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Chapter 6

Environmental Effects of Endocrine-Disrupting Chemicals: A Special Focus on Phthalates and Bisphenol A

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Additional information is available at the end of the chapter

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Abstract

Several environmental chemicals are classified as endocrine-disrupting chemicals (EDCs). Many of them have an impact on reproductive functions and sex hormones because of their estrogenic and/or antiandrogenic properties. Phthalates and bisphenol A (BPA) are two well-known EDCs. They are abundant in the environment. Phthalates are usually classified as antiandrogens, whereas BPA is considered as estrogen-like EDC and xenoestrogen. Other than their endocrine-disrupting effects, these two chemicals are also known to have genotoxic and epigenetic effects. Besides, they are hepatotoxic and have substantial effects on other organs/systems (thyroid, kidney, neuroendocrine system, immune system, etc.). In this chapter, we will mainly focus on the toxic effects of different phthalate esters and BPA by discussing their availability in the environment, mechanism and mode of actions, their biotransformation and reproductive effects, and their effects on other systems (hepatic, renal, etc.). Besides, we discuss epidemiological studies that are conducted to reveal their effects on the reproductive and endocrine systems. This chapter provides the readers a compact piece of knowledge on these abundant substances and helps them to understand the action of these substances at the molecular and cellular levels.

Keywords: endocrine-disrupting chemical, antiandrogen, xenoestrogen, phthalate, bisphenol A

1. Introduction

Exposure to environmental chemicals, particularly in early life, is among the substantial risks for developmental programming of different diseases in adult life of humans. In a report by

World Health Organization (WHO), it was estimated that more than 13 million deaths were caused by environmental exposures each year. Moreover, this report also proposed that nearly one third of mortality and morbidity can be due to environmental causes in underdeveloped or developing countries [1].

Many environmental exposures to different chemical, physical, or biological agents can interact with genetic and epigenetic mechanisms and affect the normal growth and development. Among those exposures, endocrine-disrupting chemicals (EDCs) are of particular concern, as humans are abundantly exposed to these chemicals by various means in every period of life. According to the U.S. Environmental Protection Agency (EPA), an EDC was defined as "an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural bloodborne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process" [2]. Several well-known environmental chemicals are classified as EDCs. Many of them act on reproductive functions because of their estrogenic and/or antiandrogenic properties.

In the present chapter, we will mainly focus on the toxic effects of different phthalate esters and bisphenol A (BPA), which are the most abundant environmental chemicals. We will discuss their availability in the environment, mechanism and mode of actions, biotransformation, and effects on reproductive systems and other organs (hepatic, renal, etc.) in different periods of life. Besides, we will address the epidemiological studies that are conducted on these chemicals.

2. Availability of endocrine disrupting chemicals in the environment

EDCs are available in polyvinyl chloride (PVC) plastics, polycarbonate materials (type 7 plastics), epoxy resins, medical devices [intravenous (i.v.) bags, dialysis bags, surgical implants, dental fillings sealants], pharmaceuticals (enteric coatings of pharmaceutical pills and nutritional supplements), consumer products (make-up products, fragrances, nail polish, lotions, creams, baby products, soaps, liquid soaps shampoos, conditioners, hair sprays), children's toys, children products (modeling clay, waxes, paints), printing inks, paints, household products (detergents, softeners, surfactants), construction materials (including floorings and PVC windows, wood floor finishes, cements, caulking in buildings), insulating fluids (transformer oils) for transformers and capacitors, lubricating oils, stabilizing additives in flexible PVC coatings of electrical cables and electronic components, textiles (footwear, raincoats, picture printed shirts), vacuum pump fluids, pesticides (insecticides), and flame retardants and, most importantly, in food (packaging materials and in the inner lining of food cans) [3–5].

3. Classification of EDCs

From a toxicological perspective, EDCs can be classified according to their sources or their modes of action.

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In the first classification, EDCs can be grouped as [6, 7]:

a. natural (e.g., phytoestrogen: genistein and coumestrol) and

b. synthetic [e.g., phthalates, BPA, polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs), dioxins; dichlorodiphenyltrichloroethane (DDT), vinclozolin, and diethylstilbestrol (DES)] compounds.

In the second classifications, EDCs can be classified as [8, 9]:

a. EDCs that effect reproductive system

b. EDCs that affect pancreas

c. EDCs that affect thyroid

d. EDCs that effect Central nervous sytem

e. EDCs that affect other systems

The signifacnt effects of EDCs on hormones are suggested to be:

a. Increasing or decreasing effect on the production of hormones (these substances may mimic naturally occurring hormones such as estrogens, androgens, or thyroid hormones or they may potentially cause the overstimulation of hormonal pathways within the body),

b. Increasing or decreasing effect on the transportation of hormones,

c. Increasing or decreasing effect on the metabolism of hormones,

d. Increasing or decreasing effect on the elimination of hormones,

e. Agonistic or antagonistic effect on the target cells of the hormones (by binding to a receptor within a cell and blocking the functions of endogenous hormones; i.e., acting as antiestrogens and antiandrogens), and

f. Altering the homeostatic systems of the organisms and causing their miscommunication or irresponsiveness to their own physiology and the environment.

4. Modes/mechanisms of actions of EDCs

a. Effect on hormone, nuclear, and nonnuclear receptors: Our understanding of the mechanisms by which EDCs exert their effect has grown. EDCs were originally thought to exert actions primarily through nuclear hormone receptors [i.e., estrogen receptors (ERs), androgen receptors (ARs), progesterone receptors, thyroid receptors (TRs), and retinoid receptors]. However, recent basic and mechanistic researches show that the underlying mechanisms of their toxicity are much wider than originally envisioned. Thus, other than nuclear receptors, EDCs may also act via nonnuclear steroid hormone receptors (e.g., membrane ERs), nonsteroid receptors (e.g., neurotransmitter receptors such as serotonin

receptor, dopamine receptor, and norepinephrine receptor), and orphan receptors [e.g., aryl hydrocarbon receptor (AhR)] [9–11].

b. Effect on enzymatic pathways: These chemicals can affect the enzymatic pathways that have substantial roles in steroid biosynthesis and/or biotransformation and several other mechanisms that militate sex-specific physiology/behavior and endocrine and reproductive systems. They can disrupt and inhibit the action of enzymes involved in steroidogenesis, particularly in the metabolism of estrogens. For instance, some PCB metabolites can inhibit sulfotransferase, resulting in an increase of circulating estradiol [12–14].

c. Effects on signaling pathways: EDCs can regulate many cellular signaling pathways. For instances, both BPA and phthalates were shown to induce epithelial-to-mesenchymal transition (EMT). These chemicals can also down-regulate or up-regulate the genes involved in the regulation of signal transduction [15, 16].

