Chapter

# Effect of Endocrine Disrupting Chemicals on HPG Axis: A Reproductive Endocrine Homeostasis

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## Abstract

The hypothalamic–pituitary-gonadal (HPG) axis plays a crucial and integrative role in the mammalian endocrine regulation to maintain homeostasis. The HPG axis is primarily responsible for governing all the hormonal events related to reproductive activity. Endocrine-disrupting chemicals (EDCs) comprise a diverse group of naturally occurring and synthetic compounds that mimic and interfere with the endogenous chemical hormones. Epidemiological investigations have shown increasing evidence of altered development and detrimental effects on reproductive health during the past 50 years associated with endocrine disruptors affecting the HPG axis. The pleiotropic harmful effects of EDCs act through hormone-dependent downstream signaling pathways responsible for gonad development either through direct interaction with steroid hormone receptor or via epigenetic regulation. Hence, this chapter summarizes the biological plausibility of EDCs exposure and elucidates the mechanism of action underlying EDCs affecting the regulatory circuits of the mammalian HPG axis and reproductive function.

**Keywords:** endocrine disrupting chemicals, hypothalmic-pituitary-gonadal axis, reproduction, epigenetic, polycystic ovarian syndrome

## 1. Introduction

In the past decade, endocrine disruptor chemicals (EDCs) in human pathophysiology have gained much more attention due to their ability to affect development and reproduction in humans and wildlife. In accordance with the US Environmental Protection Agency (EPA), EDCs can be defined as 'exogenous agents that disrupt the hormone homeostasis by interfering with the synthesis, secretion, transport, metabolism, receptor binding or elimination of endogenous hormone [1–6]. EDCs comprise mainly heterogeneous compounds, including synthetic (chemical) and natural (plant products such as isoflavones) responsible for disrupting the hormone system. Endocrine disruptors may be found easily in almost every product used in our daily life, including detergents, plastic bottles, food, toys, pesticides, insecticides, and flame retardants. The criteria proposed by the European Commission for being in the process of defining any compound as EDCs should at least exhibit three

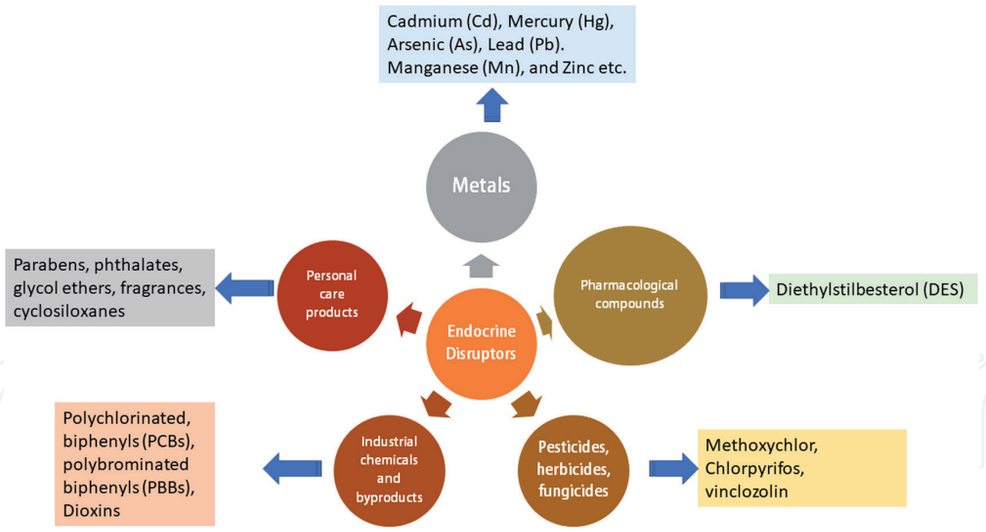


**Figure 1.**
*Sources and examples of EDCs.*

characters: (1) an endocrine activity, (2) an adverse or deleterious endocrine mediated effect in the exposed subject or its progeny or subpopulations (3) a plausible cause-effect relationship between the two [7, 8]. Most of the EDCs interfere with the endocrine system by binding the hormonal receptor or regulating genomic expression. Increasing evidence has documented that the highest risk is posed during early and postnatal development while forming organ and neural systems [9]. Sometimes, epigenetic changes (DNA methylation or acetylation) or histone modifications are also involved in endocrine disruption [10].

