In a similar study, Harvey *et al.* (2009) reported that HPTE (5–10 μM) decreased expression of *Cyp11a1*, *Hsd3b1*, and *Cyp19a1* in rat granulosa cells *in vitro*. Furthermore, HPTE (50–100 nM) has been shown to directly inhibit the catalytic activity of CYP11A1 in cultured theca–interstitial cells obtained from immature rats primed with pregnant mare serum gonadotropin (Akgul *et al.* 2008). However, no changes were detected in granulosa cells and no changes in *Cyp11a1* mRNA or protein were detected in theca-interstitial cells (Akgul *et al.* 2008). Interestingly, a microarray experiment using cultured rat granulosa cells (Harvey *et al.* 2009) revealed downregulation by HPTE (10 μM) of *Inhba* and *Inha* mRNAs, which encode subunits of the peptide hormone inhibin. HPTE treatment also downregulated the mRNA expression of ovarian paracrine factors such as kit ligand and insulin-like growth factor 1. This observation suggests HPTE may also cause paracrine disruption in the ovary. Lastly, evidence exists that MXC is capable of inducing metabolic enzymes involved in the catabolism of E_2. Specifically, MXC (200 mg/kg) has been reported to induce expression of rat hepatic *Cyp3a1* mRNA (Li & Kupfer 1998). Furthermore, MXC (100 μg/ml) induces expression of *Cyp1b1* mRNA in isolated mouse antral follicles to levels comparable to those stimulated by TCDD, a classical inducer of *Cyp1b1* transcription (Basavarajappa *et al.* 2011). Thus, MXC may not only decrease synthesis of sex steroids, but also increase their catabolism by modulating the expression of the enzymes responsible for these processes.

Plasticizers: bisphenol A and phthalates

Bisphenol A (BPA) is a plasticizer found in reusable plastic containers, food and beverage can liners, baby bottles and dental sealants among others. BPA exposure has become an important health concern based on its ability to 'leach' from these products and enter the materials contained within them. Human exposure has been confirmed in various tissues including ovarian follicular fluid, and various exposures have been linked to reproductive effects in animal models (reviewed in Hengstler *et al.* (2011)). Recent studies have been aimed at understanding the effects of BPA on ovarian steroidogenesis in various experimental systems. In mice, BPA (44–440 μM, 96 h) inhibits growth of antral follicles *in vitro* and reduces production of progesterone, DHEA, androstenedione, estrone, testosterone, and E_2. Furthermore, BPA-induced inhibition of steroidogenesis is accompanied by decreased expression of *Star*, *Hsd3b1*, and *Cyp17a1* transcripts (Peretz *et al.* 2011). Another study evaluating the effects of BPA on cultured rat theca–interstitial and granulosa cells showed dose-dependent alterations in sex steroid levels and mRNA for steroidogenic enzymes. Specifically, BPA (0.1–100 μM, 48 h) increased testosterone synthesis as well as expression of *Cyp17a1*, *Cyp11a1*, and *Star* mRNAs in theca–interstitial cells. In granulosa cells, BPA (0.1–10 μM, 48 h) increased progesterone and *Cyp11a1* levels, but decreased E_2 and *Cyp19a1* levels when given at concentrations among 1, 10, and 100 μM (Zhou *et al.* 2008). In cultured porcine granulosa cells, all doses of BPA (0.1–10 μM) decreased progesterone production, while a low dose of 0.1 μM increased E_2 levels and high doses ranging from 1 to 100 μM BPA decreased E_2 production (Grasselli *et al.* 2010). Another study with porcine granulosa cells showed that, depending on the dose given, BPA increased basal (1 μM) and FSH-induced (10 μM) progesterone production, while it inhibited (1–100 μM) FSH-induced E_2 production (Mlynarcíková *et al.* 2005). Furthermore, BPA (100 μM) decreased progesterone production, decreased expansion of cumulus cells and inhibited oocyte maturation in porcine oocyte–cumulus complexes (Mlynarcíková *et al.* 2009). BPA treatment (40–100 μM) decreased aromatase mRNA and protein, and reduced E_2 production by primary human granulosa cells and a human ovarian granulosa-like tumor cell line (Kwintkiewicz *et al.* 2010). Neonatal female caiman exposed in ovum to BPA at 1.4 and 140 ppm had increased E_2 serum levels, while 1.4 ppm BPA resulted in decreased testosterone levels compared with vehicle-treated controls (Stoker *et al.* 2008). Lastly, expression of ovarian aromatase (*Cyp19a1*) was downregulated in rare minnow juveniles treated with BPA (0.1–10 nM; Wang *et al.* 2010). Although various lines of evidence show that BPA alters ovarian steroidogenesis by decreasing expression of key steroidogenic enzymes, the mechanisms by which these alterations occur remains to be determined. Furthermore, the mechanisms underlying the ability of BPA to produce opposite effects on steroid hormone levels and steroidogenic enzyme expression may be due to differences in experimental approaches such as dose given, species and cell type used, and on the length of exposure.

Phthalates are synthetic chemicals used in polyvinyl chloride plastics, beauty and infant products, medical devices, and the enteric coating of some medications. Several groups have documented the ability of phthalates to cause female reproductive toxicity and the subject has been reviewed previously (Lovekamp-Swan & Davis 2003). *In vivo* studies in rats showed that di-(2-ethylhexyl) phthalate (DEHP; 300 and 600 mg/kg) significantly decreased levels of E_2, and aromatase mRNA and protein (Xu *et al.* 2010). In mice, DEHP (1–100 μg/ml) and its metabolite mono-(2-ethylhexyl) phthalate (MEHP; 0.1–10 μg/ml) inhibit antral follicle growth, reduce *in vitro* E_2 production, and decrease *Cyp19a1* expression. Furthermore, DEHP- and MEHP-induced inhibition of follicle growth and

downregulation of *Cyp19a1* mRNA levels were prevented with E_2 co-treatment (1–10 nM; Gupta *et al.* 2010). In isolated mouse small preantral follicles, MEHP treatment (10–200 μM) resulted in increased inactivation of E_2 to estrone and a precocious increase in progesterone synthesis (Lenie & Smitz 2009). In human granulosa-lutein cells, MEHP (0.6–500 μmol/l) suppressed basal and FSH-, hCG-, and 8Br-cAMP-stimulated E_2 production, and decreased aromatase mRNA and activity (Reinsberg *et al.* 2009). In porcine oocyte–cumulus complexes, DEHP increased progesterone production when given at 1 μM (Gunnarsson *et al.* 2008). However, MEHP was found to increase progesterone without altering cAMP levels produced by the granulosa tumor cell line KK-1 (Gunnarsson *et al.* 2008). A study in rats described the ability of DEHP (500 mg/kg) to alter ovarian steroidogenesis and production of the gonadotropins by gonadotropes. Specifically, *ex vivo* production of LH was increased in gonadotropes obtained from DEHP-treated immature rats while serum progesterone levels were decreased (Svechnikova *et al.* 2007). However, a different study showed that inhaled DEHP (5 and 25 mg/m^3) increased levels of LH and E_2 in serum of prepubertal rats, and it increased ovarian *Cyp19a1* expression (Ma *et al.* 2006), the latter two in disagreement with more recent findings. Perhaps this difference is due to differences in the experimental approaches used (*in vivo* versus *in vitro*) as well as exposure routes and doses given. In cultured rat granulosa cells, MEHP (50 and 100 μM) decreased *Cyp19a1* mRNA and protein (Lovekamp & Davis 2001), induced the expression of 17β-hydroxysteroid dehydrogenase IV (*Hsd17b4*), *Ahr* and *Cyp1b1* and epoxide hydrolase (*Ephx*; Lovekamp-Swan *et al.* 2003). Thus, MEHP may alter E_2 levels by decreasing synthesis (decreased *Cyp19a1*) and by increasing its catabolism by *Cyp1b1* and *Hsd17b4*, which are involved in E_2 conversion to catechol estrogens and estrone respectively. Finally, although the majority of studies evaluating the effects of phthalates on ovarian function have focused on DEHP and MEHP, one study demonstrated that dioctylphthalate (DOP), diisodecylphthalate (DiDP), and diisononylphthalate (DiNP) possess endocrine-disrupting ability because they increase progesterone production by cultured porcine granulosa cells (Mlynarcíková *et al.* 2007). These findings indicate that phthalates, other than DEHP and MEHP, may alter ovarian processes and merit further investigation.

