Pure Appl. Chem., Vol. 75, Nos. 11–12, pp. 2099–2123, 2003.

Topic 3.10

Critical evaluation of observed adverse effects of endocrine active substances on reproduction and development, the immune system, and the nervous system*

John C. O'Connor‡,[1] and Robert E. Chapin[2]

[1]DuPont Haskell Laboratory for Health and Environmental Sciences, P.O. Box 50, Elkton Road, Newark, DE 19714, USA; [2]Pfizer, Inc., Drug Safety Evaluation, MS 8274-1336, Eastern Point Road, Groton, CT 06340-8014, USA

Abstract: The last 40 years have seen many reports that man-made chemicals and environmental pollutants cause adverse effects in humans and wildlife; however, actually linking an exposure with a mechanism and an effect has yet to be done for endocrine disruption. Certainly, studies in experimental animals have shown that sufficient doses of select compounds can disrupt the endocrine system and produce the attendant adverse outcomes. The purpose of this contribution is to evaluate some of the recent reports of the adverse effects on reproduction and development, the immune system, and the nervous system that have been observed in experimental animals after treatment with man-made chemicals and environmental pollutants. Space limitations prevent us from presenting a comprehensive review of all reported endocrine active chemicals and their effects. Instead, we have focused on drawing conclusions as to the scope and etiology of the adverse effects in experimental animals using examples from the scientific literature, and on suggesting a path forward for further work.

INTRODUCTION

The purpose of this contribution is to evaluate the adverse effects on reproduction and development, the immune system, and the nervous system that have been observed in experimental animals after treatment with man-made chemicals and environmental pollutants. Space limitations prevent a comprehensive review of all reported endocrine active chemicals (EACs) and their effects; we have focused on drawing conclusions as to the scope and etiology of the adverse effects in experimental animals, and on suggesting a path forward for further work.

REGULATION OF REPRODUCTION AND DEVELOPMENT IN MAMMALS

The endocrine and nervous systems are the major mechanism(s) by which the body communicates information between cells and/or organ systems, and both are critical for the regulation of growth and development, reproduction, and maintaining metabolic processes. The endocrine system is a highly com-

*Report from a SCOPE/IUPAC project: Implication of Endocrine Active Substances for Human and Wildlife (J. Miyamoto and J. Burger, editors). Other reports are published in this issue, *Pure Appl. Chem.* **75**, 1617–2615 (2003).

‡Corresponding author: Tel: (302) 366-6169; Fax: (302) 366-5003; E-mail: john.c.oconnor@usa.dupont.com

plex and integrated system of glands that secrete hormones into the circulatory system, ultimately regulating the function of specific target tissues/organs. The main components of the endocrine system are the hypothalamus, pituitary, and a variety of endocrine glands (e.g., testis, ovary, thyroid, adrenals, pancreas) that each participates in regulating numerous physiological processes. The inherent design of the endocrine system allows the body to react to acute changes in homeostasis through the positive and negative feedback loops that control hormone production and release. However, the complexity of the endocrine system also provides many potential sites for endocrine disruption (ED) to occur. Figure 1 summarizes the basic regulation of the reproductive axes in males and females, and illustrates several potential sites of ED. Examples of potential mechanisms of ED include [1,2]:

- alterations in receptor-mediated signaling (e.g., agonism and antagonism);
- alterations in hormone synthesis;
- alterations in hormone storage and/or release;
- alterations in hormone transport;
- alterations in hormone metabolism; and
- alterations in post-receptor activation.

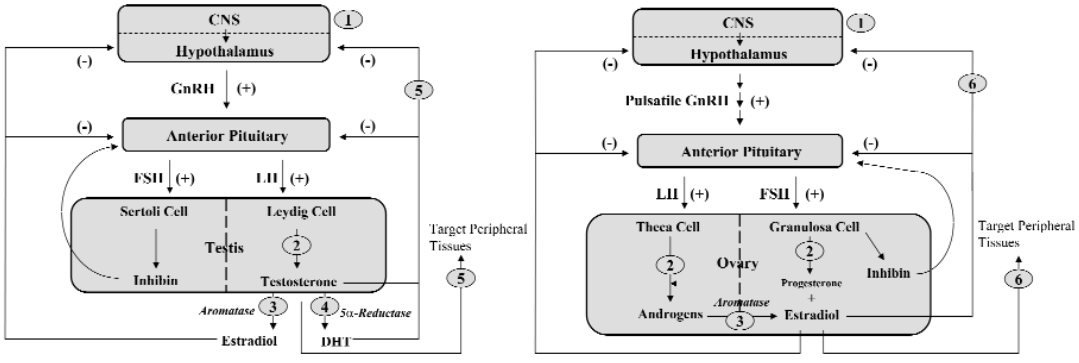

Fig. 1 *Regulation of the hypothalamic-pituitary-gonadal axis in male (a) and female (b) mammals.* Abbreviations: CNS: central nervous system; GnRH: gonadotropin releasing hormone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; DHT: dihydrotestosterone. Potential sites of ED include: (1)dopamine agonists would act on the CNS to affect GnRH release; (2)steroid biosynthesis inhibitors would inhibit testosterone production, reducing the amount of testosterone and perhaps DHT or estradiol; (3) aromatase inhibitors would inhibit the conversion of testosterone to estradiol; (4) 5α-reductase inhibitors would inhibit the conversion of testosterone to DHT; (5) androgen receptor blockers would interfere with the normal androgen feedback to the pituitary and brain, as well as decreasing androgen action peripherally; (6) estrogen receptor blockers would interfere with the normal estrogen feedback to the pituitary and brain, as well as decreasing estrogen action peripherally. Inhibin has not yet been rigorously evaluated in environmental toxiciology.

Unfortunately, for most compounds, the specific mechanism of action is unknown, or is confounded by the ability of the substance to affect multiple sites of endocrine control (e.g., binding to more than one receptor). Although not all-inclusive, Table 1 summarizes some of the EACs that have been identified in experimental animals.

Although adult animals are susceptible to ED (as described below), the developing fetus is uniquely sensitive to alterations in endocrine status, and the spectrum of effects that are observed after in utero exposure to EACs is a reflection of the complexities of reproductive development and differentiation. For this reason, selected aspects of mammalian differentiation will be summarized here; many thorough reviews are available on the subject of mammalian differentiation, to which the reader is referred [3,4].

Table 1 Examples of known or suspected endocrine active substances.

ER agonists
DDT (*o,p′*-DDE)
Methoxychlor
Chlordecone
Bisphenol A
PCBs
Endosulfan
Dieldrin
Dicofol
Chlordane
Toxaphene
Lindane
Butyl benzyl phthalate (BBP)
Alkylphenols (nonylphenol, octylphenol)
Endogenous estrogens (estradiol, estrone)
Pharmaceuticals (DES, ethiynl estradiol)
Phytoestrogens (coumestrol, genistein)
Mycoestrogens (zearalenone)
ER antagonists
ICI-182,780
ICI-182,164
AR antagonists
Vinclozolin
(DDT) *p,p′*-DDE
PCBs
Linuron
Cyproterone acetate
Procymidone
Steroid biosynthesis inhibitors
Finasteride
Fenarimol
Exemestane
Ketoconazole
Di-*n*-butyl phthalate (antiandrogen-like)
Other
Dioxin (TCDD, Ah receptor agonist)

In rats, development of the endocrine system starts at approximately gestation day 8 with the first steps in the differentiation of the bipotential gonad into the testis or the ovary (Fig. 2). The activation of the *Sry* gene on the Y chromosome triggers a cascade of events that result in the development of the male phenotypic traits, whereas in the absence of the *Sry* gene, the embryo develops into a female. In this respect, the female phenotype is considered the "default" pathway for reproductive development in mammals. Activation of *Sry* induces the differentiation of the bipotential embryonic gonads into the testes. As the testes develop, two main cell types produce hormones that ultimately drive reproductive development and differentiation. The Sertoli cells, the cells that will ultimately support the maturation of the germ cells, produce Müllerian inhibiting substance (MIS), also known as anti-Müllerian hormone (AMH), which causes regression of the Müllerian ducts. In the absence of MIS, the Müllerian ducts give rise to the female genitalia, uterus, and vagina. Concurrently, the Leydig cells of the embryonic testes secrete testosterone (T), which supports the differentiation of the Wolffian ducts, which give rise to the male epididymis, seminal vesicles, and vas deferens. Dihydrotesterone (DHT), the major metabolite of

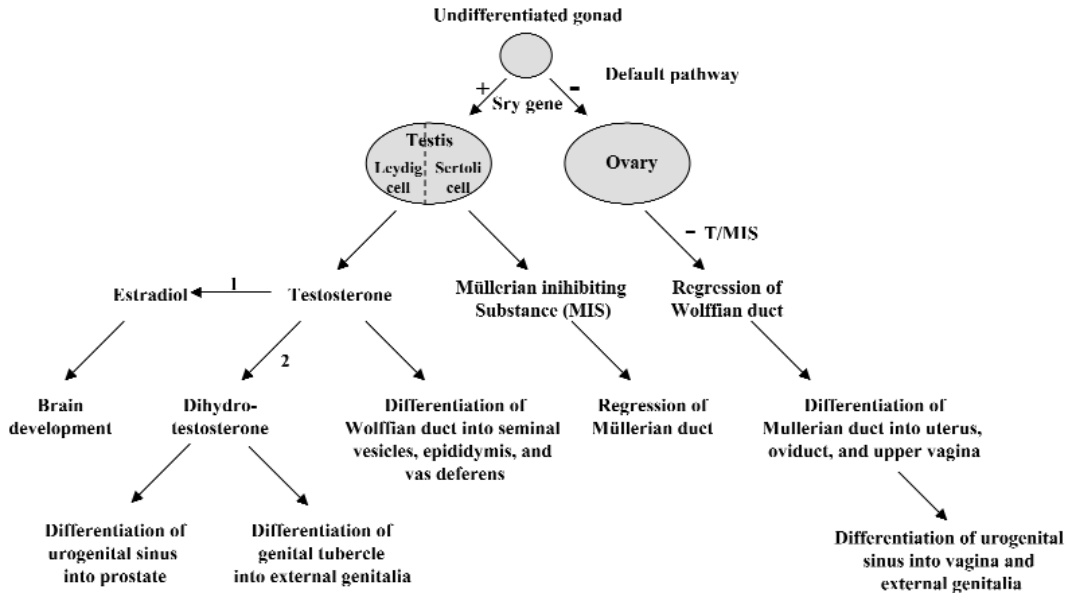

Fig. 2 *Regulation of mammalian reproductive differentiation and development.* Abbreviations: T: testosterone; MIS: Müllerian inhibiting substance; 1 (aromatase enzyme); 2 (5α-reductase enzyme).

T, induces the formation of the external genitalia and also participates in the descent of the testes into the scrotum (along with MIS). Estradiol, the aromatization product of T, is required for normal brain sexual/behavioral development (see discussion below). Each of these complex steps is under the control of androgens, and requires proper androgen signaling for normal development to occur.

In both males and females, differentiation of the reproductive organs continues throughout gestation, while reproductive maturation (e.g., masculinization of external genitalia, anogenital distance, behavioral development) continues throughout the first few weeks of postnatal life [3,4] until puberty, which is under hormonal control (i.e., androgen-dependent in males and estrogen-dependent in females). After puberty, processes such as spermatogenesis [5,6] or ovarian function remain under hormonal control [3,4]. This is a greatly simplified description of the very complex processes of mammalian sexual development, a process rife with many potential sites for ED. As described below, perturbations in the endocrine-signaling pathways during development lead to very distinct developmental abnormalities.