EDCs are highly heterogeneous and can be classified based on their origin: Industrial and household chemicals (dioxins, phthalates, polychlorinated biphenyls (PCBs), alkylphenols, plasticizers, fire retardants), agricultural (insecticides, pesticides, herbicides, fungicides, phytoestrogens), residential {bisphenol A (BPA), polybrominated biphenyls (PBBs), phthalates} and some pharmaceuticals agents {parabens, diethylstilbestrol (DES)} [3, 5, 11]. Heavy metals such as lead, mercury, cadmium, and arsenic are also included in the EDCs long list (**Figure 1**) [10, 12]. According to the Stockholm Convention (2001), both production and usage of persistent organic pollutants (POPs) was restricted [13]. Guidelines for a list of chemicals were developed to store and eliminate them, which was happened in 2008 and 2014 [14]. Initially, almost twelve POPs were designated as "dirty dozen" due to their severe adverse effects on humans and the ecosystem. Hence, their production and use were banned. The dirty dozen included industrial chemicals, pesticides, and by-products such as aldrin, chlordane, dieldrin, endrin, dichlorodiphenyltrichloroethane (DDT), heptachlor, mirex, toxaphene, PCBs, hexachlorobenzene, polychlorinated dibenzo-p-dioxins, and polychlorinated dibenzofurans [9].

## 2. Human exposure and route of entry of EDCs

Endocrine-disrupting chemicals may exhibit various exposure routes to enter the human body. Generally, inhalation (e.g., Plasticizers), dietary intake (e.g., Foods), dermal absorption (e.g., Cosmetics), and embryonic exposure (e.g., Transfer from mother) represent the main exposure pathways (**Figure 2**) [5, 15]. Following any of these pathways, EDCs may enter the food chain and accumulate in different tissues [16]. Mostly EDCs are highly lipophilic in nature and hence accumulate in the adipose tissue having a long half-life. These aforementioned

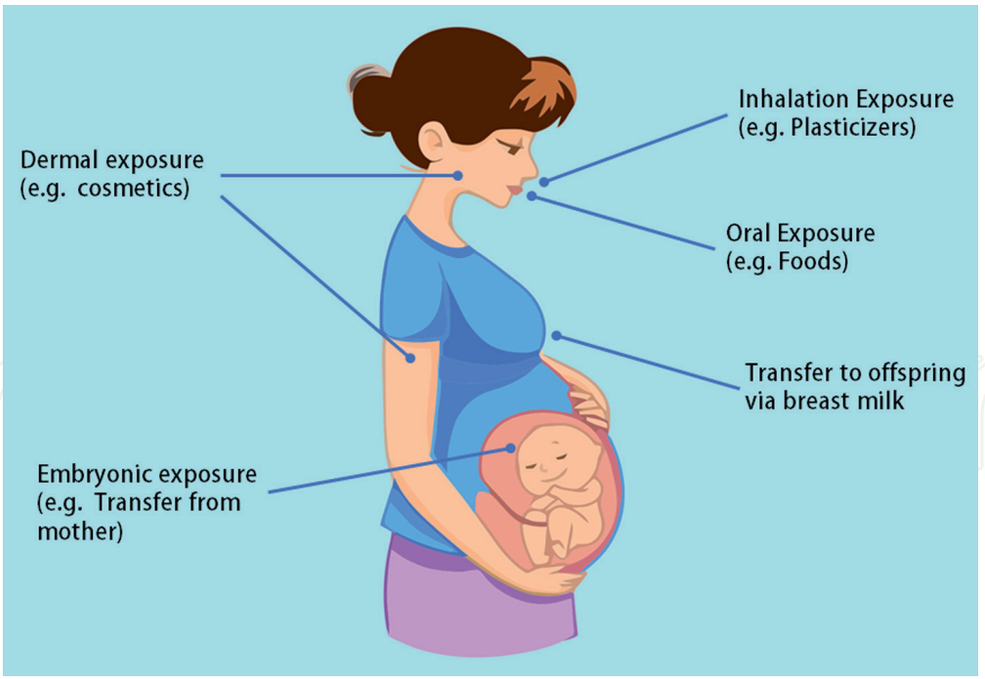


**Figure 2.**
*Route of entries of EDCs.*

features explain the exact reasons for being the accumulation of EDCs for years in the adipose tissue. Humans and other top predators are at the top of the food chain. Hence, due to bioaccumulation and biomagnification, they may store many EDCs, ultimately leading to various adverse consequences. Even lifelong exposure or fetal or neonatal stage exposure may bring about cumulative or additive or synergic effects. Therefore, the timing of exposure is of utmost importance in evaluating adverse effects on the endocrine system.

Endocrine disruptors such as dioxins, PCBs, perfluorinated compounds, and DDT are commonly found in pesticide-contaminated soil or groundwater or industrial waste quickly enter the human body via oral consumption of food or water. Some of the commonly used EDCs (DDT, vinclozolin, pyrethroids, chlorpyrifos) in households, agriculture, or public disease vector control may come in contact with human skin or through inhalation. In addition to this, cosmetics, personal care products, sunscreens, medications (triclosan, paraben, phthalates) that we apply on our skin are also responsible for their uptake into our body [17]. Professional workers using fungicides, pesticides, and chemicals are most prone and at high risk of EDCs exposure.