Polyhalogenated arylhydrocarbons

The subject of the molecular actions of polyhalogenated arylhydrocarbons, such as polychlorinated dibenzo-*p*-dioxins (PCDDs; dioxins) and polychlorinated biphenyls (PCBs) on female reproduction has been reviewed previously (Petroff *et al.* 2001, Hombach-Klonisch *et al.* 2005, Kotwica *et al.* 2006).

Therefore, this section focuses on recent findings on the ability of these chemicals to reduce the availability of ovarian hormones as a mechanism for ovarian endocrine disruption.

Dioxins are a class of environmental chemicals exemplified by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The effects of TCDD on porcine steroidogenesis have been described by others and the subject has been reviewed previously (Gregoraszczuk 2002). Therefore, this section will focus on providing an updated and brief overview of the subject including data obtained using other species. A study in rats investigated whether maternal exposure to TCDD (1 μg/kg) damages gonadotropin-regulated steroidogenesis by fetal gonads and found that it decreased the expression of LH in the pituitaries, and decreased *Star* and *Cyp17a1* mRNAs in the ovaries of the offspring (Takeda *et al.* 2009). Another study evaluated the effects of gestational and lactational exposure to TCDD (0.04–1 μg/kg) on ovarian steroidogenesis in rats, and observed that plasma progesterone levels were significantly increased in the absence of decreased steroidogenic enzyme expression when animals were given a dose of 1 μg/kg TCDD (Pesonen *et al.* 2006). *In utero* and lactational exposure of rats to TCDD (0.04–1 μg/kg) resulted in decreased E_2 levels in serum of dams and offspring, decreased levels of *Star* and increased levels of FSH when given at 1 μg/kg. A lower dose of 0.2 μg/kg resulted in decreased *Cyp19a1* mRNA in that same study (Myllymäki *et al.* 2005b). In cultured immature rat granulosa cells, TCDD (10 pM) reduced FSH-induced LH receptor mRNA expression (Minegishi *et al.* 2003). Furthermore, in whole rat ovary microarray studies, chronic TCDD exposure (200 ng/kg per week) induced *Cyp1b1*, and downregulated *Cyp17a1* and growth differentiation factor 9 (*Gdf9*), a paracrine factor involved in folliculogenesis (Valdez *et al.* 2009). Thus, evidence suggests that TCDD, such as other endocrine disruptors, decreases sex steroid hormone synthesis, but at the same time also increases catabolism of E_2, the major product of ovarian steroidogenesis.

PCBs are organic compounds widely used in dielectric fluids such as those in transformers, capacitors, and coolants. They exist in various forms termed PCB congeners. The idea of PCB exposure as a concern to humans is supported by studies reporting levels of various congeners in ovarian follicle fluid of women (total PCB 0.37 ng/g wet weight; De Felip *et al.* 2004) and in farm animals (0.45–3.05 depending on species; Kamarianos *et al.* 2003). Various studies have used different species and experimental models to evaluate the ability of PCBs to cause endocrine disruption in the ovary. In bovine luteal cells, PCBs 126, 77, and 153 (each given at 1–100 ng/ml) did not alter basal luteal steroidogenesis, but were able to decrease LH-stimulated secretion of progesterone in the luteal phase (Mlynarczuk & Kotwica 2006). A 2 days exposure

to PCB 153 (50 and 100 ng/ml) decreased progesterone and testosterone secretion by porcine ovarian follicular cells. Interestingly, longer exposures increased progesterone secretion, accompanied by decreased testosterone and a tendency for decreased E_2 secretion (Gregoraszczuk & Wójtowicz 2002). In porcine luteal cells, PCB 126 (5–100 ng/ml) was shown to decrease progesterone secretion, while the effect of PCB 153 was dependent on length of exposure. Specifically, between 48 and 72 h, PCB 153 decreased progesterone secretion, but after 72 h, it induced a dose-dependent increase in progesterone secretion (Augustowska et al. 2001). A later study in the same in vitro model evaluated the effects of PCBs 153, 118, 180, and 138, and showed that congeners 153 (8 µg/ml), 118 (3 µg/ml), and 180 (3 µg/ml) increased E_2 secretion, while congener 138 (8 µg/ml) increased testosterone secretion (Mlynarcíková et al. 2009). Although various studies have described the ability of PCBs to alter ovarian steroidogenesis, the molecular mechanisms underlying these alterations remain to be determined.

Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are potent lipophilic pollutants that consist of fused aromatic rings. These chemicals can be formed during the incomplete combustion of carbon-containing substances such as wood, coal, and tobacco. This class of chemicals includes various members of which benzo[a]pyrene (BAP), dimethylbenz[a]anthracene (DMBA), phenanthrene, and chrysene are examples. Humans are exposed to PAHs mainly through tobacco smoking. Although PAHs are recognized as potent toxicants, not many studies have evaluated their ability to alter levels of ovarian steroids directly. However, several members of this family have been described as potent ovotoxicants (e.g. DMBA and BAP) that indirectly may alter levels of hormones by depleting follicles before they reach the steroid-producing antral stage. The topic of PAH-induced depletion of preantral follicles has been briefly reviewed elsewhere (Miller et al. 2004). One study using isolated preantral follicles from rats showed that treatment with BAP ($\geq$1.5 ng/ml) inhibited follicular growth, and decreased E_2 and anti-Müllerian hormone output (Neal et al. 2010). In rats, BAP (5 and 10 mg/kg) has been shown to decrease levels of E_2 and decrease aromatase mRNA and protein (Xu et al. 2010). Furthermore, BAP, phenanthrene, and chrysene inhibited androstenedione and E_2 secretion by ovarian tissues of flounder treated with each chemical individually at 15 µM (Monteiro et al. 2000, Rocha Monterio et al. 2000) and phenanthrenequinone (100–1000 nM), a metabolite of phenanthrene, reduced LH-stimulated progesterone in rat luteal cells (Nykamp et al. 2001). Despite the limited data currently available, the influence of PAHs on steroidogenesis is evident and, thus, it is reasonable to predict that studies aimed at understanding their mechanisms of action are forthcoming.

Chemicals that alter hormone receptor binding and action

Estrogen receptors

E_2 plays critical roles in female reproduction. The biological functions of estrogens are mainly mediated by two estrogen receptors: ESR1 and ESR2. The two ESR subtypes have distinct tissue expression patterns, ligand specificities, and functions (Pelletier 2000). In the ovary, ESR1 is expressed primarily in theca and interstitial cells and its main function is to regulate steroidogenesis in theca cells (Britt & Findlay 2002). In contrast, ESR2 is predominantly expressed in granulosa cells and its main roles are FSH-directed granulosa cell differentiation, follicle maturation and ovulation (Drummond & Fuller 2010). Many EDCs interfere with female reproductive function by activating or antagonizing ESRs. Different EDCs show distinct effects in the ovary depending on their binding affinity to different ESRs.