5. Toxicity mechanisms of EDCs

There are a number of toxicity mechanisms/pathways that are suggested to be caused by estrogenic and antiandrogenic EDCs [17, 18]. These mechanisms are associated with but are not limited to:

1. Genotoxicity,
2. Epigenetic mechanisms (hormonal dysregulation, peroxisome proliferation, cytotoxicity, oxidative stress, DNA methylation, histone modification, RNA interfering, apoptosis, and imprinting), and
3. Other mechanisms.

The effects of EDCs on cellular metabolism or functions, cellular organelles (peroxisomes, mitochondria, cytoskeleton), DNA damage, chromosomal aberrations, cell cycle checkpoints, translational control, cell death (apoptosis, autophagy, necrosis), immunology/inflammation response, neurological pathways, and development/differentiation are now being studied extensively. However, their effects were also shown to extend beyond these mechanisms/pathways and may include multiple functions, tissues, and organs such as the liver, kidney, and spleen. Thus, they may have broader impacts—most of them yet to be identified—on disrupting signaling webs and cellular communication [17, 18].

6. Phthalates

The word "plastic" originates from the Greek word "plasticos," which expresses "a material's being capable of molding into different shapes." The first plasticizer was synthesized in 1860. By the progression of technology and increase in the global population growth, plastic materials are now widely used and have very different application fields [19]. Phthalates are dialkyl or alkyl aryl esters of phthalic acid and are abundantly used to make plastic materials

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more flexible. They are synthesized by reacting phthalic anhydride with alcohol(s) [methanol and ethanol (C1/C2) up to tridecyl alcohol (C13)] either as a straight chain or with some branching. Their main use is for the softening of rigid plastics and polymers. Di(2-ethylhexyl)phthalate (DEHP), the most abundant phthalate derivative, is used 1% to 40% in plastics by weight [20]. Almost 90% of DEHP is used to soften PVC plastics, and in the European Union, 95% of DEHP is used in polymer products as a plasticizer [21].

Phthalates were first synthesized in the 1930s. DEHP was first synthesized in 1933 in Japan and in 1939 in United States in commercial quantities. DEHP was first used in 1949 in United States and has been the most abundantly used phthalate derivative in the Twentieth century. In 1999, the consumption of phthalates were 3.25 million tons and DEHP accounted for 2.1 million tons of the total production. The European Commission reported that 1 million tons of DEHP were used in 2000 [22].

6.1. Occurrence, uses, and exposure to phthalates

Phthalates migrate out PVC-containing items into food, air, dust, water, and soils and cause human exposure in various ways [23]. Several studies were conducted in different parts of the world, and human blood and urine (mostly spot urine samples) were used as biological fluids to evaluate the exposure to phthalates. The results of these studies revealed that humans are ubiquitously exposed to different phthalates, mostly in industrialized countries [24–27]. On the contrary, workplace inhalation is also of concern as phthalates; particularly, DEHP has low vapor pressure [28].

Diet is the main source of phthalate exposure in the general population. Particularly, fatty food (e.g., fish and oils) can cause high phthalate exposure if contaminated [29, 30]. One other major source is medical exposure by blood storage bags and blood transfusion equipment during receiving blood transfusion [31, 32] or hemodialysis (dialysis bags) [33, 34].

Based on the number of carbon atoms in their alcohol chain, phthalates are divided into two distinct groups, with very different applications, toxicological properties, and classification: high molecular weight (MW) phthalates and low MW phthalates. Their use largely depends on their MW. Higher MW phthalates, such as DEHP, are used in construction materials and in numerous PVC products, including clothing, food and beverage packaging, children products (toys, grip bumpers), and biomedical equipment (e.g., blood transfusion bags, dialysis bags, and umbilical catheterization devices), whereas relatively lower MW phthalates such as dimethyl phthalate (DMP), diethyl phthalate (DEP), and dibutyl phthalate (DBP), are mainly used as odor/color fixatives or as solvents and in cosmetics, textiles, and pharmaceuticals [35].

DEHP has a very low degree of acute toxicity, with oral lethal dose 50 (LD_{50}) values ranging from 26 to >34 g/kg in a variety of species. In a study by Lawrence et al. [36], the lethal effect of this compound appeared to be cumulative, because the LD_{50} value for intraperitoneal (i.p.) administration to mice five times weekly for 10 weeks was 1.36 g/kg in comparison to a single-dose value of 37.8 g/kg. Autian [37] concluded that this was because biotransformation was required before DEHP produces toxic effects. In rats, lethal concentration 50 (LC_{50}) by inhala-

tion (1 h) was found to be >23.670 mg/m^3 (1457 ppm) and inhalation LC_{50} (6 h) value was >600 mg/m^3 (37 ppm) [37]. The oral administration LD_{50} value for rats (Wistar, male) was suggested to be between 26.000 and 34.000 mg/kg body weight and the LD_{50} value for i.p. administration in rats was found to be between 30.600 to 49.000 mg/kg body weight [38].

6.1. Biotransformation of phthalates

Phthalates are not covalently bound to plastic products and therefore may leak out to contaminate blood or food products and can be ingested. When administered orally to humans and rodents, phthalates are rapidly hydrolyzed by esterases in the gut and other tissues to produce the corresponding active monoesters and their further oxidized metabolites. During phase I biotransformation, the relatively polar and low MW phthalates (e.g., DEP) are primarily metabolized to their hydrolytic monoesters by hydrolysis of one of the ester bonds. In contrast, the high MW phthalates are first metabolized to their respective hydrolytic monoesters and then, after enzymatic oxidation of the alkyl chain, to more hydrophilic, oxidative metabolites [39]. For example, DEHP is metabolized to its monoester metabolite, mono-2-ethylhexyl phthalate (MEHP), which is more toxic than the parent compound. MEHP is further metabolized to secondary oxidative metabolites [40]:

- Mono-(2-ethyl-5-hydroxyhexyl)phthalate (5OH-MEHP, MEHHP), which is then metabolized to mono-(2-ethyl-5- oxohexyl) phthalate (MEOHP, 5oxo-MEHP). MEHP can also be metabolized to different structural isomers of MEHHP, which also have further metabolism to different monoethylphthalate structures.
- Mono-(2-ethyl-5-carboxypentyl)phthalate (5cx-MEPP)
- Mono-[2-(carboxymethyl)hexyl]phthalate (2cx-MMHP)

Some phthalates are subject to phase II (particularly to glucuronidation and, to a lesser extent, sulfation) metabolic reactions. Glucuronidation not only provided higher urinary excretion of phthalate metabolites but also can reduce their biological activity. Monoesters and the oxidative metabolites of phthalates are excreted in urine (95%) and, to a very lesser extent, eliminated by feces (5%) [39].

6.2. Genotoxic effects of phthalates

The biological effects of phthalates are of major concern but so far elusive. Phthalates are shown to cause cytogenetic damage to animals and humans. In 1980s, phthalates were evaluated as epigenetic carcinogens because of their peroxisome proliferative effects. However, in the 1990s and in the Twenty first century, several studies confirmed their genotoxic effects [41–43].

