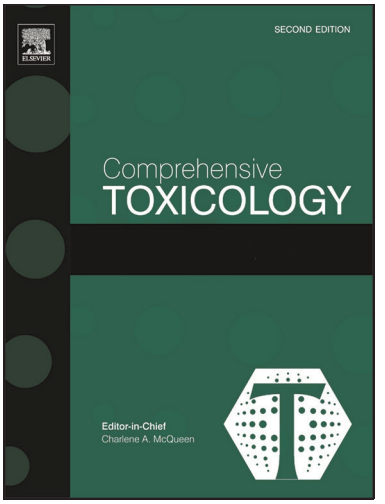

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11.11 Environmental Endocrine Disruptors and Male Reproductive Toxicology

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Abbreviations

27HC	27-hydroxysterol
AhR	aromatic hydrocarbon receptor
BPA	bisphenol A
BPH	benign prostatic hyperplasia
BzBP	benzylbutyl phthalate
DBP	dibutyl phthalate
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DEHP	di-2-ethylhexylpthalate
DES	diethylstilbestrol
DHT	5α-dihydrotestosterone
DINP	di-isononyl phthalate
E2	17β-estradiol
EDC	endocrine disrupting chemical
ER	estrogen receptor
ERα	estrogen receptor alpha
FSH	follicle-stimulating hormone
GEN	genistein
GnRH	gonadotropin-releasing hormone
INSL3	insulin-like factor 3
LH	luteinizing hormone
PCB	polychlorinated biphenyl
SERM	selective estrogen receptor modulator
SSC	spermatogonial stem cell
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TDS	testicular dysgenesis syndrome

11.11.1 Introduction

Endocrine communication is the process by which hormones are produced in an endocrine organ, released into the circulation, transported to target tissues, and bind to receptors in target cells to affect their subsequent activity. Endocrine signaling regulates reproduction, growth, metabolism, and many other critical processes. Over the last several decades, there has been steadily increasing evidence that many natural and synthetic chemicals, now commonly referred to as endocrine disruptors or endocrine disrupting chemicals (EDCs), can alter this process by mimicking or inhibiting the actions of endogenous hormones (Colborn *et al.* 1993). Each step of the process for any hormone signaling system is potentially vulnerable to disruption by an external agent. Indeed, there are known examples of endocrine disruption that result from effects at a wide variety of sites of action encompassing almost all aspects of hormone production, transport, and action. Convincing data links these EDCs to serious health effects in wildlife and experimental animals. This has repeatedly raised the critical question of whether these compounds could have effects on human health, but here the evidence has been both less extensive and conclusive.

In this chapter, we review the historical development of work on endocrine disruption, and attempt to provide a concise description of the state of our present knowledge with regard to the ability of environmental chemicals to disrupt the male reproductive system. The large and increasing volume of scientific literature related to endocrine disruption by environmental chemicals precludes a comprehensive review of all literature, even in a specialized area of this field. Instead, in this chapter we give an overview of current work and understanding in this area, and include appropriate citations to the large number of excellent reviews which detail past and current work in various areas of this field and will give readers more detailed information about specialized topics.

11.11.1.1 History of Endocrine Disruption

Initial scientific data indicating that compounds in the environment could alter endocrine processes in animals first appeared over 50 years ago, with the demonstration that consumption of a certain type of red clover produced estrogenic effects that disrupted reproduction of sheep in Australia (Bennetts and Underwood 1951). Similarly, other studies in subsequent years began to document that the widely used insecticide dichlorodiphenyltrichloroethane (DDT) had estrogenic effects in various types of experimental paradigms (Colborn *et al.* 1993).

Both public and scientific attention was focused on this area by the publication of Rachel Carson's classic book *Silent Spring* (Carson 1962). This book described the developing scientific and other literature indicating that environmental chemicals such as DDT could disrupt the endocrine system of birds and other wildlife by acting as estrogens and by altering such parameters as eggshell thickness in birds. This book fueled the rise of the environmental movement during the 1960s and brought increased focus to the idea that environmental chemicals disrupting endocrine systems could potentially have deleterious reproductive effects on a wide array of species.

The field continued to progress and expand over the next three decades, although both scientific and public focus on this area abated several years after publication of *Silent Spring*. By the early 1990s, it had become clear that although compounds in the environment that disrupted estrogen signaling had been the initial focus of work in this area, many types of endocrine signals, involving both steroid and nonsteroid hormones, were vulnerable to disruption by environmental chemicals. It had also become clear that although the initial focus was on synthetic chemicals such as pesticides and industrial pollutants in the environment that disrupted endocrine activity, a wide variety of chemicals, including those in food, could alter endocrine signaling. Initial concern in this area related to potential effects of environmental chemicals on reproduction. Indeed, the reproductive processes in males and females are extremely susceptible because of their obligatory dependence on steroid hormones, well-known targets of EDCs. However, the expanding focus of work in this area clearly illustrated that environmental endocrine disruptors could affect many other critical processes such as growth, metabolism, adipose deposition, and immune function.

Against this background of rising scientific documentation of the ubiquity of environmental endocrine disruption, Theo Colborn, who had become interested in this area as a result of her work looking at the disruption of endocrine and reproductive systems in Great Lakes wildlife by environmental chemicals (Colborn 1991), organized a conference on EDCs in the environment.

Analogous to publication of *Silent Spring* 30 years before, this conference and the resultant publications on EDCs (Colborn *et al.* 1993) again heightened and catalyzed concern about the potential health impacts of these chemicals in man and other animals in both the scientific community and general public.

In the ensuing one and a half decades, the breadth and depth of scientific work in all areas of this field has continued to expand. Despite persistent debates over all areas of this field, certain critical questions, such as the magnitude of human health consequences resulting from exposure to these chemicals, remain to be definitively established. However, based on the current weight of evidence, even the most skeptical must acknowledge the potential for human health effects and the need for more extensive data to accurately assess the impacts of these chemicals.

11.11.1.2 Endocrine Disruption and the Male Reproductive Tract

The discussion of the effects of EDCs will focus on the male reproductive tract, specifically the testis and prostate due to their clinical relevance. Many cell types in the testis are estrogen targets, and the testis is both the major site of androgen production and a primary target of these hormones, as androgens are essential for spermatogenesis. An extensive literature related to EDC effects on testis development and function exists. Likewise, male infertility is a significant clinical problem, and testicular cancer and cryptorchidism are major and increasing human health concerns around the world. A link between environmental EDCs and human health problems such as cryptorchidism and testicular cancer therefore looks likely, although unequivocal data establishing such a link has not yet appeared (Skakkebaek *et al.* 2003).