ADVERSE EFFECTS OF ENDOCRINE ACTIVE SUBSTANCES ON REPRODUCTION AND DEVELOPMENT

In *adult* animals, adverse effects to EACs are typically transient; that is, the effects subside if chemical treatment is withdrawn. This is a function of an endocrine system that developed normally, and that can maintain hormonal homeostasis via the built-in feedback loops in the presence of external challenges. Transient effects can include changes in weight and morphology of target organs, and alterations in reproductive capacity. For example, exposure of adult rats to high levels of endogenous estrogens such as 17β-estradiol or estrone [7–12]; synthetic estrogens such as diethylstilbestrol (DES) [13–16]; or environmental estrogens such as methoxychlor, chlordecone, and octylphenol [17–23] leads to decreased reproductive capacity in both males and females. In males, these effects are characterized by decreased reproductive organ weights and abnormal reproductive tract morphology, often accompanied by impaired spermatogenesis resulting in decreased sperm count, decreased sperm motility, and/or altered sperm morphology. In female rats, alterations in estrous cyclicity and evidence of ovarian malfunction

(e.g., decreased corporea lutea, decreased ova count) are commonly reported. In both cases, reproductive capacity is compromised due to disruption of the hormone feedback loops resulting in decreased gonadotropin release from the pituitary, and ultimately altered function of the male and female gonads. Activation of estrogen receptor (ER)-mediated events likely contributes to the effects. While these examples represent compounds that act via binding to the ER, there are similar reports in adult animals exposed to EACs with other mechanisms of action such as antiandrogens [1,2], aromatase inhibitors [24,25], and testosterone biosynthesis inhibitors [26,27]; and the list of suspected EACs continues to grow. In cases where prolonged exposures occur, EACs often induce neoplasia of hormone-responsive tissues. The particular type of neoplasia is dependent upon the mechanism of action of the EAC [28].

While sustained alterations in hormonal homeostasis at any point during life can result in adverse effects as discussed above, even small transient alterations in hormonal homeostasis *during development* can be detrimental since the developing organism is uniquely sensitive to hormonal perturbations. The inherent sensitivity of the fetus is due to the reproductive and behavioral "programming" that occurs during development of the endocrine system in the fetus and neonate [3,4,29,30]. Even small perturbations in the endocrine axes during this period of development may result in permanent alterations in the way the affected cells respond to hormones at any time in the future. Thus, there may be lasting impacts on the reproductive and/or behavioral capacity of the animal. Table 2 summarizes some examples of chemicals that are known EACs in experimental animals when administered in utero, and the adverse effects that are associated with each.

Overall, male progeny of pregnant xenobiotic-treated dams seem to be more susceptible than females to perturbations in endocrine signaling during reproductive development, whereas both males and females are equally susceptible to alterations in behavioral development (see below). This is true in both the scope and severity of the effects, as well as the number of EACs that have been shown to adversely impact experimental animals. This is not surprising when one considers the series of events that is required for reproductive development of males versus females (Fig. 2). In general, any EAC with the ability to alter androgen signaling has the potential to cause adverse effects in males. Three classes of EACs appear particularly important in male reproductive development: ER agonists, androgen receptor (AR) antagonists, and arylhydrocarbon (Ah) receptor agonists [1]. Any EAC with the ability to disrupt steroidogenesis also has the potential to induce adverse effects in males. Insufficient androgen signaling during reproductive development manifest in a pseudohermaphrodite condition of the male offspring, the scope and severity dependent upon a variety of factors including the period (i.e., gestation days) and duration of exposure, the mechanism of action of the EAC, and the level of exposure (i.e., dose). Surprisingly, given the different mechanisms of action of the EACs that have been evaluated in experimental animals, the scope of effects induced by different EACs on male reproductive development are remarkably similar.

One of the most well-studied disruptors of androgen signaling in developing males is vinclozolin, which is metabolized to two chemicals that have been shown to act as AR antagonists [31,32]. The sequelae of effects induced by vinclozolin exposure include decreased (i.e., female-like) anogenital distance (AGD), delayed puberty (delayed preputial separation), presence of female reproductive tissues (e.g., vaginal pouch), decreased sperm production, and a variety of malformations of the reproductive tract, from small/atrophied to completely absent male reproductive organs [31,33–36]. These effects impair the reproductive performance and success of the affected animal. All of the effects that are observed in the male progeny are the result of insufficient androgen exposure during development as a result of blockage of the AR by vinclozolin. T- and DHT-dependent events are both adversely effected (Fig. 2), resulting in incomplete differentiation of the male reproductive tissues and/or incomplete regression of the female reproductive tissues. The results observed with vinclozolin are representative of those observed for a variety of other AR antagonists including flutamide, procymidone, linuron, and *p,p′*-DDE [31,33,36–40]. The effects are likely the result, at least in part, of the attenuated transcription of AR-mediated genes during development and/or perturbations in other signaling pathways as a result of permanent alterations in the hormone-feedback loops during reproductive imprinting [4].

Table 2 Examples of endocrine active substances and their effects on reproduction and development in experimental animals exposed in utero.

Compound (mode of action)	Exposure	Effect	Ref.
Testosterone (AR agonist)	Rats; subcutaneous injection; 0 (corn oil), 0.1, 0.5, 1, 2, 5, 10 mg/0.1 ml on GD14–19.	Males: ↓ anogenital distance; ↓ glans penis weight (persisted at adulthood). Females: ↑ anogenital distance (persisted at adulthood); delayed puberty (vaginal opening); ↓ number and/or absent areolas/nipples; presence of male reproductive tissues and/or no vaginal orifice; altered estrous cyclicity.	[84]
Vinclozolin (AR antagonist)	Rats; gastric gavage; 0 (corn oil), 100, 200 mg/kg/day on GD14–PND3.	Males: ↓ anogenital distance; retained areolas/nipples (persisted at adulthood); cleft phallus with hypospadias; ectopic/undescended testes; blind vaginal pouch; small or absent accessory sex glands; ↓ sperm concentration. Females: no effects.	[33,34]
	Rats; gastric gavage; 0 (corn oil), 10, 30, 100 mg/kg/day from age 22–56 days.	Males: delayed puberty (age of preputial separation); ↓ organ weights (accessory sex gland unit and epididymis weight); hormonal alterations (increased testosterone and luteinizing hormone).	[35]
Flutamide (AR antagonist)	Rats; gastric gavage; 0 (corn oil), 6.25, 12.5, 25, 50 mg/kg/day on GD12–21.	Males: ↓ anogenital distance (persisted at adulthood); retained nipples/areolas (persisted at adulthood); hypospadias; ectopic/undescended testes; prostate agenesis; ↓ organ weights (seminal vesicles, testes, epididymides, levator ani/bulbocavernosus); epididymal malformations.	[36]
	Rats; subcutaneous injection; 0 (ethanol/corn oil), 18, 24, 50, 75, 100, 200, 250, 300 mg/kg/day on GD12–21.	Males: severe feminization of external genitalia; absent vas deferens and prostate; malformation of epididymidis.	[37]
Procymidone (AR antagonist)	Rats; gastric gavage; 0 (corn oil), 25, 50, 100, 200 mg/kg/day on GD14–PND3.	Males: ↓ anogenital distance; hypospadias; retained areolas/nipples (persisted at adulthood); ectopic/undescended testes; vaginal pouch; ↓ organ weights (ventral prostate, levator ani/bulbocavernosus, Cowper's glands, seminal vesicles, glans penis); histological lesions in accessory sex glands.	[38]
	Rats; gastric gavage; 0 (corn oil) or 100 mg/. kg/day on GD14–PND3	Males: ↓ anogenital distance; hypospadias; retained areolas/nipples (persisted at adulthood); vaginal pouch; ↓ weights (ventral prostate and seminal vesicles); prostate agenesis.	[33]
Linuron (AR antagonist)	Rats; gastric gavage; 0 (corn oil) or 100 mg/kg/day on GD14–18.	Males: delayed puberty; hypospadias; ↓ anogenital distance; retained areolas/nipple; malformations of epididymis and testes; ↓ organ weights (testes, epididymis, seminal vesicles); agenesis of testes and epididymis.	[33]

Table 2 (*Continued*).

Compound (mode of action)	Exposure	Effect	Ref.
p,p′-DDE (AR antagonist)	Rats; gastric gavage; 0 (corn oil), 100 mg/kg/day on GD14–18 or PND25–57, or 200 mg/kg/day for 4 days (adult).	Males (GD14-18): retained areolas/nipples; ↓ anogenital distance. Males (PND25-57): delayed puberty. Males (adult): ↓ organ weights (seminal vesicle, ventral prostate).	[39]
	Rats; gastric gavage; 0 (corn oil), 10, 100 mg/kg/day on GD14–18.	Males: retained areolas/nipples, ↓ anogenital distance. Females - no effects.	[40]
	Rats; gastric gavage; 0 (corn oil) or 100 mg/kg/day on GD14–PND3.	Males: ↓ anogenital distance; hypospadias; retained areolas/nipples (persisted at adulthood); ↓ organ weights (ventral prostate, levator ani/bulbocavernosus, epididymis).	[33]
Diethylstilbestrol (ER agonist)	Mice; gastric gavage; 0 (corn oil), 0.1, 1, 10, 100 mg/kg/day on GD9-16.	Males: ↓ fertility; abnormal sperm motility and morphology; ↓ sperm number; ectopic/undescended testes; retained Mullerian duct remnants; small phallus; hypospadias; histological lesions of the reproductive tract; neoplasia of reproductive tissues at adulthood.	[15]
	Mice; subcutaneous injection; 0 (corn oil), 0.01, 1, 2.5, 5, 10, 100 μg/kg/day on GD9-16.	Females: ↓ fertility; ovarian malfunction (decreased ova count); structural abnormalities of reproductive tract (oviduct, uterus, cervix, vagina); altered estrous cyclicity; hypospadias; neoplasia of reproductive organs at adulthood.	[16]
	Mice; subcutaneous injection; 0 (corn oil), 0.01, 1, 2.5, 5, 10, 100 μg/kg/day on GD9–16.	Females: abnormalities of reproductive tract (vagina, cervix, uterus, ovaries, oviduct); hypospadias; neoplasia of reproductive organs at adulthood.	[14]
	Mice; subcutaneous injection; 0 (corn oil) or 100 μg/kg/day on GD9–16.	Males: sterility; ectopic/undescended testes; histological lesions of testes, epididymis, seminal vesicles.	[13]
17β-Estradiol (ER agonist)	Rats; dietary; 0, 0.05, 2.5, 10, 50 ppm; one-generation reproduction study.	Males: ↓ sperm counts; delayed puberty; feminization of male reproductive organs (mammary gland); atrophy of reproductive organs; hormonal alterations. Females: accelerated puberty; ↓ fertility; altered estrous cyclicity; ovarian malfunction (↓ corporea lutea); hormonal alterations.	[10–12]
Nonylphenol (ER agonist)	Rats; gastric gavage; 0 (peanut oil), 3, 15, 75 mg/kg/day on GD11–18	Males: ↓ epididymal weight.	[53]
	Rats; dietary; 0, 200, 650, 2000 ppm; three-generation reproduction study.	Females: accelerated puberty; altered estrous cyclicity; ↓ fertility; ↓ ovary weight. Males: ↓ sperm number in F1 adults (questionable effect).	[54] [55]

(*continues on next page*)

Table 2 (*Continued*).