Chromosomal aberration test, unscheduled DNA synthesis (UDS), Ames test, micronucleus test, and hypoxanthine guanine phosphoribosyltransferase (HPRT) mutation test were applied to evaluate the genotoxic potentials of phthalates. DEHP was shown to induce single chromatid aberrations and sister chromatid exchange (SCE) in human lymphocytes [44]. Besides, DEHP caused lymphatic mitotic inhibition after 4 h of exposure and caused an increase in the doubling time of human lymphocytes [44]. Later, Stenchever et al. [45] reported that DEHP

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caused chromosomal breaks in human lymphocytes; mitotic rate decreased and DEHP caused polyploidy and aneuploidy in human fetal lung cells.

Phillips et al. [46] reported that, in Chinese hamster ovary (CHO) cells, MEHP caused chromosome damage without affecting in the SCE and HPRT mutation test. However, after DEHP treatment in rat hepatocytes, Astill et al. [47] did not find a change in Ames test, mouse lymphoma activation assay, micronucleus test, UDS, and cell transformation tests. A study performed on both CHO cells and RL4 liver cells showed that MEHP caused chromosomal aberrations. However, S-9 mix (a mix of cytosolic and microsomal drug-metabolizing enzymes and cofactors) had no effect on the chromosome damage produced by MEHP in CHO cells [48].

A study using mouse hepatocytes evaluated the genotoxicity of these compounds (i.e., DNA repair or UDS). No changes were observed in DNA repair capacity. However, UDS of the hepatocytes obtained from mice treated with DEHP containing diet showed significantly higher UDS compared to control cells [49]. Lindahl-Kiessling et al. [50] showed that DEHP induced SCE in human lymphocytes, which were co-cultured with rat liver cells. Müller-Tegethoff et al. [51] observed that peroxisome proliferators (nafenopin, Wy-14,643) and DEHP did not induce any changes in micronucleus test in rat hepatocytes. Kim et al. [52] showed that DBP caused both chromatid and chromosomal type chromosomal aberrations (break and exchange) in the lymphocytes of B6C3F1 mice and this aberrations showed marked increases and these alterations show marked elevations dependent on the time of exposure. McKee et al. [53] reported that di(isononyl) phthalate (DINP) was not found to be mutagenic in Ames test, *in vitro* cytogenetic assay, and mouse micronucleus assay. Using Ames test, Lee and Lee [54] observed that the phthalic acid and terephthalic acid did not produce any mutagenic responses in the absence or presence of S9 mix on the *Salmonella typhimurium* strains in Ames test. Besides, phthalic acid and terephthalic acid did not show any significant cytogenetic effect on CHO cells in the chromosomal aberration test and in the mouse micronucleus test [54].

Many studies are performed on the genotoxicity of phthalates using Comet assay in the last 30 years. Anderson et al. [55] showed that both DEHP and its major metabolite MEHP induced DNA damage in human leukocytes as evidenced by increases in tail moment in Comet assay. Kleinsasser et al. [56] compared susceptibilities to DBP and di-iso-butyl-phthalate (DiBP) in nontumor patients to those in patients with squamous cell cancer (SCC) of the oropharynx or larynx using Comet assay and indicated that DBP and DiBP produced significant differences in the Olive tail moment (OTM) between oropharynx (TO), larynx (TL), and TO plus TL groups and the nontumor donors. The same researchers also determined the correlation between the genotoxic sensitivities to DBP and its isomer DiBP in mucosal epithelial cells or lymphocytes using Comet assay, and both phthalates showed significant genotoxicity on both cells and lymphocytes where the genotoxic effect of DiBP was higher than DBP in both cell types [57]. Biscardi et al. [58] reported that DEHP can leach out of polyethylene terephthalate (PET) bottles in time, especially after almost 10 months of storage, and this phenomenon can cause increases in both total tail length and number of cells in human leukocytes *in vitro*.

DEHP, BPA, nonylphenol, and paraquat dichloride were tested for their genotoxicity potentials on HeLa cells. DEHP showed genotoxicity (>90 μM) with significant increases in tail moment [59]. In a recent study performed on HepG2 cells exposed to various concentrations

of DEHP for 24 or 48 h, DNA damage increased significantly in a dose-dependent manner [60]. Throughout our studies on DEHP and MEHP, we determined that both DEHP and MEHP were both cytotoxic and genotoxic in LNCaP cells (human prostate cancer cell line) and Leydig cells (mouse Leydig carcinoma cell line). We also observed that selenium supplementation in either organic form (selenomethionine at 10 μM) or inorganic form (sodium selenite at 30 nM) was highly protective against the cytotoxicity and genotoxicity exerted by these particular phthalate derivatives [42, 43].

The correlation between urinary phthalate levels and sperm DNA damage is being investigated by several studies. Duty et al. [61] analyzed semen and urine samples of 141 subjects were for five phthalate metabolites using neutral Comet assay and DNA was only correlated with monoethyl phthalate (MEP) levels, although there was no correlation with other phthalate metabolites [monobenzyl phthalate (MBzP), mono-*n*-butyl phthalate (MBP), MEHP, and monomethyl phthalate (MMP)]. Hauser et al. [62] determined the urinary concentrations of phthalate metabolites among men (n=379) who were admitted to an infertility clinic. Sperm DNA damage was associated with MEP and MEHP. Recently, Ahbab et al. [63] determined the possible genotoxicity of two different phthalate esters, namely, di-*n*-hexyl phthalate (DHP) and dicyclohexyl phthalate (DCHP), at different concentrations using Comet assay in male rat pups from gestational day (GD) 6 to GD19 at different doses [0 (vehicle), 20, 100, and 500 mg/kg/day]. Male rats were allowed to grow until different ages (prepubertal, pubertal, and adulthood). The Comet assay was performed on blood lymphocytes and testes samples of adult male rats and the results showed that DHP significantly induced genotoxicity at doses of 100 and 500 mg/kg/day versus control; however, DCHP did not show the same effect [63].

6.3. Epigenetic effects of phthalates

The results of many studies strongly point out that that EDC exposure can caused by epigenetic mechanisms, which can lead to cumulative adverse effects on future generations. The epigenetic marks can induce up/down alterations in gene expression that may persist throughout a lifetime. These permanent changes will result in adverse health effects, such as neural and immune disorders, infertility, and late-onset complex diseases (cancers and diabetes) [64].

Phthalates are long suggested to be epigenetic carcinogens because of their peroxisome proliferator effects. The *in utero* and neonatal exposure to phthalates [particularly to DEHP, MEHP, benz-butyl phthalate (BBP), DBP, and MBP] may cause methylation changes in DNA at CpG islands near gene promoter regions, different histone modifications (acetylation, methylation, phosphorylation, ubiquitination, sumoylation, and ADP ribosylation), and alterations in the expression of noncoding RNAs, including microRNAs (miRNAs) [65].