Like the testis, the prostate has been a major research focus for many years due to its clinical significance and the prevalence of two major diseases that affect this organ. Prostatic cancer and benign prostatic hyperplasia (BPH) are important human health concerns, especially in Western countries. The prostate contains abundant estrogen receptor (ER) both during development and adulthood and is obligatorily dependent on androgen signaling. EDCs that disrupt one or both of these endocrine systems have been at least suspected to have a role in the etiology or progression of major prostatic diseases.

Estrogen receptors are widely distributed in male reproductive organs other than the testis and prostate both during development and adulthood (Cooke *et al.* 1991). Furthermore, the efferent ducts have the highest expression of estrogen receptor alpha (ERα) in the male tract, and critical effects of estrogen on functional parameters in this tissue such as fluid resorption have been described (Hess *et al.* 1997a). Similarly, estrogenic effects on other male reproductive organs such as the ductus deferens, epididymis, seminal vesicles, and bulbourethral glands have been documented. However, these organs have limited clinical relevance and will not be the present focus, but excellent reviews of early estrogen effects on reproductive tract development are available (Hotckiss *et al.* 2008; McLachlan 2001; Newbold 1995).

11.11.1.3 Environmental Estrogens and Antiestrogens

The field of endocrine disruption began with the discovery of environmental chemicals that had estrogenic actions. Since then, a wide variety of natural and man-made chemicals, both exogenous and recently endogenous, have been described which can disrupt estrogen signaling (**Table 1**). The initial descriptions of environmental estrogens occurred almost concomitantly with the pioneering studies describing ER. The two fields have evolved and developed together over the intervening decades. For example, the discovery of a second ER in mammals, named ERβ to differentiate it from the original ER, now known as ERα (Kuiper *et al.* 1996), and the existence of three ERs in fish (Hawkins *et al.* 2000), has necessitated taking the potential effects of an EDC on more than one ER into account when seeking to understand the effects of these chemicals.

One striking example of how progress in understanding basic estrogen action has shaped aspects of the field of environmental endocrine disruption relates to the soy phytoestrogen genistein (GEN). Human populations consuming diets rich in soy, such as the Japanese, take in up to 1 mg of isoflavones/kg of body weight/day (Adlercreutz 1998), and there appears to be significant health benefits associated with this level of consumption. In contrast to adults, who take in modest amounts of isoflavones even when eating soy-rich foods in their diets, the relatively recent use of soy formula over the past 40 years for infant nutrition has led to exposure of large numbers of newborns and infants to unprecedented

Table 1 Endocrine disruptors that affect male reproductive system

Estrogens (ER-mediated)	*Toxicants that inhibit hormone production*
Methoxychlor	TCDD (inhibits testosterone synthesis in adult male rats)
Bisphenol A	Endosulfan (reduces plasma testosterone, FSH, and LH in male rats)
Soy phytoestrogens (genistein, daidzen, equol)	Azole fungicides (reduces testosterone levels)
Coumestrol, Biochanin	Atrazine (increases aromatase activity)
DES	Prochloraz
Ethinylestradiol	***Toxicants affecting reproduction via the CNS***
Lignans	Carbon disulfide, lead, manganese, mercury, etc.
Lindane	***Toxicants affecting endocrine cells***
Certain hydroxylated PCB metabolites	Benomyl (Sertoli cell)
Octylphenols, nonylphenols, alkylphenol ethoxylates	Dinitrobenzene (Sertoli cell)
Parabens	Phthalates (Sertoli cell)
Perillyl alcohol (Lavender)	***Toxicants that alter hormonal status by depleting germ cells***
Heavy metals	Benzidine-based dyes (kills stem cells)
Zearalenone	Dibromochloropropane (kills germ cells)
Antiestrogens (ER-mediated or aromatase inhibition)	***Antithyroid endocrine disruptors***
27-hydroxysterol	Perchlorate
Drugs like tamoxifen, nafoxidine	Persistent organic pollutants such as dioxins and PCBs
Fenarimol	Polybrominated diphenyl ethers (PBDEs)
TCDD (downregulates ER and increases estrogen metabolism)	Nitrofen
Androgens (AR-mediated)	Hexachlorobenzene
Trenbolone	Lead
Antiandrogens (AR-mediated or 5a-reductase inhibition)	Phthalic acid esters
Vinclozolin metabolites	Mirex
p,p′-DDE	Polychlorinated dibenzofurans
Permethrin	Spironolactone
Procymidone	TCDD
Saw Palmetto	Thiocarbamide- and sulfonamide-based pesticides (ethylenethiourea, linuron)
Some environmental estrogens	**Cytochrome P450 activation**
American dwarf palm extract (Permixon)	St. John's wort
AhR agonists (AhR-mediated)	
TCDD	
3,3′,4,4′,5,5′-Hexachlorobiphenyl	
Other planar polyhalogenated dibenzo-*p*-dioxin, dibenzofuran, and biphenyl congeners	

Representative examples, rather than a complete list, are given for appropriate classes of compounds

levels of GEN and another isoflavone present in soy, daidzein. Approximately 15% of all infants in the United States, or about 750 000 infants per year (Strom *et al.* 2001), use soy formula. Since both cow's milk and human breast milk contain low amounts of isoflavones, infants fed soy-based formula consume high levels of isoflavones during developmental periods when humans have normally been exposed to low amounts of these substances and estrogens in general. Furthermore, because soy formula is initially the infant's sole nutritional source, they ingest approximately 10-fold more isoflavones on a per-weight basis than adults eating a high soy diet, and they have similar 10-fold increases in serum isoflavones (Setchell *et al.* 1997). Although there is presently no conclusive data indicating estrogenic effects of early soy formula usage during critical periods of neonatal development can produce deleterious effects in infants or adults, this remains an area of concern. In total, it is necessary to consider the age of exposure and the relative level of consumption in discussions about benefits and risks of phytoestrogen consumption.

GEN has been shown to have a variety of deleterious effects on male reproduction in primate and other animal models (Sharpe *et al.* 2002; Tan *et al.* 2006). Early work on estrogenic GEN effects assumed its actions were through the only ER, initially known as

ERα, but the potency of GEN to signal through ERα was at least two to three orders of magnitude less than the primary endogenous ER ligand, 17β-estradiol (E2). GEN has significantly higher affinity for ERβ over ERα (Kuiper *et al.* 1997), and especially at low doses functions primarily as an ERβ agonist, although at higher doses it can signal through ERα as well. The greater affinity of GEN for ERβ over ERα contrasts with E2, which has equal or greater affinity for ERα compared to ERβ (Kuiper *et al.* 1997). Thus, environmental estrogens such as GEN have qualitative as well as quantitative differences from E2 that complicate analysis of their biological actions, and our understanding of GEN effects has been irrevocably tied to progress in the broader field of estrogen action.