## 3. HPG axis: the central regulator

The mammalian reproductive cycles are mainly controlled by an intricate play between hypothalamus pituitary and gonads [18]. The hypothalamic Gonadotropin-Releasing Hormone (GnRH) neurons regulate reproductive functions in all vertebrates. The hypothalamic–pituitary-gonadal (HPG) axis plays a crucial role in the normal development of the reproductive system by controlling the ovarian as well as uterine cycles in females and also spermatogenesis in males [18, 19]. In response to GnRH release from the hypothalamus, pituitary gonadotropic cells synthesize and release Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH), which travel via the bloodstream to the target organs. LH and FSH are essential as regulators of both ovarian and testicular function. In males, LH stimulates

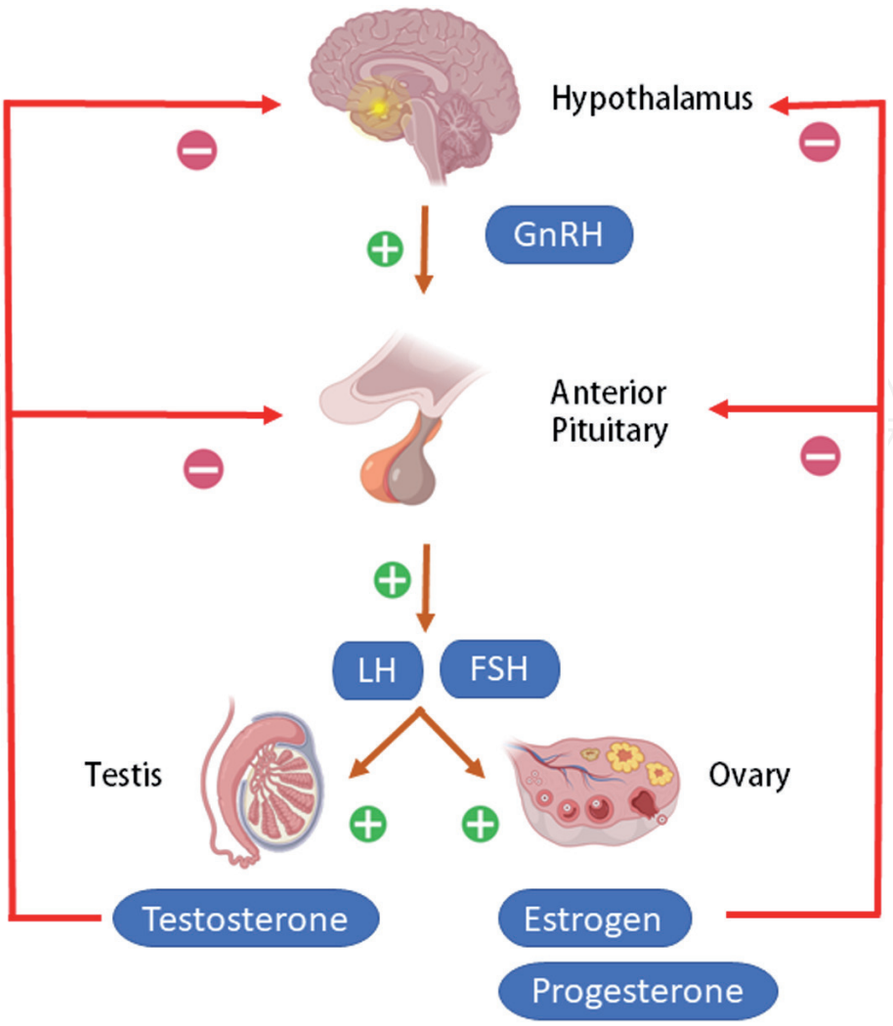


**Figure 3.**
*Regulation of HPG axis.*

testicular Leydig cells to synthesize and secrete testosterone, which in turn maintains spermatogenesis in Sertoli cells through its paracrine action and exerts sexual and anabolic actions. While FSH acts on Sertoli cells to produce androgen-binding protein, which is critical for spermatogenesis initiation and ultimately augments sperm production [20]. In females, the production of GnRH, LH, and FSH via the HPG axis are similar, but the actions of these hormones are different. LH and FSH exert their function on the ovaries to promote follicular maturation, ovulation, corpus luteum development, and estrogen and progesterone production [21, 22]. These hormones also have a role in regulating the uterine (menstrual) cycle to prepare for ovulation and embryo implantation [23]. These gonadal steroid hormones secreted by ovaries and testis can inhibit GnRH's hypothalamic synthesis via a feedback loop, hence playing a vital role in regulating reproductive function. In most mammals, 17β- Estradiol, testosterone, and progesterone are the primary estrogen, androgen, and progestin, respectively, and each of their receptors are expressed abundantly in the hypothalamus (**Figure 3**) [24].