The treatment of human breast cancer MCF-7 cells with BBP led to the demethylation of ERα promoter-associated CpG islands, indicating that altered ERα mRNA expression by BBP can induce aberrant DNA methylation in the promoter region of this gene. Maternal exposure to DEHP was shown to induce DNA methylation and different DNA methyltransferase expressions in mouse testis. Fetal testis is suggested to be the main target for DEHP. DEHP can lead to testicular dysgenesis syndrome (TDS) due to a reduction in insulin-like hormone 3 (INSL3) expression and testosterone production [66]. During the period of embryonic sex

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determination, transient exposure to a plastic mixture (BPA and phthalates) of gestating female rats was shown to promote early-onset puberty transgenerationally (F_3 generation) and decrease the pool size of ovarian primordial follicles in female pups. On the contrary, in male pups, spermatogenic cell apoptosis was also affected transgenerationally, and differential DNA methylation of the F_3 generation sperm promoter regions was also observed [67].

6.4. Carcinogenicity of phthalates

Phthalates are well-known peroxisome proliferators that can alter gene and protein expressions. This capability may result in the promotion of hepatic carcinogenesis in rodents [68]. On the contrary, there are data in the literature that indicate that phthalates increase oxidative stress in the rodent liver even before peroxisomal oxidases are induced. In addition, Kupffer cells have been suggested to be a potential source of oxidants in rodent liver after treatment with DEHP [69, 70]. It appears that molecular events, which may be a consequence of increase oxidative stress, could interact with other pathways activated by peroxisome proliferation in rodent liver [69, 70]. Although several studies including the studies by our group (in the last decade) pointed out that DEHP can induce reactive oxygen species (ROS) production and lead to increased cellular oxidative stress both *in vivo* and *in vitro*, there are no convincing data to prove whether the induction of ROS production is a one of the major pathways or whether ROS elimination is not efficiently achieved after a series of molecular events induced by phthalates, particularly by DEHP [42, 43, 70, 71].

In the 1980s, the hepatocarcinogenic effects of DEHP, due to its peroxisome proliferator effect, was shown by several studies. Back then, some concerns started rising about the safety of this substance. In 2000, the International Agency for Research on Cancer (IARC) classified DEHP as a Group III carcinogen (not classifiable as to its carcinogenicity to humans), as peroxisome proliferation has not been documented in human hepatocyte cultures exposed to DEHP nor in the liver of exposed nonhuman primates [71]. Therefore, the mechanism by which DEHP increases the incidence of hepatocellular tumors in rats and mice is not relevant to humans. However, later in 2013, DEHP was classified as a Group IIB carcinogen due to some concerns [72]. On the contrary, butyl benzyl phthalate (BBP) is classified as a Group III carcinogen in 1999 [73]. Blom et al. [74] showed that exposure to different phthalate esters, particularly to DBP, can lead to high proliferation of human breast cancer cell lines, which was explained in part by the potency of phthalates in terms of a "xenoestrogenic impact," although phthalates are usually classified as "antiandrogens" and not "xenoestrogens" [74, 75]. This effect is suggested to be related to a direct ER binding of some, but not all, phthalates [76, 77].

6.5. Reproductive toxicity of phthalates

Recent *in vivo* and *in vitro* studies on phthalates are mainly focusing on their reproductive toxicity potential. Phthalates were suggested to target mainly male reproductive system. The "TDS hypothesis" proposes that a proportion of the male reproductive disorders—cryptorchidism, hypospadias, decline infertility (or loss of fertility), and testicular cancer—may be symptoms of TDS, which is most likely a result of disturbed gonadal development in the embryo. There is a decline in male fertility and increase in the number of cases with TDS in

the last decades, and phthalates are suggested to be the major underlying factors. Several studies have shown that fetal exposures to DEHP or DBP induce TDS-like effects and reduce anogenital distance (AGD) in rodents [78].

In testis, Leydig and Sertoli cells are the main targets of phthalates. Many researchers observed that DEHP caused disruption in the function of both cell types. The administration of MEHP to Wistar rats at a single oral dose (400 mg/kg body weight) was toxic to Sertoli cells and caused detachment of germ cells [79]. In fact, Richburg and Boekelheide [80] demonstrated histopathological disturbances and alterations of cytoplasmic distribution of vimentin in Sertoli cells in testis of 28-day-old Fisher rats after a single oral dose of MEHP (2000 mg/kg). Tay et al. [81] also observed a correlation between the increase in TUNEL-positive cells and the vimentin disruption in treated mice. We also determined that DEHP exposure caused disruption and collapse of vimentin filaments and significantly induced apoptotic death of germ cells [82].

Exposure to phthalates, particularly to DEHP, resulted in decreased testicular testosterone production in rodents, and most of the reprotoxic effects are suggested to be related to their antiandrogenic potential [83, 84]. In our studies, we determined that DEHP caused abnormal sperm production, decreases in sperm count, and motility when administered to 10-week-old rats at 1000 ppm dose for 10 days. Moreover, we also observed that DEHP caused decreases in testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH) levels [84]. Moreover, DEHP induced oxidative stress in rat testis, as evidenced by the significant decrease in GSH/GSSG redox ratio, marked increase in lipid peroxidation, and a significant decrease in GPx4 activity [85].

6.6. Hepatotoxic effects of phthalates

DEHP and other phthalates, such as di-(2-ethylhexyl) adipate (DEHA) and DINP, are shown to be hepatocarcinogenic in both sexes in mice and rats. These substances were shown to cause both hepatocellular carcinomas and adenomas [86–88]. There are a number of molecular events that underlie the hepatocarcinogenic potential of these substances: Their genotoxicity, peroxisome proliferative property, and epigenetic effects are the most studied mechanisms. Collectively, it appears that, in rodent liver, oxidative stress-related molecular events could interact with other pathways that can be activated by peroxisome proliferation. Previously, we have also shown that DEHP caused peroxisome proliferation, alterations in antioxidant enzyme activities (decreases in glutathione peroxidase 1, glutathione peroxidase 4, superoxide dismutase, and glutathione *S*-transferase activities; increase in thioredoxin reductase activity), and liver enzymes when administered to 10-week-old rats at 1000 ppm dose for 10 days. Besides, DEHP caused cellular disorganization, increases in catalase activity/immunoreactivity, and lipid peroxidation [89].

6.7. Effects of phthalates on other organs/systems

Other than testis and liver, phthalates were suggested to be toxic to kidneys and thyroid [90, 91]. Moreover, phthalates were also shown to affect the neuroendocrine system and the hypothalamus-pituitary-ovarian/testicular axis in rats [92–94].