Diethylstilbestrol (DES) is a non-steroidal synthetic estrogen that was prescribed from the 1940s to 1970s to pregnant women to prevent miscarriages. By the 1970s, numerous studies showing multi-generational effects of DES on reproductive, cardiovascular, and immune systems lead to a ban on the use of this drug during pregnancy (Goldberg & Flacone 1999). DES mimics the natural estrogen, E_2, through conserved protein-ligand contact and binds to both ESR1 and ESR2 with higher affinity than that of E_2 (Kuiper et al. 1997, le Maire et al. 2010). Animal studies have shown that neonatal exposure to DES (3 µg) induces abnormalities in the mouse ovary, including delayed folliculogenesis and polyovular follicles (Kim et al. 2009b). Interestingly, further studies on ESR deficient mice (αERKO and βERKO) have shown that DES (3 µg) induces ovarian abnormalities through different ESRs. Specifically, DES inhibits follicle formation and development through ESR1 (Kim et al. 2009b), whereas it induces polyovular follicles through ESR2 (Kirigaya et al. 2009). In mice, in utero exposure to DES (100 µg/kg) was shown to change ovarian morphology and alter ex vivo ovarian steroidogenesis in the offspring of the dosed dams (Haney et al. 1984). Furthermore, in vitro studies have demonstrated that DES can disrupt steroidogenesis by rat ovarian follicles. For example, DES (1 µM) was shown to decrease E_2 and testosterone secretion by ovarian follicles without affecting cAMP levels or aromatase activity (Myllymäki et al. 2005a). These observations highlight the fact that some EDCs not only alter the availability of endogenous hormones (i.e. synthesis and metabolism), but also interfere with hormone signaling via their specific receptors.

Phytoestrogens are plant compounds with estrogenic biological activity. They have a phenolic A ring, which is

crucial for ESR binding. Since they have much higher binding affinity for ESR2 compared with ESR1, they are thought to signal predominantly via ESR2 (Drummond & Fuller 2010). Genistein, for example, has a 20-fold higher binding affinity for ESR2 compared with ESR1. Studies have shown that neonatal exposure to environmentally relevant doses of genistein (3 µg) leads to abnormalities in female reproductive functions in rodents, including reduced fertility, polyovular follicle formation, altered estrous cycles, and delayed parturition (Cimafranca *et al.* 2010). The effects of genistein on the ovary are comparable to the ovarian phenotype of βERKO mice, suggesting that the action of genistein is predominantly mediated via ESR2 (Jefferson *et al.* 2002). Like DES, genistein has also been shown to alter steroidogenesis. Specifically, genistein (0.1–1 µM) decreased *ex vivo* production of cAMP and testosterone by increasing aromatase activity in rat ovarian follicles (Myllymäki *et al.* 2005a).

The pesticide MXC is another example of an EDC that affects ovarian function through ESR. Studies using transfected cell lines have demonstrated that the active metabolites of MXC are agonists of ESR1, but antagonists for both ESR2 and AR (Gaido *et al.* 2000). Other studies have shown that fetal and neonatal exposure to MXC (20 µg/kg and 100 mg/kg) alters methylation on the promoter region of ESR2 and therefore suppresses the expression of ESR2, causing ovarian dysfunction in the rat (Armenti *et al.* 2008, Zama & Uzumcu 2009). Interestingly, antral follicles overexpressing ESR1 have been shown to be more sensitive to toxicity by MXC and its metabolites in both *in vivo* and *in vitro* experiments (Tomic *et al.* 2006, Paulose *et al.* 2011). Increased sensitivity to MXC and metabolites in this model has been proposed to be due to differential mRNA expression of other receptors such as ESR2, AR, progesterone receptor (PGR), and AHR, as well as that of the xenobiotic-metabolizing enzyme *Cyp3a41a* (Paulose *et al.* 2011).

Early studies with BPA showed that it has estrogenic properties and its binding affinity to ESRs is lower than E_2 or DES (Zama & Uzumcu 2009). *In vitro*, BPA acts as an agonist for both ESR1 and ESR2 on a tissue-specific basis (Kurosawa *et al.* 2002), although *in vivo* findings suggest that it can also antagonize ESR2 function (Susiarjo *et al.* 2007). Furthermore, receptor binding assays show that BPA has higher affinity for ESR2 than ESR1 (Matthews *et al.* 2001). Also, receptor binding assays and X-ray crystal structure analyses have demonstrated that BPA binds strongly to human estrogen-related receptor γ (ESRRG/ ERRγ; Takayanagi *et al.* 2006, Matsushima *et al.* 2008). Animal studies have shown that prenatal and neonatal exposure to low environmentally relevant doses of BPA (25 and 250 mg/kg) cause numerous abnormalities in the ovary and uterus (Maffini *et al.* 2006). BPA causes defects in synapsis and recombination in the homologous chromosomes in the fetal ovary, which are similar to the meiotic defects in the oocytes of βERKO fetal gonads,

suggesting that BPA disrupts early oogenesis in mouse ovary via an ESR2 signaling pathway (Hunt *et al.* 2003, Susiarjo *et al.* 2007).

Aryl hydrocarbon receptor

The AHR is a ligand-activated nuclear transcription factor, which acts as an intracellular mediator of xenobiotic signaling pathways. The AHR and its nuclear partner AHR nuclear translocator (ARNT) are present in various ovarian cell types (oocytes, granulosa cells, and theca cells) in rodents, primates, and humans (Hernandez-Ochoa *et al.* 2010). In general, xenobiotics bind to the AHR, triggering the nuclear translocation and formation AHR–ARNT heterodimers. The ligand-AHR–ARNT active complex binds to the xenobiotic-responsive element on the promoter region of AHR-regulated genes and therefore alters their transcription (Pocar *et al.* 2005). These genes include *Cyp1a1* and *Cyp1b1*, as well as genes that regulate cell proliferation and differentiation (Okey *et al.* 1994, Dasmahapatra *et al.* 2002, Barnett *et al.* 2007). Gene-depletion studies in mice have suggested that the AHR controls folliculogenesis by inducing apoptosis, which leads to germ cell nest breakdown and formation of primordial follicles (Benedict *et al.* 2000). It also regulates ovarian follicular growth and steroidogenesis in antral follicles (Baba *et al.* 2005, Barnett *et al.* 2007, Hernandez-Ochoa *et al.* 2010).

Several studies indicate that the AHR mediates the ovarian toxicity of xenobiotics, such as TCDD and PCBs. TCDD has been shown to act through the AHR and affect follicle development, steroidogenesis, and ovulation. Animal studies have shown that chronic exposure to TCDD (50 and 200 ng/kg per week) induces premature reproductive senescence and decreases estradiol levels in female rats, without depletion of ovarian follicular reserves (Shi *et al.* 2007). In the porcine ovary, TCDD (32 pg/ml) induces the expression of AHR in granulosa, but not theca cells (Wojtowicz *et al.* 2005). In the rat ovary, TCDD (32 µg/kg) inhibits ovulation by suppressing the expression of prostaglandin-endoperoxide synthase 2 (PTGS2, also known as cyclooxygenase-2 (COX-2)), a critical ovulation factor, via the AHR signaling pathway (Mizuyachi *et al.* 2002).

Finally, epidemiological studies of smokers have provided evidence suggesting that PAHs are ovotoxic in women (Weinberg *et al.* 1989). Animal studies also showed that PAHs destroy ovarian follicles via the AHR signaling pathway in rat and mice (Hombach-Klonisch *et al.* 2005). Maternal exposure to PAHs before pregnancy and/or during lactation (cumulative dose of 12 mg/kg), compromises ovarian reserve of female offspring (Jurisicova *et al.* 2007). Further studies show that PAHs activate the AHR signaling pathway and induce the expression of pro-apoptotic genes, thus, accelerating germ cell depletion in mice (Pru *et al.* 2009).