## 4. Molecular mechanisms of EDCs

It is widely known that EDCs molecule, either natural or synthetic, follows the classical mechanism of action to mimic or interfere with the action of an endocrine regulated network of vertebrates. This interference can happen through different

mechanisms, either through genomic or non-genomic actions. So before understanding the mechanism of endocrine disruption, we must have a clear picture of the endocrine system that relies on the synthesis and release of hormones from various endocrine glands and its transport via the bloodstream to the required distant cells and tissues [25]. This process involves complex interacting signaling pathways and hormone receptors to control normal body function. Estrogen receptors (ERs) regulate the transcription of their target genes via multiple pathways, either directly or indirectly. Any changes in ER signaling may lead to adverse consequences such as hormone-dependent cancer, abnormal fetal growth and development, altered metabolism, and sometimes impaired fertility. Although the effect of EDCs is not limited to only the ligand-dependent ER signaling pathway, it is the best-studied of ER targeted endocrine disruption. ER in response to ligand signals through both genomic as well as a non-genomic pathway. Briefly, ER mediates its signal in the genomic pathway by binding directly to estrogen-responsive elements (ERE) or indirectly through coactivators such as SP-1 or AP-1. Although the best well-studied nuclear receptor cofactors belong to the p160 family of coactivators (e.g., SRC-1, SRC-2, and SRC-3) but the cofactor complex that mediates ER signaling is more complicated.

The non-genomic pathway has a rapid response as within minutes of ligand binding, signal transduction occurs. During estrogen ligand, a G-protein coupled receptor (GPCR-30) activates and mediates the signal independent of ERs and stimulates cAMP production, fluctuates intracellular calcium, or lead to MAPK or PI3K signaling cascades events. BPA and DES are extensively studied EDCs, which also induce rapid estrogen signaling via the non-genomic pathway (**Figure 4**).

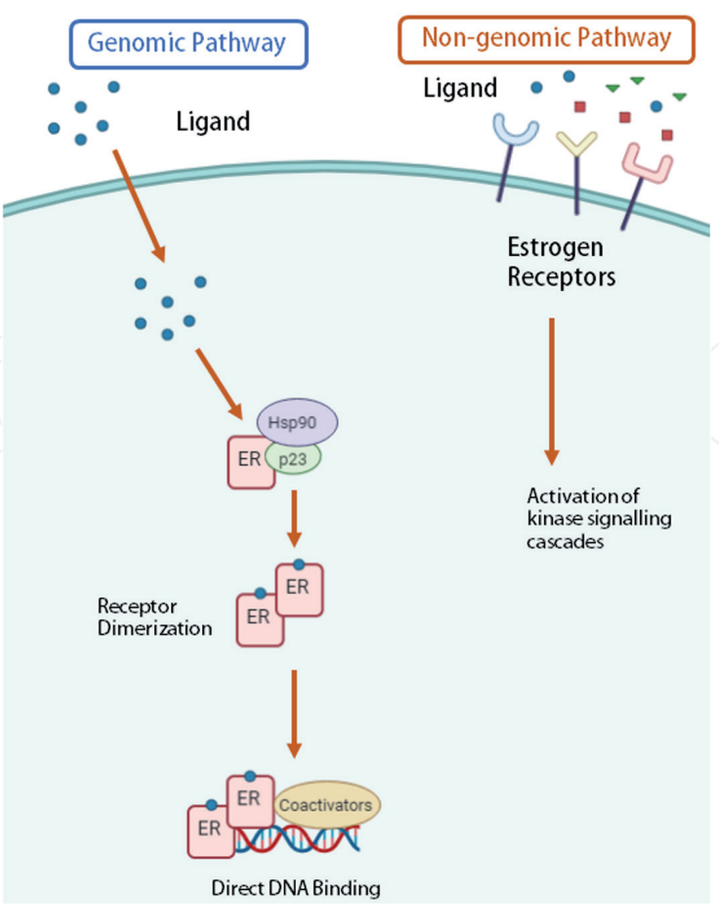


**Figure 4.**
*Genomic and non-genomic pathway of estrogen signaling.*

Steroid hormones are a group of lipophilic molecules that regulate a wide variety of physiological functions starting from fetal life to adulthood [26]. All these steroid hormones are biosynthesized from cholesterol, including glucocorticoids, mineralocorticoids, progestin, estrogen, and androgen, regulating sexual and reproductive development. Steroidogenic enzymes involved in the steroid hormone biosynthesis pathway are considered as the crucial targets for EDCs action. For example, Researches have demonstrated that exposure of Nonylphenol (NP) concentration $10^{-4}$ to $10^{-1}$ M at several ages with multiple rat generations (N = 3-4 per group) can decrease P450scc activity in testes [27]. Phthalates exposure, which consists of a particular class of pesticides, inhibits testosterone synthesis in Leydig cells due to direct CYP17 inhibition and hence exerts anti-androgenic effects [28]. Furthermore, BPA of different concentrations ($10^{-8}$ to $10^{-4}$ M) have been shown to inhibit the enzymatic activity of 3β-HSD, CYP17A1, and 17β-HSD3 in a dose-dependent manner in both human and rat testicular microsomes [29]. EDCs exposure also inhibits the activity of 5-α reductase, which is one of the main enzymes involved in dihydrotestosterone production from testosterone and hence in the regulation of masculinization of the external genitalia and the prostrate [30].