Compound (mode of action)	Exposure	Effect	Ref.
	Rats; gastric gavage; 0, 2, 10, 50 mg/kg/day; two-generation reproduction study.	Males: ↑ pituitary weight. Females: accelerated puberty; ↓ ovary weight; ↓ number of implantation sites.	[55]
Butylbenzyl phthalate (ER agonist)	Rats; gastric gavage; 0, 20, 500 mg/kg/day; two-generation reproduction study.	Males: ↓ anogenital distance; delayed puberty; testicular atrophy; ↓ sperm counts; hormonal alterations. Females: ↑ anogenital distance, ↓ ovary weight.	[55]
Methoxychlor (ER agonist/ AR antagonist)	Rats; gastric gavage; 0 (corn oil), 25, 50, 100, 200 mg/kg/day; various study designs.	Males: delayed puberty; ↓ organ weights (seminal vesicles, testes, epididymis); ↓ sperm count; histological lesions in testes. Females: accelerated puberty (age at vaginal opening); accelerated time to first estrus; ↓ fertility; altered estrous cyclicity; ↓ ovary weight.	[82]
	Rats; review paper.	Males: ↓ organ weights (seminal vesicles, prostate, epididymis); delayed puberty; ↓ sperm count; testicular atrophy. Females: accelerated puberty; accelerated time to first estrous; ↓ fertility; altered estrous cyclicity; ↓ implantation sites; ↓ corporea lutea; ↓ serum progesterone.	[159]
	Rats; 0, (corn oil), 5, 50, 150; maternal gavage GD14–PND7 and direct dose PND7–42.	Males: delayed puberty; ↓ organ weights (seminal vesicles, testes, epididymis, prostate); ↓ sperm count and sperm motility Females: accelerated puberty; altered estrous cyclicity; ↓ corporea lutea; ↓ fertility	[23]
Ketoconazole (steroidogenesis inhibitor)	Rats; 0 (corn oil), 12.5, 25, 50 mg/kg/day on GD14–PND3.	Males: ↓ organ weights (testes, seminal vesicles, epididymis); no effect on areolas/nipples, reproductive tract malformations, or anogenital distance.	[33]
	Rats; review paper.	Females: ↓ implantation sites; ↑ ovary weight; ↓ serum progesterone	[88]
Di-*n*-butyl phthalate (steroidogenesis inhibitor)	Rats; gastric gavage, corn oil vehicle; various exposures from GD3–PND20; doses from 0.5–750 mg/kg/day.	Males: retained areolas/nipples; delayed puberty; malformations of epididymis; malformations or missing prostate and seminal vesicles; histological lesions in testes; ↓ anogenital distance; ectopic/undescended testes; hypospadias. Females: no effects.	[50,160, 161]
	Rats; 0 (corn oil) or 500 mg/kg/day on GD14–PND3.	Males: retained areolas/nipples; hypospadias; ↓ anogenital distance; ↓ organ weights (ventral prostate, epididymis, testes, levator ani/bulbocavernosus); delayed puberty; ↓ sperm count.	[33]

Table 2 (*Continued*).

Compound (mode of action)	Exposure	Effect	Ref.
	Rats; dietary; 0, 0.1, 0.5, 1 %; continuous breeding protocol.	Males: ↓ fertility; ↓ organ weights (testes, seminal vesicles); ↓ sperm count; ectopic/undescended testes; reproductive tract malformations (epididymis, external genitalia). Females: no effects.	[162]
Finasteride (5α-reductase inhibitor)	Rats; gastric gavage; 0.5 % methylcellulose vehicle; various exposures from GD6–20; doses from 0.03–300 mg/kg/day.	Males: ↓ anogenital distance (reversed at adulthood); hypospadias; retained areolas/nipples (reversed at adulthood). Females: no effects.	[41,42]
	Rats; subcutaneous injection; 0 (ethanol/corn oil), 25, 50, 120, 160, 320 mg/kg/day on GD 12–21.	Males: feminization of external genitalia; ↓ organ weights (seminal vesicles and prostate); ↓ anogenital distance; hypospadias	[37]
Fenarimol (Aromatase inhibitor)	Rats; dietary and gastric gavage; various exposures–reproduction studies; doses from 5–350 ppm (diet) or 35 mg/kg/day (gavage).	Males: ↓ fertility due to feminized behavior.	[24,25]
Exemestane (Aromatase inhibitor)	Rats; gastric gavage; various exposures–reproduction & developmental studies; doses from 52–1000 mg/kg/day.	Females: ↑ anogenital distance; no effects on fertility; delayed parturition.	[89,163]
TCDD (Ah receptor agonist)	Rats; review paper.	Males: ↓ anogenital distance; ectopic/undescended testes; impaired spermatogenesis (persisted at adulthood); delayed puberty; ↓ organ weights (prostate and seminal vesicle); feminization of male sexual behavior. Females: cleft phallus; hypospadias; incomplete vaginal opening; ↓ ovary weight.	[164]
	Rats; 0 (DMSO/corn oil); various exposures and doses.	Males: ↓ organ weights (ventral prostate, testis, epididymis); ↓ anogenital distance.	[60]
	Rats; gastric gavage; 0 (corn oil), 0.05, 0.2, 0.8 μg/kg/day on GD15.	Males: ↓ fertility; delayed puberty; ↓ sperm count; ↓ organ weights (ventral prostate and seminal vesicles). Females: cleft phallus; hypospadias; incomplete vaginal opening; ↓ ovary weight; delayed puberty; malformations of the reproductive tract at adulthood.	[59]

Abbreviations: GD: gestational day; PND: postnatal day.

As stated previously, the severity of the effects is dependent on several factors. In some instances, many of the effects are irreversible (e.g., AGD, retained areolas/nipples, reproductive tract malformations, hormonal alterations) [31,33,34,36]. For example, the AR antagonists flutamide, vinclozolin, *p,p′*-DDE, and procymidone all induce permanent retention of areolas/ nipples at adulthood in male offspring [31,33,34,36,38], and flutamide permanently decreases AGD in male offspring [36]. In contrast, the vinclozolin-induced effects on AGD were reversible in a dose- and time-dependent manner if treatment was stopped on postnatal day 3 [31]. It has been hypothesized that the reversibility of the AGD effects after vinclozolin exposure may be due to a decrease in cell number in the AGD region of male rats during development that is not reversible, but the remaining cells may grow in size enough to regrow the region, and allow room for scrotal development [31]. Regardless of the molecular events that control reversibility/irreversibility of the secondary sex characteristics described above, in the most severe cases, the affected males permanently resemble phenotypic females, although they still possess testes (undescended) and do not have a full compliment of female reproductive organs.

The spectrum of the effects that is observed also reflects specific events that are interrupted during mammalian development. For example, AR antagonists (e.g., flutamide or vinclozolin), which decrease both T- and DHT-dependent signaling pathways and reproductive processes, induce adverse effects (i.e., malformations) of the reproductive structures arising from the Wolffian ducts, urogenital sinus, and genital tubercle (Fig. 2) [31,33–37], while also resulting in incomplete Müllerian duct regression [31,33,34]. In contrast, the effects induced by 5α-reductase inhibitors primarily reflect attenuation of the DHT-dependent pathways (i.e., the urogenital sinus and genital tubercle) [37,41–46]. Compounds that have more broad inhibitory effects on steroid hormone synthesis can induce a similar profile of effects on both T- and DHT-dependent pathways [33,47–52]. Interestingly, ER agonists [10,11,13,15,23,53–56] and Ah receptor agonists (e.g., TCDD) [57–60] also induce a very similar pattern of effects to compounds that interfere with androgen signaling, although the exact mechanism of action of TCDD on the reproductive tract remains elusive. This underscores the complexity of mammalian reproductive development, and illustrates the importance that both estrogens and androgens play in the developmental process, a hypothesis that is supported by the presence of both receptor types throughout the reproductive tract in males and females [61–63].

Thyroid hormones control multiple physiological processes, and compounds that alter thyroid hormone homeostasis have the ability to affect both reproductive and behavioral development. A wide range of compounds (e.g., PCBs, dioxins, thionamides, phenobarbital) have been shown to alter thyroid homeostasis in experimental animals [64,65]. In fact, thyroid disorders are among the most common of endocrine-related disorders. Disruption of thyroid homeostasis during mammalian development exerts its most striking effects on behavioral development [66,67], however, effects on reproductive development have also been observed. For example, altered thyroid hormone homeostasis (either hypo- or hyperthyroidism) during male sexual development can result in altered testicular development, and as a result, quantitatively altered spermatogenesis at adulthood [5,68–72]. Surprising given the role of thyroid involvement in many physiological processes, few reports of altered reproductive development are found in the scientific literature.

Other EACs can affect reproduction and/or development without inducing any noticeable lesions of the reproductive tract. For example, aromatase inhibitors such as fenarimol cause no noticeable effects on the reproductive tract, but due to alterations in brain development, the sexual behavior of the males is affected and fertility is compromised (see additional discussion below) [24,25,73,74]. In addition, other compounds (e.g., atrazine) may induce adverse effects, while the exact mechanism(s) of action remains elusive [75]. Compounds that depress the central nervous system (CNS) will also have the potential to impact reproduction and development, as well as behavioral development. For example, phenobarbital, a pharmaceutical agent that is used as a sedative, induces a spectrum of effects in male rats that is consistent with a general depression of the CNS: decreased AGD, decreased seminal vesicle weights, delayed testicular descent, and altered reproductive hormone levels [76–78]. These examples illustrate that although receptor agonists and antagonists have received the most attention regarding

their potential to disrupt reproduction and development, there are clearly EACs that will effect reproduction and development through nontraditional mechanisms of toxicity.

In females, a more limited number of EACs have been shown to induce adverse effects on reproduction and development. This is in part a reflection of the fact that differentiation of the female phenotype occurs in the absence of androgens; hence, EACs that impact androgen signaling do not *usually* manifest in noticeable effects in females, although androgens are involved in regulating ovarian function [79–81]. With the exception of alterations in the age of puberty, ED in females often results in long-term alterations (e.g., altered estrous cyclicity and ovarian function) that are not easily observed in the neonate, and therefore are typically not detected in short-term studies.

The effects of EACs on female reproduction and development have primarily been evaluated after exposure to ER agonists, of which DES is probably the most well-studied example [14,16]. Adult effects of perinatal exposure to DES, and other estrogens, appear to be primarily on the CNS systems controlling gonadotropin secretion. These permanent alterations in gonadotropin secretion and control produce numerous downstream effects, first in the ovary (i.e., decreased ovary weight, ova count, and corporea lutea), and subsequently in the various estrogen or progesterone-responsive tissues [11,12,14,16,54–56,82]. These impacts are most easily identified as decreased reproductive success in mating studies with the gestationally exposed females. In addition, some instances of reproductive tract malformation (structural abnormalities of oviduct, uterus, cervix, vagina; hypospadias) have also been observed with DES [14,16], indicating that estrogens can also derange reproductive tract development in both female and male offspring.

In females, AR agonists produce masculinizing effects, both morphologically and behaviorally [83–85]. Adverse effects associated with androgenic exposure in females include increased AGD (i.e., male-like), delayed puberty, altered estrous cyclicity, and masculinization (e.g., decreased number of areolas/nipples, presence of male reproductive tissues) [83–85]. Numerous documented cases of alterations in female sexual behavior are also common [83,86,87]. In contrast, prenatally administered antiandrogens do not typically cause adverse effects on reproduction or development in females (Table 2).

Depending on specificity, steroid biosynthesis inhibitors can also affect females, and in cases where female sex steroid production is attenuated (e.g., ketoconazole treatment), alterations in ovarian function, estrous cyclicity, and/or delayed puberty occur [88,89]. The possibility exists for several other mechanisms to alter reproductive capacity and/or development in females, although documented examples are limited. For example, the herbicide atrazine has been shown to alter estrous cyclicity and gonadotropin release in females via a neuroendocrine mechanism [90–92]. Similar to the effects observed in males, phenobarbital induces a spectrum of effects in female rats that is consistent with a general depression of the CNS: lowered gonadotropin release leading to reduced steroid levels and delayed puberty, altered estrous cyclicity, and infertility [76–78]. Prolactin, an endogenous hormone, or compounds that alter dopamine signaling and therefore prolactin levels, can also affect female reproductive development (e.g., enhanced puberty) [93]. The number of compounds with nontraditional mechanisms of action (i.e., not receptor-mediated) continues to grow [1].