The initial reports of estrogenic effects of EDCs such as pesticides and industrial chemicals were in wildlife and domestic and laboratory animals (reviewed in Colborn *et al.* 1993; Crews *et al.* 2003; Hotckiss *et al.* 2008; McLachlan 2001; Newbold 1995; Zala and Penn 2004). However, the ability of estrogenic compounds to produce serious and long-lasting effect in humans was inadvertently discovered following widespread treatment of pregnant women with the pharmaceutical diethylstilbestrol (DES). DES was a synthetic estrogen that had been prescribed to women beginning in the 1940s to prevent miscarriages. A landmark study by Arthur Herbst in 1971 (Herbst *et al.* 1971) detailed the high incidence of a previously rare clear cell vaginal adenocarcinoma in women whose mothers had taken DES during pregnancy, and led to extensive studies documenting abnormalities in both sons and daughters of women who took DES during gestation (Herbst *et al.* 1971). Our understanding of the potential effects of DES on both male and female fetuses has been facilitated by the development of rodent models of early DES exposure (McLachlan 2001; Newbold 1995). These rodent studies have greatly advanced our understanding of the DES target tissue and the mechanism of the effects, and in many cases served as effective predictive tests for the ultimate phenotypic and functional abnormalities that this type of exposure could lead to in humans. These original findings related to effects of early DES exposure have led to the field now known broadly as "the fetal (developmental) basis of adult disease," which postulates that the developing organism is particularly vulnerable to perturbations of the environment that may predispose it to the expression of a disease or dysfunctional phenotype much later in life (Barker 2003).

Recent interest in environmental estrogens and their potential health effects has been heightened by the demonstration that some municipal water supplies have measurable levels of residues from various prescription drugs (e.g., ethinyl estradiol from birth control pills, etc.) that could act as EDCs, and earlier work had shown that exposure to sewage effluents could have estrogenic effects in wildlife (Knudsen *et al.* 1997). Although drug residues are typically low in water supplies, the potential of these compounds to add to the overall burden of EDCs exposure and exacerbate potential deleterious effects exists.

Although the most common endocrine disruptors are compounds that are estrogenic, a substantial literature documenting antiestrogenic effects of certain environmental chemicals also exists. The highly toxic environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces its antiestrogenic and other biological effects primarily through binding to the aromatic hydrocarbon receptor (AhR). Similarly, a number of polychlorinated biphenyl (PCB) congeners also have significant affinity for AhR and act primarily through AhR to induce antiestrogenic effects. For example, TCDD treatment decreased uterine responses to estrogen (Astroff *et al.* 1991) and mammary cell proliferation and gland development in pubertal rats, and also inhibited the E2-induced growth and function of the human breast tumor cell line MCF-7 *in vitro* (Brown and Lamartiniere 1995; Gierthy *et al.* 1993). Furthermore, TCDD treatment also inhibited development and growth of mammary tumors in rodent models, indicating that the antiestrogenic effects seen in MCF-7 cells may translate to similar effects in mammary tissue *in vivo* (Brown and Lamartiniere 1995). No clear consensus has emerged for how TCDD and related compounds induce antiestrogenic activities through AhR, although data from a variety of *in vivo* and *in vitro* systems suggest that this effect may involve alterations of ER signaling at various steps in the signaling cascade normally initiated by the binding of a ligand to ER (Safe and Wormke 2003).

Liganded AhR may not function simply and exclusively as an antiestrogen. Other work has shown that TCDD exposure is associated with increases in endometriosis and certain types of estrogen-dependent tumors, an effect more consistent with an estrogenic action. In addition, some recent work suggests that some AhR ligands may induce estrogenic effects by binding to AhR and then subsequently inducing activation of unliganded ER,

ultimately producing phenotypic effects similar to that seen with estrogen exposure, although this is controversial (Safe and Wormke 2003). Further complicating the elucidation of the cross talk between these two signaling pathways, some AhR ligands, including the major ligand used in the studies indicating that liganded AhR can induce activation of unliganded ER, are themselves ER agonists (Safe and Wormke 2003). In conclusion, TCDD and related chemicals can induce antiestrogenic effects through AhR, but their overall endocrine disrupting effects are not yet completely understood.

11.11.2 Estrogen Effects on Males

Many studies over the years have documented the effects of neonatal exposure to DES and environmental estrogens on the male reproductive tract (McLachlan 2001; Newbold 1995). Early DES exposure causes reduced circulating testosterone, disruption in spermatogenesis, functional abnormalities of Sertoli cells, increased incidence of testicular hypoplasia, and functional and morphological alterations of the accessory sex glands, as well as secondary effects through actions on other trophic hormones. A critical question that is still being addressed is how early estrogen exposure induces these abnormalities.

11.11.2.1 ER in Testis

A growing body of evidence suggests that estrogens play an important role in the normal development of the male tract, although initial literature in this area was almost entirely devoted to deleterious effects of exogenous estrogen exposure. ER is widely distributed in the developing and adult testis. ERβ is the predominant receptor expressed in the cells of the seminiferous epithelium, while peritubular and Leydig cells express both ERα and ERβ (**Table 2**).

11.11.2.1.1 ER signaling in the testis

The role of estrogen actions in regulating testicular function in males has been reviewed (Akingbemi 2005; Couse *et al.* 2001; Delbes *et al.* 2006; Hess *et al.* 1997a; Sierens *et al.* 2005). Transgenic technology has facilitated the understanding of the role of estrogen in the male, and the generation of ERα and ERβ knockout mice have led to a much fuller understanding of estrogen signaling in regulation of the development and function of the testis, prostate, and other male organs. ERα null male mice are infertile (Lubahn *et al.* 1993), due to effects on the testis itself as well as the efferent ductules (Hess *et al.* 1997b). Conversely, ERβ null mice are fertile (Krege *et al.* 1998), although reproductive tract changes in these animals have been described.

The direct effects of estrogens on testicular cells are critical for the eventual pathologies that result, but some effects of early estrogen treatment are indirect and caused by changes in other hormones, which secondarily induce abnormalities. Insulin-like factor 3 (INSL3) is synthesized by Leydig cells and works with androgens to promote testicular descent. Male mice exposed to DES or estrogen had impaired steroidogenic function and INSL3 secretion by Leydig cells, and this was mediated by ERα (Cederroth *et al.* 2007). When rats were neonatally exposed to DES there was a permanent impairment in the functional maturation of Sertoli cells, which was suggested to be partly due to the suppression of follicle-stimulating hormone (FSH), as well as direct effect of estrogens on the Sertoli cells mediated through ERβ (Sharpe *et al.* 1998).

