

Endocrine disrupting chemicals

Multiple effects on testicular signaling and spermatogenesis

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In the past 200 years, an enormous number of synthetic chemicals with diverse structural features have been produced for industrial, medical and domestic purposes. These chemicals, originally thought to have little or no biological toxicity, are widely used in our daily lives as well as are commonly present in foods. It was not until the first World Wildlife Federation Wingspread Conference held in 1994 were concerns about the endocrine disrupting (ED) effects of these chemicals articulated. The potential hazardous effects of endocrine disrupting chemicals (EDCs) on human health and ecological well-being are one of the global concerns that affect the health and propagation of human beings. Considerable numbers of studies indicated that endocrine disruption is linked to "the developmental basis of adult disease," highlighting the significant effects of EDC exposure on a developing organism, leading to the propensity of an individual to develop a disease or dysfunction in later life. In this review, we intend to provide environmental, epidemiological and experimental data to associate pollutant exposure with reproductive disorders, in particular on the development and function of the male reproductive system. Possible effects of pollutant exposure on the processes of embryonic development, like sex determination and masculinization are described. In addition, the effects of pollutant exposure on hypothalamus-pituitary-gonadal axis, testicular signaling, steroidogenesis and spermatogenesis are also discussed.

Industrialization, Chemical Contamination and Human Health

In the past century, the drastic advancement in industrialization and technology and the growth in human population have driven a change to the environment to a scope that is unprecedented in human history. The production of large amounts of synthetic industrial and biomedical chemicals, as well as unwanted pollutants pose destructive consequences to our ecosystem and impose negative health effects to wildlife and humans.¹⁻⁴ A recent review highlighted that about 40% of human death (62 million per

year) is attributed to the exposure of chemical pollutants.⁵ In the past 60 years, more than 140,000 synthetic chemical compounds were made and approximately 1,000–2,000 new chemicals are produced each year.⁶ These chemicals are ubiquitous and are dispersed in air, water, soil and food. A study from the US Center for Disease Control (CDC) reported that Americans of all ages have accumulated over 116 extraneous chemicals into their bodies.⁷ Over 358 industrial chemicals and pesticides have been detected in the cord blood of American infants.⁸ Some of the more damaging chemical contaminants are classified as endocrine disrupting chemicals (EDCs) since they can interfere with the synthesis, metabolism and action of endogenous hormones (Fig. 1).² They are known to exert different biological effects via a diverse mechanism of actions.⁹ Most of the understanding of the EDC elicited-effects is derived from experimental studies conducted on animals and/or cell culture, but little direct evidence of effects has been compiled for humans. Nevertheless, the potential hazardous effects of EDCs on human health are currently strengthening via epidemiological studies and clinical observations¹⁰ and have been shown to impose long-term effects on metabolism, immune system defects, cancer development, decreased fertility and reproductive health.^{9,11-15}

Environmental Pollution and Reproductive Health

Exposure to environmental pollutants is suggested to be one of the culprits to reproductive problems worldwide. This exposure-effect relationship has long been established in wildlife and in laboratory animal studies.¹⁶⁻²³ Adverse biological effects to male reproductive functions were first reported in wild animals where an accidental exposure to pollutants caused feminization or a change in reproductive behavior in the animals.²⁴ In the 1980s, the adult male alligators in Apopka Lake that were exposed to agricultural wastes, produced low testosterone levels and presented micro-penis and disorganized testes.²⁵⁻²⁷ Effects of mercury exposure on reproductive behavior and sexual preference of white ibises were reported in reference 28.

In humans, increased incidences of birth defects, precocious puberty, reproductive cancers and infertility have been reported in reference 29–32. From the data of World Bank 2005, total fertility rate had decreased from 1970 to 2002 in both developed and industrialized countries.³³ According to the 2001