The following paragraphs summarize the state of the science regarding the effects of EACs on reproduction and development:

- There is clear evidence that man-made chemicals and environmental pollutants induce adverse effects in experimental animals. While most of the focus has centered on receptor-mediated mechanisms of ED, and more specifically on ER agonist and AR antagonists, a large number of potential mechanisms of ED exist. As research on ED continues, the number of EACs and the variety of mechanisms of action are certain to increase.
- The data collected for most of the compounds evaluated to date suggests that males are more susceptible to ED than females due to the events involved in mammalian sexual differentiation and development. In males, adverse effects generally include decreased reproductive organ weight and function, altered morphology, altered age of puberty, and compromised reproductive capac-

ity (i.e., spermatogenesis); with alterations in androgen signaling, incomplete masculinization (e.g. retention of the female Müllerian ducts) also occurs. Interestingly, compounds with a variety of endocrine mechanisms (i.e., AR antagonists, ER agonists, Ah receptor agonists) induce a common profile of effects in males. In females, adverse effects generally include altered age of puberty, ovarian function (e.g., altered estrous cyclicity), and in some cases, morphological alterations of the reproductive tract. Prenatal exposures can result in permanent alterations in reproduction (e.g., morphology and behavior) and development. In both male and female animals, early changes that produce long-term increases in gonadotropin levels often produce neoplasia.

- Current guideline studies, for example, the current U.S. Environmental Protection Agency (USEPA) multigeneration reproduction study design, have endpoints recently added for evaluating potential ED, and many proposed screening studies will also evaluate potential endocrine activity of man-made chemicals. These data will prove critical in evaluating the potential effects of a wide variety of compounds on reproduction and development in experimental animals.
- While numerous cases of the adverse effects of EACs have been documented in experimental animals and wildlife species, data showing altered human reproductive system structure or function after environmental exposures are still lacking. The pharmaceutical DES is, of course, the best example of a human endocrine disruptor, although not an "environmental" exposure. In addition, most of the documented cases of ED in experimental animals involve doses greater than those encountered in the environment, or are high-level exposure for long durations.
- In addition, physiological differences between species can confound human risk assessment. The nascent National Children's Study, currently being planned in the United States, could be the first study to really address the issue of early exposures and reproductive system function in humans. Until these data come in, epidemiological studies on existing populations and exposures should be performed to determine whether ED is a true health problem for humans.

ADVERSE EFFECTS OF ENDOCRINE ACTIVE SUBSTANCES ON THE IMMUNE SYSTEM

The reproductive system is not the only body system affected by developmental exposure to EACs. As is clear from Table 3, the immune system is also a target, and these changes can last for extended durations (more than half of the total lifespan). This interaction between the reproductive and immune systems has been well known for several decades (Fig. 3). There appear to be several ways these interactions can occur. There are several recent reviews of this topic, to which the reader is referred for more in-depth analysis and examples [94,95].

To begin with, the immune system carries significant sexual dimorphism: numerous characteristics of the immune system vary significantly between adult males and adult females. Broadly speaking, compared to males, females (humans and rodents) generate a stronger immune response, are more resistant to immune tolerance, have greater levels of immunoglobulins, and have a higher incidence of certain forms of autoimmune diseases, including induced experimental forms [96]. While these statements seem to imply that female hormones are supportive of immune function, it is interesting to note that ovariectomy allows thymic hypertrophy to occur, while administration of estradiol causes thymic involution [97,98]. This involution appears to be more long-lasting than that produced by hydrocortisone. Thus, estradiol has both inhibitory and stimulatory effects upon different parts of the immune system. Meanwhile, male rats have greater thymic weight and thymocyte cellularity than females [99], T administration generally causes less thymic involution than estrogen [94], and T also seems to limit the immune responsiveness of males [96]. Thus, even at the basic descriptive level, there are differences between the genders in terms of basal functioning and set-points in the adult immune system.

Aside from sex differences in immune system measures, we should also note the apparent dichotomy in sex hormone effects: estradiol both involutes the thymus and allows for increased immune

Table 3 Examples of endocrine active substances and their effects on the immune system in experimental animals exposed in utero.

Compound	Exposure	Effect	Ref.
Lead	Rats; lead acetate in drinking water; 0, 25, 50 ppm; dams exposed 7 weeks prior to mating, during gestation, and lactation; offspring exposed to same levels from weaning to evaluation on PND35–45.	↓ thymus weight; ↓ mitogen-stimulated splenic lymphocyte proliferation.	[165]
	Sprague–Dawley rats; lead acetate in drinking water; 500 ppm; GD3–9 or 15–21; evaluated 12 weeks postpartum (adult).	Males, late gestation exposure: ↑ IL-12 production, ↓ IL-10 production. Females, late gestation exposure: reduced DTH, ↑ IL-10 production, ↑ monocyte count.	[166]
Chlordane	Mice; analytical chlordane free-fed in peanut butter during gestation only; 0.16 or 8.0 mg/kg/day; pups randomized within treatment groups for rearing; evaluated on PND101.	Males and females: ↓ contact hypersensitivity (high dose); no change in primary antibody response or secondary antibody response.	[167]
	BALB/c mice; technical chlordane free-fed in peanut butter during gestation; 4 or 16 mg/kg/day; no postpartum randomization; evaluated on PND30 and 100.	Males: DTH measures NS; splenic lymphocyte proliferation in response to ConA NS. Females: ↓ DTH at PND100, at other times NS; splenic lymphocyte proliferation in response to ConA increased at PND30.	[168]
	Mice; technical chlordane free-fed in peanut butter during gestation; 4 or 8 mg/kg/day; evaluated on PND100 and 200.	Both doses had ↓ colony-forming units in bone marrow and spleen at both time points; same 18-day treatment in adults gave no effect; indicates altered myeloid lineage rather than T-cell function.	[169]
TCDD	Rats; TCDD (>99 % pure) dissolved in acetone/diluted in corn oil; gastric gavage; 0, 1, 5 μg/kg on GD11, 18, PND4, 11, 18; evaluated on PND25.	Complete mortality at 5 μg/kg; ↓ organ weights (thymus and adrenal) at PND25 at 1 μg/kg; ↓ thymic PHA stimulation at PND25.	[170]
	Rats; TCDD (>99 % pure) dissolved in acetone/diluted in corn oil; gastric gavage; 0, 5 μg/kg on PND0, 7, 14; evaluated on PND25.	↓ organ weights (thymus and spleen) PND25; ↓ thymic PHA stimulation; ↓ host-resistance at PND25.	[170]
	Mice; 0, 2, 5 μg/kg on GD14, 17, PND1, 8, 15; evaluated on PND23.	↓ host rejection on PND23.	[170]
	F344 rats; gastric gavage; 5 μg/kg in corn oil on GD18 and/or PND 0, 7, 14; evaluated on PND25, 39, 59, 145.	↓ thymus weight at all time points; ↑ spleen weight at PND25 and 39; ↓ response to mitogens in vitro, ↓ DTH responses at all time points.	[171]

(*continues on next page*)

Table 3 (*Continued*).

Compound	Exposure	Effect	Ref.
	BALB/c mice; gastric gavage; 10 µg/kg on GD14; evaluated on PND4, 11, 18.	↓ TdT synthesis in liver and bone marrow.	[172]
	C57Bl/6N mice; gastric gavage; 0, 1.5, 3 µg/kg on GD6–14; cross-fostered after birth; evaluated on GD18, PND6, 14, 21	↓ thymus weight on GD18 at both doses; ↓ thymic cellularity on GD18 and PND6 at both doses; altered T-cell differentiation.	[113]
	C57Bl/6 mice; gastric gavage; 0, 1.5, 3 µg/kg on GD6–14; evaluated on GD18.	↓ thymus weight and cellularity on GD18; inhibited prenatal thymocyte differentiation.	[173]
	F344 rats; 1 or 3 µg on GD14; evaluated on GD19 or PND1.	↓ thymus weight on GD19 at 3 µg; changes in surface markers on t hymocytes on PND1; ↑ liver weight; surface marker changes both dose groups.	[174]
	F344 rats; (a) 3 µg/kg on GD14; evaluated DTH in males and females at 4, 8, 12, and 19 months old. (b) 0.1, 0.3, or 1 µg/kg on GD14; evaluated at 4 or 14 months old.	(a) Males: ↓ DTH at all time points. Females: *↓ DTH at 4 months only; ↓ thymic cellularities and phenotypic changes. (b) Males: ↓ DTH at all doses. Females: ↓ DTH at 0.3 and 1 µg, greater response at 14 months than at 4; response to both KLH and BSA was reduced.	[114]
Heptachlor	Sprague–Dawley rats; 0, 30, 300, or 3000 µg/kg/day; maternal gavage GD12–PND7 and direct dose PND8–42; evaluated PND46 and 156.	Males: ↓ primary antibody response to sheep red blood cells at all dose levels at 8 weeks and at the middle dose at 21 weeks.	[175]
Methoxychlor	Sprague–Dawley rats; 5, 50, 150 mg/kg/day; maternal gavage GD12–PND7 and direct dose PND8–42; evaluated PND46 and [a]156.	Males: ↓ thymus weight at PND46. Females: ↓ thymus weight at PND46 and 156; reduced plaque forming cells/ spleen at PND156.	[23]
DMSA (meso-2,3-dimercapt-osuccinic acid)	Fisher 344 rats; 30 or 60 mg/kg/day; gastric gavage on GD6–21; evaluated females only at 13 weeks of age.	Males: ↑ IL-2 after ConA stimulation in vitro. Females, high dose: ↓ DTH response to KLH and ↓ serum levels of monocyte chemoattractant protein-1; ↑ IL-2 after ConA stimulation in vitro.	[176]

Abbreviations: GD: gestational day; PND: postnatal day; NS: not significant; PHA: phytohemagglutinin; TdT: terminal deoxynucleotidyl transferase; DTH: delayed type hypersensitivity; KLH: keyhole lympet hemocyanin; ConA: concanavalin A.

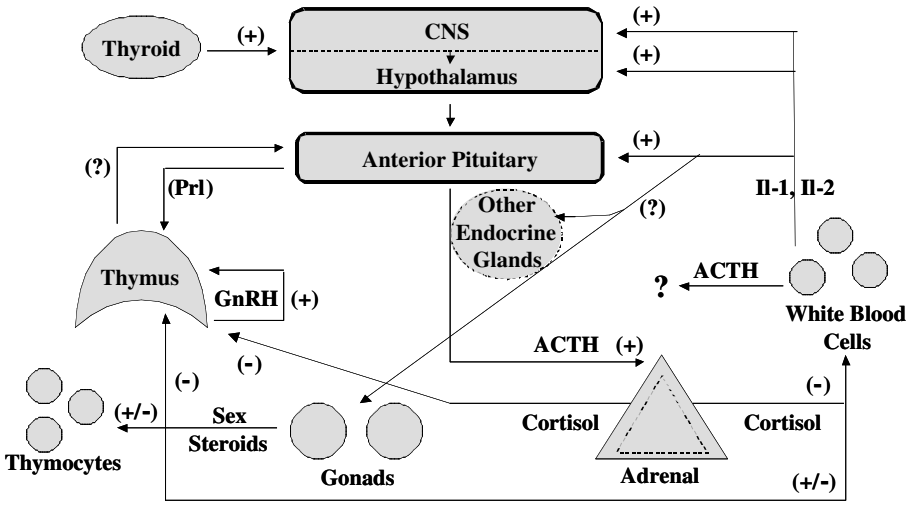

Fig. 3 *Interaction of the endocrine, immune, and nervous systems in mammals.* Overview of interrelation of endocrine, immune, and nervous signaling pathways.

responses, and T can be immunosuppressive but also confers more immunocompetence [100]. This illustrates the complex responses that could be seen after developmental exposure to EACs. That is to say, two different EACs, both with apparent estrogen-like activity, may produce different effects on the immune system, which, upon further investigation may be entirely consistent with their estrogenic activities.