Aromatase (cyp19a) is known as the potential target action of EDC, and any modulation in its expression and function alters the estrogen production rate and hence disrupts the estrogen-dependent process [31]. A study suggested that BPA exposure in rat testis R2C Leydig cells stimulate aromatase activity and is correlated with upregulation in COX-2 in R2C cells [32].

In addition to steroidogenic enzymes and hormone metabolism, EDCs are also known to affect hormone-related receptor and their expression. EDCs, due to their similarity in structural features with the endogenous estrogen hormones, bind easily with the estrogen receptors and thus modify estrogen-responsive gene expression. EDCs that display binding with ERs include industrial bisphenolics, pharmaceutical chemicals, phytoestrogens, and organochlorine pesticides. Methoxychlor (MTX) and DDT are examples of organochlorine pesticides that exhibit estrogenic activity through binding with ERα and ERβ ligand-binding domains [33]. Thus, as reported, both pesticides adversely affect the female reproductive trait by impairing normal follicle development and stimulating uterine proliferation [34]. The insecticide endosulfan has a similar estrogenic activity that causes ovarian regression in both in vitro and in vivo studies [35]. Endosulfan competes with estradiol for interacting with the estrogen receptor but with lower affinity and, in turn, affects sex-specific gene expression [36]. Endosulfan can also affect the male reproductive system by decreasing the gene expression of testis-related transcription factors (sox9a and wt1) [37]. BPA possesses a binding affinity for ERs subtypes (ERα and ERβ) and is categorized as a prototypical non-steroidal ER [38].

It is also important to note that EDCs exhibit multiple hormone-binding actions regardless of their binding to hormonal receptors. For instance, DDT is an agonist for the estrogen receptor, but one of the metabolites of DDT shows anti-androgenic activity [39]. Similarly, BPA shows estrogenic and androgenic activity but exhibits an antagonist nature for thyroid hormone [40, 41].

## 5. Effect of EDCs on female reproductive system

The female reproductive system's development is credited to folliculogenesis, where adverse biological effects of EDCs can be observed. The primordial follicles finally become primary, pre-antral, and the antral follicle. Environmental toxicants such as BPA, MTX, and phthalates may interfere at any developmental stage of the

aforementioned antral follicle growth and hinder the reproduction, sometimes causing infertility. BPA exposure during in-vitro studies has been shown to inhibit mouse antral follicle development [42]. Follicle growth is dependent on the proliferation of theca and granulosa cells [43].

Estrogens are well-known as the gatekeepers of the female reproductive system. The sudden increase during puberty opens the gate to enter the reproductive life, and at menopause, the gate gets closed due to their decreased level. Thus, the environmental chemicals that behave as agonists or antagonists to estrogen hormone may play a role in precocious puberty, polycystic ovary syndrome, delay in menopause, and premature ovarian failure [44]. Increased growth of the endometrium and breast cancer are some of the unwanted side effects in females that can also occur due to EDCs exposure.

### 5.1 Puberty and breast development

The growing evidence of EDCs affecting puberty or early breast development in the female has dramatically increased. It has been observed from the studies that the age of menarche has been decreased from 16-17 years to less than 13 years [5]. The increased estrogen-to-androgen ratio due to overexpression of aromatase activity has been shown to cause early or premature breast development and sometimes gynecomastia in boys [45, 46].

### 5.2 Breast cancer

Breast cancer is a multifactorial disease [47] and mainly results from time-related complex interactions between internal and external factors [48]. Although endogenous estrogen has a role in breast tumor genesis, but estrogen-mimicking exogenous EDCs such as PCBs, BPA, DES, and phthalates have substantial impacts on breast development during the perinatal period and also on carcinogenesis in adults [48]. Studies have reported that women with prenatal exposure to DES, a synthetic estrogen, have an increased risk of breast cancer in their later age (≥40 years) [49]. Perinatal BPA exposure at environmentally relevant concentration alters breast development in both outbred mice and rats. The mode of action of estrogen-mimicking EDCs is two-fold; firstly, their action is on the proliferation of stromal cells and, secondly, concerned with epigenetic mechanisms [50]. EDCs affect the stromal cells by interfering with the estrogen signal pathway. HOXB9 is a homeobox-containing gene that plays a vital role in mammary gland development and is associated with breast cancer. Reports have shown that BPA competes with ER and leads to activation of this gene through histone modification and acetylation [50].