11.11.2.1.2 ER expression in prostate

The rodent prostate expresses both ERα and ERβ, although ER status varies developmentally and in stromal and epithelial cell compartments (**Table 3**).

Table 2 Distribution of ER in the testis

Cell type	*ERα*	*ERβ*	*Reference*
Fetal Leydig cell	+	+	(Greco *et al*. 1992; Jefferson *et al*. 2000)
Adult Leydig cell	+	+	(Fisher *et al*. 1997; Rosenfeld *et al*. 1998)
Peritubular cell	+	+	(Zhou *et al*. 2002)
Sertoli cell	−	+	(Pelletier *et al*. 2000; Saunders *et al*. 1998)
Gonocytes	−	+	(Jefferson *et al*. 2000)
Adult germ cell	−	+	(Saunders *et al*. 1998)
Efferent ducts	+	+	(Hess *et al*. 1997a,b)

Table 3 Distribution of ER in the prostate

Cell type	*Receptor*	*Expression*	*Reference*
Neonatal mesenchymal cells in the ventral and dorsolateral prostate	ERα	High	(Prins *et al*. 2001)
Adult prostatic stromal cells	ERα	High	(Prins and Birch 1997)
Neonatal prostatic epithelium	ERβ	Low	(Prins *et al*. 1998)
Adult prostatic epithelial cells	ERβ	High	(Kuiper *et al*. 1996; Omoto *et al*. 2005)

11.11.2.1.3 ER signaling in prostate

Androgen is the major endocrine regulator of prostatic development and function (Cunha and Donjacour 1987). The physiological significance of ER signaling in normal prostatic development is not clear. Mice in which ERα or ERβ has been knocked out lack clear histological abnormalities in their prostates (Kuiper *et al*. 1996; Prins *et al*. 2001; Omoto *et al*. 2005), although some data suggest that signaling through ERα could regulate ductal branching and elongation (Berman *et al*. 2004; Donjacour *et al*. 2003). Despite its unclear role in the normal prostate, an extensive literature documents the clear role of exogenous estrogen in inducing prostatic abnormalities, especially given during development (Heldring *et al*. 2007; McPherson *et al*. 2007; Prins *et al*. 2006).

Prostatic abnormalities have been seen with both prenatal (days 9–16 of gestation) and neonatal (birth to postnatal 5) estrogen treatment (Prins *et al*. 2006). The alterations brought about by estrogen exposure neonatally reflect both suppression of the hypothalamic-pituitary-gonadal axis and consequent changes in androgen levels as a result of estrogen administration, as well as direct estrogen effects on the prostate. Early estrogen treatment appears to alter the differentiation pathway of prostatic epithelial cells and cause permanent differentiation defects. There is suppression of the formation of the distal prostatic ducts, and the proximal ductal phenotype is extended through a larger portion of the prostatic duct. This proximal ductal phenotype includes a thick layer of periductal fibroblasts and a continuous layer of basal epithelial cells which block ductal branching and cell–cell interactions that are needed for normal morphogenesis. In the prostate, estrogen imprinting acts via ERα signaling and not through ERβ (Prins *et al*. 2001). There is a growing body of evidence that suggests that estrogen imprinting is associated with prostatic hyperplasia and cancer – this area has been reviewed elsewhere (Carruba 2007; Harkonen and Makela 2004; Ricke *et al*. 2007).

11.11.3 Environmental Estrogens/ Antiestrogens and Effects on the Hypothalamic-Hypophyseal System

Reproductive function is controlled by the tightly regulated interactions of hypothalamus, pituitary, and gonad. The primary driving force upon this system is a small group (about 800–1000) of neurons in the hypothalamus that synthesize and secrete the decapeptide hormone, gonadotropin-releasing hormone (GnRH) (Gore 2002). As is the case for all hypothalamic-releasing hormones, GnRH is transported to the anterior pituitary gland via the hypothalamic-hypophyseal portal capillary vasculature, and then binds GnRH receptors on the pituitary gonadotropes. The gonadotropins, luteinizing hormone (LH) and FSH, are released upon this stimulus into the periphery, and subsequently act upon their respective receptors in the testis to promote spermatogenesis and steroidogenesis.

From the neuroendocrine perspective, the hypothalamus and pituitary are important targets of estrogenic and antiestrogenic chemicals (reviewed in Dickerson and Gore 2007; Gore 2008). This is not surprising considering the abundance of hypothalamic ERα and ERβ. However, in the case of the hypothalamus, this concept is complicated by reports showing that GnRH neurons do not coexpress most nuclear steroid hormone receptors. Although they express ERβ, they do not express ERα, androgen receptor (AR), and progesterone receptor (Hrabovszky *et al*. 2000; Wintermantel *et al*. 2006). Therefore, most feedback to the hypothalamic GnRH system is indirectly mediated by inputs from other neurons and glia that express steroid hormone receptors (Yin and Gore 2006). In the context of estrogenic and antiestrogenic endocrine disruptors, the circuitry that regulates GnRH release is potentially sensitive to these substances, either directly via ERβ or indirectly via both ERα and ERβ. Indeed, estrogenic endocrine disruptors such as PCBs,

organochlorine pesticides (e.g., chlorpyrifos, methoxychlor), and phytoestrogens have significant effects on GnRH neurons in the *in vitro* GT1 cell model, and *in vivo* on GnRH release and neuron numbers (reviewed in Gore 2008). In some of these cases, the observed effect occurs via a U- or inverted U-shaped dose–response curve (Welshons *et al.* 2003), consistent with effects of hormonally active substances (Gore *et al.* 2006).

Although beyond the scope of this chapter, the pituitary gland is also targeted by estrogenic and antiestrogenic EDCs (reviewed in Brevini *et al.* 2005), an effect that may be mediated by its own expression of ER (Sanchez-Criado *et al.* 2005) or through GnRH-stimulated effects on gonadotropins. Thus, the hypothalamic-hypophyseal system controlling reproduction is a plausible target for endocrine disruption through ER-mediated processes. Although largely understudied compared to effects of EDCs on the reproductive tract and gonad, this is an important area for future investigation.