Sex steroid effects are often mediated by the nuclear steroid receptors, which are specific for each steroid [101]. Steroid receptors have been found in various parts of the immune system including the individual cells as well as the generative epithelium [95,97,102–106]. It is important to note that not all of the effects seen in the immune system correlate with the distribution of steroid receptors [107]. Steroids also have numerous effects that are independent of nuclear receptors [108,109], and are believed to be mediated by membrane-bound receptors. Nuclear receptor-mediated or not, sex steroids are likely to have direct effects on the immune system. If a xenobiotic binds to either nuclear or membrane receptors (i.e., ER, AR, or progesterone receptor) as part of its effects on the endocrine system, then effects on the immune system should also be expected.

Additionally, other nonsteroid reproductive-related hormones exert profound effects on immune functions. Prolactin has been shown to promote lymphocyte growth and differentiation [110] by acting on the thymus as well as directly on the lymphocytes themselves [111,112]. One current view is that prolactin operates as an immunomodulator: while not playing a key role in immune function, it is one of several hormones (including growth hormone, thyroid hormone, and insulin-like growth factor [1]) that appear to "fine tune" cellular responses, primarily when the organism is under stress [112].

An important point to remember is that the immune system, largely unlike the reproductive system, can be modulated up or down. A hyperactive immune system can be just as adverse for the organism as a repressed immune system. The development of autoimmune disease in women is one example of this, and any exposure-related increase in this condition would be cause for real concern. Currently, too little is known about the range of postnatal immunologic consequences of perinatal exposure to EACs to confidently predict the outcome, but the *potential* impact, given the literature reviewed above and the data in the accompanying table certainly suggests that adverse effects can occur.

Since females are more prone to autoimmune disease, since this tends to happen more in aged individuals, and since many EACs appear to carry some component of estrogenic activity, one could plausibly hypothesize that an increase in autoimmune dysfunction would be observed if studies were per-

formed on animals of sufficient age. This has been noted for TCDD [113], which increases the level of concern for estrogenic compounds. Because of the length of time required for such studies, they have been infrequently performed in the past. Given the potential health impacts in humans, more attention should be paid in the future to age-related autoimmunity.

Finally, delayed-type hypersensitivity (DTH) reactions help protect against infectious and neoplastic challenges, and appear most important in the very old and very young [114]. The ability of modest amounts of TCDD (1–3 μg/kg, once on GD14) to reduce DTH reactions even in aged rats [114] shows again that long-term health consequences are possible from pre/perinatal exposures. A careful analysis of this endpoint after specifically altering sex steroid levels would help put some perspective around the ability of EACs to cause similar changes, and would help set our concern at an appropriate level.

The following paragraphs summarize the state of the science regarding the effects of EACs on the immune system:

- A review of the available papers indicates significant variability in the hypotheses being tested, the periods of exposure, the ages of the animals at evaluation, and the methods of evaluation. The issue of EAC-induced changes in immune structure and function would benefit greatly from a consensus about the important questions to be addressed, followed by a focus on generating a common database for several EACs. This would be an excellent project for a multinational collaboration, one that could take advantage of existing networks of professional collaborations and organizations.
- The field will be hampered by the current uneven definition of the roles of sex steroids in setting baseline values for the immune system cells and tissues. A thorough and systematic evaluation of the effects of specific ER and AR agonists and antagonists will set the stage for a cleaner interpretation of toxicant exposures and greatly facilitate the identification of possible mechanisms. This is not an open-ended call for more research, but for work tightly focused on the short- and long-term effects of prenatal steroid administration on every aspect of immune system function.
- Alterations that either reduce immunocompetence or cause hyper-reactivity are of concern. Such effects are biologically plausibly after prenatal exposure, yet no studies to date were found that have evaluated both possible changes in the same study.
- There are multiple known mechanisms that EACs could use to impinge on the immune system. The implications are that: (1) more mechanisms will be found with further work in this area, (2) more effects will be found, and (3) a logical approach to generating a comprehensive data set, instituted very soon, would save years of environmental damage, regulatory uncertainty, and industrial and public anxiety.

ADVERSE EFFECTS OF ENDOCRINE ACTIVE SUBSTANCES ON THE NERVOUS SYSTEM

The CNS is not only a key part of reproduction, but it also has much in common with the immune system. Cytokines play a central role in the function of both the CNS and immune systems, and both are targets for steroid action (Fig. 3) [115–117], which makes them readily susceptible to xenobiotics that modulate steroid activity.

The determination of effects on the function of the CNS after prenatal exposure can be considered functional, or behavioral, teratology. Indeed, the field of behavioral teratology is a recognized specialty, with its own methods [118,119], society, and journal (*Neurotoxicology and Teratology*). The evaluation of endocrine-mediated effects on CNS organization and/or function after developmental exposure is really a subspecialty of this complicated area.

Like the immune system, many parts of the brain have steroid hormone receptors, which make the brain a target for xenobiotics that interact with receptors or alter hormone concentrations [120–122].

The forebrain (association), hippocampus (memory), most areas of the hypothalamus (endocrine physiology and control), as well as the midbrain (integrative function) and cortex (cognition and processing) possess steroid receptors at some point during development. Both glucocorticoids and sex steroids (as exemplified by estradiol) appear to act on multiple cell types, and through both nuclear receptors and non-receptor-mediated mechanisms [120,121]. Thyroid hormones also act on most parts of the adult and developing brain, and appear to act as each part of the brain passes through a specific developmental window [123,124]. All these hormones modulate the differentiation of neurons by affecting cellular migration, death, and synapse formation and pruning [123–125]. These fundamental changes in cellular structure impact neuronal function, and thus overall CNS output. This structure–function relationship is slightly more challenging to prove in the CNS, where output is an amalgamated result of the output of millions of neurons, modulated by an additional overwhelming number of cells, but there is considerable support for this structure–function link. For example, the increased neuronal spine density in neurons in the male hippocampus, and the fewer spines in females, has been shown to correlate positively with spatial learning ability (i.e., more synapses on spines confers greater spatial ability). This is corroborated by the data from males castrated neonatally (who have decreased spine density and reduced spatial learning ability), and the increases in spine density in T-treated females (who have increased spatial learning capabilities) [126]. These correlations are one example that supports the relationship between microscopic structure and overall CNS function. Thus, hormonally induced changes in neuronal structure will have some effect on function, at the cellular and/or the organismal level.

While receptors are dispersed differentially throughout the brain, several areas appear particularly sexually dimorphic (different in males and females). These include the sexually dimorphic nucleus of the preoptic area (SDN-POA), the ventromedial nucleus of the hypothalamus, and the hippocampus. The SDN and hippocampal dentate gyrus are larger in males. The hypothalamic nucleus appears important in sexual behavior, while the hippocampus subserves spatial learning and memory [126]. The hippocampus is also vulnerable to changes in thyroid hormone levels and glucocorticoids during development [126,127]. Levels of these hormones correlate positively with the number of cells and the number of synapses per cell in these areas.

Not only can steroids modify neuronal structure and function, but neurotransmitters, the means by which neurons communicate and process depolarizations, biochemically converge with steroid receptors to control neuron function and signaling [128,129]. Thus, compounds that alter neurotransmitter activity can produce effects on gene transcription and neuronal activity that are seen in other situations to be caused by hormones, and endocrine alterations can perturb neurotransmitter-mediated functions. This demonstrates a nonhormonal means by which an exposure can produce a change in the brain that may appear hormonally mediated.

One counter-intuitive fundamental that underlies gender differences in brain development is the nature of the developmental steroid dependence. Prenatally, estradiol is primarily bound to α-fetoprotein; very little is free to diffuse into cellular compartments. T, which is unbound, is taken up by the developing CNS cells and converted by the enzyme aromatase to estradiol, which subsequently *masculinizes* the brain. Increasing the circulating levels of T or estradiol will masculinize that animal, through the result of increased local levels of estradiol, while decreasing levels of T will feminize the brain and behaviors of that animal [126]. This paradox of increasing estradiol resulting in a more masculinized brain exemplifies again the complexities in this area.

Effects on the CNS can manifest at multiple levels, from cellular fine structure and light-microscope-level morphology, to electrophysiology, control of secretion, sensory control and perception, biochemical processes, or that most integrative of measures, behavior. The best evaluation of exposure-related effects will include all of these levels. Some themes emerge from the recent literature that is discussed below.

EACs affect sex-specific CNS endpoints. The SDN-POA is larger in males than in females, because it contains more cells due to the rescue effect of fetal T, whose unbound concentrations in plasma

are greater than estradiol because of its inability to bind to α-fetoprotein [130]. Fetal treatment with T, high levels of estradiol, or DES will increase the size of the SDN-POA [131,132]. Females have higher levels of dopamine (DA) in their striatum, and estradiol increases the amount of DA released by striatal neurons [133]. Although exposure to 500 parts per million (ppm) genestein during gestation and into adulthood did not change baseline release of striatal DA, amphetamine-stimulated DA release was significantly increased in males but not females. TCDD, administered to pregnant rat dams on gestation day (GD) 15, selectively affected female brains, reducing the amount of gamma-aminobutyric acid decarboxylase gene expression in female POA to the male level, with no detectable change in the male POA, or in other brain areas examined [134]. Methoxychlor, whose metabolites are estrogenic and antiandrogenic, reduces follicle-stimulating hormone (FSH) release in only perinatally treated adult female rats [23], while it decreases mating behavior in male rats and increases those behaviors in perinatally exposed females [135,136]. Bisphenol A, when administered to the dams prenatally and lactationally at 1.5 mg/kg/day, eliminated gender differences normally seen in rats in open field and passive avoidance tests [137]. Bisphenol A also eliminated the gender differences in the size of the locus coeruleus. All the CNS effects described above were seen in the absence of detectable changes in male or female reproductive organ weights or serum concentrations of luteinizing hormone (LH), FSH, T, or estradiol.

Gender-specific behaviors can be affected by EACs. Weiss [138] provides a useful review of the benefits of evaluating gender-specific behaviors that are not related to reproduction, using the polychlorinated aromatics TCDD and PCBs using spatial learning and operant behaviors as measures of effect. Play behavior is organized by postnatal T action mediated via AR in the amygdala [83]. Administering T to female rats increases the amount of male-specific rough-and-tumble play [83]. Exposure to bisphenol A (an estrogenic mimic at high doses) during either development or adulthood (but curiously, not both) reduced maternal care and nursing behavior in treated mice [139], though this was without detectable effect on the offspring using the measures employed by the authors. A large dose of bisphenol A given to mice on GD11–19, masculinized some peripubertal behaviors in females, as did estradiol [140]. Sociosexual play and exploration was reduced in male rats and increased in females after perinatal bisphenol A exposure [141]. This can also be demonstrated by the work of Roegge and coworkers [142] who showed that radial arm maze performance was impaired by Aroclor 1254 in male rats, but not in females.