6.8. Epidemiological studies concerning the effects of phthalates on reproductive/endocrine systems

In the last five decades, a gradual decline in global semen quality has been reported [95]. The effects of phthalate exposure on male fertility has been attracting the attention of researchers for a long period of time, although phthalates exert serious health effects on different organs/systems. Semen quality defines the sperm count, motility, and morphology. An early study described sperm concentration and DBP measured in the seminal fluid of American students (N=21) recruited and these students were classified as low metabolizers (n=12) and high metabolizers (n= 9). Seminal DBP levels were associated with decreased sperm concentration, and an overall analysis indicated a positive association between DBP and sperm concentration [95]. Total semen phthalates were higher among 21 men with an infertility diagnosis compared to 32 men without (p<0.05) in a cross-sectional study of infertility clinic patients in India. Among the infertile men, seminal phthalates were associated with increased sperm abnormality but not with sperm count or motility [96]. Later, in a cross-sectional study of 234 Swedish military conscripts aged 18 to 21 years, Jönsson et al. [97] observed that urinary MEP concentrations were associated with decreased sperm motility. No associations were determined between semen quality and MBP, MBzP, or MEHP. A positive association between sperm motility and phthalic acid was found, and this finding suggested that an increased ability to metabolize phthalates may be protective. Besides, the researchers reported a cross-sectional association between urinary MEP concentrations and decreased LH levels when comparing the quartile of highest exposure to the lowest [97].

Among 52 Chinese men attending a reproduction clinic in Shanghai, no associations were detected for sperm count or morphology and semen concentrations of DEP, DEHP, and DBP [98]. The majority of studies of phthalate exposure and semen quality have been conducted among infertile populations. In 2006, an American study was conducted on 443 men undergoing infertility therapy. Higher urinary MBP levels were associated with decreased sperm concentration and motility after adjusting for age, abstinence time, and smoking status. No associations with semen quality were suggested for MEP, MMP, MEHP, MEOHP, or MEHHP [99].

Pant et al. [100] measured the phthalate diesters levels in the semen of 300 Indian men, and correlations were detected between DBP, DEHP, and decreased motility in unadjusted analyses. Unadjusted associations were also reported between DBP, DEHP, DEP, and decreased sperm concentration and for DEHP and increased abnormal morphology. No associations were reported for semen DMP or di-*n*-octyl phthalate (DnOP) levels and sperm parameters [100]. Wirth et al. [101] determined the urinary phthalate levels in 45 men who were admitted to an infertility clinic. Urinary MEP levels were associated with decreased sperm concentration (after adjusting for race and for urine specific gravity) and abnormal morphology (after adjusting for urine specific gravity). Urinary mono-3-carboxypropyl phthalate (MCPP) levels were also associated with an increased proportion of morphologically abnormal sperm, and an association was suggested for urinary MEHP with low sperm concentration. However, no associations were indicated for MMP, MBP, MiBP, MBzP, MEHP, MEOHP, and MEHHP [101].

Another study on 349 men who were recruited from a German andrology clinic reported no associations between urinary DEHP metabolites (MEHP, MEHHP, MEOHP, and 5cx-MEPP) and any semen parameters after adjusting for age, smoking, abstinence period, and urine creatinine [102]. In another cross-sectional study, the median semen DBP concentration was higher in oligoasthenospermic men (*n*=65) compared to fertile men (*n*=50), and median DEHP concentration was also higher in oligoasthenospermic men versus in fertile men. In addition, sperm motility was inversely associated with DBP and DEHP in oligoasthenospermic (*n*=65) and asthenospermic (*n*=65) men, respectively. However, the associations were not adjusted for potential confounding variables [103]. Another cross-sectional study recruited 97 men undergoing infertility treatment in China and found the top tertile of urinary MBP to be strongly associated with decreased sperm concentration after adjusting for age, abstinence time, body mass index (BMI), smoking, alcohol consumption, and education. There were no associations reported for sperm parameters and MMP, MEP, MBzP, MEHP, or MEOHP [104]. A more recent work suggested that urinary MBP levels were inversely associated with sperm concentrations in a general population sample of 232 men residing in a heavily industrialized urban area in China. However, no such associations were found for MEP (median=3.10 ng/mL) or MEHP (1.10 ng/mL) or for motility and morphology [105]. A Polish study on men (n=269) under 45 years of age and attending a infertility clinic measured urinary MEHP, MEHHP, monoisononyl phthalate (MiNP), MBzP, MBP, and MEP levels. Inverse associations were detected between sperm motility and log-transformed MEHP, MEHHP, and MiNP levels after adjusting for age, smoking, abstinence period, past diseases, and creatinine as confounding covariates. No adjusted associations were detected between sperm concentration and morphology and urinary MBzP and MEP levels [106].

There are several studies that were conducted to understand the relationship between sex or reproductive hormones and different phthalate derivatives. One cross-sectional study in China quantified exposure using urinary metabolites in occupationally exposed workers (*n*=74) and unexposed referent workers (*n*=63) matched by age and smoking. Decreases in free testosterone, but not FSH, were associated with MBP exposure in all the men after adjusting for age and alcohol consumption [107]. An earlier work performed on 295 men reported an association between an interquartile range (IQR) increase in urinary MBzP concentrations and decreased FSH levels and a nonsignificant association between an IQR increase in MEHP and decreased testosterone after adjusting for age, BMI, and time of specimen collection [108]. Furthermore, MBP was nonsignificantly associated with increased inhibin B after adjustment for covariates. The results of this study are in contradiction with the results obtained from a study that recruited 118 men seeking infertility treatment in China. Sex steroid hormones were assessed in association with urinary MEP, MBP, MBzP, MEHP, MEHHP, and MEOHP levels among 425 men. The molar sum of DEHP metabolites and percentage MEHP were assessed, and an IQR increase for MEHP was associated with decreased serum estradiol and testosterone levels after adjusting for age, BMI, smoking, and time of specimen collection. A significant association between percentage MEHP and an increase in the testosterone/estradiol ratio was also determined. Adjusted decreases in the free androgen index [FAI; describes the ratio of testosterone to sex hormone binding globulin (SHBG)] were also reported for MEHHP, MEOHP, and the sum of DEHP metabolites. No associations were reported between MBP or

MBzP and serum prolactin, FSH, or LH. However, no associations between concentrations of the other phthalate metabolites and concentrations of the other hormones, including testosterone, estradiol, FSH, inhibin B, and SHBG, were found [109]. Another study performed on 425 men recruited through a U.S. infertility clinic found limited inverse association between MEHP and FAI by the proportion of DEHP metabolites in the urine measured as MEHP (MEHP%), a phenotypic marker of less efficient metabolism of DEHP to its oxidized metabolites. Finally, the ratio of testosterone to estradiol was positively associated with MEHP and MEHP%, suggesting potential relationships with aromatase suppression [110]. In a cross-sectional study of 363 fertile men participating in a multicenter U.S. study, inverse associations were described for urine DEHP metabolites (MEHP, MEHHP, MEOHP, and MECPP) and FAI after adjusting for age, BMI, smoking, ethnicity, urine creatinine, and time of sample collection. A positive correlation was also found between MEHP and SHBG, and the FAI/LH ratio after adjustment for covariates. However, no associations were reported for FSH, estradiol, or LH [111]. A recent study compiled data from two investigations of phthalate exposure and reproductive hormones, combining men from the Massachusetts General Hospital and multicenter U.S. studies. In a combined total of 783 men, there were no associations with urinary concentrations of MEP, MBP, or MBzP and any reproductive hormone measured. However, metabolites of DEHP (MEHP, MEHHP, and MEOHP) were associated with decreased free testosterone and increased SHBG levels [112].