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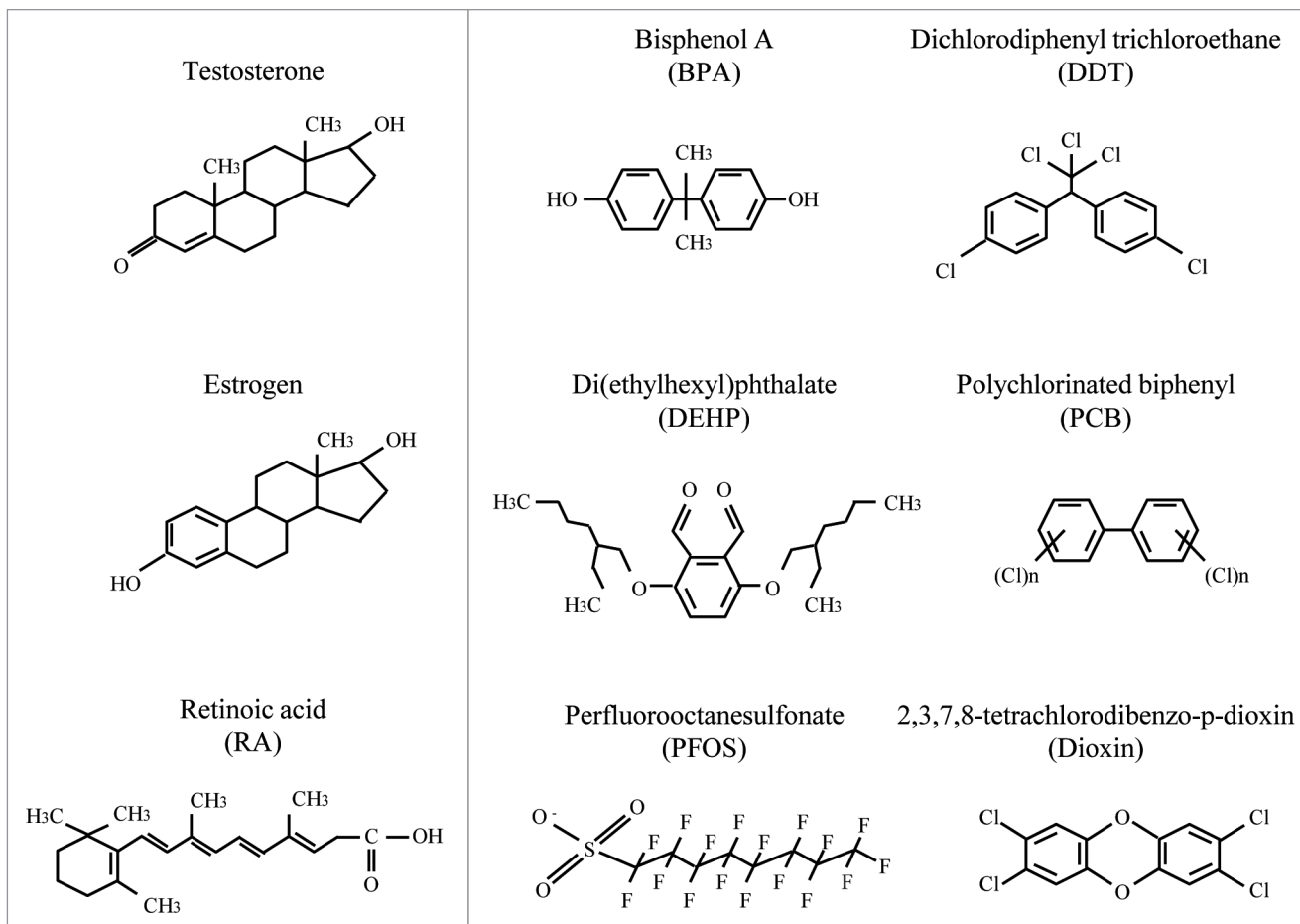


Figure 1. Chemical structures of sex steroid hormones (testosterone, estrogen), natural metabolite (retinoic acid) and some common EDCs (BPA, DDT, DEHP, PCB, PFOS, dioxin).

World Health Organization (WHO) report “Current practices and Controversies in Assisted Reproduction,” at least 80 million people worldwide were estimated to be affected by infertility, of which the most common cause has been identified to be the “male factor”.³⁴ Among all the infertility cases, over 10% of infertility cannot be explained medically. Given the adverse effects of EDC exposure on wildlife and laboratory animals, negative effects of environmental pollutants/chemicals on human fecundity are extrapolated. This postulation was supported by a study from Carlsen and coworkers in 1992, highlighting that the estrogenic-like activity of EDC was the cause of the decline in male fertility.³⁵ However the scientific accuracy of the paper remains questionable, as numerous flaws have been recognized in the study.³⁶⁻³⁸ Although the accuracy of the Carlsen’s paper is controversial, the paper motivated many follow-up investigations to re-analyze the data or to identify putative causative agents responsible for the declined human fecundity. Since there are variations in the quality of the methodologies and the great heterogeneity of the recruited subjects (i.e., different in age, behaviors and lifestyles), the general outcomes of these investigations are still not conclusive.^{39,40} At present the possible involvement of EDCs in human fecundity can neither be confirmed nor rejected.⁴⁰ However it is generally agreed that in utero chronic

exposure to environmental pollutants might impose significant effects on fetal development.^{11,41-43}