11.11.4 Effects on Wildlife

The literature describing the effects of environmental estrogens and antiestrogens on wildlife is rich and diverse. As discussed above, beginning nearly 50 years ago with *Silent Spring* (Carson 1962), evidence has expanded to include a long list of chemicals and species (see Crews 2003; McLachlan 2001; Zala and Penn 2004 for reviews of this literature). Bird species continue to be favored in the literature due to substantial evidence for a relationship between brain morphology and behavior. For example, the observation that DDT skews the sex ratio in natural populations of adult gulls, leading to female–female pairing, was one of the first demonstrations of the behavioral consequences of EDCs (Fry *et al.* 1987; Hunt 1977). In the American robin, increasing levels of DDT and dichlorodiphenyldichloroethylene (DDE) in the yolk are correlated with a reduction in the size of song nuclei in males (Iwaniuk *et al.* 2006). A particularly interesting recent study with the European starling (Markman *et al.* 2008) relates the potential long-term consequences of EDC contamination. In England, starlings forage in the winter on the worms in sewage effluent filter beds that, in turn, contain high levels of synthetic and natural estrogens. Hypothesizing that these contaminants might influence both the behavior and brain morphology, captive starlings were fed mealworms containing E2 or a mixture of E2 and dioctylphthalate, bisphenol A (BPA), and dibutylphthalate (EDCs also found in worms in contaminated sites). The following spring both males and females were assessed for the amount and complexity of song and the size of song nuclei. Male song and song control nucleus volume increased in individuals receiving the mixture; males receiving E2 alone did not differ from control (peanut oil) in any of these trait measures. In a separate experiment the attractiveness of the male's song was measured by the time females spent on the perch adjacent to a speaker playing the song. There was a strong preference for the more complex song of males that had received the EDC mixture. However, both the males receiving E2 alone and those receiving the mixture had significantly lower cell-mediated immune function and secondary humoral response. Thus, by selecting males with more complex song, the females were also selecting males who were immunocompromised.

Among reptiles, the now classic work of Louis Guillette and colleagues on the American alligator in Lake Apopka in Florida effectively documents how environmental contamination can affect reproduction of animals in nature (Orlando and Guillette 2007). The resemblance of gonadal and penile abnormalities of the alligators in this lake to those described in mice treated with DES led to detailed studies documenting that chronic pollution by agricultural runoff – exacerbated by a chemical spill of dicofol – was the most likely cause of the observed reproductive abnormalities (Guillette *et al.* 2007). Dicofol and its components have been shown to bind ER from the alligator, thereby mimicking estrogens early in development. In addition to dicofol, DDE/DDT, PCBs, and a variety of pesticides have been detected in alligator eggs, including dieldrin, toxaphene, *cis*/*trans* nonachlor, chlordane, and *p,p′*-DDD (dichlorodiphyenyldichloroethane) (Heinz *et al.* 1991).

The red-eared slider turtle is another powerful biological marker system for examining endocrine disruption at the organismal (sex determination), physiological (circulating steroid hormone levels), and now genetic levels (regulation of gene networks), and is particularly useful for studying EDCs singly, in mixtures, and in low doses (Willingham and Crews 2000). Indeed, work with the slider turtle established two of three foundational principles in EDC actions, namely the synergistic actions of ecologically relevant levels of mixtures of individual PCB compounds (Bergeron *et al.* 1994) and the absence of a threshold for EDC compounds (Sheehan *et al.* 1999). When

applied individually, environmentally relevant dosages of commonly used compounds and mixtures such as chlordane, *trans*-nonachlor, *cis*-nonachlor, DDE, and the PCB mixture Aroclor 1242 can alter sex ratio outcomes in the slider turtle. Aroclor 1242 produced the most powerful effects, shifting the ratio of females almost twofold, while chlordane had the greatest effect when combined with E2 (Willingham and Crews 1999). The combined effect of all eight compounds also significantly altered the sex ratio in the female direction. Further work indicated that when administered at even lower dosages (0.25 ng *trans*-nonachlor, 7 ng DDE, and 0.125 ng chlordane), these EDCs were effective in overriding a male-producing incubation temperature (Willingham 2004).

Declines in amphibian populations have been related to EDC contamination, in particular atrazine, a herbicidal contaminant found in groundwater. Atrazine affects aromatase, the enzyme that converts testosterone to E2, by acting on the SF-1 gene, thereby changing the relative production of androgen and estrogen. Atrazine exposure during reproductive development induces morphological abnormalities in amphibians (Fan *et al.* 2007a,b; Hayes *et al.* 2006a,b). Exposure to both atrazine and PCB inhibits development of the larynx (Hayes *et al.* 2002; Qin *et al.* 2007), a sexually dimorphic structure important in male calling behavior in *Xenopus* (Kelley and Brenowitz 2002).

A clear example of endocrine disruption of the aquatic environment comes from the studies documenting the relationship between concentrations of wastewater effluent in rivers in England and the incidence of intersex male fish (Jobling *et al.* 2002; Tyler *et al.* 1998). In a recent study, Kidd *et al.* (2007) exposed fathead minnows in an experimental lake to sustained levels of 17α-ethinylestradiol typical of concentrations found in wastewater effluent (5–6 ng l^{-1}). As shown by previous researchers, the males produced vitellogenin (a biomarker of estrogen exposure), often with intersex gonads, and in females oogenesis was affected. After 2 years the population had crashed to near extinction and only after cessation of the treatment did the population recover.

11.11.5 Disruption of Androgen Signaling by Environmental Chemicals

Initial work in the area of endocrine disruptors was primarily focused on estrogenic chemicals, and there was also a significant amount of work related to chemicals that disrupted thyroid hormone signaling. A critical development in the evolution of our thinking about endocrine disrupting chemicals was the unexpected demonstration by Kelce, Gray, and others (Kelce *et al.* 1995; Hotckiss *et al.* 2008) that a number of environmental chemicals could function as antiandrogens (**Table 1**). Androgens are essential for the adult function of the testis, prostate, epididymis, and other male reproductive organs. Androgens also play essential roles in male reproductive tract development as well. The fungicide vinclozolin has affinity for AR but does not stimulate typical transcriptional changes seen with androgen (Kelce *et al.* 1995) and thus induces antiandrogenic effects by blocking AR signaling. This was directly demonstrated when it was shown that vinclozolin administration to developing males *in vivo* resulted in antiandrogenic effects and reproductive tract abnormalities. Vinclozolin has limited affinity for AR, but its two major metabolites, M1 and M2, compete strongly for AR binding yet do not activate subsequent transcriptional steps as normal androgens would (Kelce *et al.* 1995). Similarly, although DDT has well-known estrogenic effects, its metabolite *p,p′*-DDE is not a potent estrogen but does bind AR and inhibit androgen-induced transcriptional activity. Since the initial work with vinclozolin and *p,p′*-DDE, other compounds that bind AR and have antiandrogenic effects have been identified, such as procymidone, prochloraz, linuron, and polybrominated diphenyl ethers (for current review, see Hotckiss *et al.* (2008)).