A study performed by our group recruited 40 newly diagnosed pubertal gynecomastia cases and 20 controls. Plasma DEHP and MEHP levels were found to be statistically significantly higher in the pubertal gynecomastia group compared to the control group. There was a statistically significant correlation between plasma DEHP and MEHP levels (r=0.58; P<0.001). In the pubertal gynecomastia group, no correlation was determined between plasma DEHP and MEHP levels and any of sex hormone levels [24]. However, as a part of the Copenhagen Puberty Study, Mieritz et al. [113] did not find any difference between the urinary phthalate levels of Danish boys (n=555) versus control.

7. Bisphenol A

BPA is used to harden plastics and to manufacture polycarbonate plastics and epoxy resins. It has high abundance in the environment and considered as estrogen-like EDC or as a xenoestrogen. It was shown that BPA acts similarly to 17-β estradiol [114, 115]. BPA induces ERs (weakly to ERα and ERβ and strongly to ERRγ) but in the concentrations approximately 1000 higher (10^{-6}–10^{-4} M) in comparison to estradiol [116]. BPA was classified as a weak environmental estrogen before. However, newer studies determined that, even in very low concentrations (picomolar and nanomolar), BPA exerted divergent effects on the physiology of different cells and tissues and can bind to both nuclear and nonnuclear receptors [117]. Furthermore, most of BPA metabolites were shown to exert stronger estrogenic activities than the main compound [118]. Apart from the estrogenic effects, BPA was shown to bind to ARs, which can be related to excessive stimulation prostate and can lead to cancer. BPA was

suggested to modulate androgen-dependent prostate cancer cell proliferation even in the concentrations corresponding to its level determined in human blood [119].

Metal food and beverage cans have a thin coating of BPA on the interior surface, which is essential to prevent corrosion of the can and contamination of food. Moreover, fetuses and young infants are commonly exposed to BPA by transplacental transfer of maternal BPA and through ingestion of maternal milk or formula in BPA containing plastic bottles [120].

BPA exhibits moderate acute toxicity to vertebrates. Through the oral, i.p., and i.v. routes, LD_{50} doses of BPA in rats were found to be 3250, 841, and 35.26 mg/kg body weight, respectively [121, 122]. On the contrary, LD_{50} doses in mice were found to be 2400 and 150 mg/kg via oral and i.p. routes, respectively. Moreover, the U.S. EPA estimated the reference dose as 50 μg BPA/kg body weight/day [123]. Studies concerning the toxic effects of BPA suggested that humans are more susceptible than rodents to the deleterious action of this substance [124]. Recently, intensive investigations are being realized that refer to toxic, teratogenic, carcinogenic, and particularly estrogenic mechanisms of BPA action.

7.1. Biotransformation of Bisphenol A

BPA can be biotransformed in vertebrates, invertebrates, and plants and it is also biodegraded by microorganisms including bacteria, fungi, and algae. There are differences of species or strain in the metabolism of BPA. It was reported that, in primates, BPA can more easily be absorbed orally or subcutaneously (s.c.) compared to rats, and primates need a longer period of time to eliminate BPA from serum than rats [125]. Moreover, human liver microsomes do not have the ability to glucuronidate BPA as extensively as rat liver microsomes [126]. The metabolism of BPA is faster in female rats than in male rats [127]. It was suggested that gender differences in serum BPA concentrations of adult humans may be caused by differences in the androgen-related metabolism of BPA [128]. In addition, Kim et al. reported higher levels of BPA glucuronide in men than in women [129]; however, the levels of BPA sulfate were higher in women than in men [130].

BPA glucuronide is the major metabolite of BPA. Other metabolites (BPA sulfate conjugate, BPA diglucuronide, 5-hydroxy BPA, and the corresponding sulfate conjugate) were also reported. BPA glucuronide has lower estrogenic effect compared to the main compound [131–134]. 5-Hydroxy BPA is also less estrogenic than BPA [132, 133]. The sulfate metabolite of BPA does not show an estrogenic effect up to 1 mM [134].

7.2. Genotoxic effects of Bisphenol A

BPA did not show any mutagenic effect in Ames test even after metabolic activation [135]. However, BPA was shown to cause DNA damage in eukaryotic cells, that is, BPA induced DNA strand breaks in L5178Y mouse lymphoma cells [136] and induced aneuploidy and structural chromosomal aberrations in ER-positive MCF-7 cells and in CHO-K1 cells [132, 133, 137]. The genotoxic potential of BPA was also evaluated by Lee et al. [138]. In mutant chicken DT40 cell lines (deficient in DNA repair pathways), researchers determined chromosomal aberrations and double-strand breaks [138]. BPA was shown to induce ROS generation, which

in turn caused an induction of the production of DNA oxidative bases [139]. However, Audebert et al. [140] did not observe any genotoxicity when BPA was administered to three human cell lines [human intestinal cell line (LS174T), hepatoma cell line (HepG2), and renal cell line (ACHN)]. On the contrary, BPA metabolites (BPA-quinone) were shown to induce DNA damage by forming covalent adducts with DNA and adducts with deoxyguanosine [131, 141].

BPA was also suggested to alter gene and protein expression. Recently, Fernandez et al. [142] showed that BPA increased the expression of some genes (i.e., BRCA1, BRCA2, BRCC3, and BCL2L11) that are involved in DNA repair and apoptosis in human breast epithelial cells. The authors suggested that women who have BRCA1 or BRCA2 mutations may be more susceptible to such effects of BPA [142].

7.3. Epigenetic effects of Bisphenol A

In utero or neonatal exposure to low doses of BPA may cause alterations in DNA methylation, modifications in histones, and changes in the expression patterns of noncoding RNAs. These changes can up-regulate or down-regulate different gene expressions, which in turn may result in permanent health effects such as neural and immune disorders, infertility, and late onset of complex diseases. BPA induced permanent alterations in DNA methylation patterns of different genes that are responsible for cellular signaling [64, 143].

The epigenetic effect of BPA was clearly demonstrated in viable yellow mice [144]. By decreasing CpG methylation in the IAP retrotransposable sequence inserted upstream of the Agouti gene, the maternal exposure to BPA shifted the coat color distribution of viable yellow mouse offspring toward yellow. This effect was completely prevented by maternal supplementation with folic acid or the phytoestrogen genistein, both of which are sources of methyl group [145].