Androgen receptor

The AR is expressed in the ovaries of several species, including rodents, primates, and humans. The expression occurs in granulosa cells, oocytes and to a lesser extent in theca/interstitial cells (Pelletier 2000). Androgens and the AR have well known roles in male reproduction, but less is known about their roles in female reproduction. Studies of AR knockout mice have revealed that AR function is essential for maintaining female fertility, by optimizing follicular growth, final follicle development, and ovulation (Walters *et al.* 2010). While toxicity studies related to androgens and the AR have focused on the effects of EDCs on male reproductive system, limited *in vitro* reporter gene assays have shown that the herbicide chlornitrofen exhibits antiandrogenic activity in Chinese hamster ovary cells (Kojima *et al.* 2003) while HPTE (500 mg/kg) was shown to be antiandrogenic in mouse ovarian and uterine tissues (Waters *et al.* 2001).

Other hormone receptors

In addition to the nuclear receptors discussed above, other hormone receptors, including PGR, FSHR, and LHR, might play roles in EDC-induced ovarian toxicity. Several studies have shown that exposure to EDCs can alter the downstream signaling pathways of these hormone receptors by suppressing their expression. *In vitro* gene profiling studies have shown that exposure to HPTE (5–10 µM), an active metabolite of MXC, affects the FSH signaling pathway and suppresses the expression of PGR and LHR in rat granulosa cells (Harvey *et al.* 2009). TCDD (10 pM) suppresses the expression and mRNA stability of FSH-induced LH receptors in rat granulosa cells, suggesting that TCDD disrupts the signaling pathway that responds to LH-induced ovulation (Minegishi *et al.* 2003). However, there is limited evidence showing that EDCs cause ovarian toxicity by directly affecting the binding and/or activation of these hormone receptors. Thus, more work needs to be done to define the mechanism of action of EDCs on receptors such as the PGR, FSHR, and LHR.

Summary

Many EDCs can interact with the female reproductive system and lead to endocrine disruption in the ovary. Although originally thought to exert their effects via binding transcription factor receptors, EDCs can alter endocrine function through a variety of mechanisms. In this review, we have discussed two major mechanisms by which EDCs may alter ovarian function. In the first mechanism discussed, chemicals may alter the expression and/or activity of enzymes required for synthesis and/or catabolism of ovarian sex steroids. Secondly, chemicals may alter the expression of

hormone receptors and/or their ability to bind their endogenous ligands. There is no doubt that both mechanisms lead to altered ovarian function by altering endocrine signaling within the ovary and the rest of the reproductive organs. More studies, however, are needed to further understand the mechanisms of action of currently known EDCs, identify and characterize new EDCs, and expand toxicological research beyond commonly studied receptors and pathways.

Declaration of interest

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

Funding

References

Akgul Y, Derk R, Meighan T, Rao K & Murono E 2008 The methoxychlor metabolite, HPTE, directly inhibits the catalytic activity of cholesterol side-chain cleavage (P450scc) in cultured rat ovarian cells. *Reproductive Toxicology* **25** 67–75. (doi:10.1016/j.reprotox.2007.10.007)

Al-Saleh I, Coskun S, El-Doush I, Billedo G, Mashhour A, Jaroudi K, Al-Shahrani A, Al-Mayman H & Mohamed G 2009 Outcome of *in-vitro* fertilization treatment and DDT levels in serum and follicular fluid. *Medical Science Monitor* **15** BR320–BR330.

Armenti A, Zama A, Passantino L & Uzumcu M 2008 Developmental methoxychlor exposure affects multiple reproductive parameters and ovarian folliculogenesis and gene expression in adult rats. *Toxicology and Applied Pharmacology* **233** 286–296. (doi:10.1016/j.taap.2008.09.010)

Augustowska K, Wójtowicz A, Kajta M, Ropstad E & Gregoraszczuk E 2001 Polichlorinated biphenyls (PCB126 and PCB153) action on proliferation and progesterone secretion by cultured *in vitro* porcine luteal cells. *Experimental and Clinical Endocrinology & Diabetes* **109** 416–418. (doi:10.1055/s-2001-18996)

Baba T, Mimura J, Nakamura N, Harada N, Yamamoto M, Morohashi K & Fujii-Kuriyama Y 2005 Intrinsic function of the aryl hydrocarbon (dioxin) receptor as a key factor in female reproduction. *Molecular and Cellular Biology* **25** 10040–10051. (doi:10.1128/MCB.25.22.10040-10051.2005)

Barnett K, Tomic D, Gupta R, Miller K, Meachum S, Paulose T & Flaws J 2007 The aryl hydrocarbon receptor affects mouse ovarian follicle growth via mechanisms involving estradiol regulation and responsiveness. *Biology of Reproduction* **76** 1062–1070. (doi:10.1095/biolreprod.106.057687)

Basavarajappa MS, Craig ZR, Hernández-Ochoa I, Paulose T, Leslie TC & Flaws JA 2011 Methoxychlor reduces estradiol levels by altering steroidogenesis and metabolism in mouse antral follicles *in vitro*. *Toxicology and Applied Pharmacology* **253** 161–169. (doi:10.1016/j.taap.2011.04.007)

Benedict J, Lin T, Loeffler I, Peterson R & Flaws J 2000 Physiological role of the aryl hydrocarbon receptor in mouse ovary development. *Toxicological Sciences* **56** 382–388. (doi:10.1093/toxsci/56.2.382)

Britt K & Findlay J 2002 Estrogen actions in the ovary revisited. *Journal of Endocrinology* **175** 269–276. (doi:10.1677/joe.0.1750269)

Brosens J 2004 Steroid receptor action. *Best Practice & Research. Clinical Obstetrics & Gynaecology* **18** 265–283. (doi:10.1016/j.bpobgyn.2004.01.006)

Carson RS, Findlay J, Burger H & Trounson A 1979 Gonadotropin receptors of the ovine ovarian follicle during follicular growth and atresia. *Biology of Reproduction* **21** 75–87. (doi:10.1095/biolreprod21.1.75)

Channing C & Kammerman S 1974 Binding of gonadotropins to ovarian cells. *Biology of Reproduction* **10** 179–198. (doi:10.1095/biolreprod10.2.179)

Chedrese P & Feyles F 2001 The diverse mechanism of action of dichlorodiphenyldichloroethylene (DDE) and methoxychlor in ovarian cells *in vitro*. *Reproductive Toxicology* **15** 693–698. (doi:10.1016/S0890-6238(01)00172-1)

Christenson L & Strauss J III 2000 Steroidogenic acute regulatory protein (StAR) and the intramitochondrial translocation of cholesterol. *Biochimica et Biophysica Acta* **1529** 175–187. (doi:10.1016/S1388-1981(00)00147-5)

Cimafranca M, Davila J, Ekman G, Andrews R, Neese S, Peretz J, Woodling K, Helferich W, Sarkar J, Flaws J *et al.* 2010 Acute and chronic effects of oral genistein administration in neonatal mice. *Biology of Reproduction* **83** 114–121. (doi:10.1095/biolreprod.109.080549)

Craig Z, Leslie T, Hatfield K, Gupta R & Flaws J 2010 Mono-hydroxy methoxychlor alters levels of key sex steroids and steroidogenic enzymes in cultured mouse antral follicles. *Toxicology and Applied Pharmacology* **249** 107–113. (doi:10.1016/j.taap.2010.09.001)

Crellin N, Rodway M, Swan C, Gillio-Meina C & Chedrese P 1999 Dichlorodiphenyldichloroethylene potentiates the effect of protein kinase A pathway activators on progesterone synthesis in cultured porcine granulosa cells. *Biology of Reproduction* **61** 1099–1103. (doi:10.1095/biolreprod61.4.1099)