### 5.3 Uterine disease

Endometriosis and uterine fibroids are the most common female reproductive disorders, having an estimated combined incidence of up to 70% of women. Due to their cryptic nature, many women with either endometriosis or fibroids may remain asymptomatic or undiagnosed and are more likely caused due to environmental endocrine-active compounds. For instance, non-human primates exposed to environmental contaminant TCDD (dioxin) have a higher endometriosis rate. The onset of fibroids occurs mainly after puberty, and this benign uterine tumor regresses after menopause. Fibroids are found to be more sensitive to the estrogen effect [51]. Hence, due to its dependency on estrogen for its growth, the role of environmental estrogen-mimicking EDCs in fibroid disease should be considered.

Several reports have documented that developmental exposure to EDCs such as MTX, PCBs, DDT, DES have been implicated in the development of uterine fibroid disease [52].

### 5.4 Primary ovarian failure (POF)

About 1% of the female population under 40 years of age suffers from POF, leading to other comorbidities related to reproductive disorder or early menopause [53]. The possible mechanism of POF development includes premature activation of the follicle, blockage of follicle maturation, and acceleration of apoptosis. Several cases of POF have been reported, and EDCs might have some association with its occurrence. Many EDCs are related to multi-oocyte follicles (MOF) mediated by EDCs-induced ERβ agonist action. Administration of BPA (0.1-1,000 μg/kg) to pregnant mice between the critical periods of differentiation (9th-16th day), ovarian cysts appeared in adulthood, which was significantly more in number in the group that received 1 μg/kg BPA [54]. Paraben is another example of EDC that affects folliculogenesis by stimulating anti-mullerian hormone (AMH) mRNA expression and inhibits the early stage of ovarian follicle in newborn rats [44]. Similarly, MTX inhibits folliculogenesis and increases AMH expression in the pre-antral and early-antral follicles [55]. Recent studies have demonstrated that neonatal exposure of DES (3 μg/kg) induces MOF [56].

### 5.5 Menstrual irregularity

Studies have shown that fetal and neonatal exposure to EDCs exposure in humans may interfere with hormonal regulation and result in irregular or long cycles of the menstrual cycle [57]. The irregularities in the menstrual cycle may ultimately reduce fecundity. In-utero exposure to estrogenic compounds such as phytoestrogens or BPA in an animal model such as adult mice increases estrous cycle duration. In comparison, perinatal exposure to BPA results in early suspension and irregular cyclic activity [58] that are likely due to a change in LH secretion's hypothalamic control and ovulation [59].

### 5.6 Polycystic ovarian syndrome (PCOS)

This disease is a more prevalent endocrine disorder in women, characterized by anovulation and hyperandrogenism. This syndrome is associated with a higher

| Reference | Chemicals | Organism | Results |
|---|---|---|---|
| [5] | EDCs | Human | Decreased age of menarche |
| [54] | BPA | Mice | Induces PCOS |
| [44] | Paraben | Rats | Inhibit early stage of ovarian follicle |
| [55] | MTX | Rats | Increase the expression of AMH in the pre-antral and early-antral follicles |
| [56] | DES | Mice | Induces MOF |
| [57] | PBD, PCB | Human | Irregular menstrual cycle |
| [58] | BPA | Human | Irregular menstrual cycle |
| [60] | BPA | Human | Development of PCOS |

**Table 1.**
*A summary of the remarkable studies on effects of EDCs on female reproductive systems.*

prevalence of obesity, insulin resistance, and other metabolic comorbidities. However, this disease's pathogenesis is still not exact, but evidence shows that genetic and environmental factors such as EDCs may contribute to PCOS's clinical development. BPA, a well-known estrogenic and androgenic endocrine disruptor, acts differently to interfere in reproductive functions. In vitro studies have shown that BPA exposure in rat ovarian thecal interstitial cells increases testosterone synthesis.

In contrast, in male rats, BPA competes with androgens to bind on sex hormone-binding globulin (SHBG), increasing serum-free androgen level. BPA exposure during neonatal conditions could lead to PCOS development. Besides, the estrogenic effect of environmental contaminant BPA enhances the risk of hypertension, type2 diabetes, and dyslipidemia (**Table 1**) [60].

## 6. Effect of EDCs on male reproductive system

A large number of studies reported the toxic effects of environmental contaminants on male reproductive health. Numerous EDCs present in our environment may have a causative role in testicular dysgenesis syndrome (TDS) in humans. Impaired spermatogenesis, decrease in semen quality, sperm anomalies, hypospadias, ectopic testes, cryptorchidism, and testicular cancer are important risk factors responsible for the symptoms of developmental disorder, TDS and ultimately causing male infertility. During the normal condition, a functional hormonal feedback loop regulates and ensures proper homeostasis. Sometimes, at the time of sexual development, exposure to certain chemicals may disrupt this tightly-regulated hormonal balance. Even short-term exposures can also have adverse effects and may cause infertility [61].