BPA was suggested to induce mainly breast and prostate cancer in both animals and humans. Keri et al. [146] suggested that BPA may increase the risk of such cancers by affecting various cell processes such as DNA methylation and chromatin remodeling during development. BPA exposure was shown to cause epigenetic alterations in rodent prostate and have been postulated to be the underlying cause of neoplastic development in later life [147]. Neonatal exposure of rats to BPA resulted in an increased incidence of prostate intraepithelial neoplasia, and the prostate tissues showed consistent methylation changes. For example, the phosphodiesterase type 4 variant 4 (Pde4d4) gene was found to be hypomethylated at the regulatory CpG island and started to have an elevated expression in the adult rat prostate [148, 149]. On the contrary, neonatal BPA exposure was also reported to alter the promoter methylation and expression of nucleosome binding protein-1 (Nsbp1) and hippocalcin-like 1 (Hpcal1) genes in rats [150]. Also, the neonatal exposure to BPA was shown to induce hypermethylation of ER promoter regions in rat testis. This phenomenon mediated epigenetic changes that in turn induced adverse effects on spermatogenesis and fertility [151].

Concerning miRNAs, BPA exposure of human placental cell lines has been shown to alter miRNA expression levels; particularly, miR-146a was strongly induced after BPA application.

Induction of miR-146a caused slower cellular proliferation rates and higher sensitivity to bleomycin, which strongly induces DNA strand breaks [152]. In mouse Sertoli cell line (TM4), 24 h BPA exposure leads to up-regulation or more often to down-regulation of 37 miRNAs [153].

7.4. Carcinogenicity of Bisphenol A

Currently, there is no evaluation of the IARC for BPA. However, "bisphenol A diglycidyl ether" is classified as a Group III carcinogen (not classifiable as to its carcinogenicity to humans) by the IARC [154].

At low doses, BPA was shown to alter mammary gland development and increased incidence of tumors in Sprague-Dawley rats [155]. Besides, at the comparable amounts to a reference dose, BPA caused development of breast, prostate, and nipple cancers in both mice and rats [146]. Acevedo et al. [156] recently evaluated the malignant potential of BPA in rats and showed that suggested that BPA acted as a mammary gland carcinogen at doses comparable to those present amounts in human urine and blood determined by epidemiological studies. BPA was also suggested to cause the development of hematopoietic cancers and induced testicular cancer in mice and rats [157].

It was also shown that BPA promoted proliferation of human epithelial ovarian cancer cells (OVCAR-3), increased the susceptibility of endometrial cells to the effects of estradiol, and inhibited the 17-β-estradiol-induced genomic activity of ERβ in DLD-1 colon cells [158–161].

7.5. Reproductive toxicity of Bisphenol A

Perinatal exposure to environmentally relevant BPA doses may predispose the tissue to earlier onset of disease, reductive fertility, and mammary and prostate cancers, as BPA may cause morphological and functional alterations in both male and female genital tracts and mammary glands. The estrogenicity of BPA has been shown by many rodent studies. When pregnant mice were exposed to environmentally relevant doses of BPA, earlier vaginal opening and earlier first estrous cyclicity in their offspring were observed. BPA elevated prostate weight in mice. An advanced reproductive aging was also observed [162]. BPA was also shown to disturb the development of reproductive organs, testosterone excretion, and sperm production in mice [163].

Some studies have suggested that BPA may not alter estrogenic function in rodents. Ryan et al. [164] showed that BPA exposure of male and female rats (2–200 μg/kg body weight/day) did not affect maternal pregnancy or weight gain or F_1 female birth weight as well as reproductive morphology, fertility, fecundity, or sexual dimorphic behaviors. Recently, Ziv-Gal et al. [165] suggested that BPA at low doses (110–438 μM) decreased estradiol levels and inhibited growth of follicles isolated from wild-type and AhR knockout mice and that AhR signaling pathways may be significantly involved in the modulatory effect of BPA on follicular growth.

7.6. Hepatotoxic effects of Bisphenol A

Atkinson and Roy [130] found that the BPA metabolite, bisphenol-*o*-quinone, could bind DNA *in vitro* and *in vivo*, leading to the induction of hepatotoxicity. Besides, BPA was also shown to cause damage to hepatocytes by oxidative stress. BPA markedly decreased antioxidant enzymes and glutathione *S*-transferase activities as well as depleted reduced glutathione levels in rats. Moreover, BPA (50 mg/kg) significantly increased the biochemical levels of liver enzymes and reduced the expression of hepatic antioxidant genes. The authors concluded that BPA generated ROS and reduced the antioxidant gene expression causing hepatotoxicity.

Huc et al. [166] observed that low BPA doses of BPA led to hepatic (HepG2) cell damage and this effect might be due to significant mitochondrial dysfunction including ROS production, mitochondrial transmembrane hyperpolarization, lipid peroxidation, and release of proinflammatory cytokines. Moon et al. [167] also observed that BPA exposure caused oxidative and proinflammatory damage in rat hepatocytes. The researchers observed a decrease in glutathione peroxidase activity, an increase in lipid peroxidation, and decreases in the proinflammatory cytokines [i.e., interleukin (IL)-6 and tumor necrosis factor-α]. Moreover, the researchers showed that, at low doses, BPA decreased the oxygen rate and ATP production and caused mitochondrial dysfunction. Based on these studies, they concluded that BPA induced hepatic mitochondrial dysfunction below the no observed effect (NOEL) value (5 mg BPA/kg body weight/day) [167].

7.7. Effects of Bisphenol A on other organs/systems

BPA was proven to affect not only estrogenic system but also functions of androgens, prolactin, insulin, and thyroid hormones [117, 168]. Gentilcore et al. [169] observed that BPA at low doses (10^{-9} M) affected the expression of the genes involved in thyroid hormone synthesis, thyroid follicular cells, and altered thyroid-specific transcriptional factors in zebrafish.

In vitro experiments showed that BPA may cause toxicity in nervous system cells. BPA in high concentrations (>100 μM) was shown to induce apoptosis of hippocampal neuronal cells by increasing calcium and ROS levels and then by activating caspase-3 and mitogen-activated protein kinases (MAPK) [170]. In rat embryonic midbrain cells, at relatively low concentrations (10^{-12} to 10^{-4} M), BPA caused S- and G_2/M-phase arrests and elevated the percentage of apoptotic cells. BPA also lowered the phosphorylation of c-Jun N-terminal kinase and increased the mRNA expression level of proapoptotic proteins (i.e., Bax and p53) [171].

BPA did not cause morphological and neurobehavioral changes in F_1 offspring of rats treated with different doses (0.15–2250 ppm) [172]. BPA (administrated 2–200 μg/kg/body weight/day by gavage) did not have any impact on the sensory system and neurobehavioral activity in Long-Evans rats [163]. However, some studies showed that BPA may have an effect on the neurotransmitter levels of rodents. Nakamura et al. (2010) observed that BPA (20 μg/kg s.c.) had increased brain levels of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC), whereas the levels of serotonin and its derivative, 5-hydroxyindoleacetic acid (5-HIAA), decreased in pregnant mice [173].