Crellin N, Kang H, Swan C & Chedrese P 2001 Inhibition of basal and stimulated progesterone synthesis by dichlorodiphenyldichloroethylene and methoxychlor in a stable pig granulosa cell line. *Reproduction* **121** 485–492. (doi:10.1530/rep.0.1210485)

Cummings A & Laskey J 1993 Effect of methoxychlor on ovarian steroidogenesis: role in early pregnancy loss. *Reproductive Toxicology* **7** 17–23. (doi:10.1016/0890-6238(93)90005-R)

Dasmahapatra A, Trewin A & Hutz R 2002 Estrous cycle-regulated expression of CYP1B1 mRNA in the rat ovary. *Comparative Biochemistry and Physiology. Part B, Biochemistry & Molecular Biology* **133** 127–134. (doi:10.1016/S1096-4959(02)00119-7)

De Felip E, di Domenico A, Miniero R & Silvestroni L 2004 Polychlorobiphenyls and other organochlorine compounds in human follicular fluid. *Chemosphere* **54** 1445–1449. (doi:10.1016/j.chemosphere.2003.10.040)

Diamanti-Kandarakis E, Bourguignon J-P, Giudice L, Hauser R, Prins G, Soto A, Zoeller R & Gore A 2009 Endocrine-disrupting chemicals: an endocrine society scientific statement. *Endocrine Reviews* **30** 293–342. (doi:10.1210/er.2009-0002)

Drummond A & Fuller P 2010 The importance of ERbeta signalling in the ovary. *Journal of Endocrinology* **205** 15–23. (doi:10.1677/JOE-09-0379)

Drummond A, Britt K, Dyson M, Jones M, Kerr J, O'Donnell L, Simpson E & Findlay J 2002 Ovarian steroid receptors and their role in ovarian function. *Molecular and Cellular Endocrinology* **191** 27–33. (doi:10.1016/S0303-7207(02)00052-7)

Falck B 1959 Site of production of oestrogen in rat ovary as studied in microtransplants. *Acta Physiologica Scandinavica* **47** 1–101. (doi:10.1111/j.1748-1716.1960.tb01823.x)

Foster W, Neal M & Younglai E 2004 Endocrine disrupters and ovarian function. *International Congress Series* **1266** 126–132. (doi:10.1016/j.ics.2004.01.066)

Furusawa N 2002 Transferring and distributing profiles of *p,p′*-(DDT) in egg-forming tissues and eggs of laying hens following a single oral administration. *Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine* **49** 334–336. (doi:10.1046/j.1439-0442.2002.00432.x)

Gaido K, Maness S, McDonnell D, Dehal S, Kupfer D & Safe S 2000 Interaction of methoxychlor and related compounds with estrogen receptor α and β, and androgen receptor: structure–activity studies. *Molecular Pharmacology* **58** 852–858.

Gold B & Brunk G 1982 Metabolism of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane in the mouse. *Chemico-Biological Interactions* **41** 327–339. (doi:10.1016/0009-2797(82)90109-0)

Goldberg J & Flacone T 1999 Effect of diethylstilbestrol on reproductive function. *Fertility and Sterility* **72** 1–7. (doi:10.1016/S0015-0282(99)00153-3)

Grasselli F, Baratta L, Baioni L, Bussolati S, Ramoni R, Grolli S & Basini G 2010 Bisphenol A disrupts granulosa cell function. *Domestic Animal Endocrinology* **39** 34–39. (doi:10.1016/j.domaniend.2010.01.004)

Gregoraszczuk E 2002 Dioxin exposure and porcine reproductive hormonal activity. *Cadernos de Saúde Pública* **18** 453–462. (doi:10.1590/S0102-311X2002000200010)

Gregoraszczuk E & Wójtowicz A 2002 *In vitro* exposure of porcine ovarian follicular cells to PCB 153 alters steroid secretion by not their viability – preliminary study. *Scientific World Journal* **2** 261–267. (doi:10.1100/tsw.2002.90)

Guillette L, Gross T, Gross D, Rooney A & Percival H 1995 Gonadal steroidogenesis *in vitro* from juvenile alligators obtained from contaminated or control lakes. *Environmental Health Perspectives* **103** 31–36.

Guillette L, Pickford D, Crain D, Rooney A & Percival H 1996 Reduction in penis size and plasma testosterone concentrations in juvenile alligators living in a contaminated environment. *General and Comparative Endocrinology* **101** 32–42. (doi:10.1006/gcen.1996.0005)

Gunnarsson D, Leffler P, Ekwurtzel E, Martinsson G, Liu K & Selstam G 2008 Mono-(2-ethylhexyl) phthalate stimulates basal steroidogenesis by a cAMP-independent mechanism in mouse gonadal cells of both sexes. *Reproduction* **135** 693–703. (doi:10.1530/REP-07-0460)

Gupta RK, Singh JM, Leslie TC, Meachum S, Flaws JA & Yao HH 2010 Di-(2-ethylhexyl) phthalate and mono-(2-ethylhexyl) phthalate inhibit growth and reduce estradiol levels of antral follicles *in vitro*. *Toxicology and Applied Pharmacology* **242** 224–230. (doi:10.1016/j.taap.2009.10.011)

Haney A, Newbold R & McLachlan J 1984 Prenatal diethylstilbestrol exposure in the mouse: effects on ovarian histology and steroidogenesis *in vitro*. *Biology of Reproduction* **30** 471–478. (doi:10.1095/biolreprod30.2.471)

Hanukoglu I 1992 Steroidogenic enzymes: structure, function, and role in regulation of steroid hormone biosynthesis. *Journal of Steroid Biochemistry and Molecular Biology* **43** 779–804. (doi:10.1016/0960-0760(92)90307-5)

Harvey C, Esmail M, Wang Q, Brooks A, Zachow R & Uzumcu M 2009 Effect of the methoxychlor metabolite HPTE on the rat ovarian granulosa cell transcriptome *in vitro*. *Toxicological Sciences* **110** 95–106. (doi:10.1093/toxsci/kfp089)

Hayes C, Spink D, Spink B, Cao J & Walker N 1996 17β-Estradiol hydroxylation catalyzed by human cytochrome P450 1B1. *PNAS* **93** 9776–9781. (doi:10.1073/pnas.93.18.9776)

Hengstler J, Foth H, Gebel T, Kramer P-J, Lilienblum W, Schweinfurth H, Völkel W, Wollin K-M & Gundert-Remy U 2011 Critical evaluation of key evidence on the human health hazards of exposure to bisphenol A. *Critical Reviews in Toxicology* **41** 263–291. (doi:10.3109/10408444.2011.558487)

Hernandez-Ochoa I, Barnett-Ringgold K, Dehlinger S, Gupta R, Leslie T, Roby K & Flaws J 2010 The ability of the aryl hydrocarbon receptor to regulate ovarian follicle growth and estradiol biosynthesis in mice depends on stage of sexual maturity. *Biology of Reproduction* **83** 698–706. (doi:10.1095/biolreprod.110.087015)

Hombach-Klonisch S, Pocar P, Kietz S & Klonisch T 2005 Molecular actions of polyhalogenated arylhydrocarbons (PAHs) in female reproduction. *Current Medicinal Chemistry* **12** 599–616.

Hunt PA, Koehler KE, Susiarjo M, Hodges CA, Ilagan A, Voigt RC, Thomas S, Thomas BF & Hassold TJ 2003 Bisphenol A exposure causes meiotic aneuploidy in the female mouse. *Current Biology* **13** 546–553. (doi:10.1016/S0960-9822(03)00189-1)

Imai H & Coulston F 1968 Ultrastructural studies of absorption of methoxychlor in the jejunal mucosa of the rat. *Experimental and Molecular Pathology* **8** 135–158. (doi:10.1016/0014-4800(68)90012-9)

Jarrell J, Villeneuve D, Franklin C, Bartlett S, Wrixon W, Kohut J & Zouves C 1993 Contamination of human ovarian follicular fluid and serum by chlorinated organic compounds in three Canadian cities. *CMAJ: Canadian Medical Association Journal* **148** 1321–1327.