However, gender-specific behaviors can also be untouched by otherwise active EACs. For example, prenatal and developmental genestein exposure caused only very modest effects on the consumption of salt water (a sexually dimorphic behavior), and there was no treatment-by-gender effect [143]. Many other gender-specific behaviors remained unaffected: open field activity, play behavior, running wheel activity, and saccharin fluid consumption were untouched by genestein. Using a similar paradigm, these same investigators noted that *p*-nonylphenol exposure during gestation, maturation, and testing increased the intake of a sodium solution in females but not males, while other sexually dimorphic behaviors were unchanged [144]. These and other related studies were recently reviewed by these authors [145].

EACs also affect non-gender-specific endpoints in the nervous system. Neurite formation and MAPK-pathway activation were reduced by hexachloro-biphenyl and endosulfan in neuronal stem cells in vitro [146]. Aroclor 1254 can induce a low-frequency hearing loss in adult rats after perinatal exposure [147]. There were no effects in other sensory organ systems, as measured by evoked potentials. The effect on hearing loss has been recently shown to be due to fewer outer hair cells in the organ of Corti [148]. This is believed to be due to the hypothyroidism experienced by these animals during treatment with the Aroclor 1254 [147].

Gender-specific behaviors can also be affected by endocrine-**in**active substances. Locomotor activity is one example: treatment with a variety of endocrine inactive substances (e.g., nitrofen, cytosize, arabinoside) can increase locomotor activity in both genders in the absence of any discernible endocrine change [149].

There can be occasional disconnects between biochemistry and output that resolve with further investigation. For example, levels of thyroid hormone during perinatal brain development are known to stimulate cerebellar granule cell proliferation, cell migration, synapse formation and densities, myelination, and glial cell proliferation and maturation [123]. From this, one would expect that anything that reduced circulating thyroid hormone levels in the dam or fetus would adversely impact brain function. Interestingly, developing rats exposed to Aroclor 1254 (a PCB), which reduced fetal thyroid hormone levels, had no effect on spatial learning or baseline electrophysiologic measures in the hippocampal dentate gyrus [150]. They were able to identify changes in long-term potentiation, but did not observe the expected effect on spatial activity in a water maze. Similarly, Taylor and coworkers [151] exposed developing rats to a polybrominated diphenyl ether, which also reduces neonatal thyroid hormone levels, and found no change in motor activity or auditory startle. Recently, Zoeller and coworkers [124] have proposed a possible mechanism by which PCBs might reduce thyroid hormone yet not impact many thyroid-dependent processes: they review evidence that is consistent with the ability of certain PCBs to bind to the thyroid receptor. This would explain the data of Crofton's group [150,151], and could comprehensively explain many of the seemingly contradictory effects seen after PCB exposure, where low thyroid hormone levels are not automatically followed by a complete hypothyroid-like picture.

Behavior is complex, and must be approached thoughtfully. Behavioral scientists assert that behavior is uniquely sensitive to ED because it integrates the output from many cells and organizational levels in the brain and periphery, and so provides more targets for toxicant action. It is also affected by a wide variety of external factors [152]. In addition, this integration implies that these multiple inputs bring along many ways to change gender-specific behaviors that have no detectable link to endocrine mechanisms.

Ethologists would have us believe that it is more appropriate to assess the behavioral effects of a compound using a behavior that is relevant to the species in question [153]. This leaves an investigator or regulator trying to interpret whether a change in grooming behavior, though statistically significant, is meaningful to any other species, including humans. Reduced ability to solve problems is a clear adverse effect; a delay in the achievement of a righting reflex that becomes equivalent to controls a few days later is less demonstrably adverse. Although the ethological approach appeals to one's sense of the appropriate, and while clearly the right approach in identifying ecologic hazards, it also leaves more ambiguity in the risk assessment application of such data.

In characterizing the toxicity of any suspected EAC, the evaluation of behavioral changes is likely to occur relatively late in the process. The authors are partial to an approach where one first queries steroid- or thyroid-dependent CNS functions, and only later moves on to hormone-independent functions. This maximizes the chances of finding the most likely effects first. Conversely, it also means that a compound that affects cognitive development but not, say, mating behavior, would be less likely to be identified. Still, such an approach has been productive in the past [73,154,155]. The prior determination of actual changes in a hormone-related endpoint means that one can then go on to ask what those changes would mean to other functions of the animal, such as behavior. However, because of the complexity involved, changes in behavior should not be expected to initially identify a compound as an endocrine disruptor.

SUMMARY

The reproductive, immune, and central nervous systems all depend on endogenous steroids for correct prenatal programming and postnatal function. Therefore, exogenous treatments that interfere with such signaling systems also disrupt the structure and function of all these systems. We are just now learning the scope and dose-response nature of these effects. Numerous pressing questions remain: Which of these organ systems is more sensitive? Are humans being affected by environmental levels of EACs?

What are the full impacts of such exposures on the lifespan of both test species and wildlife? Have we found all the effects of EACs?

The small amount of data that have been generated to specifically address the relative organ system sensitivity issue (the National Institute of Environmental Health Sciences [NIEHS]/USEPA collaborative Juvenile Pesticide Studies) indicates that the most sensitive body system varies by compound when tested in rats. It is also likely that the most sensitive system will vary in different species. This greatly complicates any testing strategy, and suggests that the initial evaluations be apical tests of integrated function performed for several organ systems within one study. Further studies are warranted only if a change is seen in these more integrative initial tests.

While there are a large number of studies in experimental animals that link exposure to EACs to reproductive, developmental, and immune abnormalities and increased cancer incidence, the link to human health is still unclear. There is still no conclusive evidence that EACs that produce adverse effects in experimental animals will also do so in humans and/or wildlife. However, the conserved function of endocrine signaling pathways across numerous species, including humans, raises a legitimate concern that effects in laboratory species or wildlife, and humans, will be comparable [156,157].

Only by looking at function throughout the lifespan of an animal will we really be able to determine the "reach" of neonatal alterations. This affects all the organ systems described here: the reproductive system could cease functioning earlier, leading to premature menopause and shortened reproductive lifespan; there could be an increase in late-onset autoimmune diseases like lupus; and the changes that occur in aging brain function [158] and structure (i.e., plaque aggregation in Alzheimer's) could be exacerbated by neonatal exposure to EACs. These lifecycle studies will be done in rodents first, and should be planned as holistic investigations and commenced without delay.

The surprises of the past suggest that we have not found all the effects of neonatal exposure to EACs. Not only are effects in other organ systems likely, but other hormonal activities are probable. For example, while the presence of environmental androgens has not been well studied or substantiated, recent cases in wildlife suggest that environmental androgens do exist [83–85]. A logical, prospective design that systematically evaluates those tissues that we know are steroid responsive would be necessary and not overly difficult. This, too, should be commenced without delay.

In conclusion, the last 40 years have seen many reports that man-made chemicals and environmental pollutants cause adverse effects in humans and wildlife; however, few instances of anthropogenic ED have been scientifically substantiated. Certainly, studies in experimental animals have shown the potential for adverse effects in humans and wildlife. The task before us is to understand how big the issue of ED is to human health and the environment. Science and ignorance created this issue; ultimately, science and wisdom will solve it.

REFERENCES

1. T. M. Crisp, E. D. Clegg, R. L. Cooper, W. P. Wood, D. G. Anderson, K. P. Baetcke, J. L. Hoffmann, M. S. Morrow, D. J. Rodier, J. E. Schaeffer, L. W. Touart, M. G. Zeeman, Y. M. Patel. *Environ. Health Perspect.* **106**, 11–56 (1998).
2. R. J. Kavlock, G. P. Daston, C. DeRosa, P. Fenner-Crisp, L. E. Gray, S. Kaattari, G. Lucier, M. Luster, M. J. Mac, C. Maczka, R. Miller, J. Moore, R. Rolland, G. Scott, D. M. Sheehan, T. Sinks, H. A. Tilson. *Environ. Health Perspect.* **104**, 715–740 (1996).
3. E. Knobil and J. D. Neill. *The Physiology of Reproduction*, Raven Press, New York (1994).
4. L. J. DeGroot. *Endocrinology*, W. B. Saunders, Philadelphia (1995).
5. R. M. Sharpe. In *The Physiology of Reproduction,* E. Knobil and J. D. Neill (Eds.), pp. 1363–1434, Raven Press, New York (1994).
6. B. R. Zirkin. *Cell Mol. Biol. Testis* 166–188 (1993).
7. J. Yochim and V. J. D. Feo. *Endocrinology* **72**, 317–326 (1963).
8. J. Yochim and V. J. D. Feo. *Endocrinology* **71**, 134–142 (1962).

9. V. Haddad and M. M. Ketchel. *Int. J. Fert.* **14**, 56–63 (1969).
10. J. C. Cook, L. Johnson, J. C. O'Connor, L. B. Biegel, C. H. Krams, S. R. Frame, M. E. Hurtt. *Toxicol. Sci.* **44**, 155–168 (1998).
11. L. B. Biegel, J. A. Flaws, A. N. Hirshfield, J. C. O'Connor, G. S. Elliott, G. S. Ladics, E. K. Silbergeld, C. S. VanPelt, M. E. Hurtt, J. C. Cook, S. R. Frame. *Toxicol. Sci.* **44**, 116–142 (1998).
12. L. B. Biegel, J. C. Cook, M. E. Hurtt, J. C. O'Connor. *Toxicol. Sci.* **44**, 143–154 (1998).
13. J. A. McLachlan, R. R. Newbold, B. Bullock. *Science* **190**, 991–992 (1975).
14. J. A. McLachlan, R. R. Newbold, B. C. Bullock. *Cancer Res.* **40**, 3988–3999 (1980).
15. R. R. Newbold and J. A. McLachlan. In *Estrogens in the Environment II,* J. A. McLachlan (Ed.), pp. 288–318, Elsevier, New York (1985).
16. J. A. McLachlan, R. R. Newbold, H. C. Shah, M. D. Hogan, R. L. Dixon. *Fertil. Steril.* **38**, 364–371 (1982).
17. P. S. Guzelian. *Annu. Rev. Pharmacol. Toxicol.* **22**, 89–113 (1982).
18. H. C. Hodge, E. A. Maynard, J. F. Thomas, H. J. Blanchet, Jr., W. E. Wilt, Jr., K. E. Mason. *J. Pharmacol. Exptl. Therap.* **99**, 140–148 (1950).
19. S. J. Harris, H. C. Cecil, J. Bitman. *J. Agric. Food Chem.* **22**, 969–973 (1974).
20. H. S. Bal. *Proc. Soc. Exp. Biol. Med.* **176**, 187–196 (1984).
21. F. R. Boockfor and C. A. Blake. *Biol. Reprod.* **57**, 267–277 (1997).
22. C. A. Blake and F. R. Boockfor. *Biol. Reprod.* **57**, 255–266 (1997).
23. R. E. Chapin, M. W. Harris, B. J. Davis, S. M. Ward, R. E. Wilson, M. A. Mauney, A. C. Lockhart, R. J. Smialowicz, V. C. Moser, L. T. Burka, B. J. Collins. *Fundam. Appl. Toxicol.* **40**, 138–157 (1997).
24. K. S. Hirsch, E. R. Adams, D. G. Hoffman, J. K. Markham, N. V. Owen. *Toxicol. Appl. Pharmacol.* **86**, 391–399 (1986).
25. K. S. Hirsch, D. E. Weaver, L. J. Black, J. F. Falcone, N. J. MacLusky. *Toxicol. Appl. Pharmacol.* **91**, 235–245 (1987).
26. S. Bhasin, S. Sikka, T. Fielder, U. Sod-Moriah, H. B. Levine, R. S. Swerdloff, J. Rajfer. *Endocrinology* **118**, 1229–1232 (1986).
27. W. R. Heckman, B. R. Kane, R. E. Pakyz, M. J. Cosentino. *J. Androl.* **13**, 191–198 (1992).
28. NRC. In *Hormonally Active Agents in the Environment*, pp. 210–242, National Academy Press, Washington DC (2000).
29. K. D. Dohler. *Int. Rev. Cyt.* **131**, 1–57 (1991).
30. R. A. Gorski. In *Pharmacology, Biology and Clinical Applications of Androgens,* A. Bhasin (Ed.), pp. 159–168, Wiley-Liss, New York (1996).
31. L. E. Gray, Jr., J. Ostby, E. Monosson, W. R. Kelce. *Toxicol. Ind. Health* **15**, 48–64 (1999).
32. W. R. Kelce, E. Monosson, M. P. Gamcsik, S. C. Laws, L. E. Gray, Jr. *Toxicol. Appl. Pharmacol.* **126**, 276–285 (1994).
33. L. E. Gray, Jr., C. Wolf, C. Lambright, P. Mann, M. Price, R. L. Cooper, J. Ostby. *Toxicol. Ind. Health* **15**, 94–118 (1999).
34. L. E. Gray, J. Ostby, W. R. Kelce. *Toxicol. Appl. Pharmacol.* **129**, 46–52 (1994).
35. E. Monosson, W. R. Kelce, C. Lambright, J. Ostby, L. E. Gray, Jr. *Toxicol. Ind. Health* **15**, 65–79 (1999).
36. B. S. McIntyre, N. J. Barlow, P. M. D. Foster. *Toxicol. Sci.* **62**, 236–249 (2001).
37. J. Imperato-McGinley, R. S. Sanchez, J. R. Spencer, B. Yee, E. D. Vaughan. *Endocrinology (Baltimore)* **131**, 1149–1156 (1992).
38. J. Ostby, W. R. Kelce, C. Lambright, C. J. Wolf, P. Mann, L. E. Gray, Jr. *Toxicol. Ind. Health* **15**, 80–93 (1999).
39. W. R. Kelce, C. R. Stone, S. C. Laws, L. E. Gray, J. A. Kemppainen, E. M. Wilson. *Nature* **375**, 581–585 (1995).