BPA was suggested to both stimulate and inhibit the activity of immune system cells. It is postulated that BPA can modulate the immune activity by affecting ERs, AhR, and probably peroxisome proliferator-activated receptor (PPAR) [174]. Youn et al. [175] showed that BPA (in drinking water) caused increases in interferon-γ and decreases in IL-4 production in T lymphocytes of mice, whereas Lee and Lim [176] observed that BPA elevated IL-4 and IL-8 levels in mouse T lymphocytes. Exposure of mouse splenic lymphocytes to low BPA concentrations (1 μM) could inhibit mitogenesis, particularly the mitogenesis of B lymphocytes [177]. Goto et al. [178] observed that BPA produced lymphocytes with higher amounts of immunoglobulin A (IgA) and IgG2a in mice. Sugita-Konishi et al. [179] reported that BPA caused depletion in neutrophil activity and inhibited IL-6 formation in mice infected with nonpathogenic *Escherichia coli*.

Roy et al. [180] showed that offspring of female mice exposed to BPA were more susceptible to infection by influenza A virus because of the modulation of their innate immunity by BPA. However, the researchers did not observe impairment in antiviral adaptive immune response, which is a crucial response for virus clearance and survival.

7.8. Epidemiological studies concerning the effects of Bisphenol A on reproductive/endocrine systems

Most of the epidemiological studies on BPA mainly focus on its effects on endocrine and reproductive systems. Meeker et al. [181] analyzed urinary BPA concentrations of men (n=167) who were admitted to an infertility clinic. The researchers reported that their urinary BPA levels were inversely correlated with their estradiol/testosterone ratio [181]. In a cross-sectional study, Melzer et al. [182] determined the urinary BPA levels of subjects from Chianti, Italy (n=1453, age=20–102 years), and observed positive associations between higher urinary BPA concentrations and higher expression of two estrogen-responsive genes, encoding ERβ and estrogen-related receptor α (ERRα). Li et al. [183] examined the urinary BPA levels of 218 men with and without BPA exposure in the workplace. Increasing urinary BPA levels were statistically significantly associated with decreased sperm concentration, total sperm count, sperm vitality and sperm motility after adjustment for potential confounders using linear regression. Men with detectable urinary BPA concentrations had more than risk of decreased sperm concentration, sperm vitality, count, and motility compared to men who had undetectable urinary BPA levels. However, urinary BPA levels were not associated with semen volume or abnormal sperm morphology [183].

Takeuchi and Tsutsumi [127] investigated serum BPA concentrations of men (n=11), women (n=14), and women with polycystic ovary syndrome (PCOS; n=16). Serum BPA levels were significantly higher in normal men and in women with PCOS. There were significant positive correlations between serum BPA and total testosterone and free testosterone concentrations in all subjects and between serum BPA and total testosterone (r 5 0.559; P<0.01) and free testosterone concentrations in all female subjects; however, there was no association between serum BPA and other sex-related hormone concentrations in any group. The researchers determined that there were gender differences in serum BPA concentrations, where men had higher serum BPA levels (1.49 ± 0.11 ng/mL in men, 1.04 ± 0.10 ng/mL in women with PCOS,

and 0.646; 0.10 ng/mL in women) possibly due to the differences in the androgen-related metabolism of BPA [27].

Miao et al. [184] conducted a cross-sectional study among female workers from BPA-exposed (n=106) and unexposed (*n*=250) factories in China. They observed a significant positive association between increased urine BPA concentration and higher prolactin and progesterone levels. Among exposed workers, a positive association between urine BPA and estradiol was also determined. In addition, a statistically significant inverse correlation between urinary BPA concentration and FSH was found among unexposed group. The researchers suggested that BPA exposure may lead to alterations in female reproductive hormone levels [184]. Mínguez-Alarcón et al. [185] conducted a prospective cohort study at the Massachusetts General Hospital Fertility Center, which included 256 women (*n*=375 *in vitro* fertilization cycles) who provided up to two urine samples before oocyte retrieval (total *N*=673) between 2004 and 2012. Urinary BPA concentrations were not associated with endometrial wall thickness, peak estradiol levels, proportion of high-quality embryos, or fertilization rates. The researchers did not observe any correlation between urinary BPA concentrations and implantation, clinical pregnancy, or live birth rates per initiated cycle or per embryo transfer. Women older than 37 years had thinner endometrial thickness across increasing quartiles of urinary BPA concentrations, whereas women younger than 37 years had thicker endometrial thickness across increasing quartiles of urinary BPA concentrations [185]. A cross-sectional study was carried out by Liu et al. [186] to determine the associations between urinary BPA levels serum reproductive hormone levels among male Chinese adults (*n*=592). A multiple linear regression and log-binomial model was used to examine the associations between urinary BPA level and hormone levels after controlling for age and smoking status. Increased urinary BPA levels were positively and significantly correlated with prolactin, estradiol, and SHBG levels and were negatively and significantly associated with androstenedione and free androgen index levels. The researchers suggested that high urinary BPA levels were associated with increased prolactin, estradiol, and SHBG level in males and these associations may contribute to male infertility [186]. Liu et al. [187] conducted a cross-sectional study to investigate the correlations between maternal phenolic exposure and cord sex steroid hormones and AGD in male newborns. Mother-infant pairs from each of two hospitals [one in a polluted town (Guiyu; *n*=77) and the other in a cleaner town (Haojiang; *n*=60)] were recruited in the study. Maternal urinary BPA concentrations (log_2 transformed) were negatively correlated with testosterone levels and testosterone/estradiol ratio in male fetal cord blood samples (after adjustment for potential confounders in linear regression models). No significant associations between AGD or anogenital index (AGI) and BPA or cord hormone levels were found [187].

Another study by our group recruited nonobese girls newly diagnosed with idiopathic central precocious puberty (ICPP; *n*=28; ages 4–8 years) and 25 healthy age-matched girls with no history of ICPP or any other endocrine disorder. Urinary BPA levels were significantly higher in ICPP group compared to the control group. There were no significant associations between urinary BPA levels and serum LH, FSH, and estradiol levels [188].

8. Conclusion

EDCs are widely available in the environment. Although exposure to these chemicals can be reduced by not using plastics, the total prevention of exposure is not feasible. Serious measures must be taken to reduce the availability of EDCs and regulatory authorities must be aware of their toxic effects and the outcomes. Parents should avoid using plastic materials and EDC-containing materials (phthalate containing gum shields, toys, and paints; BPA-containing feeding bottles and water bottles) for children, particularly for newborns. Therefore, the reduction of the use of phthalates and BPA must seriously be taken into concern, and chemical companies must be encouraged to synthesize and produce nontoxic alternatives of these substances.

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