The ability of several chemicals to bind to AR and disrupt the ability of endogenous ligands to signal normally is analogous to environmental estrogens, many of which bind to ER. However, in most cases those compounds induce transcription in a manner similar to endogenous estrogens and thus are agonists rather than antagonists. As discussed above, there are several chemicals that disrupt estrogen signaling by mechanisms other than the most obvious one involving ER binding. Similarly, there also appear to be compounds that have antiandrogenic effects, but do so by mechanisms other than binding AR. Some chemicals (e.g., linuron, prochloraz) that bind AR also decrease testosterone production, and their overall antiandrogenic actions represent the sum of their actions through both pathways.

Another group of chemicals that have aroused extensive concern is the phthalates (**Table 1**), due to both their ubiquity and their potential negative reproductive effects. Phthalates are used extensively

as plasticizers in a wide variety of products, including baby bottles. Phthalates are major environmental contaminants and in particular dibutyl phthalate (DBP), benzylbutyl phthalate (BzBP), di-2-ethylhexyl pthalate (DEHP), and di-isononyl phthalate (DINP) have been shown to be antiandrogenic and disrupt reproductive development in male rodents (Hotckiss *et al.* 2008). The testicular target of phthalates is Sertoli cells (Hotckiss *et al.* 2008), although the mechanism of its antiandrogenic effects remains unclear; these chemicals act through a mechanism that does not appear to involve AR and is distinct from antiandrogens previously described or inhibitory effects on steroidogenesis previously reported.

Phthalates may also be of concern in human reproduction. In a groundbreaking but controversial epidemiological study by Swan *et al.* (2005), it was suggested that prenatal exposure to environmental levels of phthalates decreased anogenital distance, a sensitive marker of antiandrogen action. The biological implications of a change in anogenital distance in humans are not known. However, the ability of phthalates to alter this parameter in humans as they do in rodents suggests that other reproductive effects reported in rodent models following phthalate exposure could also occur in humans exposed to high levels of these chemicals.

In contrast to potential antiandrogenic effects of environmental chemicals, recent literature indicates that environmental androgens may also be a concern. Pulp mill effluent has androgenic activity *in vitro* (Hotckiss *et al.* 2008), and this is consistent with reports of masculinized female fish in waterways that have high levels of this environmental contaminant. The synthetic androgen trenbolone is anabolic and is used extensively in animal feeding operations, and effluents from these operations have been shown to be androgenic using both *in vitro* and *in vivo* assays (Hotckiss *et al.* 2008).

Early exposure of mouse fetuses to TCDD inhibits the formation of prostatic epithelial buds and results in striking decreases in adult prostatic size (Ko *et al.* 2002; Lin *et al.* 2002). This inhibitory TCDD effect is mediated by AhR, and a natural hypothesis is that TCDD signaling through AhR disrupts androgen signaling at some level. However, TCDD does not appear to decrease testicular testosterone content or impair conversion of testosterone to the active androgen in the prostate (5α-dihydrotestosterone, DHT). Other work has indicated that TCDD does not inhibit androgen-dependent gene expression in the urogenital sinus, the fetal organ from which the prostate develops. Therefore, TCDD produces phenotypic effects that are consistent with an antiandrogenic effect, but does so without impairing the AR signaling pathway, or at least the facets of this pathway that have been assessed so far. The mechanism by which TCDD inhibits prostatic development thus remains to be established and may not be an antiandrogenic effect in the strict sense, but it is included here because phenotypic changes are consistent with those arising from antiandrogenic effects.

In summary, several environmental antiandrogens that act through a variety of mechanisms are known, and in some cases their exact mode of action remains to be established. Recent evidence suggests that environmental androgens may also be a concern, although the specific compounds involved here are not clear. Thus, the androgen signaling system is an important target for EDCs, and as discussed below may be involved in the epigenetic transmission of traits that are altered by early life exposures to EDCs.

11.11.6 Endocrine Disruptors and the Testicular Dysgenesis Syndrome

The known deleterious effects of estrogens on the male reproductive tract of wildlife and experimental animals suggested that adult or especially developmental exposure to these chemicals could compromise testicular function and sperm production in humans. However, direct evidence of this was lacking. In 1992, Skakkebaek and coworkers (Carlsen *et al.* 1992) published a provocative meta-analysis of sperm counts in men over the past 50 years, and they reached the alarming and unexpected conclusion that sperm counts had fallen 40–50% over this time. Although no cause was known for the putative decline, EDCs were obvious candidates. This controversial finding again ignited a worldwide burst of concern over potential effects of EDCs on human health. Subsequent data have not all been consistent with this initial hypothesis and there appear to be clear geographical and population differences in the putative decline in human sperm production, but this work has galvanized worldwide interest in endocrine disruptors and their potential human reproductive effects.

In the years since this initial paper appeared, Skakkebaek and coworkers have broadened their original hypothesis. Based on well-documented large increases in testicular cancer, hypospadias,

and cryptorchidism in some areas of the world (Skakkebaek *et al.* 2003), this group has suggested that the original putative decline in semen quality may be linked to these other male reproductive maladies and all may be manifestations of one problem, which they term the testicular dysgenesis syndrome (TDS). Although the etiology of TDS remains controversial, EDCs are likely involved. The ability of developmental phthalate exposure in rodents to mimic many features of TDS has led to the hypothesis that impaired androgen signaling may be critical (Sharpe and Skakkebaek 2008), but EDCs that alter estrogen or other signaling pathways may also be involved.

11.11.7 Male Reproductive Effects of Thyroid Hormone Signaling Disruption by Environmental Chemicals

Thyroid hormone, although not commonly thought of as a reproductive hormone, has critical effects on Sertoli (Cooke and Meisami 1991; van Haaster *et al.* 1992) and Leydig cell (Teerds *et al.* 1998) development in the testis. Thus, environmental chemicals that disrupt thyroid hormone signaling can function as EDCs of the male reproductive tract through this mechanism (**Table 1**). The case of PCBs illustrates this point. Some PCB congeners are estrogenic, while others are AhR agonists that produce antiestrogenic effects, as well as other developmental abnormalities in males through unknown mechanisms (Ko *et al.* 2002). In addition, PCBs induce hypothyroidism through effects on thyroid hormone carrier proteins, and developmental exposure to PCBs has been shown to alter testicular development in a rodent model through antithyroid effects (Cooke *et al.* 1996).

Another group of environmental chemicals that may have reproductive effects through effects on thyroid hormone are the isoflavones, whose reproductive actions related to their estrogenic effects were previously discussed. Isoflavones such as GEN inhibit thyroid peroxidase *in vitro* and *in vivo*. Thus, they could potentially inhibit thyroid hormone signaling, although significant effects on overall thyroid hormone status and functional deficits in thyroid hormone action have not been demonstrated *in vivo* as a consequence of phytoestrogen exposure (Doerge and Chang 2002). Some literature has also suggested that dietary soy can increase thyroid hormone levels in cats (White *et al.* 2004). There are also data suggesting that GEN has significant affinity for thyroid hormone transport proteins (Radovic *et al.* 2006), and can potentially affect thyroid hormone status in this way. Therefore, current literature in this area is not entirely clear, and a definitive understanding of what effects, if any, that GEN may induce through thyroid hormone changes remains to be established.