40. L. You, M. Casanova, S. Archibeque-Engle, M. Sar, L.-Q. Fan, H. d. A. Heck. *Toxicol. Sci.* **45**, 162–173 (1998).
41. R. L. Clark, J. M. Antonello, S. J. Grossman, L. D. Wise, C. Anderson, W. J. Bagdon, S. Prahalada, J. S. MacDonald, R. T. Robertson. *Teratology* **42**, 91–100 (1990).
42. C. A. Anderson and R. L. Clark. *Teratology* **42**, 483–496 (1990).
43. B. S. McIntyre, N. J. Barlow, P. M. D. Foster. *Toxicol. Sci.* **65**, 62–70 (2002).
44. J. Imperato-McGinley, Z. Binienda, A. Arthur, D. T. Mininberg, E. D. Vaughan, Jr., F. W. Quimby. *Endocrinology (Baltimore)* **116**, 807–812 (1985).
45. J. Imperato-McGinley, Z. Binienda, J. Gedney, E. D. Vaughan, Jr. *Endocrinology (Baltimore)* **118**, 132–137 (1986).
46. F. W. George and K. G. Peterson. *Endocrinology* **122**, 1159–1164 (1988).
47. E. Bloch, M. Lew, M. Klein. *Endocrinology* **89**, 16–31 (1971).
48. A. S. Goldman, R. D. Eavey, M. K. Baker. *J. Endocrinol.* **71**, 289–297 (1976).
49. J. M. Goldman, L. E. Gray, R. L. Cooper. *Principles and Processes for Evaluating ED in Wildlife, Proceedings from a Conference on ED in Wildlife, Kiawah Island, S. C., Mar. 1996*, 311–333 (1998).
50. E. Mylchreest, R. C. Cattley, P. M. Foster. *Toxicol. Sci.* **43**, 47–60 (1998).
51. E. Mylchreest, M. Sar, D. G. Wallace, P. M. D. Foster. *Reprod. Toxicol.* **16**, 19–28 (2002).
52. L. G. Parks, J. S. Ostby, C. R. Lambright, B. D. Abbott, G. R. Klinefelter, N. J. Barlow, L. E. Gray, Jr. *Toxicol. Sci.* **58**, 339–349 (2000).
53. A. Hossaini, M. Dalgaard, A. M. Vinggaard, H. Frandsen, J.-J. Larsen. *Reprod. Toxicol.* **15**, 537–543 (2001).
54. R. E. Chapin, J. Delaney, Y. Wang, L. Lanning, B. Davis, B. Collins, N. Mintz, G. Wolfe. *Toxicol. Sci.* **52**, 80–91 (1999).
55. T. Nagao, R. Ohta, H. Marumo, T. Shindo, S. Yoshimura, H. Ono. *Reprod. Toxicol.* **14**, 513–532 (2000).
56. T. Nagao, K. Wada, H. Marumo, S. Yoshimura, H. Ono. *Reprod. Toxicol.* **15**, 293–315 (2001).
57. R. L. Cooper and R. J. Kavlock. *J. Endocrinol.* **152**, 159–166 (1997).
58. L. S. Birnbaum. *Environ. Health Perspect.* **102**, 676–679 (1994).
59. L. E. Gray, Jr., J. S. Ostby, W. R. Kelce. *Toxicol. Appl. Pharmacol.* **146**, 11–20 (1997).
60. S. Ohsako, Y. Miyabara, M. Sakaue, R. Ishimura, M. Kakeyama, H. Izumi, J. Yonemoto, C. Tohyama. *Toxicol. Sci.* **66**, 283–292 (2002).
61. R. M. Sharpe. *Pure Appl. Chem.* **70**, 1685–1701 (1998).
62. R. M. Sharpe. *Trends in Endocrinology and Metabolism* **9**, 371–377 (1998).
63. K. Williams, C. McKinnell, P. T. K. Saunders, M. Walker, J. S. Fisher, K. J. Turner, N. Atanassova, R. M. Sharpe. *Hum. Reprod. Update* **7**, 236–247 (2001).
64. C. C. Capen. In *Casarett and Doull's Toxicology: The Basic Science of Poisons,* C. D. Klaassen (Ed.), pp. 617–640, McGraw-Hill, New York (1996).
65. C. C. Capen. *Toxicol. Pathol.* **25**, 39–48 (1997).
66. S. Darbra, F. Balada, A. Garau, P. Gatell, J. Sala, M. A. Marti-Carbonell. *Behav. Brain Res.* **68**, 159–164 (1995).
67. E. A. Attree, A. K. Sinha, M. J. Davey, M. R. Pickard, F. D. Rose, R. P. Ekins. *Med. Sci. Res.* **20**, 197–199 (1992).
68. E. A. Jannini, S. Ulisse, M. D'Armiento. *Endocr. Rev.* **16**, 443–459 (1995).
69. J. D. Kirby, A. E. Jetton, P. S. Cooke, R. A. Hess, D. Bunick, J. F. Ackland, F. W. Turek, N. B. Schwartz. *Endocrinology* **131**, 559–565 (1992).
70. P. S. Cooke, Y.-D. Zhao, L. G. Hansen. *Toxicol. Appl. Pharmacol.* **136**, 112–117 (1996).
71. D. Canale, M. Agostini, G. Giorgilli, C. Caglieresi, G. Scartabelli, V. Nardini, E. A. Jannini, E. Martino, A. Pinchera, E. Macchia. *J. Androl.* **22**, 284–288 (2001).

72. S. Francavilla, G. Cordeschi, G. Properzi, L. Di Cicco, E. A. Jannini, S. Palmero, E. Fugassa, B. Loras, M. D'Armiento. *J. Endocrinol.* **129**, 35–42 (1991).
73. L. E. Gray and J. Ostby. *Toxicol. Ind. Health* **14**, 159–184 (1998).
74. Y. Takahashi, H. Yamanaka, I. Akiyama, Y. Sakuma. *Endocrinol. Jpn.* **36**, 29–36 (1989).
75. T. E. Stoker, S. C. Laws, D. L. Guidici, R. L. Cooper. *Toxicol. Sci.* **58**, 50–59 (2000).
76. C. Gupta, B. H. Shapiro, B. R. Sonawane, S. J. Yaffe. *Colloq. - Inst. Natl. Sante Rech. Med.* **89**, 89–93 (1980).
77. C. Gupta, B. H. Shapiro, S. J. Yaffe. *Pediatr. Pharmacol.* **1**, 55–62 (1980).
78. C. Gupta and S. J. Yaffe. *Pediatr. Res.* **15**, 1488–1491 (1981).
79. C. Beyer, M. L. Cruz, V. L. Gay, R. B. Jaffe. *Endocrinology* **95**, 722–727 (1974).
80. G. L. Kumari, J. K. Datta, R. P. Das, S. Roy. *Horm. Res.* **9**, 112–120 (1978).
81. J. P. Louvet, S. M. Harman, J. R. Schreiber, G. T. Ross. *Endocrinology* **97**, 366–372 (1975).
82. L. E. Gray, J. Ostby, J. Ferrell, G. Rehnberg, R. Linder, R. Cooper, J. Goldman, V. Slott, J. Laskey. *Fundam. Appl. Toxicol.* **12**, 92–108 (1989).
83. A. K. Hotchkiss, Jr. *Environ. Health Perspect.* **110**, 435–439 (2002).
84. C. J. Wolf, A. Hotchkiss, J. S. Ostby, G. A. LeBlanc, L. E. Gray, Jr. *Toxicol. Sci.* **65**, 71–86 (2002).
85. S. J. McCoy and B. A. Shirley. *Life Sci.* **50**, 621–628 (1992).
86. R. W. Rhees, B. A. Kirk, S. Sephton, E. D. Lephart. *Dev. Neurosci. (Basel)* **19**, 430–437 (1997).
87. L. Huffman and S. E. Hendricks. *Physiol. Behav.* **26**, 773–778 (1981).
88. A. M. Cummings, J. L. Hedge, J. Laskey. *Fundam. Appl. Toxicol.* **40**, 238–246 (1997).
89. D. Beltrame, E. DiSalle, E. Giavini, K. Gunnarsson, M. Brughera. *Reprod. Toxicol.* **15**, 601–602 (2001).
90. J. C. Eldridge, D. G. Fleenor-Heyser, P. C. Extrom, L. T. Wetzel, C. B. Breckenridge, J. H. Gillis, L. G. Luempert, III, J. T. Stevens. *J. Toxicol. Environ. Health* **43**, 155–167 (1994).
91. R. L. Cooper, T. E. Stoker, J. M. Goldman, M. B. Parrish, L. Tyrey. *Reprod. Toxicol.* **10**, 257–264 (1996).
92. R. L. Cooper, T. E. Stoker, W. K. McElroy, J. Hein. *Toxicologist* **42**, 160 (1998).
93. J. P. Advis and S. R. Ojeda. *Endocrinology* **103**, 924–935 (1978).
94. H. O. Besedovsky and A. DelRey. *Endocr. Rev.* **17**, 64–102 (1996).
95. D. Tomaszewska and F. Przekop. *J. Physiol. Pharmacol.* **48**, 139–158 (1997).
96. S. C. Wilcoxen, E. Kirkman, K. C. Dowdell, S. A. Stohlman. *J. Immunol.* **164**, 6237–6243 (2000).
97. A. Martin, L. M. Alonso, M. Gomez del Moral, A. G. Zapata. *Tissue Cell* **26**, 169–179 (1994).
98. A. E. Silverstone, D. E. Frazier, Jr., N. C. Fiore, J. A. Soults, T. A. Gasiewicz. *Toxicol. Appl. Pharmacol.* **126**, 248–259 (1994).
99. G. Leposavic, B. Karapetrovic, S. Obradovic, B. Vidic Dankovic, D. Kosec. *Pharmacol. Biochem. Behav.* **54**, 269–276 (1996).
100. A. Peters. *Proc. Roy. Soc. London.* **267**, 883–889 (2000).
101. M. A. Carson-Jurica, W. T. Schrader, B. W. O'Malley. *Endocr. Rev.* **11**, 201–220 (1990).
102. I. Screpanti, A. Gulino, J. R. Pasqualini. *Endocrinology* **111**, 1552–1561 (1982).
103. P. T. Pearce, B. A. K. Khalid, J. W. Funder. *Endocrinology* **113**, 1287–1291 (1983).
104. W. J. Kovacs and N. J. Olsen. *J. Immunol.* **139**, 490–493 (1987).
105. A. Gulino, I. Screpanti, M. R. Torrisi, L. Frati. *Endocrinology* **117**, 47–54 (1985).
106. T. C. Chao, P. J. Van Alten, R. J. Walter. *Am. J. Reprod. Immunol.* **32**, 43–52 (1994).
107. C. J. Grossman. *Endocr. Rev.* **5**, 435–455 (1984).
108. M. Wehling. *Trends Endocrinol. Metab.* **5**, 347–353 (1994).
109. A. Revelli, M. Massobrio, J. Tesarik. *Endocr. Rev.* **19**, 3–17 (1998).
110. A. R. Buckley. *Lupus* **10**, 684–690 (2001).
111. V. De Mello-Coelho, W. Savino, M.-C. Postel-Vinay, M. Dardenne. *Dev. Immunol.* **6**, 317–323 (1998).
112. K. Dorshkind and N. D. Horseman. *Endocr. Rev.* **21**, 292–312 (2000).