### 6.1 Semen and sperm quality

Semen parameters are used to measure sperm quality and are sometimes considered indicators of compromised male fertility [62]. Recently, the adverse health effects of the male reproductive system, especially due to BPA's estrogenic property, have attracted much more attention. BPA is well-known as a testicular toxicant in animal models as it results in decreased sperm quality and motility, oxidative stress increases, and alters steroidogenesis [42]. Few studies were done in occupationally exposed men, and infertile men have also reported a negative correlation between semen quality and urinary BPA levels. The underlying mechanism may be related to increased oxidative stress and disruption in the steroidogenesis pathway [63].

Functional anomalies of sperm may play a crucial role in male infertility. As reported, in comparison with fertile controls, infertile men have higher seminal reactive oxygen species (ROS) levels; hence studies have suggested ROS might have some role in male infertility. Sperm cells do not contain any cytoplasmic defense enzymes that can serve as ROS scavengers. Therefore, a change in lipid peroxidation (LPO) impairs the plasma membrane fluidity and integrity and ultimately leading to loss of sperm function and movement. Exposure to carbaryl causes low LPO concentrations with an increase in ROS and hence may be associated with altered semen quality, especially sperm motility [64].

Furthermore, BPA was also associated with DNA damage, lower sperm count, abnormal sperm morphology, and motion [65]. Regarding the hormonal level, BPA caused higher FSH, lower inhibin B level, and a lower estradiol-to-testosterone ratio [65]. In a study comprising 375 fertile men, BPA was further associated with decreased free androgen index and higher testosterone level [66].

| Reference | Chemicals | Organism | Results |
|---|---|---|---|
| [42] | BPA | Human | Decreased sperm quality and motility, altered steroidogenesis |
| [63] | BPA | Rat | Disrupted steroidogenic pathway |
| [64] | Carbaryl/naphthalene and chlorpyrifos | Human | Altered semen quality, sperm motility |
| [65] | BPA | Human | Lower sperm count, abnormal sperm morphology, higher FSH, lower inhibin B level, lower estradiol-to-testosterone ratio |
| [67] | Dioxin | Human | Higher percentage of oligospermia and abnormal sperm morphology |
| [10] | Phthalate, Vinclozolin, PBDE | Human | TDS |

**Table 2.**
*A summary of the remarkable studies on effects of EDCs on male reproductive systems.*

Mixtures of polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), shortly named 'dioxins', produce adverse health effects reported in several studies. For instance, A study have reported a potential association between chemical dioxins exposure and semen quality. Exposed men have a higher percentage of oligospermia and abnormal sperm morphology [67].

### 6.2 Testicular cancer, cryptorchidism and hypospadias

Testicular cancer is the most common disorder found in most young men in many countries. A worldwide trend can be observed towards the increasing number of testicular cancers. Exposure to environmental contaminants such as phthalates causes low semen quality, which ultimately may coincide with the development of cryptorchidism, testicular cancer, and hypospadias. The combination of the three aforementioned disorders of the male reproductive system can be termed as "so-called" testicular TDS. Experimental and epidemiological evidence has demonstrated that TDS is an outcome of interference during embryonal physiological programming and gonadal development in fetal life [68, 69]. EDCs such as phthalates, vinclozolin, polybrominated diphenyl ethers (PBDE), and acetaminophen have shown a significant etiopathogenetic role towards the onset of TDS [10]. Despite all these studies, the exact contribution and mechanism behind pesticide and EDC exposure towards testicular cancer development have not been elucidated to date. However, genetic and environmental factors have been documented for the reduced sperm count and disease formation (**Table 2**) [70].

## 7. Effect of EDCs on fetal and neonatal stages

Although adult exposure towards EDCs is an essential factor, the duration of EDCs exposure in fetus and neonate is also of primary concern. It plays a crucial factor in determining its fate. For instance, Due to early EDCs exposure, development is compromised because EDCs at the neonatal stage are extremely sensitive and affect the same brain regions, circuits, hormone-sensitive pathways, and receptors like the endogenous hormones. Such effects have been reported for several EDCs across various species and have profound detrimental consequences in developing organisms compared to adults [71, 72]. The extreme sensitivity of