Jefferson W, Couse J, Padilla-Banks E, Woodling K, Kissling G & Newbold R 2002 Neonatal exposure to genistein induces estrogen receptor (ER)alpha expression and multioocyte follicles in the maturing mouse ovary. *Biology of Reproduction* **67** 1285–1296. (doi:10.1095/biolreprod67.4.1285)

Jefferson W, Doerge D, Padilla-Banks E, Woodling K, Kissling G & Newbold R 2009 Oral exposure to genistin, the glycosylated form of genistein, during neonatal life adversely affects the female reproductive system. *Environmental Health Perspectives* **117** 1883–1889. (doi:10.1289/ehp.0900923)

Jurisicova A, Taniuchi A, Li H, Shang Y, Antenos M, Detmar J, Xu J, Matikainen T, Benito Hernandez A, Nunez G *et al.* 2007 Maternal exposure to polycyclic aromatic hydrocarbons diminishes murine ovarian reserve via induction of *Harakiri. Journal of Clinical Investigation* **117** 3971–3978. (doi:10.1172/JCI28493)

Kamarianos A, Karamanlis X, Goulas P, Theodosiadou E & Smokovitis A 2003 The presence of environmental pollutants in the follicular fluid of farm animals (cattle, sheep, goats and pigs). *Reproductive Toxicology* **17** 185–190. (doi:10.1016/S0890-6238(02)00118-1)

Kapoor IP, Metcalf RL, Nystrom RF & Sangha GK 1970 Comparative metabolism of methoxychlor, methiochlor, and DDT in mouse, insects, and in a model ecosystem. *Journal of Agricultural and Food Chemistry* **18** 1145–1152. (doi:10.1021/jf60172a017)

Kim H, Hayashi S, Chambon P, Watanabe H, Iguchi T & Sato T 2009*a* Effects of diethylstilbestrol on ovarian follicle development in neonatal mice. *Reproductive Toxicology* **27** 55–62. (doi:10.1016/j.reprotox.2008.10.005)

Kim H, Nakajima T, Hayashi S, Chambon P, Watanabe H, Iguchi T & Sato T 2009*b* Effects of diethylstilbestrol on programmed oocyte death and induction of polyovular follicles in neonatal mouse ovaries. *Biology of Reproduction* **81** 1002–1009. (doi:10.1095/biolreprod.108.070599)

Kirigaya A, Kim H, Hayashi S, Chambon P, Watanabe H, Lguchi T & Sato T 2009 Involvement of estrogen receptor beta in the induction of polyovular follicles in mouse ovaries exposed neonatally to diethylstilbestrol. *Zoological Science* **26** 704–712. (doi:10.2108/zsj.26.704)

Kojima H, Iida M, Katsura E, Kanetoshi A, Hori Y & Kobayashi K 2003 Effects of a diphenyl ether-type herbicide, chlornitrofen, and its amino derivative on androgen and estrogen receptor activities. *Environmental Health Perspectives* **111** 497–502. (doi:10.1289/ehp.5724)

Kotwica J, Wróbel M & Mlynarczuk J 2006 The influence of polychlorinated biphenyls (PCBs) and phytoestrogens *in vitro* on functioning of reproductive tract in cow. *Reproductive Biology* **6** 189–194.

Kuiper G, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S & Gustafsson J 1997 Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* **138** 863–870. (doi:10.1210/en.138.3.863)

Kurosawa T, Hiroi H, Tsutsumi O, Ishikawa T, Osuga Y, Fugiwara T, Inoue S, Muramatsu M, Momoeda M & Taketani Y 2002 The activity of bisphenol A depends on both the estrogen receptor subtype and the cell type. *Endocrine Journal* **49** 465–471. (doi:10.1507/endocrj.49.465)

Kwintkiewicz J, Nishi Y, Yanase T & Giudice L 2010 Peroxisome proliferator-activated receptor-γ mediates bisphenol A inhibition of FSH-stimulated IGF-1, aromatase, and estradiol in human granulosa cells. *Environmental Health Perspectives* **118** 400–406. (doi:10.1289/ehp.0901161)

Lenie S & Smitz J 2009 Steroidogenesis-disrupting compounds can be effectively studied for major fertility-related endpoints using *in vitro* cultured mouse follicles. *Toxicology Letters* **185** 143–152. (doi:10.1016/j.toxlet.2008.12.015)

Li H-C & Kupfer D 1998 Mechanism of induction of rat hepatic CYP2B and 3A by the pesticide methoxychlor. *Journal of Biochemical and Molecular Toxicology* **12** 315–323. (doi:10.1002/(SICI)1099-0461(1998)12:6<315::AID-JBT1>3.0.CO;2-6)

Lovekamp T & Davis B 2001 Mono-(2-ethylhexyl) phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells. *Toxicology and Applied Pharmacology* **172** 217–224. (doi:10.1006/taap.2001.9156)

Lovekamp-Swan T & Davis BJ 2003 Mechanisms of phthalate ester toxicity in the female reproductive system. *Environmental Health Perspectives* **111** 139–145. (doi:10.1289/ehp.5658)

Lovekamp-Swan T, Jetten A & Davis B 2003 Dual activation of PPARalpha and PPARgamma by mono-(2-ethylhexyl)phthalate in rat ovarian granulosa cells. *Molecular and Cellular Endocrinology* **201** 133–141. (doi:10.1016/S0303-7207(02)00423-9)

Ma M, Kondo T, Ban S, Umemura T, Kurahashi N, Takeda M & Kishi R 2006 Exposure of prepubertal female rats to inhaled di(2-ethylhexyl)phthalate affects the onset of puberty and postpubertal reproductive functions. *Toxicological Sciences* **93** 164–171. (doi:10.1093/toxsci/kfl036)

Maffini M, Rubin B, Sonnenschein C & Soto A 2006 Endocrine disruptors and reproductive health: the case of bisphenol-A. *Molecular and Cellular Endocrinology* **254–255** 179–186. (doi:10.1016/j.mce.2006.04.033)

Magoffin D & Erickson G 1982 Primary culture of differentiating ovarian androgen-producing cells in defined medium. *Journal of Biological Chemistry* **25** 4507–4513.

le Maire A, Bourguet W & Balaguer P 2010 A structural view of nuclear hormone receptor: endocrine disruptor interactions. *Cellular and Molecular Life Sciences* **67** 1219–1237. (doi:10.1007/s00018-009-0249-2)

Matsushima A, Teramoto T, Okada H, Liu X, Tokunaga T, Kakuta Y & Shimohigashi Y 2008 ERRγ tethers strongly bisphenol A and 4-α-cumylphenol in an induced-fit manner. *Biochemical and Biophysical Research Communications* **373** 408–413. (doi:10.1016/j.bbrc.2008.06.050)

Matthews J, Twomey K & Zacharewski T 2001 *In vitro* and *in vivo* interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. *Chemical Research in Toxicology* **14** 149–157. (doi:10.1021/tx0001833)

Meeker J, Missmer S, Altshul L, Vitonis A, Ryan L, Cramer D & Hauser R 2009 Serum and follicular fluid organochlorine concentrations among women undergoing assisted reproduction technologies. *Environmental Health* **32**. (doi:10.1186/1476-069X-8-32)

Miller W 1988 Molecular biology of steroid hormone synthesis. *Endocrine Reviews* **9** 295–318. (doi:10.1210/edrv-9-3-295)