Effects of EDC on Sex Ratio and Early Testicular Development

In human epidemiologic studies, significant reductions in the ratio of “male birth to total number of birth” were recorded in highly polluted areas. The incidences of low male-to-female sex ratio at birth were reported in Aamjiwnaang First Nation community (areas close to industrial areas) in Canada,⁴⁴ Seveso Italy,⁴⁵ the Austrian chloracne cohort,⁴⁶ and the victims in the Yucheng oil disaster, Taiwan.⁴⁷ Possible explanation for the change in sex ratio at birth has not been elucidated. In mammals, sex development in embryonic stage depends on a delicate balance between male and female sex determining pathways.⁴⁸⁻⁵⁰ It is generally believed that the development of ovary from genital ridges is a default mechanism while the development of testis depends on the activity of Y chromosome testis-determining gene (*Sry*) and its downstream/associated factors [i.e., SRY-box containing gene 9 (*Sox9*), doublesex and mab-3 related transcription factor 1 (*Dmrt1*), prostaglandin D synthase, anti-Müllerian hormone (*Amh*) and testosterone].⁵¹⁻⁵⁴ The spatiotemporal action

of SRY to switch the supporting cells of genital ridges from the female to male pathway is essential and has to be undertaken within a critical programming time window.⁵⁵ Any disruption in the early steps of the male pathway would lead to the mal-development of testes or an engagement of ovary development. Other subtle effects of EDCs on the masculinization process resulted in reproductive disorders similar to testicular dysgenesis syndrome (TDS). In the Study for Future Families in the United States, a correlation of prenatal exposure to several phthalates with shortening of the anogenital index and incomplete testicular descent were observed.^{56,57} Using rat models, adverse effects of phthalates on the male offspring such as absent/underdeveloped epididymis and germ cell loss were demonstrated.^{58,59} Epidemiological studies reported an increased risk of genital malformations and cryptorchidism in children of workers who were exposed to pesticides.^{60,61} The effects observed in laboratory animal studies seems to correlate with increased incidences of malformation of genital tracts in both European and US populations,⁶²⁻⁶⁴ however the exploitation of animal data to human cases is still controversial.^{65,66}

Effects of EDC on HPG Circuitry Signaling in Neonatal and Pubertal Development

Mammalian spermatogenesis is a complicated cascade process that is under the tight control of the hypothalamus-pituitary-gonadal (HPG) axis as well as the de novo auto/paracrine circuit.⁶⁷ The primary role of the hormones involved is to enable a coordinated regulation of the process that allows the development of highly differentiated spermatozoa within the seminiferous tubules. The process depends on a functional hypothalamic-pituitary-testicular (HPT) axis. The hypothalamic kisspeptin-1 (KiSS-1) and its G protein-coupled receptor (GPR54) act as the gatekeeper to control the secretion of gonadotrophin releasing hormone (GnRH), which regulates the anterior pituitary hormones—luteinizing hormone (LH) and follicle stimulating hormone (FSH), and testicular hormones—testosterone, activin and inhibin B.^{41,68,70,71} Since neuroendocrine actions of EDCs have been shown,⁷²⁻⁷⁴ HPG circuitry signaling can be the EDC target during perinatal development. Any interruption on the hypothalamic circuitry, hormonal mediated regulation or on the constituents at the microenvironments in seminiferous tubules may result in a transient/long-term modification of the hormonal feedback circuitry, leading to the disturbance of spermatogenesis.^{15,75} In the following sections, some examples of the HPG-related systems that have been shown to be affected by EDCs are discussed.

Considerable numbers of studies have revealed effects of EDCs on the hypothalamic KiSS-1/GPR54 system and the HPG axis.^{15,76} The alteration of the HPG axis upon EDCs exposure [i.e., polychlorinated biphenyls (PCBs), lead, cadmium] has been shown from piscine to rodent,⁷⁷⁻⁸⁰ although their actions may vary in different development stages.⁸¹ Using nonhuman primate and mouse models, Leranath and coworkers demonstrated the effects of BPA exposure on spine synapse formation in brains.^{12,82} The observation provides profound insights on the effects of