113. S. D. Holladay, P. Lindstrom, B. L. Blaylock, C. E. Comment, D. R. Germolec, J. J. Heindell, M. I. Luster. *Teratology* **44**, 385–393 (1991).
114. B. C. Gehrs and R. J. Smialowicz. *Toxicology* **134**, 79–88 (1999).
115. D. A. Weigent and J. E. Blalock. *Immunol. Rev.* **100**, 79–108 (1987).
116. M. Dardenne. *Ann. Med. (Helsinki)* **31**, 34–39 (1999).
117. R. Mentlein and M. D. Kendall. *Immunol. Today* **21**, 133–140 (2000).
118. E. P. Riley and C. V. Vorhees. *Behavioral Toxicol.* 522 (1986).
119. W. P. Weisenburger. In *Toxicology Testing Handbook,* D. Jacobson-Kram, K. A. Keller (Eds.), pp. 255–290, Marcel Decker, New York (2001).
120. E. R. De Kloet, S. A. B. E. Van Acker, R. M. Sibug, M. S. Oitzl, O. C. Meijer, K. Rahmouni, W. De Jong. *Kidney Intl.* **57**, 1329–1336 (2000).
121. B. McEwen, K. Akama, S. Alves, W. G. Brake, K. Bulloch, S. Lee, C. Li, G. Yuen, T. A. Milner. *Proc. Natl. Acad. Sci. USA* **98**, 7093–7100 (2001).
122. P. J. Shughrue and I. Merchenthaler. *Frontiers Neuroendocrinol.* **21**, 95–101 (2000).
123. K. L. Howdeshell. *Environ. Health Perspect.* **110**, 337–348 (2002).
124. R. T. Zoeller, A. L. S. Dowling, C. T. A. Herzig, E. A. Iannacone, K. J. Gauger, R. Bansal. *Environ. Health Perspect.* **110**, 355–361 (2002).
125. N. J. MacLusky, D. A. Bowlby, T. J. Brown, R. E. Peterson, R. B. Hochberg. *Neurochem. Res.* **22**, 1395–1414 (1997).
126. S. L. Schantz and J. J. Widholm. *Environ. Health Perspect.* **109**, 1197–1206 (2001).
127. E. S. Sher, X. M. Xu, P. M. Adams, C. M. Craft, S. A. Stein. *Toxicol. Ind. Health* **14**, 121–158 (1998).
128. S. K. Mani, J. M. C. Allen, J. H. Clark, J. D. Blaustein, B. W. O'Malley. *Science* **265**, 1246–1249 (1994).
129. K. D. Dohler. *Arch. Toxicol.* **20**, 131–141 (1998).
130. A. C. Scallet and J. M. Meredith. *Neurotoxicol. Teratol.* **24**, 81–85 (2002).
131. K. A. Faber and C. L. Hughes, Jr. *Reprod. Toxicol.* **7**, 35–39 (1993).
132. K. A. Faber, L. Ayyash, S. Dixon, C. L. Hughes, Jr. *Biol. Reprod.* **48**, 947–951 (1993).
133. J. B. Becker. *Pharmacol. Biochem. Behav.* **64**, 803–812 (1999).
134. L. E. Hays. *Environ. Health Perspect.* **110**, 369–376 (2002).
135. L. E. Gray, Jr., J. Ostby, R. L. Cooper, W. R. Kelce. *Toxicol. Ind. Health* **15**, 37–47 (1999).
136. L. E. Gray, Jr., J. S. Ostby, J. M. Ferrell, E. R. Sigmon, J. M. Goldman. *Toxicol. Appl. Pharmacol.* **96**, 525–540 (1988).
137. K. Kubo, O. Arai, R. Ogata, M. Omura, T. Hori, S. Aou. *Neurosci. Lett.* **304**, 73–76 (2001).
138. B. Weiss. *Environ. Health Perspect.* **110**, 387–391 (2002).
139. P. L. Palanza. *Environ. Health Perspect.* **110**, 415–422 (2002).
140. M. Sato, M. Shimada, Y. Sato. *Congenital Anomalies* **41**, 187–193 (2001).
141. F. Dessi-Fulgheri, S. Porrini, F. Farabollini. *Environ. Health Perspect.* **110**, 403–407 (2002).
142. C. S. Roegge, B.-W. Seo, K. M. Crofton, S. L. Schantz. *Toxicol. Sci.* **57**, 121–130 (2000).
143. K. M. Flynn, S. A. Ferguson, K. B. Delclos, R. R. Newbold. *Toxicol. Sci.* **55**, 311–319 (2000).
144. S. A. Ferguson, K. M. Flynn, K. B. Delclos, R. R. Newbold. *Neurotoxicol. Teratol.* **22**, 583–591 (2000).
145. S. A. Ferguson, A. C. Scallet, K. M. Flynn, J. M. Meredith, B. A. Schwetz. *Neurotoxicology* **21**, 947–956 (2000).
146. K.-S. Kang, J.-E. Park, D.-Y. Ryu, Y.-S. Lee. *J. Vet. Med. Sci.* **63**, 1183–1190 (2001).
147. E. S. Goldey and K. M. Crofton. *Toxicol. Sci.* **45**, 94–105 (1998).
148. K. M. Crofton, D. L. Ding, R. Padich, M. Taylor, D. Henderson. *Hearing Res.* **144**, 196–204 (2000).
149. L. E. Gray, Jr., R. J. Kavlok, J. Ostby, J. Ferrell, J. Rogers, K. Gray. *Neurotoxicology* **7**, 449–462 (1986).

150. M. E. Gilbert, W. R. Mundy, K. M. Crofton. *Toxicol. Sci.* **57**, 102–111 (2000).
151. M. Taylor, J. Hedge, M. DeVito, K. Crofton. *Toxicologist* **66**, 133 (2002).
152. P. Clausing, H. K. Mothes, B. Opitz. *Neurotoxicol. Teratol.* **22**, 113–123 (2000).
153. P. Palanza, F. Morellini, S. Parmigiani, F. S. vom Saal. *Neurosci. Behav. Rev.* **23**, 1011–1027 (1999).
154. F. D. Farabollini. *Environ. Health Perspect.* **110**, 409–414 (2002).
155. E. D. Lephart, T. W. West, K. S. Weber, R. W. Rhees, K. D. R. Setchell, H. Adlercreutz, T. D. Lund. *Neurotoxicol. Teratol.* **24**, 5–16 (2002).
156. D. O. Norris, *Vertabrate Endocrinology*. Academic Press, San Diego (1996).
157. G. Ankley, E. Mihaich, R. Stahl, D. Tillitt, T. Colborn, S. McMaster, R. Miller, J. Bantle, P. Campbell, N. Denslow, R. Dickerson, L. Folmar, M. Fry, J. Giesy, L. E. Gray, P. Guiney, T. Hutchinson, S. Kennedy, V. Kramer, G. Leblanc, M. Mayes, A. Nimrod, R. Patino, R. Peterson, R. Purdy, R. Ringer, P. Thomas, L. Touart, G. Van Der Kraak, T. Zacharewski. *Environ. Toxicol. Chem.* **17**, 68–87 (1998).
158. L. Helmuth. *Science* **296**, 2131–2133 (2002).
159. A. M. Cummings. *Crit. Rev. Toxicol.* **27**, 367–379 (1997).
160. E. Mylchreest, M. Sar, R. C. Cattley, P. M. Foster. *Toxicol. Appl. Pharmacol.* **156**, 81–95 (1999).
161. E. Mylchreest, D. G. Wallace, R. C. Cattley, P. M. Foster. *Toxicol. Sci.* **55**, 143–151 (2000).
162. R. N. Wine, L.-H. Li, L. H. Barnes, D. K. Gulati, R. E. Chapin. *Environ. Health Perspect.* **105**, 102–107 (1997).
163. D. Beltrame, E. DiSalle, E. Giavini, K. Gunnarsson, M. Brughera. *Reprod. Toxicol.* **15**, 195–213 (2001).
164. L. S. Birnbaum. *Environ. Health Perspect.* **103**, 89–94 (1995).
165. R. E. Faith, M. I. Luster, C. A. Kimmel. *Clin. Exp. Immunol.* **35**, 413–420 (1979).
166. T. L. Bunn, P. J. Parsons, E. Kao, R. R. Dietert. *Toxicol. Sci.* **64**, 57–66 (2001).
167. J. M. Spyker-Cranmer, J. B. Barnett, D. L. Avery, M. F. Cranmer. *Toxicol. Appl. Pharmacol.* **62**, 402–408 (1982).
168. J. B. Barnett, L. S. F. Soderberg, J. H. Menna. *Toxicol. Lett.* **25**, 173–183 (1985).
169. J. B. Barnett, B. L. Blaylock, J. Gandy, J. H. Menna, R. Denton, L. S. F. Soderberg. *Fundam. Appl. Toxicol.* **14**, 688–695 (1990).
170. J. G. Vos and J. A. Moore. *Int. Arch. Allergy Appl. Immunol.* **47**, 777–794 (1974).
171. R. E. Faith and J. A. Moore. *J. Toxicol. Environ. Health* **3**, 451–464 (1977).
172. J. S. Fine, T. A. Gasiewicz, A. E. Silverstone. *Mol. Pharmacol.* **35**, 18–25 (1989).
173. B. L. Blaylock, S. D. Holladay, C. E. Comment, J. J. Heindel, M. I. Luster. *Toxicol. Appl. Pharmacol.* **112**, 207–213 (1992).
174. B. C. Gehrs, M. M. Riddle, W. C. Williams, R. J. Smialowicz. *Toxicology* **130**, 219–228 (1998).
175. R. J. Smialowicz, W. C. Williams, C. B. Copeland, M. W. Harris, D. Overstreet, B. J. Davis, R. E. Chapin. *Toxicol. Sci.* **61**, 164–175 (2001).
176. S. Chen, K. A. Golemboski, F. S. Sanders, R. R. Dietert. *Toxicology* **132**, 67–79 (1999).