Miller K, Borgeest C, Greenfeld C, Tomic D & Flaws J 2004 *In utero* effects of chemicals on reproductive tissues in females. *Toxicology and Applied Pharmacology* **198** 111–131. (doi:10.1016/j.taap.2003.07.016)

Minegishi T, Hirakawa T, Abe H, Kishi H & Miyamoto K 2003 Effect of IGF-1 and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on the expression of LH receptors during cell differentiation in cultured granulosa cells. *Molecular and Cellular Endocrinology* **202** 123–131. (doi:10.1016/S0303-7207(03)00073-X)

Mizuyachi K, Son D, Rozman K & Terranova P 2002 Alteration in ovarian gene expression in response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin: reduction of cyclooxygenase-2 in the blockage of ovulation. *Reproductive Toxicology* **16** 299–307. (doi:10.1016/S0890-6238(02)00024-2)

Mlynarcíková A, Kolena J, Ficková M & Scsuková S 2005 Alterations in steroid hormone production by porcine ovarian granulosa cells caused by bisphenol A and bisphenol A dimethacrylate. *Molecular and Cellular Endocrinology* **244** 57–62. (doi:10.1016/j.mce.2005.02.009)

Mlynarcíková A, Ficková M & Scsuková S 2007 The effects of selected phenol and phthalate derivatives on steroid hormone production by cultured porcine granulosa cells. *Alternatives to Laboratory Animals* **35** 71–77.

Mlynarcíková A, Nagyová E, Ficková M & Scsuková S 2009 Effects of selected endocrine disruptors on meiotic maturation, cumulus expansion, synthesis of hyaluronan and progesterone by porcine oocyte–cumulus complexes. *Toxicology In Vitro* **23** 371–377. (doi:10.1016/j.tiv.2008.12.017)

Mlynarczuk J & Kotwica J 2006 Influence of polychlorinated biphenyls on LH-stimulated secretion of progesterone and oxytocin from bovine luteal cells. *Polish Journal of Veterinary Sciences* **9** 101–108.

Monteiro P, Reis-Henriques M & Coimbra J 2000 Plasma steroid levels in female flounder (*Platichthys flesus*) after chronic dietary exposure to single polycyclic aromatic hydrocarbons. *Marine Environmental Research* **49** 453–467. (doi:10.1016/S0141-1136(99)00085-9)

Myllymäki S, Haavisto T, Vainio M, Toppari J & Paranko J 2005*a* *In vitro* effects of diethylstilbestrol, genistein, 4-*tert*-butylphenol, and

4-*tert*-octylphenol on steroidogenic activity of isolated immature rat ovarian follicles. *Toxicology and Applied Pharmacology* **204** 69–80. (doi:10.1016/j.taap.2004.08.009)

Myllymäki S, Haavisto T, Brokken L, Viluksela M, Toppari J & Paranko J 2005*b In utero* and lactational exposure to TCDD; steroidogenic outcomes differ in male and female rat pups. *Toxicological Sciences* **88** 534–544. (doi:10.1093/toxsci/kfi308)

Neal M, Mulligan Tuttle A, Casper R, Lagunov A & Foster W 2010 Aryl hydrocarbon receptor antagonists attenuate the deleterious effects of benzo[a]pyrene on isolated rat follicle development. *Reproductive BioMedicine Online* **21** 100–108. (doi:10.1016/j.rbmo.2010.03.025)

Nims R, Lubet R, Fox S, Jones C, Thomas P, Reddy A & Kocarek T 1998 Comparative pharmacodynamics of CYP2B induction by DDT, DDE, and DDD in male rat liver and cultured rat hepatocytes. *Journal of Toxicology and Environmental Health. Part A* **53** 455–477.

Nykamp J, Bols N & Carlson J 2001 Phenanthrenequinone disrupts progesterone production in rat luteal cells. *Reproductive Toxicology* **15** 393–398. (doi:10.1016/S0890-6238(01)00140-X)

Okey A, Riddick D & Harper P 1994 The Ah receptor: mediator of the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-diozin (TCDD) and related compounds. *Toxicology Letters* **70** 1–22. (doi:10.1016/0378-4274(94)90139-2)

Oktem O & Urman B 2010 Understanding follicle growth *in vivo*. *Human Reproduction* **25** 2944–2954. (doi:10.1093/humrep/deq275)

Paulose T, Hernández-Ochoa I, Basavarajappa M, Peretz J & Flaws J 2011 Increased sensitivity of estrogen receptor alpha overexpressing antral follicles to methoxychlor and its metabolites. *Toxicological Sciences* **120** 447–459. (doi:10.1093/toxsci/kfr011)

Pelletier G 2000 Localization of androgen and estrogen receptors in rat and primate tissues. *Histology and Histopathology* **15** 1261–1270.

Penning T 1997 Molecular endocrinology of hydroxysteroid dehydro-genases. *Endocrine Reviews* **18** 281–305. (doi:10.1210/er.18.3.281)

Peretz J, Gupta R, Singh J, Hernández-Ochoa I & Flaws J 2011 Bisphenol A impairs follicle growth, inhibits steroidogenesis, and downregulates rate-limiting enzymes in the estradiol biosynthesis pathway. *Toxicological Sciences* **119** 209–217. (doi:10.1093/toxsci/kfq319)

Pesonen S, Haavisto T, Viluksela M, Toppari J & Paranko J 2006 Effects of *in utero* and lactational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on rat follicular steroidogenesis. *Reproductive Toxicology* **22** 521–528. (doi:10.1016/j.reprotox.2006.03.007)

Petroff B, Roby K, Gao X, Son D, Williams S, Johnson D, Rozman K & Terranova P 2001 A review of mechanisms controlling ovulation with implications for the anovulatory effects of polychlorinated dibenzo-*p*-dioxins in rodents. *Toxicology* **158** 91–107. (doi:10.1016/S0300-483X(00)00367-X)

Pickford D & Morris I 1999 Effects of endocrine-disrupting contaminants on amphibian oogenesis: methoxychlor inhibits progesterone-induced maturation of *Xenopus laevis* oocytes *in vitro*. *Environmental Health Perspectives* **107** 285–292. (doi:10.1289/ehp.99107285)

Pickford D & Morris I 2003 Inhibition of gonadotropin-induced oviposition and ovarian steroidogenesis in the African clawed frog (*Xenopus laevis*) by the pesticide methoxychlor. *Aquatic Toxicology* **62** 179–194. (doi:10.1016/S0166-445X(02)00082-6)

Pocar P, Fischer B, Klonisch T & Hombach-Klonisch S 2005 Molecular interactions of the aryl hydrocarbon receptor and its biological and toxicological relevance for reproduction. *Reproduction* **129** 379–389. (doi:10.1530/rep.1.00294)

Pru J, Kaneko-Tarui T, Jurisicova A, Kashiwagi A, Selesniemi K & Tilly J 2009 Induction of proapoptotic gene expression and recruitment of p53 herald ovarian follicle loss caused by polycyclic aromatic hydrocarbons. *Reproductive Sciences* **16** 347–356. (doi:10.1177/1933719108327596)

Reinsberg J, Wegener-Toper P, van der Ven K, van der Ven H & Klingmueller D 2009 Effect of mono-(2-ethylhexyl) phthalate on steroid production of human granulosa cells. *Toxicology and Applied Pharma-cology* **239** 116–123. (doi:10.1016/j.taap.2009.05.022)

Richards J 1980 Maturation of ovarian follicles: actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. *Physiological Reviews* **60** 51–89.