EDC on neuronal circuit development. This presumption has been supported by other studies where prenatal PCBs exposure interrupted neuronal development and receptor expression in rat hypothalamus.⁸³ In rodent models, bisphenol A (BPA) exposures were found to affect hypothalamic kisspeptin fiber density, KiSS-1 and estrogen receptor- α (ER α) mRNA expression.⁸⁴⁻⁸⁸ In our recent study, we demonstrated that prenatal exposure to BPA exerted considerable effects on the functional circuitry of HPG axis in mice.⁸⁴ The disruption of the normal functioning of the hypothalamic circuitry may lead to an interruption of GnRH, LH and FSH release for the regulation of sexual development and gametogenesis (Fig. 2 and arrow A).⁸⁹ In addition to the modulation of hormone release from hypothalamus-pituitary level, the decrease in testosterone and sperm production can be the consequence of the reduced testicular expression levels of receptors for gonadotrophin, as demonstrated in perfluorooctanesulfonic acid (PFOS) exposed mice (Fig. 2 and arrow B).⁹⁰

Although the biological and physiological outcomes of EDC exposures have been reported, the possible molecular targets at the HPG axis have not been elucidated. Since some of the EDCs bear very similar chemical structures to the endogenous hormones,⁹¹ the interaction between nuclear hormone receptors (NHRs) and EDCs has been proposed as the elementary action on endocrine disruption.^{92,93} It is generally believed that EDCs can affect the hormonal system via (but not limited to) estrogenic, androgenic, anti-androgenic and anti-thyroid mechanisms.^{30,94} The mechanistic aspects of endocrine disruption may be via the roles as (1) agonist or antagonist, (2) selective modulators in the recruitment of coactivators/co-repressor in transcriptional complex or/and (3) in cross-talk between NHRs.⁹⁵ Among different EDCs, the molecular targets and the mechanistic actions of dioxins are well characterized. Dioxins are known to impose biological effects via the aryl hydrocarbon receptor (AHR), which belongs to a member of the basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) family of transcription factors. AHR exhibits its transcriptional activity primarily via ligand-dependent nuclear translocation.⁹⁶ Other regulatory functions mediated by dioxin/AHR complex include the modulation of other transcriptional factors, including retinoblastoma (Rb)/elongation factor-2 (E2F), nuclear factor- κ B (NF κ B) and the estrogen receptors (ER α and ER β) and androgen receptors.⁹⁷⁻¹⁰³ Comparable to the dioxin/AHR mediated actions, EDCs that possess estrogenic and/or anti-estrogenic activities, have also been shown to have striking effect on animals.¹⁰⁴ This is particularly true if we look at it from an evolutionary perspective where the DNA-binding domain and the ligand-binding domain of ER α are conserved across metazoans.^{105,106} Global environmental contaminants, persistent organic pollutants (i.e., dichlorodiphenyl-trichloroethane (DDT), hydroxylated PCBs, BPA, *p*-nonylphenol and dioxins) and heavy metals (i.e., cadmium and mercury) were shown to have either or both estrogenic and androgenic activity.^{104,107,108} In addition, BPA was found to be able to activate membrane G-protein coupled estrogen receptor.¹⁰⁹ Some newly identified emerging contaminants, like perfluorinated compounds (PFCs) and flame retardants were also reported to possess estrogenic activities.^{110,111}