Recent concern in the area of thyroid hormone disruption by environmental chemicals has focused increasingly on perchlorates used in rocket fuel that are present at significant levels in many sites in the western U.S. Perchlorate inhibits iodide uptake by the sodium–iodide symporter and thus impairs thyroid hormone production (Keverne and Curley 2008). Perchlorate exposure can thus produce significant disruptions in normal thyroid hormone signaling. Perchlorates, along with a wide array of other environmental chemicals known to disrupt thyroid hormone signaling such as thiocarbamide- and sulfonamide-based pesticides (e.g., ethylenethiourea, linuron), TCDD, hexachlorobenzene, nitrofen, lead, mirex, and phthalic acid esters (**Table 1**), may potentially all have effects on the developing male reproductive tract through this mechanism.

11.11.8 Epigenetic Effects of Environmental Endocrine Disruptors

Epigenetics is the study of how the environment can affect the genome of the individual during its development as well as the development of its descendants, all without changing the DNA sequence. The cumulative exposures to endocrine disruptors throughout an individual's life history interact with genetic predispositions to shape the individual's physiology and behavior. Recent evidence suggests that chemical exposures in past generations may also influence how an individual responds to toxicants in his or her own life history, and there is unequivocal evidence that EDCs can influence not only the exposed individual, but also subsequent generations.

11.11.8.1 Mechanism of Epigenetic Effects

It is important to distinguish the difference between context-dependent and germline-dependent epigenetic modifications (Crews 2008). Context-dependent epigenetic modifications refer to transmission within a generation, while germline-dependent epigenetic modifications refer to transmission across generations.

The best examples of context-dependent epigenetic modifications that have been shown are for early life exposures to EDCs *in utero*. In this instance, the onset of disease manifests itself later in life. The extent to which the modification is perpetuated in future generations is determined by the persistence of the environmental factors (i.e., the context) that bring about the epigenetic modification. That is, in each generation, individuals are exposed to the same environmental conditions. For example, if the diet or environmental toxicant continues to be present in the environment, then the epigenetic modification will be manifest in each generation. This type of epigenetic modification lends itself to relatively straightforward therapeutic venues such as providing methyl donors to the diet (Waterland *et al.* 2006) or removing the environmental toxicant. Hence, the environment can induce epialleles, but this epigenetic state can be reversed by other factors.

Germline-dependent epigenetic modifications are fundamentally different from context-dependent epigenetic modification in that the epigenetic imprint (i.e., the modification) has become independent of the original causative agent. Here, the epigenetic modification is transferred to subsequent generations because the epigenome change has been incorporated into the germline. Thus, effects are manifested in each generation, without the need for reexposure.

DNA methylation of heritable epialleles is passed to subsequent generations rather than being erased normally during gametogenesis and shortly after fertilization. Importantly, germline-dependent epigenetic modifications can be associated with one sex, as many behaviors and affective disorders show sex differences. It should be emphasized that germline-dependent epigenetic modifications are not equivalent to genomic imprinting in which genes are monoallelically expressed in a parent-of-origin dependent manner (Davies 2008; Keverne and Curley 2008). In the latter case of genomic imprinting, subsets of genes are silenced and influence development; silencing of genes is erased and not transmitted to the next generation.

11.11.8.2 Chemicals Known to Induce Epigenetic Effects

There is very little direct evidence for either context- or germline-dependent epigenetic modifications following exposure to EDCs. This is surprising considering that this discipline was strongly influenced by observations in the mid-1900s that alterations in the hormonal milieu during fetal development caused perturbations in adult reproductive physiology and behavior (Phoenix *et al.* 1959; reviewed in Gore 2008). The best example is the groundbreaking work of Anway, Skinner, and collaborators (2005), who showed that exposure of developing male fetuses to the antiandrogenic fungicide vinclozolin resulted in adult-onset disease and dysfunction, a phenotype that was passed to subsequent generations by the male germline.

A second example of an EDC that can cause epigenetic modifications, and one that is probably mediated through estrogenic actions, is that of BPA. Dolinoy, Jirtle, and colleagues showed that BPA exposure to the fetus altered DNA methylation of the viable yellow agouti gene, a pattern that could be assessed by coat coloration. Supplementation with methyl donors counteracted hypomethylating effects of BPA (Dolinoy *et al.* 2007). Although this group did not assess the hypothalamic-pituitary-gonadal axis *per se*, the finding of epigenetic modifications by an estrogenic EDC, and counteractive effects of phytoestrogens, indicate the potential for context-dependent modifications of neuroendocrine traits.

11.11.9 New Frontiers in Endocrine Disruption

Are stem cells targets of endocrine disruptors? Stem cells are undifferentiated cells that are capable of dividing and self-renewing, and also can give rise to cells that differentiate into one or more specialized cell lineages. The spermatogonial stem cell (SSC) has been studied for many years, and indeed many important concepts regarding stem cell biology arose from SSC studies. In contrast, prostatic epithelial stem cells have received much less attention, although recent progress in this area has led to the identification of a prostatic epithelial stem cell and a rapidly increasing understanding of this cell's role in normal and pathological prostatic epithelium (Lawson *et al.* 2007). Because the intense research interest in stem cells is relatively recent, the role of stem cells as targets of various types of toxicants has not been extensively considered. Thus, there are few studies on toxicants or EDCs that even consider stem cell effects. This reflects the fact that the endocrine disruptor literature evolved prior to the current wave of stem cell interest and may also be partially due to

the fact that stem cells are not as vulnerable to toxicological insult as other cells, as discussed below.

Stem cells by definition divide slowly, and in the case of the seminiferous and prostatic epithelium, the stem cells are less dependent on the trophic hormones that regulate these organs. This argues that stem cells in the seminiferous epithelium and prostatic epithelium may not be as susceptible to EDCs that affect the trophic hormones for these tissues and alter proliferation of more differentiated cells. However, adult stem cells are potential targets of toxicants as they have comparatively relaxed DNA repair functions, which may lead to mutagenesis when exposed to toxicants (Trosko and Tai 2006). In support of this hypothesis, estrogen treatment of human mammary epithelial cells increased hypermethylation of 0.5% of the CpG islands (Cheng *et al.* 2008) and it was suggested that this might increase breast cancer risk.