developing fetus and neonate has been described briefly in a chapter titled "The Fragile Fetus" by Howard Bern [73]. All those protective mechanisms present in adults like DNA Repair Mechanism, detoxifying enzymes, liver metabolism, competent immune system, and the blood–brain barrier are not fully developed or functional during the fetus or neonatal stage. Even the metabolic rate of developing organisms is much higher than adults, which may be the cause of increased toxicity due to environmental contaminants exposures. Numerous reports have documented that developmental exposure to EDCs can lead to various adverse effects in adults and sometimes cause tumors in endocrine tissues and adverse reproductive consequences in males and females [74]. Pieces of evidence have stated that exposure to environmental triggers such as EDCs during critical stages in fetal sex differentiation and development in utero disrupts reproductive organ differentiation and sometimes leading to intersex variation (IV) conditions. IV can be defined as a morphological and physiological anomaly where an individual is born with a congenital condition such as ambiguous genitalia/ hermaphrodite or pseudohermaphroditism etc. [75]. The most evident case for endocrine disruption in utero that may lead to the onset of adult disease in the newborn is prenatal exposure to DES. Between 1958 and 1976, doctors prescribed synthetic estrogen to pregnant women to prevent miscarriages and premature delivery. It was almost a 4-6million pregnancies that were treated with DES in the US alone. In 1971, DES was linked with a rare gynecologic neoplasm in female offspring of DES-exposed pregnancies [76]. Subsequent studies have documented the link between maternal treatment with DES and cervicovaginal cancer in DES-exposed daughters, usually in their late teens or early 20s. This was the first evidence of transplacental carcinogenesis in humans [51]. Additionally, the offspring of DES-exposed mothers also had functional and anatomical abnormalities of the uterus and fallopian tubes.

## 8. Effect of EDCs on epigenetic regulations

The epigenetic modifications can be defined as "heritable and reversible chemical modifications of chromatin, resulting in an adjustment of its activity without a change in the underlying DNA sequence [77]. Notably, epigenetic effects are mediated by those transcription factors that enhance or repress any specific genes' transcription. The well-studied epigenetic modifications include DNA methylation at the cytosine base, post-translational modification of histone proteins (histone acetylation and deacetylation), and non-coding RNAs. Post-translational modification of histone protein at specific amino acids, for instance, lysine, may alter chromatin's structure and function. Generally, acetylation of histone at lysine position results in activation of transcription by relaxing the chromatin structure, while methylation of lysine depending on the position may lead to activation or repression of gene expression. However, deacetylation may lead to transcriptional repression or silencing of the genes. Non-coding RNA is the transcript of gene sequences that do not code for proteins but regulate its expression in a cis or trans manner mainly involved in some unique functions such as genomic imprinting, X-chromosome inactivation, and developmental patterning and differentiation [70].

The most commonly studied epigenetic mechanism is the EDCs effect on the enzymes that regulate epigenetic patterning, especially the DNA methyl transferase (DNMTs). The endocrine disruptor, Vincolozolin, an androgen receptor antagonist, induced increased expression of dnmt mRNA expression in an in-vivo model through an AR-mediated pathway [78]. Studies have reported that DES

can activate immediately early genes such as c-myc, c-jun, c-fos, and lactoferrin, which are upregulated during childhood [53]. This activation is possible due to the promoter region's hypomethylation of the lactoferrin gene in the adult uterus [79]; however, no such patterns were observed when an adult was exposed during adulthood at the same interval. Upon prenatal exposure to DES, tet1 mRNA expression was significantly decreased in mouse uterus and the same response found in zebrafish gonads upon BPA exposure [21, 80, 81]. Exposure to EDCs during development could alter the perturbation of the genome's epigenetic patterning and may result in adult-onset disease. The epigenetic changes in the ovary have been reported for the organochlorine pesticide MTX. In a study, MTX exposure from embryonic to postnatal days caused hypermethylation in the ERβ promoter regions by performing DNA methylation analysis using bisulfite sequencing and methylation-specific PCR [82].

## 9. Conclusion

Research over the last few years exploited the adverse effects of EDCs, which were lesser-known. Some of them were heavily used in the past decade, and some are just enlisted their names on the list of 'emerging pollutants,' and altogether, they are creating an unhealthy world to live on for us as well as for other animals. This chapter summarizes the harmful effects of EDCs exposure on male and female reproductive physiology and also elucidates the possible mechanism of actions. Though numerous articles and reports regarding these EDCs' toxicological effects are available today, still very little is known about their mechanism of action in our body. The poor understanding of their underlying mechanism in hampering one's endocrine system keeps us at bay to fight against it. Be it your morning toothpaste or your hair nourishing shampoo, and you cannot escape from the exposure of EDCs in your daily life. Even processed foods have EDCs in them. Scientists predict that there are many more of this kind of chemical whose effects are yet to be evaluated. Many countries' government bodies are actively monitoring the situations and taking actions accordingly alongside the scientists who are tirelessly working to find out ways to nullify the adverse effects. We can follow the recent resources available in the public domains and choose a healthy way to live to minimize the EDCs exposure. Extra care should be taken in choosing your packaged food materials, cosmetics, plastic made equipment, cooking utensils, fruits, and vegetables. Finally, emphasis should be on the betterment of regulatory systems for introducing new, untested chemicals alongside the continuous use of chemicals already proved for being EDCs. Utmost care should be taken for pregnant women and infants, who are most vulnerable to EDCs exposure.

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## Conflict of interest

The author declares that there is no conflict of interest.

## Author contributions

All authors listed have made a substantial contribution in this chapter. And special thanks to AM for making the required illustrations for this chapter. Thanks to RKS for reviewing the manuscript before the final submission.





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