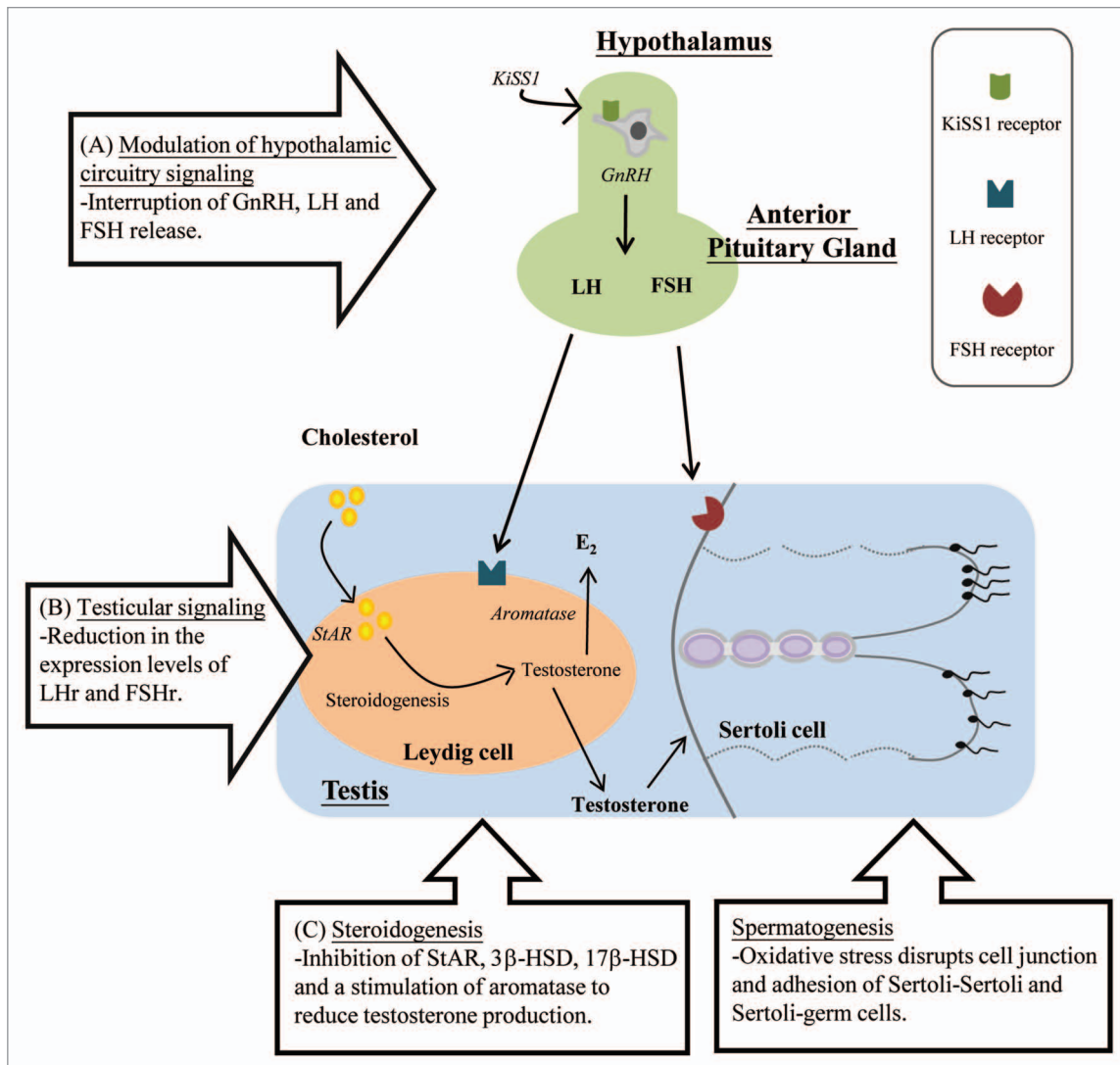


Figure 2. A schematic diagram depicts the effects of EDCs on HPG axis. (A) EDCs modulated the neural circuit in hypothalamus (KiSS-1/GPR54 and HPG axis) leading to the dysregulation of gonadotrophin hormones (FSH/LH) secretion by the pituitary. (B) In the testis, EDC exposure caused the reduction in the expression of gonadotrophin receptors (LHr and FSHr). (C) EDCs interfered with the enzymes (StAR, P450_{scc}, 3β-HSD, 17β-HSD) involved in steroidogenesis. (D) In addition to the reduction of testosterone level, oxidative stress elicited by EDCs caused disruption of cell-cell interaction and perturbed the process of spermatogenesis.

Effects of EDC on Adult Spermatogenesis

The modulation of steroidogenic enzymes. In addition to the NHR-mediated effects, recent hypothesis has highlighted that steroidogenesis is the major target for EDCs (Fig. 2 and arrow C).^{112,113} Steroidogenesis is the process for steroid hormone production. It is an enzymatic-mediated process catalyzed by several enzymes from two main categories: the cytochrome P450 enzymes (CYP11A and CYP17A), and hydroxysteroid dehydrogenase (HSD) enzymes (3β-HSD and 17β-HSD).¹¹⁴ Negative influences of EDC exposure on steroidogenesis have been reported in both in vivo and in vitro studies. Inhibitory effects of BPA, PCBs, PFCs, dioxins and some of the phthalates on the expression levels of some steroidogenic enzymes were elucidated.¹¹⁵⁻¹²² Mostly downregulation of the expression levels of