Rocha Monterio P, Reis-Henriques M & Coimbra J 2000 Polycyclic aromatic hydrocarbons inhibit *in vitro* ovarian steroidogenesis in the flounder (*Platichthys flesus* L.). *Aquatic Toxicology* **48** 549–559. (doi:10.1016/S0166-445X(99)00055-7)

Shi Z, Valdez K, Ting A, Franczak A, Gum S & Petroff B 2007 Ovarian endocrine disruption underlies premature reproductive senescence following environmentally relevant chronic exposure to the aryl hydrocarbon receptor agonist 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Biology of Reproduction* **76** 198–202. (doi:10.1095/biolreprod.106.053991)

Stocco D 2001 StAR protein and the regulation of steroid hormone biosynthesis. *Annual Review of Physiology* **63** 193–213. (doi:10.1146/annurev.physiol.63.1.193)

Stoker C, Beldoménico P, Bosquiazzo V, Zayas M, Rey F, Rodríguez H, Muñoz-de-Toro M & Luque E 2008 Developmental exposure to endocrine disruptor chemicals alters follicular dynamics and steroid levels in *Caiman latirostris*. *General and Comparative Endocrinology* **156** 603–612. (doi:10.1016/j.ygcen.2008.02.011)

Strauss J III, Kallen C, Christenson L, Watari H, Devoto L, Arakane F & Kiriakidou M 1999 The steroidogenic acute regulatory protein (StAR): a window into the complexities of intracellular cholesterol trafficking. *Recent Progress in Hormone Research* **54** 394.

Susiarjo M, Hassold T, Freeman E & Hunt P 2007 Bisphenol A exposure *in utero* disrupts early oogenesis in the mouse. *PLoS Genetics* **3** e5. (doi:10.1371/journal.pgen.0030005)

Svechnikova I, Svechnikov K & Söder O 2007 The influence of di-(2-ethylhexyl) phthalate on steroidogenesis by the ovarian granulosa cells of immature female rats. *Journal of Endocrinology* **194** 603–609. (doi:10.1677/JOE-07-0238)

Takayanagi S, Tokunaga T, Liu X, Okada H, Matsushima A & Shimohigashi Y 2006 Endocrine disruptor bisphenol A strongly binds to human estrogen-related receptor γ (ERRγ) with high constitutive activity. *Toxicology Letters* **167** 95–105. (doi:10.1016/j.toxlet.2006.08.012)

Takeda T, Matsumoto Y, Koga T, Mutoh J, Nishimura Y, Shimazoe T, Ishii Y, Ishida T & Yamada H 2009 Maternal exposure to dioxin disrupts gonadotropin production in fetal rats and imprints defects in sexual behavior. *Journal of Pharmacology and Experimental Therapeutics* **329** 1091–1099. (doi:10.1124/jpet.109.151282)

Tiemann U 2008 *In vivo* and *in vitro* effects of the organochlorine pesticides DDT, TCPM, methoxychlor, and lindane on the female reproductive tract of mammals: a review. *Reproductive Toxicology* **25** 316–326. (doi:10.1016/j.reprotox.2008.03.002)

Tomic D, Frech MS, Babus JK, Gupta RK, Furth PA, Koos RD & Flaws JA 2006 Methoxychlor induces atresia of antral follicles in ERalpha-overexpressing mice. *Toxicological Sciences* **93** 196–204. (doi:10.1093/toxsci/kfl040)

Tsuchiya Y, Nakajima M & Yokoi T 2005 Cytochrome P450-mediated metabolism of estrogens and its regulation in human. *Cancer Letters* **227** 115–124. (doi:10.1016/j.canlet.2004.10.007)

Valdez K, Shi Z, Ting A & Petroff B 2009 Effect of chronic exposure to the aryl hydrocarbon receptor agonist 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in female rats on ovarian gene expression. *Reproductive Toxicology* **28** 32–37. (doi:10.1016/j.reprotox.2009.03.004)

Walters K, Simanainen U & Handelsman D 2010 Molecular insights into androgen actions in male and female reproductive function from androgen receptor knockout models. *Human Reproduction Update* **16** 543–558. (doi:10.1093/humupd/dmq003)

Wang C, Hsueh A & Erickson G 1982 The role of cyclic AMP in the induction of estrogen and progestin synthesis in cultured granulosa cells. *Molecular and Cellular Endocrinology* **25** 73–83. (doi:10.1016/0303-7207(82)90170-8)

Wang J, Liu X, Wang H, Wu T, Hu X, Qin F & Wang Z 2010 Expression of two cytochrome P450 aromatase genes is regulated by endocrine disrupting chemicals in rare minnow *Gobiocypris rarus* juveniles. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology* **152** 313–320. (doi:10.1016/j.cbpc.2010.05.007)

Waters KM, Safe S & Gaido KW 2001 Differential gene expression in response to methoxychlor and estradiol through ERα, ERβ, and AR in reproductive tissues of female mice. *Toxicological Sciences* **63** 47–56. (doi:10.1093/toxsci/63.1.47)

Weinberg C, Wilcox A & Baird D 1989 Reduced fecundability in women with prenatal exposure to cigarette smoking. *American Journal of Epidemiology* **129** 1072–1078.

Wojtowicz A, Tomanek M, Augustowska K & Gregoraszczuk E 2005 Aromatic hydrocarbon receptor (AhR) in the porcine theca and granulosa cells: effect of TCDD, PCB126 and PCB 153 on the expression of AhR. *Endocrine Regulations* **39** 109–118.

Wójtowicz A, Kajta M & Gregoraszczuk E 2007 DDT- and DDE-induced disruption of ovarian steroidogenesis in prepubertal porcine ovarian follicles: a possible interaction with the main steroidogenic enzymes and estrogen receptor β. *Journal of Physiology and Pharmacology* **58** 873–885.

Wu Y, Foster W & Younglai E 2006 Rapid effects of pesticides on human granulosa-lutein cells. *Reproduction* **131** 299–310. (doi:10.1530/rep.1.00922)

Xu C, Chen J-A, Qiu Z, Zhao Q, Luo J, Yang L, Zeng H, Huang Y, Zhang L, Cao J *et al.* 2010 Ovotoxicity and PPAR-mediated aromatase down-regulation in female Sprague–Dawley rats following combined oral exposure to benzo[a]pyrene and di-(2-ethylhexyl) pthalate. *Toxicology Letters* **199** 323–332. (doi:10.1016/j.toxlet.2010.09.015)

Younglai E, Holloway A, Lim G & Foster W 2004*a* Synergistic effects between FSH and 1,1-dichloro-2,2-bis(*P*-chlorophenyl)ethylene (*P,P'*-DDE) on human granulosa cell aromatase activity. *Human Reproduction* **19** 1089–1093. (doi:10.1093/humrep/deh252)

Younglai E, Kwan T, Kwan C-Y, Lobb D & Foster W 2004*b* Dichlorodiphenylchloroethylene elevates cytosolic calcium concentrations and oscillations in primary cultures of human granulosa-lutein cells. *Biology of Reproduction* **70** 1693–1700. (doi:10.1095/biolreprod.103.026187)

Zachow R & Uzumcu M 2006 The methoxychlor metabolite, 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane, inhibits steroidogenesis in rat ovarian granulosa cells *in vitro*. *Reproductive Toxicology* **22** 659–665. (doi:10.1016/j.reprotox.2006.04.018)

Zama A & Uzumcu M 2009 Fetal and neonatal exposure to the endocrine disruptor methoxychlor causes epigenetic alterations in adult ovarian genes. *Endocrinology* **150** 4681–4691. (doi:10.1210/en.2009-0499)

Zhou W, Liu J, Liao L, Han S & Liu J 2008 Effect of bisphenol A on steroid hormone production in rat ovarian theca-interstitial and granulosa cells. *Molecular and Cellular Endocrinology* **283** 12–18. (doi:10.1016/j.mce.2007.10.010)