Other studies of toxicant effects on nonreproductive organs have documented effects on stem cells. For example, human hematopoetic stem cells exposed to very low doses of cadmium or hexavalent chromium show activation of autophagy, the process by which cytoplasmic components such as macromolecules and organelles are degraded by the lysosomes. This leads to chronic diseases associated with exposure to heavy metals (Di Gioacchino *et al.* 2008). Neurotoxins affecting neural stem cells not only alter development of the nervous system but also have adult effects as stem cells are responsible for brain repair and normal functions. Manganese is an essential nutrient but excess amounts affect intellectual functions in children. Tamm *et al.* (2008) have shown that when neural stem cells, both primary cultures and cell lines, are exposed to manganese there is apoptotic cell death and reactive oxygen species formation, leading to manganese toxicity.

Few toxicant studies have examined SSCs, primarily because it is difficult to define or isolate these cells and it is difficult to assess the functionality of these cells. Rodent studies have shown that some toxicants affect later stages of germ cell development, impair spermatogenesis, and lead to testicular atrophy, yet do not produce obvious effects on SSCs. For example, SSCs are not affected following exposure to irradiation (Shuttlesworth *et al.* 2000), chemotherapeutic alkylating agents such as cyclophosphamide (Meistrich *et al.* 1995) and procarbazine (Meistrich 1999), or environmental toxicants such as *n*-hexane (Boekelheide and Hall 1991) and boric acid (Ku *et al.* 1993). However, Schoenfeld and colleagues (Richburg *et al.* 2002) found that the primate SSC population was less resistant to irradiation therapy, suggesting that there might be species differences and primates and humans may be more sensitive to toxicants. There is also evidence that SSCs may be affected by environmental pollutants. Combustion of fossil fuels produces complex mixtures of chemicals that are environmental pollutants. Particulate air pollutants bring about tandem repeat DNA sequence mutations that are transmitted through the paternal germ line. Mice exposed to particulate air pollution in an urban industrial location had elevated extended simple tandem repeat mutations in sperms 10 weeks following exposure, but not earlier, indicating that SSCs were susceptible and caused germ-line mutations (Yauk *et al.* 2008). Mutation frequency in SSCs was 1.6-fold higher in these mice. The sperms were hypermethylated due to structural change in the chromatin, decreased gene expression, and reduced rates of transposon movements.

SSCs and prostatic epithelial stem cells are by definition undifferentiated, while all other germ cells in the spermatogenic epithelium and prostatic epithelial cells are committed to differentiate and are at various points along this pathway. For toxicants that act directly on germ cells and produce permanent effects, the toxicant action must involve SSCs, or otherwise the initial toxicological effect would be reversible as more differentiated spermatogenic cells that were the initial target of the endocrine disruptor/toxicant were lost and were replaced by normal cells from unaffected stem cells. Since this type of recovery from toxicological insult is rare, many permanent toxicological effects on spermatogenesis may involve SSC effects, although this has not been extensively examined. Similarly, many effects of toxicants and endocrine disruptors may involve changes in prostatic epithelial stem cells, and this should be considered in future studies.

The stem cell niche is the microenvironment that promotes self-renewal and maintenance of stem cells. In the testis, maintenance of the normal stem cell niche is dependent on glial cell-derived neurotrophic factor, a Sertoli cell secretory protein, and ets variant gene 5, a Sertoli cell transcription factor essential for SSC maintenance and self-renewal. Sertoli cells are primary targets of many toxicants such as phthalates, *n*-hexane, and haloacetic acids. Hence, some effects of environmental toxicants and endocrine disruptors on SSCs may occur by impairing the ability of Sertoli cells to support SSCs in their niche, either through altered secretion of SSC trophic factors or through

physical or functional damage to the Sertoli cells. However, this area remains unexplored.

11.11.9.1 Metabolic Products as Endocrine Modulators

The historical focus in this field has been on exogenous chemicals that perturb normal endocrine signaling. However, recent work (Umetani *et al.* 2007) has identified an endogenous compound, 27-hydroxysterol (27HC), that modulates normal estrogen signaling and is at least potentially capable of altering estrogen signaling in a variety of tissues *in vivo* (**Table 1**). These findings have clinical relevance, as discussed below, but also necessitate a reassessment of our basic definition of an endocrine disruptor. The demonstration that an endogenous compound in the body can function as an endocrine disruptor will force us to broaden our definition of this type of compound and the effects they may have in various physiological situations.

27HC is a catabolic product of cholesterol elimination in extrahepatic tissues. It is highly expressed in human atherosclerotic lesions and correlates well with cholesterol (Brown and Jessup 1999). A recent study reports that 27HC is a classic competitive antagonist of both ERα and ERβ action in HEK293 cells and antagonizes estrogen induction of inducible nitric oxide synthase (iNOS) in vascular cells (Umetani *et al.* 2007), a response that is predominately mediated by ERβ. Furthermore, 27HC inhibited the nontranscriptionally mediated effects of estrogen on endothelial nitric oxide synthase (eNOS) enzymatic activity in vascular cells, which occurs through both ERα and ERβ. These NOSs generate nitric oxide, and one way in which estrogen imparts protective vascular effects is believed to be through the upregulation of these synthases (Murphy and Steenbergen 2007; Ogita *et al.* 2003). Based on its effects on the NOSs, 27HC appears to be capable of inhibiting estrogen action in vascular cells. This was confirmed *in vivo* by showing that increased 27HC, induced through either diet-induced hypercholesterolemia or use of mice deficient in the catabolic enzyme for 27HC, decreased estrogen-mediated induction of iNOS and eNOS.

Thus, 27HC antagonism of estrogen signaling in the vasculature may be one of the means by which estrogen protection is compromised in postmenopausal women (Umetani *et al.* 2007). Atherosclerotic plaques may produce 27HC and antagonize estrogen's cardiovascular benefits (Umetani *et al.* 2007). This oxysterol may serve as a potential therapeutic target in the treatment and prevention of cardiovascular disease in postmenopausal women and men.

Despite its antiestrogenic effects in vasculature, 27HC is not simply a competitive antagonist, but actually functions as a selective estrogen receptor modulator (SERM). For example, 27HC in human breast cancer cells mimics E2 actions in terms of its effects on gene expression and stimulation of cell cycle progression, and 27HC also displayed agonist activity in hepatoma and colon cancer cell lines (Umetani *et al.* 2007). These findings again have clinical relevance that extends beyond the endocrine disruptor field.

11.11.10 Conclusions and Future Directions

The full ramifications of EDCs are still being established. New chemicals, new mechanisms of action, and new effects of existing chemicals are being added to the literature on a regular basis. The importance of this area for both human and animal health suggests that this area will be the subject of both intense research and debate in the decades to come.

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