CYP11A and CYP17A were observed, resulting in the reduction of testosterone production. This hypothesis is rational as receptor binding affinities of most EDCs are generally low as compared to the endogenous ligands.¹²³ Although the additive/synergistic effects of mixture of EDCs cannot be neglected, it seems unlikely that EDC can compete with the endogenous ligand for receptor binding. Retrospectively it is more likely that EDCs interfere with steroidogenesis and modulate the release of endogenous steroid hormones. The altered serum levels of the steroid hormones may cause subsequent reproductive dysfunction by interfering with the feedback regulatory mechanisms of the HPG axis. For example, BPA is a weak estradiol agonist, its estrogenic effect in animal bodies is probably mediated by its stimulatory action on gonadal aromatase to increase serum estradiol (E₂) levels.^{84,124} Consistently using H295R human adenocarcinoma cells, BPA

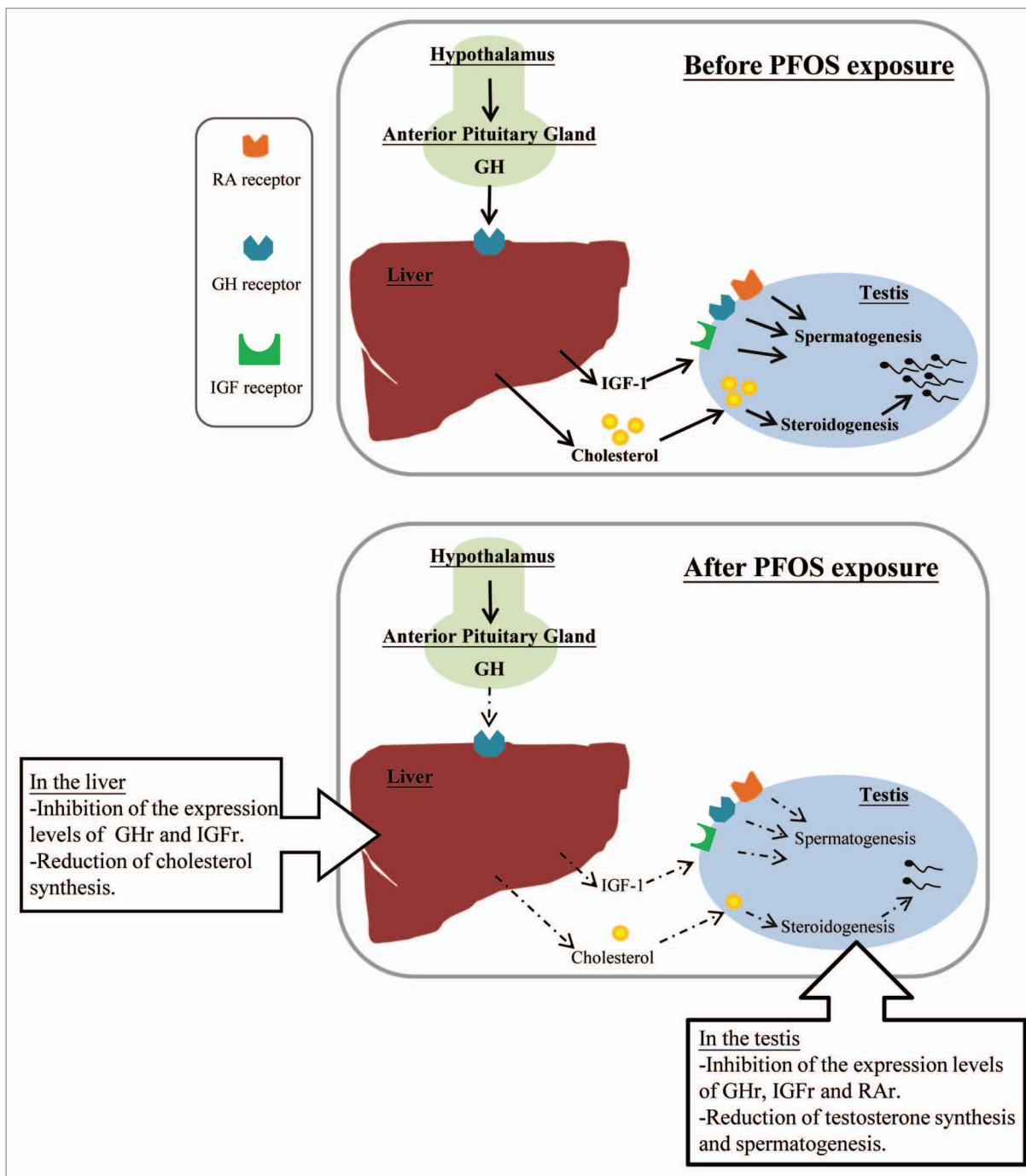


Figure 3. A schematic diagram illustrates the influence of PFOS on GH/IGF-signaling and testicular functions. Our recent data demonstrated that PFOS-induced testicular dysfunctions and metabolic disorders via the reduction in the expression levels of receptors for GH and IGF in both liver and testis, leading to the inhibition of steroidogenesis and spermatogenesis.

treatment caused an increase of E_2 production.¹²⁵ Another EDC, dioxin was shown to reduce testosterone production in rat primary Leydig cell culture via the inhibition of human chorionic gonadotrophin (hCG)-stimulated cAMP and CYP11A levels.¹²⁶ The effects of EDCs on fetal testis seem to be more striking as the disruption of steroidogenesis at this early developmental stage can also affect the proliferation of germ cells and Sertoli cells.^{113,127,128} Notably it may interfere with the formation of the

Sertoli-germ cell complex which supports the maximum number of sperm that can be produced in adulthood.^{129,130} More importantly, the effects on germ cell development can be inherited via epigenetic actions of EDCs.^{111,131} Although the underlying action of EDCs on epigenetic modification is not known, this hypothesis is supported by data from other laboratory animal studies.^{132,133} Retrospectively the effects of EDCs on fetal testis can be long-lasting and transgenerational.^{127,128,134} In contrary, the effects on

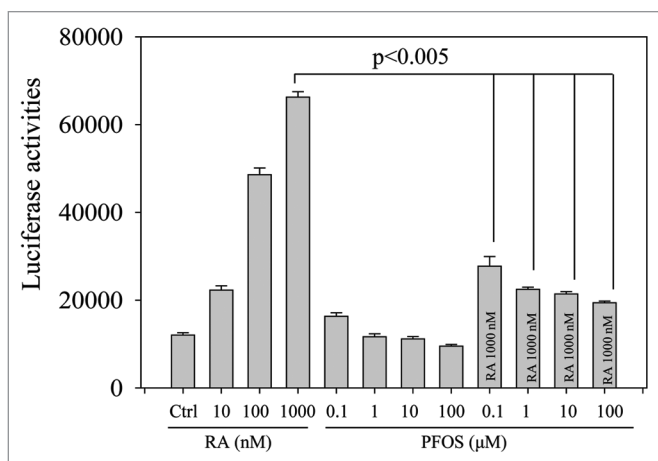


Figure 4. PFOS inhibits RA-mediated transactivation of retinoic acid response element (RARE), as illustrated using luciferase reporter assay. The day before transfection, MCF7 cells were plated into 24-well tissue culture dishes at a density reaching 70–80% confluence by the time of transfection. Transfection was performed using LipofectAMINE™ 2000 reagent (Invitrogen) and OPTI-MEM™ I medium (GIBCO) with 250 ng of retinoic acid reporter (Addgene). Six hours after transfection, the transfection medium was replaced by a complete medium and different doses of retinoic acid (10–1,000 nM) or PFOS (0.1–100 μM) or co-treatment of retinoic acid (1,000 nM) and PFOS (0.1–100 μM) were added. After 24 h incubation, the cells were then lysed in the passive lysis buffer. Firefly luciferase activities were measured using the Luciferase reporter assay system (Promega) and the multilabel reader VICTOR™X4 (PerkinElmer).

adult testis may be short-term and reversible upon the reduced exposure to the chemicals.

The induction of oxidative stress. As mentioned above, the effects of EDs are believed to be mediated by their direct and/or indirect actions on steroid hormone receptors and steroidogenesis.³⁰ However, these effects may be limited to EDCs with particular chemical structures. The effects of other heterogeneous structures of EDCs may not be accounted for.¹³⁵ Recently, oxidative stress is identified as a common mechanism of action for EDC in affecting cellular structures and functions.¹³⁶ Induction of oxidative stress was detected in epididymal sperms of rats, exposed to BPA.¹³⁷ Specifically EDC-induced oxidative stress caused disruption to tight and adherens junctions between Sertoli-Sertoli cells and Sertoli-germ cells.^{67,138} The underlying mechanism of the dysregulation is found to be associated with the modulation of phosphatidylinositol-3-kinase (PI3K)/c-Src/focal adhesion kinase (FAK) and mitogen-activated protein kinase (MAPK)-signaling, in affecting the metabolism of some polarity proteins [e.g., occludin, zonula occludens-1 (ZO-1) and N-cadherin].¹³⁹ The disruption of the junctional structures leads to the dysregulation of spermatogenesis (Fig. 2 and arrow D).^{140,141} Since there are two recent excellent reviews from Cheng's group that have covered most of the updated information on this aspect,^{139,142} the underlying mechanisms of EDC-induced oxidative stress in mediating disruption to cell junctions will not be discussed in this review.

The alternation of body metabolism. The maintenance of normal male reproductive function is not exclusively controlled

by gonadotrophin and testicular hormones/factors (i.e., testosterone, activins, inhibins).¹⁴³⁻¹⁴⁵ Possible influence from body metabolic disturbances on testicular functions is discussed in recent years.¹⁴⁶ Perturbation of the testicular steroidogenesis can be due to the inhibitory action of EDC on the gene expression levels of insulin-like growth factor-1 (IGF-1) and its receptor in rat testes as shown by perfluorododecanoic acid (PFDoA) exposure.¹¹⁷ Similarly, our recent data demonstrated that PFOS-induced testicular dysfunctions and metabolic disorders may be related to the reduction in the expression levels of receptors for growth hormone (GH) and IGF in both liver and testis of mice (Fig. 3).⁹⁰ The effects of GH and IGF-1 are known to stimulate the transcription of *CYP11A* gene encoding cytochrome P450 side-chain cleavage (P450_{scc}), for the conversion of free cholesterol into pregnenolone in the early steps of steroidogenesis.¹⁴⁷ A decrease of signal interaction between GH/IGF-1 and HPG axis would therefore affect steroidogenesis.^{144,148} Furthermore the GH/IGF-1 axis has been suggested to link with the adipocytokine signaling system.¹⁴⁹⁻¹⁵¹ Leptin plays a regulatory role on the HPG axis via leptin-kisspeptin-GnRH pathway, leading to the hormonal regulation of LH and FSH.¹⁵²⁻¹⁵⁵ Indeed, serum leptin was found to be negatively correlated with serum testosterone. Reduced interaction of leptin with Leydig or germ cells would lead to the reduction in the expression of steroidogenic enzymes^{156,157} and the impairment of sperm mobility.^{155,158-160} In gestational exposure of EDCs (i.e., phthalate), plasma leptin level was reduced and was accompanied by the reduction of anogenital distance and the expression levels of several steroidogenic enzymes such as steroidogenic acute regulatory protein (StAR), CYP11A, CYP17 in fetal male rats.¹⁵⁶ Leptin synthesis was also found to be inhibited in cadmium exposure.¹⁶¹ In addition to leptin, another adipocytokines such as adiponectin and retinol-binding protein 4 (RBP4) have been suggested to be modulated by EDC exposure. For instance, BPA treatment diminished adiponectin production in 3T3-L1 adipocytes.¹⁶² Long-term exposure of DDT caused a reduction of serum RBP4 levels, leading to an inadequate intake of vitamin A,¹⁶³ which is an important factor for the regulation of spermatogenesis.¹⁶⁴ Intriguingly using retinoic acid (RA) reporter assay, PFOS was found to inhibit RA-mediated transactivation of retinoic acid response element (RARE) (unpublished data, Fig. 4). The data indicate that PFOS may act as a RA antagonist to interfere with retinoid signaling to inhibit spermatogenesis.¹⁶⁵

Conclusion

Adverse effects of EDCs on male reproductive dysfunction are well recognized from the epidemiological and laboratory animal data. However tens of thousands of industrial chemicals or pollutants are still produced or discharged extensively on a daily basis.¹⁶⁶⁻¹⁶⁹ They are ubiquitous and the possible routes of human exposure to EDCs are from the environments, consumer products and foods. Effects of EDCs on animal reproductive function can be multi-faceted and pleiotropic. Exposures to EDCs can interfere with cell signaling via direct/indirect "hormonal" and/or oxidative stress related pathways in HPG axis and other

body tissues (i.e., liver). These direct and indirect effects disrupt the homeostasis at different levels of feedback regulatory mechanisms (neuron communication, endocrine, autocrine and paracrine) for the regulation of testicular development and functions (i.e., steroidogenesis and spermatogenesis). However current cell culture- and animal-based experiments can only produce limited data on human risk assessment in reproductive impairment.^{170,171} Since most of the adverse health responses in laboratory animals are demonstrated when animals are exposed to doses that are greater than those found in the environment, this leads to the

difficulty to assess the potential low-dose human exposure risk. Prenatal exposure to EDCs seems to be particularly critical in affecting neural circuits at hypothalamus-pituitary axis and fetal testicular development, leading to long-term, irreversible consequences in reproductive dysfunction.

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